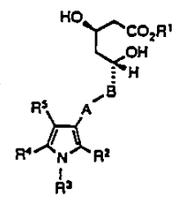
Table I. Inhibition of Solubilized Rat Liver HMG-CoA Reductase in Vitro[a] for Compounds of the General Structure 1,[c] 2,[c] and 13[d]

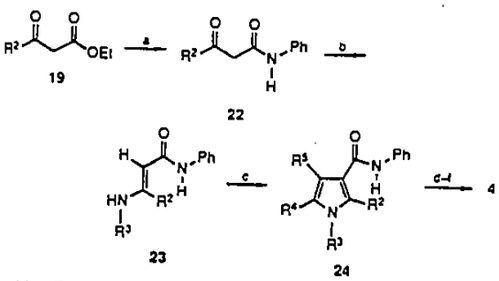


1[e], 2[c], and 13[d]

no.	R^1	R^2	R^3	R^4	R^5	A–B	formula	anal.[f]	IC_{50},[g] nM	rel[g] pot.
1a	Na	CH_3	Ph	H	*p*-C_6H_4F	CH=CH	$C_{24}H_{23}FNO_4Na$	C, H, N	65	12
1b	Na	*i*-Pr	Ph	H	*p*-C_6H_4F	CH=CH	$C_{26}H_{27}FNO_4Na$	C, H, N	7	110
2b	Na	*i*-Pr	Ph	H	*p*-C_6H_4F	CH_2CH_2	$C_{26}H_{29}FNO_4Na$	C, H, N	6	128
13b	Na	*i*-Pr	Ph	H	*p*-C_6H_4F	CH_2CH_2	$C_{26}H_{29}FNO_4Na$	C, H, N	3	257
1c	Na	CH_3	Ph	CH_3	*p*-C_6H_4F	CH=CH	$C_{25}H_{25}FNO_4Na$	C, H, N	250	3
2c	Na	CH_3	Ph	CH_3	*p*-C_6H_4F	CH_2CH_2	$C_{25}H_{27}FNO_4Na$	C, H, N	70	11
1d	Na	CH_3	*i*-Pr	H	*p*-C_6H_4F	CH=CH	$C_{21}H_{25}FNO_4Na$	C, H, N	330	2
2d	Na	CH_3	*i*-Pr	H	*p*-C_6H_4F	CH_2CH_2	$C_{21}H_{27}FNO_4Na$	C, H, N	100	9
1e	Na	*i*-Pr	*i*-Pr	H	*p*-C_6H_4F	CH=CH	$C_{23}H_{29}FNO_4Na$	C, H, N	117	6
2e	Na	*i*-Pr	*i*-Pr	H	*p*-C_6H_4F	CH_2CH_2	$C_{23}H_{31}FNO_4Na$	C, H, N	18	42
13e	Na	*i*-Pr	*i*-Pr	H	*p*-C_6H_4F	CH_2CH_2	$C_{23}H_{31}FNO_4Na$	C, H, N	9	85
1f	Na	*i*-Pr		H	*p*-C_6H_4F	CH=CH	$C_{26}H_{33}FNO_4Na$	C, H, N	69	12
2f	Na	*i*-Pr		H	*p*-C_6H_4F	CH_2CH_2	$C_{26}H_{33}FNO_4Na$	C, H, N	9	92
1g	Na	*i*-Pr	Ph	CH_3	*p*-C_6H_4F	CH=CH	$C_{27}H_{29}FNO_4Na$	C, H, N	6	125
2g	Na	*i*-Pr	Ph	CH_3	*p*-C_6H_4F	CH_2CH_2	$C_{27}H_{31}FNO_4Na$	C, H, N	5	149
13g	Na	*i*-Pr	Ph	CH_3	*p*-C_6H_4F	CH_2CH_2	$C_{27}H_{31}FNO_4Na$	C, H, N	2.5	300
mevinolin							$C_{24}H_{37}O_6Na$		8	100

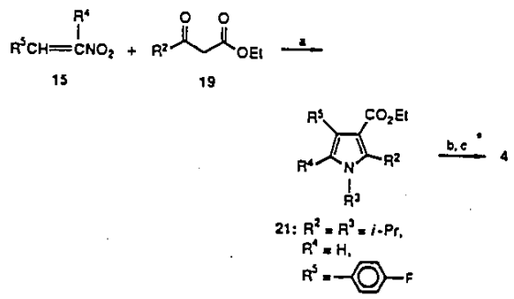
[a] The assay system described in ref 1 was used. [b] Ring-opened sodium dihydroxy carboxylate form, optically pure. [c] Racemic. [d] Optically active 3*R*,5*R* configuration. [e] Analytical results were within ±0.4% of the theoretical value. [f] IC_{50} values were determined by using four or five concentrations of each inhibitor. [g] For estimation of relative inhibitory potencies, mevinolin was assigned a value of 100. The IC_{50} value of test compound was compared with that of mevinolin, corrected for the somewhat different molecular weight.

Scheme V[a]



[a] (a) $PhNH_2$/AcOH; (b) R^3NH_2/AcOH/$-H_2O$; (c) 15/Δ; (d) NaH/CH_3I/toluene/Δ; (e) $LiAlH_4$/Δ; (f) CrO_3/pyridine.

Scheme VI[a]



[a] (a) R^3NH_2/CH_3OH/25 °C/1 day; (b) $LiAlH_4$; (c) 4 equiv of  /0.02 equiv of $(Ph_3P)_3RuCl_2$.

functionality much faster than the keto group of 19. In this case, it was necessary to preform the anilides 22 (Scheme V). Addition of aliphatic or aromatic primary amines R^3NH_2 to 22 under acid catalysis gave 23, which were cyclocondensed with nitroethenes 15 to give 3-pyrrolecarbanilides 24. While amides on LAH reduction usually lead to the corresponding amines, carbanilides 24 could be reduced to the corresponding aldehydes 4 via N-methylation, LAH treatment, and subsequent oxidation.

A new three-component coupling reaction allowed a one-pot synthesis of ethyl 1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (21, Scheme VI).

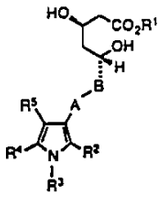
When a methanolic solution of β-nitro-*p*-fluorostyrene (15: R^4 = H, R^5 = *p*-C_6H_4F), β-keto ester 19 (R^2 = *i*-Pr), and isopropylamine was stirred at ambient temperature, the pyrrole ester 21 was obtained in 50% yield. LAH reduction followed by ruthenium(II)-catalyzed oxidation of the alcohol with *N*-methylmorpholine-*N*-oxide[11] gave the corresponding aldehyde 4. This convenient three-component coupling may also be applicable for the syntheses of pyrrole esters 21 with other substitution patterns for R^2–R^5.

Biological Results and Discussion

The racemic sodium salts (1 and 2, R^1 = Na) as well as the optically active sodium salts 13 (R^1 = Na) were evaluated for their ability to inhibit solubilized, partially purified rat liver HMG-CoA reductase in vitro (Table I) and

(11) Sharpless, K. B.; Akashi, K.; Oshima, K. *Tetrahedron Lett.* 1976, *29*, 2503.

Table II. Inhibition of Cellular HMG-CoA Reductase in Cultures of HEP G2 Cells[a] for Sodium Salts of the General Formula 1[c] and 2[c]



1[c] and 2[c]

	IC_{50},[d] μM	relative[e] potency
mevinolin[b]	0.05	100
1a	0.83	6
1b	0.014	350
2b	<0.01	>500
1c	5.0	1
2c	0.57	9
1d	6.0	1
2d	0.27	19
1e	0.05	100
2e	0.002	2500
1f	0.106	47
2f	0.018	275

[a] Assay described in the preceding paper.[1] [b] Ring-opened sodium dihydroxy carboxylate form, optically pure. [c] Racemic. For definition of R^1–R^5 and A–B see Table I. [d] IC_{50} values varied somewhat for different batches of cells. Mevinolin sodium salt averaged $IC_{50} = 5 \times 10^{-9}$ M and was used in every run as an internal standard. The measured IC's for test compounds 1 and 2 were corrected for deviations of mevinolin's IC from its average value. [e] Mevinolin was assigned a value of 100. Potencies were obtained by comparison of racemic test compounds 1 or 2 with the internal standard mevinolin.

to inhibit *cellular* HMG-CoA reductase in cultures of hepatic cells (HEP G2, a human hepatoma cell line), as determined by the inhibition of the incorporation of sodium [^{14}C]acetate into cholesterol (Table II).

Selected compounds were evaluated for their ability to inhibit hepatic cholesterol "de novo" synthesis in male rats after po administration, as determined by the inhibition of the incorporation of sodium [^{14}C]octanoate[12] into hepatic cholesterol (Table III).

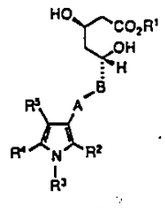
Selected compounds were further evaluated for their ability to decrease plasma cholesterol levels in normolipemic rabbits and dogs after po administration.

All tests were also conducted under the same experimental conditions with optically pure mevinolin. The respective results are included in Tables I–III. For substitution patterns "b", "e", and "g", we prepared and tested the racemic 2 as well as the optically active 3*R*, 5*R* sodium salt 13. Optically active compounds 13 proved to have twice the potency in HMG-CoA reductase inhibition than the structurally identical but racemic 2 (Table I). This result was expected, since the antipode of the configuration drawn for 1, 2, and 13, is biologically inactive.[13]

(12) Dietschy, J. M.; McGarry, J. D. *J. Biol. Chem.* 1974, *249*, 52. Andersen, J. M.; Dietschy, J. M. *J. Lipid Res.* 1979, *20*, 740. Stenge, E. F.; Dietschy, J. M. *J. Lipid Res.* 1983, *24*, 72.

(13) 3-epi, 5-epi, and 3,5-bis epi isomers of compactin and mevinolin have been reported to be biologically inactive: Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. *J. Med. Chem.* 1987, *30*, 1858. Stokker, G. E.; Rooney, C. S.; Wiggins, J. M.; Hirshfield, J. J. *J. Org. Chem.* 1986, *51*, 4931. The biological inactivity of synthetic compactin analogues with 3*S* configuration has also been reported: Lee, T.-J. *Trends Pharmacol. Sci.* 1987, *8*, 442 and references cited therein.

Table III. Inhibition of Hepatic Cholesterol "De Novo" Synthesis in Vivo (Rat, Orally)[a]



1 and 2

	% cholesterol "de novo" synthesis	relative potency
no drug	100	
mevinolin[b]	14	100
1b[c]	5.5	255
2b[c]	5.6	250
2e[c]	9	156
2f[c]	6.0	233

[a] Assay described in ref 16. [b] Lactone form, optically pure, 5 mg/kg bw. [c] Racemic sodium salts, 10 mg/kg bw. For definition of R^1–R^5 and A–B see Table I.

For better comparison of structure–activity relationships in 1 and 2 as well as with extensive work on analogues of the phenolic type (isocyclic central aromatic, A = oxygen, B = CH_2),[14,15] R^5 was kept constant as *p*-fluorophenyl.

The work on analogues of the phenolic type[14,15] has shown that alkyl substitution of the second ortho position is essential and leads to optimal biological activity for an isopropyl substituent.

We concentrated on R^2 = methyl or isopropyl, since ortho substituents smaller (methyl, ethyl, longer *n*-alkyl) or larger (cyclopentyl, *tert*-butyl) than the isopropyl group decreased activity in analogues of the phenolic type[14,15] and since halogen substituents (Cl, Br) led to good activity but increased toxicity.

Table I shows that the isopropyl derivatives were more potent than the methyl derivatives by a factor of 10–40 (e.g. 1b vs 1a, 1g vs 1c, 2g vs 2c).

There is much tolerance concerning R^3. Variation of R^3 (Ph, *i*-Pr, cyclohexyl) led to only small activity changes (e.g. 2b vs 2e vs 2f, 1b vs 1e vs 1f, 1a vs 1d).

Substitution of R^4 = hydrogen for a methyl group either slightly decreased (e.g. 1a vs 1c) or slightly increased (2b vs 2g and 1b vs 1g) activity, depending on the nature of the other substituents. Hydrogenation of the trans olefinic bridge (A–B = (*E*)-HC=CH) had little influence on the biological activity of 1 in vitro (e.g. 1b vs 2b, 1c vs 2c; 1d vs 2d, 1e vs 2e, 1f vs 2f, 1g vs 2g); however, the hydrogenated derivatives 2 were much less acid sensitive (vide supra) and much more active in vivo.

In the HEP G2 cell-test (Table II) the racemic compounds 1b, 2b, and 2e are 3.5, 5.0, and 25 times, respectively, more active than optically pure mevinolin sodium salt of the same concentration. General trends in Tables I and II are comparable. The superiority of 1b, 2b, and

(14) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Jendralla, H.; von Kerekjarto, B.; Kesseler, K.; Krause, R.; Wess, G. International Symposium on Cholesterol Control And Cardiovascular Diseases: Prevention And Therapy, Milan (Italy) July 7–9, 1987; Abstract book page 133.

(15) European Application 0216 127, 1987.

(16) Dietschy, J. M.; Spady, D. K. *J. Lipid Res.* 1984, *25*, 1469. Alfin-Slater, R. B.; Deuel, H. J., Jr.; Scholtz, M. C.; Shimoda, F. K. Group Report No. N3, 1950; University of Southern California, Consolidated Eng. Corp.

especially 2e compared with mevinolin is more pronounced in the cell test. Inhibition of hepatic cholesterol "de novo" synthesis in vivo by oral 1b or 2b is about 2.5 times stronger than that for mevinolin (Table III). In normally fed rabbits ($n = 6$), 20 mg/kg racemic 2b decreased total plasma cholesterol levels by 34% after oral administration for 10 days (optically pure mevinolin at 10 mg/kg for 10 days, 25%), while 1b was totally inactive under the same conditions. The reason for the lack of activity of 1b in the rabbit experiment is currently not known.[22] The chemically demonstrated acid sensitivity of 1b (vide supra) would suggest that, contrary to 2b, 1b may not survive the stomach passage. However this view is not consistent with the comparable activity of 1b and 2b to inhibit hepatic cholesterol "de novo" synthesis in rats after po administration (Table III). In normally fed rabbits ($n = 4$), 10 mg/kg racemic 2e decreased total plasma cholesterol levels by 42% after oral administration for 6 days (optically pure mevinolin at 10 mg/kg for 6 days, 25%).[17,18]

In normally fed male beagle dogs ($n = 4$), 20 mg/kg racemic 2b decreased LDL-cholesterol levels by 48% and increased HDL-cholesterol levels by 14% after oral administration for 14 days (optically pure mevinolin at 10 mg/kg for 19 days: LDL-cholesterol −18%, HDL-cholesterol +2%).[18]

In conclusion, some compounds of general formula 2 exceeded mevinolin in their ability to inhibit HMG-CoA reductase in vitro and to inhibit cholesterol biosynthesis in vivo. They are promising candidates for development as antiarterosclerotic agents.

Experimental Section

For general remarks see the preceding paper in this issue.[1] ^{1}H NMR spectra were recorded in $CDCl_3$, unless noted otherwise. All starting materials were commercially available unless indicated otherwise.

1-(*p*-Fluorophenyl)-2-nitropropene (15). A solution of *p*-fluorobenzaldehyde (84 g), nitroethene (69.4 g), and *n*-butylamine (4 mL) in xylol (110 mL) was refluxed for 20 h under a Dean–Stark trap. On cooling to 0 °C, 21.7 g of the product crystallized (mp 64–65 °C). To the filtrate were added nitroethene (41.4 g) and *n*-butylamine (3 mL), and the solution was refluxed for 14 h under a Dean–Stark trap. The solution was evaporated in vacuo and the residue was digerated with methanol at 0 °C, until crystallization occurred. The crystals were collected and washed with cold methanol (53.8 g, mp 65–66 °C). Anal. ($C_9H_8FNO_2$) C, H, F, N.

Ethyl 3-(Phenylamino)-but-2(*E*)-enoate (20). A solution of aniline (45.5 mL, 0.5 mol), ethyl acetoacetate (63.5 mL, 0.5 mol), and glacial acetic acid (1 mL) in toluene (100 mL) was refluxed for 4 h under a Dean–Stark trap. The solvent was evaporated and the residue was distilled to give 57.9 g of colorless oil: bp 118–120 °C (1.5 mm); MS $C_{12}H_{15}NO_2$ m/e = 205 (M^+). Anal. ($C_{12}H_{15}NO_2$) C, H, N.

***N*,*N*-Bis[3-(4-fluorophenyl)-4-(methoxycarbonyl)-5-methyl-2,3-dihydrofuran-2-yl]hydroxylamine (17).** To a stirred solution of sodium methanolate (2.92 g, 54 mmol) in methanol (54 ml) was added methyl acetoacetate (20.9 g, 180 mmol) dropwise at 0 °C followed by 4-fluoro-β-nitrostyrene[19] (30.1 g, 180 mmol). After 15 min, a thick mash formed that was allowed to stand for 2 h at 0 °C. The solid was collected by suction, washed with ice-cold methanol, and dried over P_4O_{10} in vacuo to give 22.0 g of colorless solid: mp 139–141 °C; 7.0 g of product were obtained from the mother liquor; NMR δ 2.25 (6 H, s), 3.32 (3 H, s), 3.50 (3 H, s), 4.30 (2 H, dd), 5.40 (2 H, d), 7.16 (8 H, d), 8.72 (1 H, s); MS $C_{26}H_{25}F_2NO_7$ FAB m/e = 502 (M + H$^+$), 458, 235. Anal. ($C_{26}H_{25}F_2NO_7$) C, H, F, N.

1-Phenyl-2-methyl-3-(methoxycarbonyl)-4-(4-fluorophenyl)-1*H*-pyrrole (18a). Aniline (5.59 g, 60 mmol) was added to a solution of hydroxylamino compound 17 (15 g, 30 mmol) in ethanol (600 mL). The mixture was refluxed for 24 h. Aniline (1.1 g) was added and the mixture was refluxed for 16 h. The solvent was removed in vacuo and the residue was distributed between dichloromethane and 1 N hydrochloric acid. The organic layer was washed with saturated sodium bicarbonate solution and then with brine, dried, and concentrated. The residue was chromatographed with *n*-hexane/ether/dichloromethane (16:3.5:0.5) over silica, giving 4.0 g of reddish, thick oil: NMR δ 2.43 (3 H, s), 3.70 (3 H, s), 6.70 (8 H, s), 6.87–7.66 (9 H, m); MS $C_{19}H_{16}FNO_2$ m/e = 309 (M^+), 278, 248. Anal. ($C_{19}H_{16}FNO_2$) C, H, F, N.

1-Isopropyl-2-methyl-3-(methoxycarbonyl)-4-(4-fluorophenyl)-1*H*-pyrrole (18d). Isopropylamine (3.6 g, 60 mmol) was added to a suspension of hydroxylamino compound 17 (15 g, 30 mmol) in methanol (500 mL). The suspension was heated for 2 h at 40 °C and for 5 h at 50 °C, changing to a clear solution. The solvent was removed in vacuo and the residue was chromatographed with *n*-hexane/ether (4:1) over silica to yield 7.3 g of pale reddish crystals: mp 97–99 °C; NMR δ 1.42 (6 H, d), 2.53 (3 H, s), 3.65 (3 H, s), 4.37 (1 H, sept.), 6.60 (1 H, s), 6.80–7.46 (4 H, m); MS $C_{16}H_{18}FNO_2$ m/e = 275 (M^+), 244, 202, 201. Anal. ($C_{16}H_{18}FNO_2$) C, H, F, N.

Ethyl 1-Phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (21c). A solution of 20 (23.1 g, 113 mmol) and 15 (20.5 g, 113 mmol) in ethanol (250 mL) was refluxed for 30 h. The solvent was evaporated in vacuo and the residue was chromatographed over silica (1 kg) with cyclohexane/ethyl acetate (95:5) to give 26.0 of a colorless oil: NMR δ 1.05 (3 H, t), 1.85 (3 H, s), 2.3 (3 H, s), 4.1 (2 H, q), 6.9–7.6 (9 H, m); MS $C_{21}H_{20}FNO_2$ m/e = 337 (M^+), 308, 292. Anal. ($C_{21}H_{20}FNO_2$) C, H, F, N.

Preparation of Substituted 1*H*-Pyrrole-3-carboxaldehydes 4 from Substituted 3-(Alkoxycarbonyl)-1*H*-pyrroles 18 or 21. General Procedure. A solution of ester 18 or 21 (82 mmol) in ether (150 mL) was added dropwise at 0–5 °C to the stirred suspension of lithium aluminum hydride (7.8 g, 200 mmol) in ether (300 mL). The suspension was stirred for 1 h at 0 °C and then for 2 h at room temperature. At 0 °C, 35 mL of ethyl acetate and then 16 mL of water followed by 24 mL of 2 N aqueous sodium hydroxide were added dropwise. The suspension was stirred for 30 min at room temperature and filtered. The filtrate was concentrated in vacuo and the residue was chromatographed over 1 kg of silica with cyclohexane/ethyl acetate (2:1) containing 0.2% triethylamine (yield 85–95%).

To a solution of the substituted 3-(hydroxymethyl)pyrrole (70 mmol) in ether (1.2 L) and triethylamine (12 mL) was added activated manganese dioxide (182.5 g). The suspension was stirred at room temperature under nitrogen. After 24 h, the same amount of manganese dioxide was added. After 24 h the solid was removed and washed with ether. The filtrates were concentrated in vacuo; the residue was chromatographed over silica with cyclohexane/ethyl acetate (6:1) containing 0.1% triethylamine (yield 65–85%).

1-Phenyl-2,5-dimethyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: colorless oil, crystallizing on standing; NMR δ 1.3 (1 H, br s), 2.0 (3 H, s), 2.1 (3 H, s), 4.55 (2 H, s), 6.9–7.65 (9 H, m); MS $C_{19}H_{18}FNO$ m/e = 295 (M^+), 278 (M^+ − OH). Anal. ($C_{19}H_{18}FNO$) C, H, F, N.

1-Phenyl-2-methyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: pale yellow, resinous solid; NMR δ 1.5 (1 H, br s), 2.26 (3 H, s), 4.63 (2 H, s), 6.87 (1 H, s), 6.93–7.70 (9 H, m); MS $C_{18}H_{16}FNO$ m/e = 281 (M^+), 264 (M^+ − OH). Anal. ($C_{18}H_{16}FNO$) C, H, F, N.

1-Isopropyl-2-methyl-3-(hydroxymethyl)-4-(fluorophenyl)-1*H*-pyrrole: colorless oil; MS $C_{15}H_{18}FNO$ m/e = 247 (M^+ − OH), 188. Anal. ($C_{15}H_{18}FNO$) C, H, F, N.

1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4a): yellow, resinous solid; NMR δ 2.50 (3 H, s), 6.80 (1 H, s), 6.85–7.70 (9 H, m), 10.03 (1 H, s); MS $C_{18}H_{14}FNO$ m/e = 279 (M^+), 278 (M^+ − H). Anal. ($C_{18}H_{14}FNO$) C, H, F, N.

1-Phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4c): yellow solid; NMR δ 1.94 (3 H, s), 2.35 (3 H, s), 6.95–7.7 (9 H, m), 9.85 (1 H, s); MS $C_{19}H_{16}FNO$ m/e =

(17) Hypocholesterolemic activity in rabbits was tested following the protocol described in ref 1.

(18) Hypocholesterolemic activity in animal studies will be described in detail in a future publication.

(19) Gattermann-Wieland *Die Praxis des Organischen Chemikers*, 43rd ed.; W. de Gruyter: Berlin, 1982; p 361.

293 (M^+). Anal. ($C_{19}H_{16}FNO$) C, H, F, N.

1-Isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4d): colorless oil; NMR δ 1.43 (6 H, d), 2.60 (3 H, s), 4.30 (1 H, sept), 6.68 (1 H, s), 6.9–7.56 (4 H, m), 9.92 (1 H, s); MS $C_{15}H_{16}FNO$ m/e = 245 (M^+), 202. Anal. ($C_{15}H_{16}FNO$) C, H, F, N.

3-Oxo-4-methylpentanoic Acid Anilide (22). A solution of ethyl 3-oxo-4-methylpentanoate[9] (47.4 g, 0.3 mol), aniline (27.93 g, 27.3 mL, 0.3 mol), and acetic acid (0.6 mL) in toluene (360 mL) was refluxed for 4 h with a Dean–Stark trap. The cold mixture was washed twice with 0.5 N hydrochloric acid, twice with saturated sodium bicarbonate solution, once with brine, dried, concentrated, and chromatographed with toluene/ethyl acetate (10:1) over 1 kg of silica, giving 40.5 g (66% yield) of a pale pink oil: NMR δ 1.2 (6 H, d), 2.8 (1 H, sept), 3.65 (2 H, s), 7.0–7.75 (5 H, m), 9.1–9.4 (1 H, br s); MS $C_{11}H_{13}NO_2$ m/e = 205 (M^+), 93. Anal. ($C_{11}H_{13}NO_2$) C, H, F, N.

3-(Phenylamino)-4-methylpent-2(*E*)-enoic Acid Anilide (23b). A solution of ethyl 3-oxo-4-methylpentanoate[9] (31 mL, 0.2 mol), aniline (37 mL, 0.41 mol), and acetic acid (1.0 mL) in toluene (50 mL) was refluxed for 6 h with a Dean–Stark trap. The solvent was removed in vacuo. On cooling the residue crystallized. It was recrystallized from toluene/petroleum ether (80–110 °C) (2:1) to yield 38.7 g of colorless solid: mp 147–148 °C; a second crop of crystals can be obtained from the mother liquor; NMR δ 1.1 (7 H, d + m), 2.9 (1 H, sept), 4.75 (1 H, s), 6.8–7.6 (10 H, m), 11.1 (1 H, br s). Anal. ($C_{18}H_{20}N_2O$) C, H, N.

3-(Isopropylamino)-4-methylpent-2(*E*)-enoic Acid Anilide (23e). To a solution of anilide 22 (35.7 g, 174 mmol) and acetic acid (0.6 mmol) in toluene (600 ml), refluxing under a Dean–Stark trap, was added isopropylamine (20.6 g, 348 mmol) dropwise over 3 h. The mixture was refluxed for 16 h, concentrated in vacuo, and cooled, leading to crystallization. The solid was digerated with diisopropyl ether/petroleum ether (1:1), collected with suction filtration, and washed with petroleum ether, giving 28.9 g of colorless solid: mp 152–153 °C; NMR δ 1.1 (6 H, d), 1.25 (6 H, d), 2.73 (1 H, sept), 3.8 (1 H, m), 4.43 (1 H, s), 6.7 (1 H, s), 6.9–7.6 (5 H, m), 9.1–9.6 (1 H, br s); MS $C_{15}H_{22}N_2O$ CI m/e = 247 ($M + H^+$), 154. Anal. ($C_{15}H_{22}N_2O$) C, H, N.

3-(Cyclohexylamino)-4-methylpent-2(*E*)-enoic Acid Anilide (23f). A solution of anilide 22 (31.6 g, 154 mmol), acetic acid (1.5 mL), and cyclohexylamine (30.55 g, 308 mmol) in toluene (750 mL) was refluxed for 20 h under a Dean–Stark trap. The solvent was removed in vacuo, the residue was swirled with 150 mL of diisopropyl ether, collected with suction filtration, and washed with petroleum ether to give 27.1 g of a colorless solid (an addition 8.9 g came from the mother liquor): yield 82%; mp 123–132 °C; NMR δ 1.15 (6 H, d), 1.0–2.1 (10 H, m), 2.7 (1 H, sept), 3.45 (1 H, m), 4.4 (1 H, s), 6.55 (1 H, m), 6.9–7.6 (5 H, m), 9.5 (1 H, br s) MS $C_{18}H_{26}N_2O$ m/e = 286 (M^+), 194, 93. Anal. ($C_{18}H_{26}N_2O$) C, H, N.

Preparation of Substituted 1*H*-Pyrrole-3-carboxanilides 24 from Enamino Anilides 23. General Procedure. A solution of the nitro olefin 15 (95 mmol) and enamino carboxanilide 23 (100 mmol) in ethanol (300 mL) was refluxed for 12 h under nitrogen. Most of the solvent was removed in vacuo. Cooling of the residue in an ice bath gave crystals that were swirled in cyclohexane/ethyl acetate (200 mL), collected, and recrystallized.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxanilide (24b): yield 78%; mp 192–194 °C (from methanol); NMR δ 1.30 (6 H, d), 3.14 (1 H, sept), 6.73 (1 H, s), 7.00–7.70 (10 H, m). Anal. ($C_{26}H_{23}FN_2O$) C, H, F, N.

1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxanilide (24e): yield 50%; mp 131–133 °C (not recryst); NMR δ 1.45 (6 H, d), 1.55 (6 H, d), 3.75 (1 H, sept), 4.6 (1 H, sept), 6.7 (1 H, s), 6.7–7.6 (10 H, m); MS $C_{28}H_{25}FN_2O$ m/e = 364 (M^+), 272, 230. Anal. ($C_{28}H_{25}FN_2O$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxanilide (24f): yield 52%; mp 215–216 °C (not recryst); NMR δ 0.9–2.2 (16 H, d + m), 3.5–4.3 (2 H, m), 6.65 (1 H, s), 6.8–7.6 (10 H, m) MS $C_{26}H_{29}FN_2O$ CI m/e = 405 ($M + H^+$), 312, 230. Anal. ($C_{26}H_{29}FN_2O$) C, H, F, N.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrole-3-carboxanilide (24g): yield 80%; mp 190–192 °C (from cyclohexane/ethyl acetate); NMR δ 1.3 (6 H, d), 1.83 (3 H, s), 3.2 (1 H, sept), 6.8–7.6 (15 H, m); MS $C_{27}H_{25}FN_2O$ m/e = 412 (M^+), 320 (M^+ – PhNH). Anal. ($C_{27}H_{25}FN_2O$) C, H, F, N.

Preparation of Substituted 1*H*-Pyrrole-3-carboxaldehyde 4 from Substituted 1*H*-Pyrrole-3-carboxanilides 24. General Procedure. (a) N-Methylation. To a mechanically stirred solution of anilide 24 (55 mmol) in toluene (300 mL) was added a 50% dispersion of NaH in mineral oil (5.5 g, 115 mmol) at 2 °C under a nitrogen atmosphere. The suspension was warmed for 30 min at 60 °C and for 10 min at 100 °C. The suspension was cooled to 20 °C and methyl iodide (62.5 g, 440 mmol) was added. It was refluxed (bath at 75 °C) for 4–16 h, depending on steric hindrance (TLC control). With external cooling with dry ice/methanol, first water (80 mL) was added dropwise, followed by ether (400 mL). The organic phase was separated, washed with brine, dried, and concentrated in vacuo. The residues often crystallized when swirled with *n*-hexane or diisopropyl ether to a colorless to pale yellow solid. Oily products were purified by chromatography with cyclohexane/ethyl acetate/triethylamine (8:2:0.01) over silica.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 94%; mp 126–127 °C (not recryst); MS $C_{27}H_{25}FN_2O$ m/e = 412 (M^+), 306, 262. Anal. ($C_{27}H_{25}FN_2O$) C, H, F, N.

1,2-Diisopropyl-4-(4-fluorophenyl)-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 73%; oil; NMR δ 1.40 (12 H, d) 3.23 (4 H, s + sept), 4.40 (1 H, sept), 6.50 (1 H, s), 6.5–7.5 (9 H m); MS $C_{24}H_{27}FN_2O$ m/e = 378 (M^+), 272, 91. Anal. ($C_{24}H_{27}FN_2O$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 98%; mp 102–105 °C (not recryst); NMR δ 1.35 (3 H, d), 1.50 (3 H, d), 1.1–2.2 (11 H, m) 3.25 (3 H, br s) 3.95 (1 H, m), 6.4–7.4 (10 H, m); MS $C_{27}H_{31}FN_2O$ CI m/e = 419 ($M + H^+$), 312. Anal. ($C_{27}H_{31}FN_2O$) C, H, F, N

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-*N*-methyl-1*H*-pyrrole-3-carboxanilide: yield 84%; mp 62–63 °C (not recryst); NMR δ 1.2 (3 H, d), 1.3 (3 H, d), 1.8 (3 H, s), 2.8 (1 H, sept), 3.17 (3 H, s), 6.5–7.5 (14 H, m); MS $C_{28}H_{27}FN_2O$ m/e = 426 (M^+), 320 (M^+ – $PhNCH_3$). Anal. ($C_{28}H_{27}FN_2O$) C, H, F, N.

(b) Reduction. To a suspension of lithium aluminum hydride (60 mmol) in dry THF (120 mL) under nitrogen was added dropwise a solution of *N*-methylanilides (29 mmol) in THF (120 mL). The mixture was refluxed for 20 h and then cooled to 0 °C. Ethyl acetate (15 mL) and then water (5 mL) followed by 2 N sodium hydroxide solution (10 mL) were added dropwise. The mixture was stirred for 30 min at 25 °C. The solids were removed and washed with ether.

The filtrate was concentrated in vacuo. The residues often crystallized when swirled with *n*-pentane. Oily products were purified by chromatography with toluene/ethyl acetate/triethylamine (20:1:0.01) over silica.

1-Phenyl-2-isopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: yield 92%; oil; NMR δ 1.28 (7 H, d + m), 3.03 (1 H, sept), 4.70 (2 H, s), 6.73 (1 H, s), 6.90–7.70 (9 H, m); MS $C_{20}H_{20}FNO$ m/e = 309 (M^+), 294, 276. Anal. ($C_{20}H_{20}FNO$) C, H, F, N.

1,2-Diisopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: yield 75%; pale yellow oil that slowly crystallized; NMR δ 1.2–1.6 (12 H, m), 2.35 (1 H, br s), 3.33 (1 H, sept), 4.40 (2 H, s), 4.50 (1 H, sept), 6.70 (1 H, s), 6.8–7.65 (4 H, m); MS $C_{17}H_{22}FNO$ CI m/e = 275 (M^+), 258, 242, 200. Anal. ($C_{17}H_{22}FNO$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole: yield 67%; mp 114–116 °C (not recryst); NMR δ 1.37 (6 H, d), 1.2–2.1 (10 H, m), 3.30 (1 H, sept), 3.96 (1 H, m), 4.38 (2 H, s), 6.70 (1 H, s), 6.95 (2 H, m), 7.47 (2 H, m); MS $C_{20}H_{26}FNO$ m/e = 315 (M^+), 300, 282, 200. Anal. ($C_{20}H_{26}FNO$) C, H, F, N.

1-Phenyl-2-isopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrole: yield 63%; colorless solid; NMR δ 1.25 (6 H, d), 1.9 (3 H, s), 2.8 (1 H, m), 4.35 (1 H, s), 4.55 (2 H, s), 6.85–7.75 (9 H, m); MS $C_{21}H_{22}FNO$ m/e = 323 (M^+), 308 (M^+ – CH_3), 290 (M^+ – CH_3 – H_2O). Anal. ($C_{21}H_{22}FNO$) C, H, F, N.

(c) Oxidation. Variant A. To a mechanically stirred suspension of Celite (50 g) and finely powdered CrO_3 (25 g, 250 mmol) in dry dichloromethane (250 mL) at 15 °C was added dropwise a solution of dry pyridine (39.5 g, 500 mmol) in CH_2Cl_2 (250 mL).

After stirring at room temperature (20 min), a solution of the substituted (hydroxymethyl)pyrrole (25 mmol) in CH_2Cl_2 (250 mL) was added dropwise but quickly. The reaction temperature was kept between 20 and 24 °C. After 15 min cyclohexane (500 mL) was added. The solid was suction filtered and washed with dichloromethane/cyclohexane (3:7). The filtrate was concentrated and chromatographed with cyclohexane/ethyl acetate/triethylamine (4:1:0.01) over 500 g of silica.

Variant B.[11] To a solution of *N*-methylmorpholine *N*-oxide (46.8 g, 400 mmol) in acetone (400 mL, dried over K_2CO_3) was added tris(triphenylphosphine)ruthenium(II) dichloride (3.8 g, 4.0 mmol). The mixture was stirred 20 min at 20 °C. A solution of the substituted (hydroxymethyl)pyrrole (100 mmol) in dry acetone (600 mL) was added dropwise. The mixture was stirred for 10–20 h at room temperature. After complete reaction (TLC, cyclohexane/ethyl acetate/triethylamine 4:1:0.1), the mixture was filtered through a short, thick silica pad. The pad was washed with ether (3 L); the filtrate was concentrated in vacuo. The residue, pure 4, usually crystallized, when digerated with *n*-pentane at 0 °C.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4b): yield (variant A) 35%, (variant B) 87%; pale yellow solid; mp 119–120 °C; NMR δ 1.36 (6 H, d), 3.16 (1 H, sept), 6.65 (1 H, s), 7.0–7.7 (9 H, m), 10.1 (1 H, s); MS $C_{20}H_{18}FNO$ *m/e* = 307 (M^+), 292. Anal. ($C_{20}H_{18}FNO$) C, H, F, N.

1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4e): yield (variant B) 87%; yellow oil; NMR δ 1.43 (6 H, d), 1.47 (6 H, d), 3.80 (1 H, sept), 4.57 (1 H, sept), 6.62 (1 H, s), 7.06 (2 H, m), 7.37 (2 H, m), 9.89 (1 H, s); MS $C_{17}H_{20}FNO$ *m/e* = 273 (M^+), 258, 244. Anal. ($C_{17}H_{20}FNO$) C, H, F, N.

1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4f): yield (variant B) 98%; colorless crystals; mp 134–135 °C; NMR δ 1.45 (6 H, d), 1.1–2.2 (10 H, m), 3.55–4.35 (2 H, m + sept), 6.65 (1 H, s), 6.9–7.6 (4 H, m), 9.95 (1 H, s); MS $C_{20}H_{24}FNO$ *m/e* = 313 (M^+), 298, 231, 216. Anal. ($C_{20}H_{24}FNO$) C, H, F, N.

1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrole-3-carboxaldehyde (4g): yield (variant A) 45%; pale yellow solid; NMR δ 1.3 (6 H, d), 2.1 (3 H, s), 3.1 (1 H, sept), 6.9–7.6 (9 H, m), 10.0 (1 H, s); MS $C_{21}H_{20}FNO$ *m/e* = 321 (M^+). Anal. ($C_{21}H_{20}FNO$) C, H, F, N.

Synthesis of 1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxaldehyde (4e) via Three-Component Coupling Reaction According to Scheme VI. (a) Three-Component Coupling. Ethyl 1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylate (21e). To a suspension of 4-fluoro-β-nitrostyrene[19] (209 g, 1.25 mol) in absolute methanol (500 mL) was added ethyl 3-oxo-4-methylpentanoate[9] (214 g, 1.35 mol) under ice cooling followed by isopropylamine (128 mL, 1.50 mol), both in one portion. Absolute methanol (1 L) was added, the ice bath was removed, and the reaction mixture was stirred for 48 h in a tightly stoppered flask. Volatile components were removed in vacuo. The brown, viscous oil was filtered with toluene/0.1% triethylamine through 5 kg of silica gel (70–200 μm) to give 197 g (49.7% yield) of a yellow solid: mp 72–74 °C; NMR (CD_2Cl_2) δ 1.07 (3 H, t), 1.36 (6 H, d), 1.42 (6 H, d), 3.73 (1 H, sept), 4.06 (2 H, q), 4.50 (1 H, sept), 6.60 (1 H, s), 6.80–7.40 (4 H, m); MS (DCI, posit. isobutane) $C_{19}H_{24}FNO_2$ *m/e* = 318 (M + H^+), 317, 302. Anal. ($C_{19}H_{24}FNO_2$) C, H, F, N.

(b) Reduction. 1,2-Diisopropyl-3-(hydroxymethyl)-4-(4-fluorophenyl)-1*H*-pyrrole. A solution of the ethyl ester (197 g, 0.62 mol) in ether (750 mL) was added dropwise at 0 °C to a suspension of lithium aluminum hydride (47.2 g, 1.24 mol) in ether (1.5 L). The reaction mixture was stirred for 1 h at 0 °C and for 1 h at 20 °C. At 0–10 °C ethyl acetate (150 mL) was added dropwise, and then water (38 mL) followed by 2 N sodium hydroxide solution (75 mL) was added. The mixture was stirred for 15 min at room temperature. The inorganic solids were removed by suction filtration and washed thoroughly with ether.

Triethylamine (1 mL) was added to the combined filtrate and washings and the solvent was removed in vacuo to give a yellow solid (131 g, 77% yield) that had spectra identical with those of the authentic material described above.

(c) Oxidation was performed as described above to give 4e as a yellow solid in 92% yield.

Pyrrole-Substituted Acrylonitriles 5. General Procedure. At 0 °C a solution of diisopropyl (cyanomethyl)phosphonate (13.5 g, 66.0 mmol) in dry THF (200 mL) was added dropwise to a suspension of sodium hydride (3.78 g of a 50% dispersion in mineral oil, 78.7 mmol) in dry THF (700 mL). After 40 min at 0 °C, a solution of aldehyde 4 (44.0 mmol) in THF (100 mL) was added dropwise. The mixture was stirred for 2 h at room temperature. The reaction mixture was poured into 1 L of brine. The organic phase was separated and the aqueous phase was extracted with ether. The combined organic phases were dried and concentrated in vacuo. The residue was chromatographed over silica with cyclohexane/ethyl acetate (6:1), containing 0.1% triethylamine.

β-[1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-acrylonitrile (5a): yield 78%; pale yellow solid; NMR δ 2.30 (3 H, s), 5.23 (1 H, d), 6.73 (1 H, s), 7.0–7.6 (10 H, m); MS $C_{20}H_{15}FN_2$ *m/e* = 302 (M^+). Anal. ($C_{20}H_{15}FN_2$) C, H, F, N.

β-[1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-acrylonitrile (5e): yield 91%; crystals; mp 121–123 °C (not recryst); NMR δ 1.43 (12 H, 2 × d), 3.30 (1 H, sept), 4.50 (1 H, sept), 4.93 (1 H, d), 6.60 (1 H, s), 6.9–7.4 (4 H, m), 7.53 (1 H, d); MS $C_{19}H_{21}FN_2$ *m/e* = 296 (M^+), 281, 256, 239. Anal. ($C_{19}H_{21}FN_2$) C, H, F, N.

β-[1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-acrylonitrile (5f): yield 96%; pale yellow solid; mp 130–132 °C (not recryst); NMR δ 1.40 (6 H, d), 1.2–2.1 (10 H, m), 3.30 (1 H, sept), 4.00 (1 H, m), 4.95 (1 H, d), 6.60 (1 H, s), 6.9–7.4 (4 H, m), 7.55 (1 H, d); MS $C_{22}H_{25}FN_2$ *m/e* = 336 (M^+), 321, 239. Anal. ($C_{22}H_{25}FN_2$) C, H, F, N.

Preparation of Pyrrole-Substituted Acroleins 6 from Acrylonitriles 5. General Procedure. To a solution of nitrile 5 (24 mmol) in dry THF (200 mL) was added dropwise 60 mL (72 mmol) of a 1.2 M solution of diisobutylaluminum hydride in toluene at 0 °C. The mixture was stirred for 1 h at 0 °C and then for 1.5 h at room temperature. At 0 °C, saturated aqueous sodium dihydrogen phosphate solution (100 mL) and then water (200 mL) were added dropwise. The mixture was stirred for 1 h at room temperature and then saturated with sodium chloride and extracted with ether. The combined organic phases were washed with saturated aqueous sodium bicarbonate and then dried and concentrated in vacuo. The residue was chromatographed over silica with cyclohexane/ethyl acetate (5:1), containing 0.1% triethylamine.

3-[1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6a): yield 70%; pale yellow solid; NMR δ 2.36 (3 H, s), 6.26 (1 H, dd), 6.97 (1 H, d), 7.15–7.70 (10 H, m), 9.54 (d, 1 H); MS $C_{20}H_{16}FNO$ *m/e* = 305 (M^+), 290, 276, 264. Anal. ($C_{20}H_{16}FNO$) C, H, F, N.

3-[1,2-Diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6e): yield 70%; crystals; mp 119–121 °C; NMR δ 1.45 (12 H, 2 × d), 3.45 (1 H, sept), 4.53 (1 H, sept), 6.00 (1 H, d), 6.65 (1 H, s), 6.9–7.5 (4 H, m), 7.63 (1 H, d), 9.45 (1 H, d); MS $C_{19}H_{22}FNO$ *m/e* = 299 (M^+), 256, 214. Anal. ($C_{19}H_{22}FNO$) C, H, F, N.

3-[1-Cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6f): yield 81%; pale yellow crystals; mp 124 °C (not recryst); NMR δ 1.46 (6 H, d), 1.3–2.2 (10 H, m), 3.50 (1 H, sept), 4.00 (1 H, m), 6.05 (1 H, dd), 6.65 (1 H, s), 6.9–7.5 (4 H, m), 7.65 (1 H, d), 9.50 (1 H, d); MS $C_{22}H_{26}FNO$ *m/e* = 339 (M^+), 296, 214. Anal. ($C_{22}H_{26}FNO$) C, H, F, N.

Synthesis of Pyrrole-Substituted Acroleins 6 from Aldehydes 4 with the Wollenberg Reagent. General Procedure. To a solution of 1-ethoxy-2-(tri-*n*-butylstannyl)ethylene[20] (3.46 g, 9.6 mmol) in dry THF (110 mL) was added a solution of *n*-butyllithium in *n*-hexane (6.25 mL of a 1.6 M solution, 10 mmol) at −70 °C under nitrogen. After 2 h at −73 °C, a solution of the aldehyde 4 (8 mmol) in THF (12 mL) was added dropwise. During this operation, the reaction temperature rose to −66 °C. After 2 h at −73 °C and 10 min at −50 °C, a saturated aqueous ammonium chloride solution (18.6 mL) was added dropwise at −40 °C. The mixture was allowed to warm to room temperature. The organic layer was separated; the aqueous layer was extracted twice

(20) Leusink, A. J.; Budding, H. A.; Drenth, W. *J. Organomet. Chem.* 1967, *9*, 285.

with ether. The combined organic layers were washed with brine and then dried and concentrated in vacuo. The residue was taken up in THF (93 mL) and water containing *p*-toluenesulfonic acid (18 mL) and stirred for 1 h at room temperature. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine and then dried and concentrated. The residue was chromatographed with cyclohexane/ethyl acetate/triethylamine (3:1:0.1) over 450 g of silica.

3-[1-Phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6a): yield 98%; spectra, see above.

3-[1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6b): yield 50% (46% recovered starting material); NMR δ 1.35 (6 H, d), 3.16 (1 H, sept), 6.05 (1 H, dd), 6.63 (1 H, s), 7.0–7.5 (9 H, m), 7.75 (1 H, d), 9.50 (1 H, d); MS $C_{22}H_{20}FNO$ DCI m/e = 334 (M + H$^+$), 290. Anal. ($C_{22}H_{20}FNO$) C, H, F, N.

3-[1-Phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6c): yield 88%; amorphous solid; NMR δ 1.9 (3 H, s), 2.2 (3 H, s), 6.07 (1 H, dd), 6.9–7.7 (10 H, m), 9.45 (1 H, d); MS $C_{21}H_{18}FNO$ m/e = 319 (M$^+$), 290 (M$^+$ – CHO). Anal. ($C_{21}H_{18}FNO$) C, H, F, N.

3-[1-Isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-(*E*)-propenal (6d): yield 94%; colorless solid; NMR δ 1.47 (6 H, d), 2.43 (3 H, s), 4.42 (1 H, sept), 6.20 (1 H, dd), 6.72 (1 H, s), 6.9–7.5 (4 H, m), 7.50 (1 H, d), 9.48 (1 H, d); MS $C_{17}H_{18}FNO$ m/e = 271 (M$^+$), 256, 242, 200. Anal. ($C_{17}H_{15}FNO$) C, H, F, N.

3-[1-Phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]-(*E*)-propenal (6g): yield 91%; yellow solid; NMR δ 1.3 (6 H, d), 2.0 (3 H, s), 3.1 (1 H, sept), 6.1 (1 H, dd), 7.0–7.6 (10 H, m), 9.5 (1 H, d); MS $C_{23}H_{22}FNO$ DCI m/e = 348 (M + H$^+$). Anal. ($C_{23}H_{22}FNO$) C, H, F, N.

β-Keto-δ-hydroxy Esters 7. General Procedure. To a suspension of sodium hydride (12.7 mmol) in THF (86 mL) was added dropwise a solution of methyl acetoacetate (1.43 g, 12.33 mmol) in THF (10 mL) at −15 °C during 5 min. The solution was stirred for 50 min at −15 °C. A solution of *n*-butyllithium in hexane (7.68 mL of a 1.6 M solution, 12.26 mmol) was added during 10 min. The reaction mixture was stirred for 20 min at −15 °C. A solution of aldehyde 6 (7.0 mmol) in THF (25 mL) was added during 10 min. The reaction mixture was stirred for 45 min at −15 °C. At −10 °C, a saturated sodium dihydrogen phosphate solution (13 mL) was added dropwise. After 5 min at 0 °C, the mixture was distributed between ether and brine. The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried, concentrated, and chromatographed with cyclohexane/ethyl acetate/triethylamine (2:1:0.1) over silica, giving a pale yellow oil (76–85% yield).

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7a): NMR δ 2.27 (3 H, s), 2.55 (1 H, br), 2.80 (2 H, m), 3.50 (2 H, s), 3.74 (3 H, s), 4.69 (1 H, q), 5.65 (1 H, dd), 6.60 (1 H, d), 6.76 (1 H, s), 7.00–7.12 (4 H, m), 7.30–7.52 (5 H, m); MS $C_{25}H_{24}FNO_4$ m/e = 421 (M$^+$), 403, 345, 302. Anal. ($C_{25}H_{24}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7b): MS $C_{27}H_{28}FNO_4$ m/e = 449 (M$^+$), 432, 373, 334, 290. Anal. ($C_{27}H_{28}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7c): NMR δ 1.6 (1 H, s), 1.9 (3 H, s), 2.13 (3 H, s), 2.36 (2 H, s), 3.57 (2 H, AB), 3.73 (3 H, s), 5.99 (1 H, d), 6.16 (1 H, dd), 6.94 (1 H, d), 7.08–7.33 (5 H, m), 7.44–7.58 (4 H, m); MS $C_{26}H_{26}FNO_4$ m/e = 435 (M$^+$), 417, 320, 319, 316, 290. Anal. ($C_{26}H_{26}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7d): NMR δ 1.44 (8 H, d + m), 1.58 (1 H, br s), 2.37 (3 H, s), 3.58 (2 H, s), 3.75 (3 H, s), 4.35 (1 H, sept), 6.02 (1 H, d), 6.27 (1 H, dd), 6.67 (1 H, s), 7.06 (2 H, m), 7.23 (2 H, m); MS $C_{22}H_{26}FNO_4$ m/e = 387 (M$^+$), 369, 272. Anal. ($C_{22}H_{26}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7e): NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.42 (6 H, d), 2.37 (1 H, d), 2.68 (2 H, m), 3.30 (1 H, sept), 3.48 (2 H, s), 3.70 (3 H, s), 4.44 (1 H, sept), 4.59 (1 H, m), 5.32 (1 H, dd), 6.62 (1 H, d), 7.00 (2 H, m), 7.30 (2 H, m); MS $C_{21}H_{30}FNO_4$ m/e = 415 (M$^+$), 397, 300, 256. Anal. ($C_{24}H_{30}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7f): NMR (CD_2Cl_2) δ 1.35 (6 H, d), 1.3–2.3 (10 H, m), 2.35 (1 H, d), 2.65 (2 H, d), 3.30 (1 H, sept), 3.50 (2 H, s), 3.70 (3 H, s), 4.00 (1 H, m), 4.60 (1 H, m), 5.35 (1 H, dd), 6.65 (1 H, s), 6.65 (1 H, d), 6.85–7.50 (4 H, m); MS $C_{27}H_{34}FNO_4$ m/e = 455 (M$^+$), 437, 340, 296, 214. Anal. ($C_{27}H_{34}FNO_4$) C, H, F, N.

Methyl 5(*RS*)-hydroxy-3-oxo-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (7g): MS $C_{28}H_{30}FNO_4$ m/e = 463 (M$^+$), 446. Anal. ($C_{28}H_{30}FNO_4$) C, H, F, N.

β,δ-Dihydroxy Esters 1 (R^1 = CH$_3$). General Procedure. To a solution of β-keto-δ-hydroxy ester 7 (5 mmol) in dry THF (70 mL) was added dropwise a solution of triethylborane in THF (6 mL of a 1 M solution, 6 mmol) during 5 min. After 20 min at 20 °C, 14 mL of dry air was bubbled through the solution with a syringe. After 2 h at 20 °C, the reaction mixture was cooled to −75 °C. Sodium borohydride (246 mg, 6.5 mmol) was added at once. After 12 h at −75 °C under nitrogen, the mixture was allowed to warm to −10 °C and saturated sodium dihydrogen phosphate solution (35 mL) was added dropwise. The reaction mixture was partitioned between ether and brine. The organic layer was washed with brine, dried, and concentrated. The residue was stirred for 3 h with dry methanol (300 mL). The solvent was evaporated and the residue was chromatographed with cyclohexane/ethyl acetate/triethylamine (1:1:0.1) through silica to yield 60–85% of a thick, pale yellow oil.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1a): NMR δ 2.12 (2 H, m), 2.24 (3 H, s), 2.37 (2 H, s), 2.54 (1 H, dd), 2.75 (1 H, dd), 3.72 (3 H, s), 4.26 (1 H, m), 5.32 (1 H, m), 5.75–5.85 (2 H, m), 6.78 (1 H, s), 7.00–7.10 (2 H, m), 7.28–7.50 (7 H, m); MS $C_{25}H_{26}FNO_4$ m/e = 423 (M$^+$), 306, 264. Anal. ($C_{25}H_{26}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1b): NMR (C_6D_6) δ 1.30 (7 H, d + m), 1.57 (1 H, dt), 2.03 (1 H, dd), 2.13 (1 H, dd), 2.70 (1 H, br s), 3.09 (1 H, sept), 3.27 (3 H, s), 3.45 (1 H, br s), 4.03 (1 H, m), 4.34 (1 H, m), 5.67 (1 H, dd), 6.50 (1 H, s), 6.87–7.15 (8 H, m), 7.45 (2 H, dd); MS $C_{27}H_{30}FNO_4$ m/e = 451 (M$^+$), 433, 334, 292, 290, 276. Anal. ($C_{27}H_{30}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1c): NMR (C_6D_6) δ 1.37 (1 H, dt), 1.67 (1 H, dt), 1.90 (3 H, s), 2.08 (3 H, s), 2.05–2.12 (1 H, dd), 2.26 (1 H, dd), 2.40 (1 H, d), 3.26 (3 H, s), 3.48 (1 H, d), 4.11 (1 H, m), 4.30 (1 H, m), 5.72 (1 H, dd), 6.72 (1 H, d), 6.85–6.91 (2 H, m), 6.95–7.17 (5 H, m), 7.32–7.40 (2 H, m); MS $C_{28}H_{28}FNO_4$ m/e = 437 (M$^+$), 419, 320, 302, 278. Anal. ($C_{26}H_{23}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1d): NMR (C_6D_6) δ 0.98 (6 H, d), 1.40 (1 H, dt), 1.68 (1 H, dt), 2.05 (3 H, s), 2.09 (1 H, dd), 2.27 (1 H, dd), 3.27 (3 H, s), 3.73 (1 H, sept), 4.14 (1 H, m), 4.34 (1 H, m), 5.72 (1 H, dd), 6.50 (1 H, s), 6.73 (1 H, d), 6.98 (2 H, m), 7.43 (2 H, m); MS $C_{22}H_{28}FNO_4$ m/e = 389 (M$^+$), 272, 230. Anal. ($C_{22}H_{28}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1e): NMR (CD_2Cl_2) δ 1.35 (6 H, d), 1.42 (6 H, d), 1.50–1.70 (2 H, m), 2.45 (2 H, d), 2.62 (1 H, br s), 3.31 (1 H, sept), 3.54 (1 H, d), 3.68 (3 H, s), 4.22 (1 H, m), 4.33–4.52 (2 H, sept + m), 5.32 (1 H, d), 6.58 (1 H, d), 6.62 (1 H, s), 7.00 (2 H, m), 7.31 (2 H, m); MS $C_{24}H_{32}FNO_4$ m/e = 417 (M$^+$), 399 (M$^+$ – H_2O), 300, 258, 212. Anal. ($C_{24}H_{32}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-7-[1-cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1f): NMR (CD_2Cl_2) δ 1.25–2.05 (12 H, m), 1.34 (6 H, d), 2.45 (2 H, d), 2.62 (1 H, d), 3.30 (1 H, sept), 3.55 (1 H, d), 3.69 (3 H, s), 3.95 (1 H, tt), 4.20 (1 H, m), 4.38 (1 H, m), 5.33 (1 H, dd), 6.58 (1 H, d), 6.62 (1 H, s), 7.00 (2 H, m), 7.30 (2 H, m); MS $C_{27}H_{36}FNO_4$ m/e = 457 (M$^+$), 439 (M$^+$ – H_2O), 421 (M$^+$ – $2H_2O$), 366, 340, 298, 212. Anal. ($C_{27}H_{36}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*SR*)-dihydroxy-*i*-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (1g): NMR (C_6D_6) δ 1.3 (7 H, d + m), 1.6 (1 H, m), 1.95 (3 H, s), 2.0–2.3 (2 H, m), 2.5 (1 H, br s), 3.1 (1 H, sept), 3.3 (3 H, s), 3.5 (1 H, s), 4.1 (1 H, m), 4.3 (1 H, m), 5.7 (1 H, dd), 6.8–7.5 (10 H, m); MS $C_{25}H_{32}FNO_4$ *m/e* = 465 (M^+), 447 ($M^+ - H_2O$). Anal. ($C_{25}H_{32}FNO_4$) C, H, F, N.

Hydrogenated β,δ-Dihydroxy Esters 2 ($R^1 = CH_3$). General Procedure. Ten percent palladium on charcoal (2.2 g) was added under nitrogen to a solution of the olefinic β,δ-dihydroxy ester 1 ($R^1 = CH_3$) (70 mmol) in methanol (1.3 L) and triethylamine (13 mL). The mixture was shaken for 20 min in a hydrogen atmosphere at atmospheric pressure and room temperature. H_2 (1240 mL) was taken up (theoretical 1570 mL). The catalyst was filtered off and washed with methanol. The filtrate was concentrated in vacuo. The residue was chromatographed with cyclohexane/ethyl acetate (5:3), containing 0.1% triethylamine, through 1.3 kg of silica. The first compound eluted was the pure product 2 (yield 75–80%, pale yellow thick oil). Shortly thereafter a diastereomer of 2 (yield 8%) was eluted that stemmed either from incomplete stereoselectivity during the borane-catalyzed reduction of keto ester 6 (steps i, j) or from some isomerization during the catalytic hydrogenation. As a last fraction, the lactonized form of 2 (yield 4–5%) was obtained, containing some diastereomers. TLC (cyclohexane/ethyl acetate 1:1, silica) R_f values: 1 (starting material), 0.26; 2, 0.29; diastereomer of 2, 0.25; δ-lactone of 2, 0.19.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2b): NMR (C_6D_6) δ 1.03 (1 H, dt), 1.28–1.43 (1 H, m), 1.32 (3 H, d), 1.33 (3 H, d), 1.60–1.85 (2 H, m), 1.94 (1 H, dd), 2.12 (1 H, dd), 2.90–3.02 (1 H, m), 3.03–3.22 (3 H, m), 3.24 (3 H, s), 3.43 (1 H, br s), 3.75 (1 H, m), 3.88 (1 H, m), 6.58 (1 H, s), 6.94 (2 H, m), 7.03–7.15 (5 H, m), 7.42 (2 H, m); MS $C_{27}H_{32}FNO_4$ FAB *m/e* = 454 ($M + H^+$), 292. Anal. ($C_{27}H_{32}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2,5-dimethyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2c): NMR (C_6D_6) δ 1.10 (1 H, dt), 1.38 (1 H, dt), 1.50–1.76 (2 H, m), 1.97 (3 H, s), 2.01 (1 H, dd), 2.08 (3 H, s), 2.17 (1 H, dd), 2.77 (2 H, m), 2.66 (1 H, d), 3.27 (3 H, s), 3.50 (1 H, d), 3.72 (1 H, m), 3.95 (1 H, m), 6.90–7.13 (7 H, m), 7.28–7.36 (2 H, m); MS $C_{26}H_{30}FNO_4$ *m/e* = 439 (M^+), 407, 279. Anal. ($C_{26}H_{30}FN$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-isopropyl-2-methyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2d): NMR (C_6D_6) δ 1.02 (6 H, 2 × d), 1.38 (2 H, dt), 1.50–1.75 (2 H, m), 1.97 (1 H, dd), 2.10 (3 H, s), 2.15 (1 H, dd), 2.82 (2 H, m), 3.27 (3 H, s), 3.70 (1 H, m), 3.78 (1 H, sept), 3.93 (1 H, m), 6.58 (1 H, s), 6.98 (2 H, m), 7.39 (2 H, m); MS $C_{22}H_{30}FNO_4$ DCI *m/e* = 392 ($M + H^+$), 391, 360, 331, 230. Anal. ($C_{22}H_{30}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2e): NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.42 (6 H, d), 1.4–1.55 (4 H, m), 2.40 (2 H, d), 2.50–2.76 (2 H, m), 2.87 (1 H, br s), 3.22 (1 H, sept), 3.60 (1 H, br d), 3.68 (3 H, s), 3.76 (1 H, qui), 4.12 (1 H, qui), 4.43 (1 H, sept), 6.62 (1 H, s), 7.03 (2 H, m), 7.32 (2 H, m); MS $C_{24}H_{34}FNO_4$ DCI *m/e* = 420 ($M + H^+$), 419 (M^+), 259. Anal. ($C_{24}H_{34}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-cyclohexyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2f): NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.3–1.8 (10 H, m), 1.32–2.05 (4 H, m), 2.39 (2 H, d), 2.50–2.72 (2 H, m), 2.88 (1 H, br s), 3.22 (1 H, sept), 3.61 (1 H, br d), 3.67 (3 H, s), 3.76 (1 H, qui), 3.94 (1 H, tt), 4.12 (1 H, qui), 6.61 (1 H, s), 7.02 (2 H, m), 7.31 (2 H, m); MS $C_{27}H_{38}FNO_4$ *m/e* = 459 (M^+), 427 ($M^+ - CH_3OH$), 299, 298, 256. Anal. ($C_{27}H_{38}FNO_4$) C, H, F, N.

Methyl 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-5-methyl-1*H*-pyrrol-3-yl]heptanoate (2g): NMR (C_6D_6) δ 1.1–1.5 (2 H, m), 1.3 (6 H, d), 1.6–2.2 (7 H, m + s), 2.9–3.2 (4 H, m), 3.3 (3 H, s), 3.45 (1 H, br s), 3.8–4.1 (2 H, m), 6.8–7.5 (9 H, m); MS $C_{28}H_{34}FNO_4$ FAB *m/e* = 468 ($M + H^+$). Anal. ($C_{28}H_{34}FNO_4$) C, H, F, N.

Optically Active HMG-CoA Reductase Inhibitors of General Formula 13 via Asymmetric Synthesis According to Scheme II. (a) Diastereoselective Aldol Reaction of Enolate 8 with Aldehydes 6. General Procedure. To a solution of diisopropylamine (97 mL, 70.0 g, 692 mmol) in dry THF (500 mL), cooled with dry ice, was added a 1.6 M solution of *n*-butyllithium in hexane (430 mL, 688 mmol) via a Flex-needle.[21] The mixture was stirred for 30 min at 0 °C under nitrogen. Another 4-L four-necked flask, equipped with a mechanical stirrer, low-temperature thermometer, dropping funnel with cooling finger, and nitrogen inlet/mercury bubbler, was charged with (*S*)-(−)-phenyl 2-hydroxy-2,2-diphenylacetate[7] (104.7 g, 315 mmol) and dry THF (1 L). The suspension was cooled with dry ice.

A LDA-solution (vide supra) was transferred via a Flex-needle through a septum into the dropping funnel and added to the stirred suspension at such a rate that the reaction temperature stayed below −20 °C. The mixture was stirred for 30 min at 0 °C and became a reddish-brown, clear solution. A precooled solution of aldehyde 6 (300 mmol) in dry THF (300 mL) was added to this solution of dianion 8 at −90 °C. The reaction mixture was stirred for 1–2 h (TLC control) at this temperature. The cold mixture was poured into the mechanically stirred saturated aqueous solution of ammonium chloride (2 L) and stirred for 20 min (pH 8, 0 °C). The organic layer was separated and the aqueous layer was extracted with ether. The combined organic layers were washed with brine, dried, and filtered, and the solvent was evaporated in vacuo to give a pale yellow solid that according to TLC consisted mostly of aldol product 9 with small amounts of unreacted chiral acetate and traces of unreacted aldehyde 6. For purification, the crude solid was shaken with hot toluene/ethyl acetate (2 L, 6:4 + 0.1% triethylamine). After the suspension had come to room temperature it was filtered, and the solid after washing with toluene was discarded. Combined filtrate and washings were evaporated in vacuo, and the remaining solid residue was stirred with *n*-pentane (2 × 1 L). The resulting suspension was suction filtered. Colorless solid 9, obtained in 95–98% yield, was pure by TLC. The pentane solution contained unreacted aldehyde 6.

The diastereomeric excess (de) of the desired 3*S* isomer of 9 was 95–96% according to HPLC analysis (LiChrosorb SI 60 Merck 506467, 40 °C, 1.2 mL/min *n*-hexane/methyl *tert*-butyl ether 3:1).

(*S*)-(−)-2-Hydroxy-1,2,2-triphenylethyl (3*S*)-hydroxy-5-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]-pent-4(*E*)-enoate (9b): mp 188–190 °C; NMR (CD_2Cl_2) δ 1.22 (6 H, 2 × d), 1.53 (1 H, s), 1.57 (1 H, d), 2.38 (2 H, d), 3.00 (1 H, hept), 4.37 (1 H, m), 5.28 (1 H, dd, *J* = 16 and 7 Hz), 6.59 (1 H, s), 6.67 (1 H, dd, *J* = 16 and 1.5 Hz), 6.69 (1 H, s), 6.93–7.58 (24 H, m); MS (DCI, posit. isobutane) $C_{44}H_{40}FNO_4$ *m/e* = 665 (M^+), 648 ($M^+ - OH$), 376, 334. Anal. ($C_{44}H_{40}FNO_4$) C, H, F, N.

(*S*)-(−)-2-Hydroxy-1,2,2-triphenylethyl (3*S*)-hydroxy-5-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]pent-4-(*E*)-enoate (9e): mp 194 °C; NMR (CD_2Cl_2) δ 1.32 (6 H, d), 1.43 (6 H, d), 2.10 (1 H, d), 2.38 (2 H, d), 2.98 (1 H, s), 3.27 (1 H, sept), 4.37 (1 H, m), 4.43 (1 H, sept), 5.23 (1 H, dd, *J* = 16 and 7 Hz), 6.53 (1 H, dd, *J* = 16 and 1.5 Hz), 6.62 (1 H, s), 6.68 (1 H, s), 6.93–7.01 (2 H, m), 7.05–7.37 (15 H, m), 7.50–7.60 (2 H, m); MS (FAB, NBA/LiI) $C_{41}H_{42}FNO_4$ *m/e* = 638 ($M + Li^+$), 631 (M^+), 614 ($M^+ - OH$), 358, 342, 300. Anal. ($C_{41}H_{42}FNO_4$) C, H, F, N.

(b) Transesterification of 9 to Optically Active Methyl Esters 10. General Procedure. To a suspension of ester 9 (178 mmol) in absolute methanol (1.4 L) was added dropwise a solution of sodium (2.0 g, 89 mmol) in absolute methanol (200 mL) at 20 °C. The mixture was stirred for 3 h at room temperature. At <10 °C, the mixture was neutralized by dropwise addition of the solution of acetic acid (5.1 mL, 89 mmol) in methanol (15 mL). Triethylamine (0.5 mL) was added, and the solvent was evaporated

(21) Commercially available from Aldrich Chemical Co., Milwaukee, WI.

(22) The oral activity in the rat is an *acute* experiment, in which the hepatic cholesterol biosynthesis inhibition is measured within 3 h after po administration. Oral activities in the rabbit and dog are *chronic* experiments, in which decrease of serum cholesterol is measured. The decrease of serum cholesterol should be coupled to the hepatic cholesterol biosynthesis inhibition, but only via a long, complex chain of biochemical reactions. It seems possible that pronounced differences of the two compounds in metabolic stability and pharmacokinetics are responsible for the lack of oral activity of the unsaturated compound 1b in the rabbit model.

at <20 °C in vacuo. The solid residue was taken up in ether and half-concentrated brine. The ether phase was washed with sodium bicarbonate and then with brine. The solvent was removed in vacuo. The liberated diol was removed from methyl ester 10 by filtration with diisopropyl ether/cyclohexane (1:1) through 2 kg of silica: yield 94–100% 10; pale-yellow oil.

Methyl esters 10 decomposed quickly in solution at room temperature, especially on air contact.

Methyl (3*S*)-hydroxy-5-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]pent-4(*E*)-enoate (10b): NMR (CD_2Cl_2) δ 1.26 (6 H, d), 2.48 (2 H, AB of AB X), 3.03 (1 H, hept), 3.60–3.71 (1 H, m), 3.67 (3 H, s), 4.53 (1 H, br s), 5.37 (1 H, dd), 6.58 (1 H, s), 6.72 (1 H, dd), 7.00 (2 H, m), 7.27–7.49 (7 H, m). Anal. ($C_{25}H_{26}FNO_3$) C, H, F, N.

Methyl (3*S*)-hydroxy-5-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]pent-4(*E*)-enoate (10e): MS (DCI, posit, isobutane) $C_{22}H_{28}FNO_3$ *m/e* = 373 (M^+), 356 (M^+ – OH). Anal. ($C_{22}H_{28}FNO_3$) C, H, F, N.

(c) Transformation of δ-Hydroxy Methyl Esters 10 to β-Keto-δ-hydroxy *tert*-Butyl Esters 11. General Procedure. *tert*-Butyl acetate (81.3 g, 94 mL, 700 mmol) was added dropwise at –75 °C under N_2 to a solution of LDA (730 mmol) in THF/hexane (1:1, 1 L). After 40 min at –70 °C, the solution of methyl ester 10 (178 mmol) in THF (100 mL) was added dropwise. The mixture was stirred for 10 min at –70 °C and then for 1 h at –30 °C. The cold solution was poured into mechanically stirred, half-saturated ammonium chloride solution (2 L). After 10 min, the organic phases were washed twice with sodium bicarbonate solution and then with brine, dried, filtered, and evaporated. Toluene (100 mL) was added and then evaporated at 20 °C (to remove the excess *tert*-butyl acetate). Residual volatile components were removed in high vacuo (24 h). *tert*-Butyl esters 11 were obtained as yellow, very viscous oils in 95–100% yield.

***tert*-Butyl (5*S*)-hydroxy-3-oxo-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (11b):** NMR (CD_2Cl_2) δ 1.25 (6 H, d), 1.46 (9 H, s), 2.68 (2 H, d), 3.03 (1 H, hept), 3.37 (2 H, s), 3.68 (1 H, m), 4.60 (1 H, m), 5.37 (1 H, dd), 6.60 (1 H, s), 6.74 (1 H, dd), 7.03 (2 H, m), 7.30–7.52 (7 H, m); MS (DCI, posit, isobutane) $C_{30}H_{34}FNO_4$ *m/e* = 491 (M^+), 474 (M^+ – OH), 418 (M^+ – isobutene), 390 (M^+ – CO_2tBu), 334. Anal. ($C_{30}H_{34}FNO_4$) C, H, F, N.

***tert*-Butyl (5*S*)-hydroxy-3-oxo-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (11e):** NMR (CD_2Cl_2) δ 1.36 (6 H, d), 1.40–1.48 (15 H, s + 2 × d), 1.57 (1 H, d), 2.67 (2 H, d), 3.32 (1 H, hept), 3.36 (2 H, s), 4.45 (1 H, hept), 4.57 (1 H, m), 5.32 (1 H, dd, *J* = 16 and 7 Hz), 6.62 (1 H, dd, *J* = 16 and 1.5 Hz), 6.63 (1 H, s), 7.00 (2 H, m), 7.30 (2 H, m); MS (DCI, posit, isobutane) $C_{27}H_{36}FNO_4$ *m/e* = 457 (M^+), 440 (M^+ – OH), 397. Anal. ($C_{27}H_{36}FNO_4$) C, H, F, N.

(d) Diastereoselective Reduction of β-Keto-δ-hydroxy *tert*-Butyl Esters 11 to β,δ-Dihydroxy *tert*-Butyl Esters 12. General Procedure. Triethylborane (185 mL of a 1 M solution in THF) was added dropwise at 20 °C to a solution of 130 mL of absolute methanol in 510 mL of dry THF. A solution of crude *tert*-butyl ester 11 (177 mmol) in THF (150 mL) was added dropwise. The mixture was stirred for 1 h at –70 °C. Sodium borohydride (8.73 g, 231 mmol) was added at once. The mixture was stirred for 1.5 h at –70 °C and then poured into half-concentrated ammonium chloride solution (2 L). The mixture was stirred for 15 min and the organic phase was separated. The aqueous phase was extracted twice with ether. The combined organic layers were washed with brine, and the solvent was evaporated in vacuo. The residue was taken up several times in wet methanol and this solvent was evaporated in vacuo at <20 °C. TLC (100% diisopropyl ether) indicated the successful conversion of the unipolar boron ester of the diol (R_f ~0.57) to free diol 12 (R_f ~0.19). Pure 12 was obtained after chromatography through silica (2 kg, 70–200 μm) with diisopropyl ether as a colorless solid (yield 70–80%).

***tert*-Butyl 3(*R*),5(*S*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (12b):** mp 107–110 °C; NMR (CD_2Cl_2) δ 1.26 (6 H, d), 1.48 (9 H, s), 1.55 (2 H, m), 2.38 (2 H, d), 2.87 (1 H, t), 3.03 (1 H, hept), 3.63 (1 H, br s), 4.16 (1 H, m), 4.39 (1 H, m), 5.37 (1 H, dd), 6.6((1 H, s), 6.71 (1 H, dd), 7.03 (2 H, m), 7.30–7.52 (7 H, m); MS (DCI, posit, isobutane) $C_{30}H_{36}FNO_4$ *m/e* = 493 (M^+), 476 (M^+ – OH) 458 (M^+ – OH – H_2O). Anal. ($C_{30}H_{36}FNO_4$) C, H, F, N.

***tert*-Butyl 3(*R*),5(*S*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoate (12e):** MS (DCI, posit, isobutane) $C_{27}H_{38}FNO_4$ *m/e* = 459 (M^+). Anal. ($C_{27}H_{38}FNO_4$) C, H, F, N.

(e) Catalytic hydrogenations of *tert*-butyl esters 12 were performed in analogy to that of the corresponding methyl esters 2 (vide supra), yield 75–82%.

***tert*-Butyl 3(*R*),5(*R*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate:** mp 108–110 °C; NMR (CD_2Cl_2) δ 1.25 (6 H, d), 1.46 (9 H, s), 1.40–1.5((4 H, m), 2.33 (2 H, m), 2.63–2.91 (2 H, m), 3.02 (1 H, hept), 3.1((1 H, br s), 3.67 (1 H, br s), 3.79 (1 H, qui), 4.11 (1 H, br qui) 6.62 (1 H, s), 7.05 (2 H, m), 7.30–7.50 (7 H, m); MS (DCI, posit, isobutane) $C_{30}H_{38}FNO_4$ 496 (M + H^+), 495 (M^+), 440 (M + H^+ – isobutene), 293. Anal. ($C_{30}H_{38}FNO_4$) C, H, F, N.

***tert*-Butyl 3(*R*),5(*R*)-dihydroxy-7-[1,2-diisopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate:** mp 128–130 °C MS (DCI, posit, isobutane) $C_{27}H_{40}FNO_4$ *m/e* = 462 (M + H^+) 461 (M^+), 406 (M + H^+ – isobutene), 259. Anal. ($C_{27}H_{40}FNO_4$ C, H, F, N.

β,δ-Dihydroxy Sodium Carboxylates 1 or 2 (R^1 = Na) General Procedure. To a solution of methyl ester 1 or 2 (R = CH_3, 48 mmol) in methanol (500 mL) was added dropwise i N aqueous sodium hydroxide solution (50 mL, 50 mmol) during 1 h at 0–10 °C. The mixture was stirred for 1 h at 0 °C and fo: 1 h at room temperature. The mixture was filtered and the filtrat was evaporated in vacuo. The residue was taken up in ethano (100 mL), evaporated in vacuo, and dried in high vacuo. Th residue was stirred with ether (300 mL). The solid was collecte by suction filtration, washed with pentane, and dried for 4 h ir vacuo in a desiccator over phosphorous pentoxide and potassium hydroxide; pale yellow solid, yield 64%. The ethereal mothe: liquor was evaporated in vacuo and treated as described above to give a solid with the same melting point and ^1H NMR; yiel 31%, combined yield 95%.

Sodium 3(*RS*),5(*SR*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]hept-6(*E*)-enoat (1b): mp 232–234 °C dec; NMR (DMSO-d_6) δ 1.20 (6 H, d) 1.25–1.62 (2 H, m), 1.80–2.11 (2 H, m), 2.98 (1 H, sept), 3.72 (H, m), 4.20 (1 H, m), 4.83 (1 H, br s), 5.37 (1 H, dd), 6.52 (1 H d), 6.80 (1 H, s), 7.14 (2 H, t), 7.30 (1 H, br s), 7.40–7.60 (8 H m). Anal. ($C_{26}H_{27}FNO_4Na$) C, H, N.

Sodium 3(*RS*),5(*RS*)-dihydroxy-7-[1-phenyl-2-isopropyl-4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (2b) mp 231–233 °C dec. Anal. ($C_{26}H_{29}FNO_4Na$) C, H, N.

Sodium 3(*R*),5(*R*)-Dihydroxy-7-[1-phenyl-2-isopropyl 4-(4-fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (13b). Th corresponding *tert*-butyl ester (48 g, 97 mmol) was suspended i ethanol (250 mL) at 5 °C. Sodium hydroxide (1 N, 98.8 mL) wa added dropwise. The suspension was stirred for 20 h at roor temperature, becoming a clear solution. Solvents were remove in vacuo. The residue was washed with ether and then wit pentane to give 44.6 g (yield 99.8%) of a colorless solid: m 252–254 °C dec; NMR (DMSO-d_6) δ 1.22 (6 H, d), 1.20–1.50 (H, m), 1.83 (1 H, dd, *J* = 15 and 8 Hz), 2.04 (1 H, dd, *J* = 15 an 4 Hz), 2.50–2.67 (1 H, m), 2.71–2.87 (1 H, m), 2.96 (1 H, hept 3.61 (1 H, br s), 3.74 (1 H, m), 4.70 (1 H, br s), 6.77 (1 H, s 7.10–7.21 (2 H, m), 7.32–7.57 (7 H, m). Anal. ($C_{26}H_{29}FNO_4Na$ C, H, N.

Sodium 3(*R*),5(*R*)-dihydroxy-7-[1,2-diisopropyl-4-(4 fluorophenyl)-1*H*-pyrrol-3-yl]heptanoate (13e) was obtaine from the corresponding *tert*-butyl ester in analogy to the metho for 13b (vide supra) to give a colorless solid: mp 255 °C dec; NMI (DMSO-d_6) δ 1.30 (6 H, d), 1.37 (6 H, d), 1.82 (1 H, dd, *J* = 1 and 8 Hz), 2.03 (1 H, dd, *J* = 15 and 4 Hz), 2.32–2.48 (1 H, m 2.52–2.67 (1 H, m), 3.18 (1 H, hept), 3.57 (1 H, br s), 3.76 (1 H hept), 4.41 (1 H, hept), 4.57 (1 H, br s), 6.80 (1 H, s), 7.12 (2 H m), 7.33 (2 H, m). Anal. ($C_{23}H_{31}FNO_4Na$) C, H, N.

Biological assays: see the preceding paper in this issue

were prepared and frozen at −30 °C until used. Bovine and human tissues were used in some assays (see below). Freshly dissected (or frozen) tissue was homogenized (Polytron setting 6 for 20 s) in 30 volumes of ice-cold buffer containing 50 mM Tris·HCl (pH 7.4 at 37 °C; pH 8.0 at 4 °C), 0.5 mM Na_2EDTA, and 10 mM $MgSO_4$, and centrifuged at 30000*g* for 15 min. The supernatant was discarded; the pellet was resuspended and preincubated for 15 min at 37 °C. The homogenate membranes were washed twice by centrifugation and resuspension. The final assay buffer contained 10 μM parglyine, and 0.1% ascorbate was added last to the incubation medium. Protein determinations were made by the Lowry method.

5-HT_{1A} sites were labeled with 0.1 nM [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]OH-DPAT) (157 Ci/mmol; New England Nuclear) and 4 mg wet weight of rat hippocampal tissue. 8-OH-DPAT (1 μM) was used to determine nonspecific binding. The 5-HT_{1B} receptor was labeled with 2.0 nM [^{3}H]-5-HT (28.3 Ci/mmol; New England Nuclear) and 8 mg of rat striatal membrane homogenate. 5-HT (10^{-6} M) was used to define nonspecific binding, and 10^{-7} M 8-OH-DPAT and mesulergine were included to block 5-HT_{1A} and 5-HT_{1C} receptors, respectively. 5-HT_{1C} sites were labeled with 1 nM [^{3}H]-5-HT and 10 mg of rat frontal cortical tissue homogenate; 20 nM spiperone was used to mask 5-HT_2 sites. 5-HT_{1D} sites were labeled with 10 nM [^{3}H]-5-HT and 10 mg of bovine caudate homogenate; 1 μM pindolol was used to block 5-HT_{1A} and 5-HT_{1B} istes, and 100 nM mesulergine was used to block 5-HT_{1C} sites. 5-HT_{1E} sites were labeled with 2 nM [^{3}H]-5-HT and 10 mg of human cortical homogenate in the presence of 100 nM 5-carboxamidotryptamine to block any 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} sites and 100 nM mesulergine was used to block 5-HT_{1C} 5-HT_1 sites. 5-HT_2 binding studies were conducted as previously reported.[3]

Eleven concentrations of nonradioactive competing drugs were made fresh daily in assay buffer, and assays were performed in (at least) triplicate. Following incubation with membranes and radioligand at 37 °C for 30 min, samples were rapidly filtered over glass-fiber filters (Schleicher and Schuell) and were washed with 10 mL of ice-cold 50 mM Tris·HCl buffer. Individual filters were inserted into vials and equilibrated with 5 mL of scintillation fluid (Scinti-Verse, Fisher) for 6 h before counting at 50% efficiency in a Beckman 3801 counter. Results were analyzed with an updated version of the program EBDA[21] in order to determine IC_{50}, K_i, and Hill values.

Acknowledgment. This work was supported in part by US PHS Grant NS 23523.

Registry No. 2, 304-52-9; 3, 78263-90-8; 4, 6260-79-3; 5·HCl, 1453-99-2; 6, 18658-09-8; 7, 124224-49-3; 5-(benzyloxy)-3-(2-nitropropenyl)indole, 101731-72-0; oxalyl chloride, 79-37-8; 5-(benzyloxy)-2-methylindole, 124224-50-6; 5-methoxy-2-methylindole, 1076-74-0.

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3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Inhibitors. 6.[1] *trans*-6-[2-(Substituted-1-naphthyl)ethyl(or ethenyl)]-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-ones

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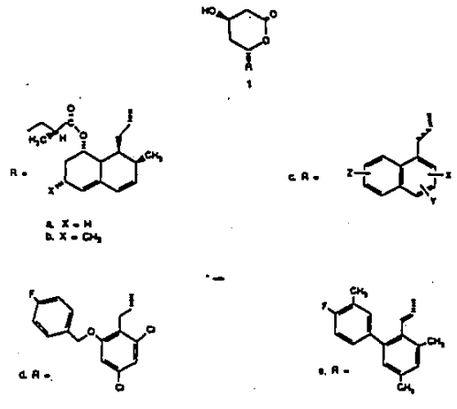
A variety of *trans*-6-[2-(substituted-1-naphthyl)ethyl(or ethenyl)]-3,4,5,6-tetrahydro-4-hydroxy-2*H*-pyran-2-ones were prepared and, upon conversion to their 3,5-dihydroxy carboxylates, were found to have good inhibitory activity against the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-determining enzyme in cholesterogenesis. The most active compounds are 2,4,6- and 2,4,7-trichloro derivatives and would be expected to display about the same potency as the standard compactin (1a) upon resolution.

The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase catalyzes the rate-determining step and point of natural regulation of cholesterogenesis. Potent inhibitors of this enzyme (e.g. 1a) have been shown to lower cholesterol blood levels in animals and man by about 30%.[2] The results of the Lipid Research Clinics Coronary Primary Prevention Trial showed that reduction in blood cholesterol by even a modest 10% results in significantly diminished risk of coronary heart disease.[3] Thus cholesterol blood level lowering by a 1a and similar inhibitors can be expected to significantly reduce the risk of coronary heart disease. In pursuit of this goal, we wanted to prepare wholly synthetic analogues of 1a and 1b without the complex stereochemistry. We began with some simple probes with modest activity.[4] Nonetheless these probes pointed the way to classes of compounds which after further ex-



† Rahway, NJ.

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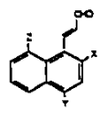
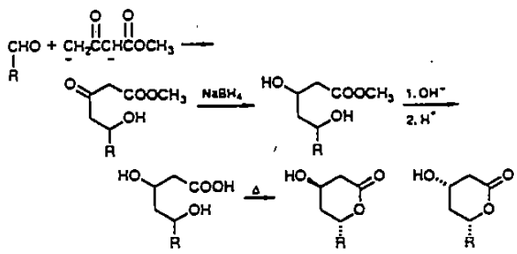
Table I. Physical Properties of 1-Naphthylpropenals

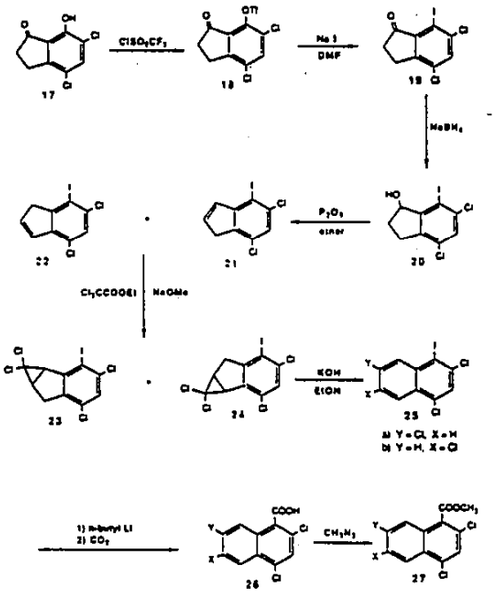


no.	X	Y	Z	recryst solvent	% yield	mp, °C	formula	anal.
14	Cl	H	H	EtOH/H_2O	44	82–84	$C_{13}H_9ClO$	C, H
15	H	Br	H	n-C_4H_9Cl	29	134–137	$C_{13}H_9BrO$	C, H
16	H	H	Br	hexane	25	96–98	$C_{13}H_9BrO$	

Scheme I



Scheme II



ploration gave benzyl ethers 1d, which display an interesting order of activity,[5] and biphenyls 1e, which are highly active.[6] Initial results[4] with the probe compound 1c (x = Y = Z = H) showed sufficient activity to merit more extensive investigation. We report herein the results of further study in the 1c series which afforded substituted naphthalene derivatives, some of which display activity similar to that of 1b.

Chemistry

The known aldehydes 2-chloro-1-naphthaldehyde[7] and 4-bromo-1-naphthaldehyde[8] were converted to propenal intermediates 14 and 15 by the method of Baker[9] (Table I), and the lactone ring was introduced with the known chemistry[4] of Scheme I to give, respectively, 2-chloro derivative 5 and 4-bromo derivative 3. The synthesis of 8-bromo propenal intermediate 16 (Table I) was accomplished by using the general method of Newman[10] and the lactone ring was introduced by using Scheme I technology. The double bond of 3 was hydrogenated with rhodium-on-carbon catalyst[11] to give 4.

Applying Parham methodology[12] produced the intermediate 2,4,6- and 2,4,7-trichloronaphthalene methyl ester derivatives 26 as outlined in Scheme II. Attempts to substitute the triflate of 18 with basic nucleophiles such as cyanide in DMF[13] were unsuccessful and gave only black tar. However, in a novel step the neutral nucleophile iodide ion smoothly displaced the triflate in DMF solvent. The remaining chemistry proceeded in a straightforward fashion to give esters 27, which were separated by HPLC and assigned structures on the basis of ^1H NMR NOE experiments (the Experimental Section). Esters 27 were converted to the intermediate propanals 31 as outlined in Scheme III. Thus treatment of chloromethyl compounds 29 with imine carbanion 30[14] followed by hydrolysis gave the desired aldehydes 31a and 31b. Introduction of the lactone ring via Scheme I technology gave final products 7 and 8.

In the chromatographic purification of 31b, an impurity (33) was isolated. Loss of the chlorine in the 2-position must have occurred during $LiAlH_4$ reduction, probably via intramolecular hydride delivery from an oxyaluminum hydride intermediate to give 32 after workup, which was then carried through the sequence undetected until the aldehyde stage. Compound 33 was then converted to 9 by using the method of Scheme I. Friedel–Crafts chemistry

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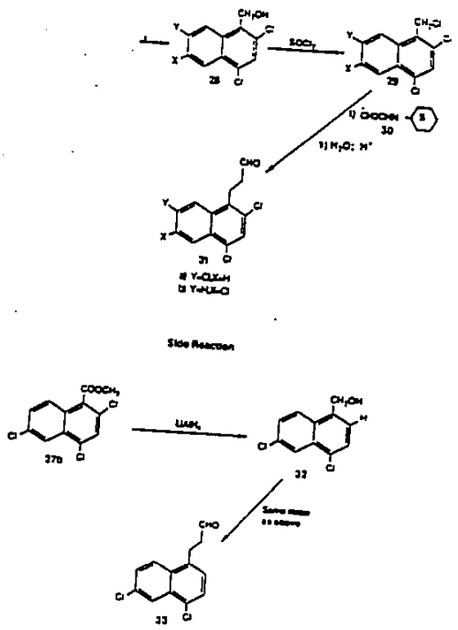
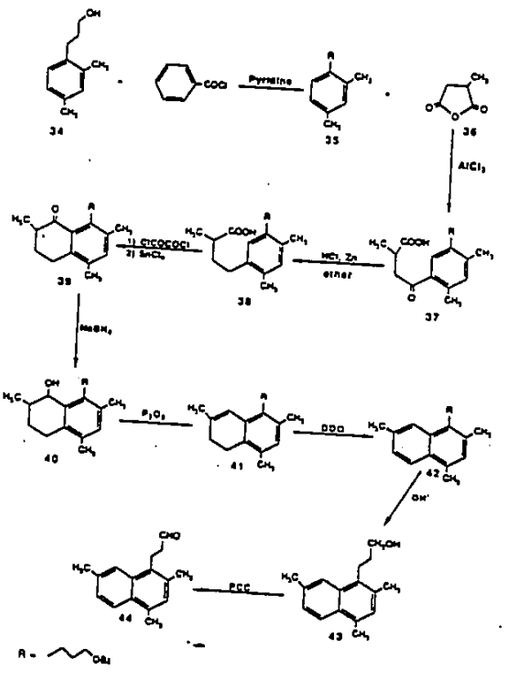


Scheme IV



was used to construct the methyl-substituted naphthalene ring via tetralones.[15] The synthesis of the needed intermediate aldehyde 44 is outlined in Scheme IV. Friedel–Crafts acylation of benzoate 35 with anhydride 36 gave 37 with high regioselectivity.[15a] The remainder of the synthesis was straightforward, giving the naphthalene aldehyde 44, which, when carried through the lactone elaboration steps of Scheme I, gave final product 10.

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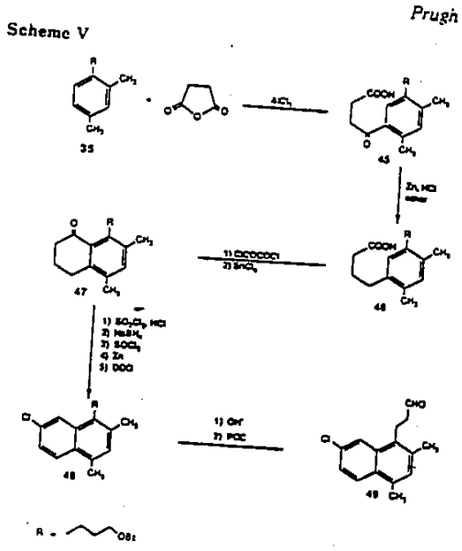
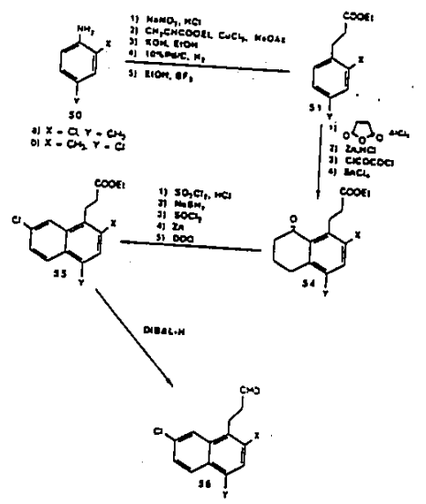
Scheme V



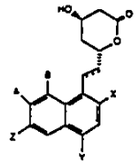
Scheme VI



Synthesis of intermediate 49, wherein the 7-methyl group has been replaced with chlorine, is outlined in Scheme V. The novel part of this scheme, the unambiguous introduction of the 7-chloro substituent to form 48 and 55 (Scheme VI) beginning with tetralones, has been reported[16] and briefly involved gem dichlorination α to the ketone using sulfuryl chloride, reduction of the ketone to the alcohol with $NaBH_4$, followed by conversion of the alcohol to chloride with thionyl chloride. After adjacent chlorines were removed with activated Zn to give a vinyl chloride, aromatization was completed with DDQ.

(16) Prugh, J. D.; Deana, A. A.; Wiggins, J. M. *Synthesis* 1989, 554.

Table II. Physical Properties and in Vitro HMG-CoA Reductase Inhibitory Activities



no.	A	B	X	Y	Z	bridge	recryst solvent	mp, °C	formula	IC_{50}, μm	relative[e] potency
2	H	H	H	H	H	sat.	none	glass	$C_{17}H_{18}O_3 \cdot {}^1/_2H_2O$	81	0.043
3	H	H	H	Br	H	ene	*a*	177–179	$C_{17}H_{13}BrO_3$[f]	4	0.96
4	H	H	H	Br	H	sat.	*a*	141–143	$C_{17}H_{17}BrO_3$	23.3	0.15
5	H	H	Cl	H	H	ene	butyl chloride	88–91	$C_{17}H_{15}ClO_3$	1.51	2.3
6	H	Br	H	H	H	ene	*a*	128–129	$C_{17}H_{15}BrO_3$	4.12	0.72
7	Cl	H	Cl	Cl	H	sat.	*b*	111–115	$C_{17}H_{15}Cl_3O_3$	0.032	47
8	H	H	Cl	Cl	Cl	sat.	*b*	123–125	$C_{17}H_{15}Cl_3O_3$	0.033	46
9	H	H	H	Cl	Cl	sat.	none	glass	$C_{17}H_{16}Cl_2O_3$	7.0	0.3
10	CH_3	H	CH_3	CH_3	H	sat.	*b*	118–120	$C_{20}H_{24}O_3$	0.36	5
11	Cl	H	CH_3	CH_3	H	sat.	none	glass	$C_{19}H_{21}ClO_3$	0.2	7
12	Cl	H	CH_3	Cl	H	sat.	*a*	111–114	$C_{18}H_{18}Cl_2O_3$[d]	0.06	30
13	Cl	H	Cl	CH_3	H	sat.	*b*	126–128	$C_{18}H_{18}Cl_2O_3$	0.13	15

[a] Acetone/hexane. [b] Ether/hexane. [c] 0.05 C_6H_{14}. [d] 0.25 Et_2O. [e] Relative to compactin = 100.

We next prepared the isomeric dichloro compounds 12 and 13. Synthesis of the intermediate aldehydes required for the straightforward elaboration of both compounds is outlined in Scheme VI. Aldehydes 56 were then transformed into target structures 12 and 13 by the chemistry shown in Scheme I.

Biological Results and Discussion

The target compounds presented in Table II as the lactones were tested as the corresponding ring-opened dihydroxy carboxylate sodium salts, the active form, in aqueous solution by using the in vitro procedure reported earlier.[4] Our investigation was limited to balogen and methyl substituents on the naphthalene ring and a brief study of the saturated or unsaturated two-carbon bridge. When comparing the bridge ene in 3 versus the saturated ethyl bridge in 4, the activities show strong enhancement with the double bond as in the biphenyl series.[6] We reported previously[4] that the two-carbon bridge between the naphthalene ring and the lactone is optimal in a series where zero, two, and three methylene units were prepared with the naphthalene ring unsubstituted.

Halogens in the 2- and 4-positions were activity enhancing as they were in the benzyl ether[5] and the biphenyl[6] series. A halogen in the 8-position also was useful. The combination of 2,4,8-trihalo substitution is an obvious objective; however, this pattern was not readily accessible synthetically. We opted rather for the more accessible 2,4,6- and 2,4,7-trichloro compounds 7 and 8, whose activity turned out to be outstanding and of a useful order of magnitude since they are racemates and, if resolved, would have activity comparable to compactin (1b). The importance of the 2-substituent was reemphasized with the nearly total loss of activity of compound 9 when compared to 8. The synthesis of these compounds was however long and inefficient. Therefore, we next prepared all-methyl compound 10, where the more readily executed Friedel–Crafts chemistry could be used. To our dismay it had very little activity. This result is contrary to the biphenyl series,[6] where replacement of chlorines with methyls was permissible. We concluded that at least one of the chlorines was needed. Accordingly, we replaced the 7-methyl substituent with chlorine, which gave only a small increase in activity. Clearly replacement of another chlorine was necessary, so we prepared both of the remaining chlorine substitutions at the 2- and 4-positions, leaving the chlorine in the 7-position (compounds 12 and 13). Although most of the activity was restored, the activity of 12 and 13 is not high enough to warrant further biological evaluation.

Conclusions

A useful order of activity has been achieved in the two trichlorinated naphthalene derivatives 7 and 8. All the permutations of a methyl substituent were not made, but those that were prepared indicate that all three chlorines are needed for a useful order of activity. The protracted and tedious chemistry of the trichlorinated compounds coupled with the inability to use Friedel–Crafts chemistry in the presence of two inactivating chlorine substituents led us to terminate this work.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) on a Varian T-60, EM-390, XL-300, or NT 360 spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as the internal standard. Elemental analysis for carbon, hydrogen, and nitrogen were determined with a Perkin-Elmer Model 240 elemental analyzer and are within ±0.4% of the theoretical values unless noted otherwise. All starting materials were commercially available and used as received unless so indicated.

4,6-Dichloro-7-[[(trifluoromethyl)sulfonyl]oxy]indan-1-one (18). 4,6-Dichloro-7-hydroxyindan-1-one[17] (21.71 g, 0.1 mol) was dissolved in DMF (80 mL) in a dry apparatus under nitrogen. Trifluoromethanesulfonyl chloride (21.60 g, 0.128 mol) was added with stirring, slowly, dropwise over a 20-min period with occasional cooling to keep the internal temperature below 30 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 30 min and then poured into ice–water with swirling. The green crystals were collected, washed with water, sucked dry, and then dried in a vacuum oven at 50 °C to give 32.7 g of product. mp: 96–100 °C. Recrystallization from hexanes gave 22.4 g. mp: 96–98 °C. A sublimed sample had the following. mp: 90–96 °C. Anal. ($C_{10}H_5Cl_2F_3O_4S$): C, H.

4,6-Dichloro-7-iodoindan-1-one (19). 4,6-Dichloro-7-[[(trifluoromethyl)sulfonyl]oxy]indan-1-one (56.0 g, 0.160 mol), sodium iodide (133.1 g, 0.8 mol), and DMF (320 mL) in a dry apparatus were stirred under nitrogen at a bath temperature of 130 °C for 4 days, cooled to room temperature, and poured into 1 L of ice–water. The crystals were collected, washed with water, dried

(17) Hokema, T.; Traxler, J. T. U.S. 4,322,414, 1982.

overnight in a vacuum oven at 50 °C, and then sublimed at 170–190 °C at 0.05 mm to give 38.3 g of crude product, which was recrystallized from toluene to give 31.5 g of product. mp 170–172 °C; ^{1}H NMR: δ 2.7–3.2 (4 H, m), 7.6 (1 H, s). Anal. ($C_9H_5Cl_2IO$): C, H.

4,6-Dichloro-7-iodoindan-1-ol (20). 4,6-Dichloro-7-iodoindan-1-one (14.71 g, 45 mmoles) was suspended and partially dissolved in ethanol (140 mL). Sodium borohydride (1.70 g, 45 mmol) was added and the mixture was stirred for 50 min. Aqueous sodium hydroxide 20% (w/v) (40 mL) was added and stirred for 10 min. The reaction mixture was poured into 700 mL of ice–water with vigorous stirring. The crystals were collected, washed with water, sucked dry, and dried in a vacuum oven at 50 °C overnight to give 14.08 g of the title compound, mp 95–100 °C. Recrystallization from acetonitrile gave material with the following data. mp: 99–102 °C. ^{1}H NMR: δ 2.1–3.3 (4 H, m), 5.2 (1 H, m), 7.3 (1 H, s). Anal. ($C_9H_7Cl_2IO$): C, H.

4,6-Dichloro-7-iodo-1-indene and 4,6-Dichloro-7-iodo-2-indene (21 and 22). 4,6-Dichloro-7-iodo-1-indanol (13.98 g, 42.50 mmol) was dissolved in ether (350 mL) and the solution was stirred mechanically. Phosphorus pentoxide (6.03 g 42.50 mmol) was added and the sealed reaction mixture was stirred vigorously overnight. The addition of phosphorus pentoxide (6.03 g, 42.5 mmol) and stirring overnight was repeated three times. The ether containing the product was decanted, washed with aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 10.76 g of a mixture of the title compounds. mp: 89–96 °C. Recrystallization from hexane gave material with the following data. mp: 95–97 °C. ^{1}H NMR: δ 3.5 (2 H, m), 6.5–6.9 (2 H, m), 7.25 (1 H, s). Anal. ($C_9H_5Cl_2I$): C, H.

1,1,3,5-Tetrachloro-1a,6a-dihydro-2-iodocycloprop[*a*]indene and 1,1,2,3-Tetrachloro-1a,6a-dihydro-5-iodocycloprop[*a*]indene (23 and 24). To a solution of a mixture of 4,6-dichloro-7-iodo-1-indene and 4,6-dichloro-7-iodo-2-indene (3.11 g, 10 mmol) and ethyl trichloroacetate (17.2 g, 12.5 mL, 90 mmol) in dry toluene (20 mL) cooled in an ice bath and stirred under nitrogen was added, in divided portions, fresh sodium methoxide (5.4 g, 100 mmol). After the addition was complete, the reaction was stirred for 2.5 h in an ice bath. When the reaction was complete, the mixture was diluted with ether and extracted with water. The ether layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated in vacuo to leave 8.1 g of crude product. The product was triturated with hexanes and filtered, and the solvent was evaporated in vacuo from the hexane-soluble product. This crude product was chromatographed on silica gel (500 g) eluting with hexanes to give, after evaporation of the solvent, in vacuo, 1.4 g of the mixture of compounds as an oil. ^{1}H NMR: δ 2.2–2.55 (1 H, m), 3.15–3.6 (3 H, m), 7.2 (1 H, s).

2,4,7-Trichloro-1-iodonaphthalene and 2,4,6-Trichloro-1-iodonaphthalene (25). A mixture of 1,1,3,5-tetrachloro-1a,6a-dihydro-2-iodocycloprop[*a*]indene and 1,1,2,4-tetrachloro-1a,6a-dihydro-5-iodocycloprop[*a*]indene (4.54 g, 11.5 mmol) was refluxed in 10% (w/v) KOH in ethanol (100 mL) for 1.5 h and cooled and approximately 80% of the ethanol was evaporated in vacuo. The remainder was dissolved in ether and extracted with water, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 3.4 g of crude product, which was flash chromatographed on a silica gel column (60 × 150 mm) by elution with hexane to give, after evaporation of the solvent in vacuo, 2.85 g of the product mixture. mp: 45–50 °C. Ratio of the two naphthalenes is 4:5 or 5:4. ^{1}H NMR: δ 7.0–7.9 (4 H, m).

2,4,6-Trichloro-1-naphthoic Acid and 2,4,7-Trichloro-1-naphthoic Acid (Ratio 5:4 or 4:5) (26). The mixture of 2,4,7-trichloro-1-iodonaphthalene and 2,4,6-trichloro-1-iodonaphthalene (7.79 g, 21.8 mmol) was dissolved in dry ether (200 mL) and cooled under nitrogen to an internal temperature of −50 °C with stirring. Butyl lithium (17.7 mL of a 1.48 M solution in hexane, 26.2 mmol) was added dropwise over about 5 min. The reaction was stirred for 30 min at −78 °C. The −78 °C reaction mixture was poured onto powdered dry ice (excess) covered with ether. The excess CO_2 was allowed to evaporate and the ether warmed to room temperature. The ether was extracted with water once and four times with dilute aqueous $NaHCO_3$ solution. The combined aqueous extracts were acidified with concentrated HCl, the product was extracted with ether four times, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 4.0 g of the product mixture. mp: 182–200 °C. Anal. ($C_{11}H_5Cl_3O_2$): C, H.

Methyl 2,4,6-Trichloro-1-naphthoate and Methyl 2,4,7-Trichloro-1-naphthoate (27): Preparation and Separation. The mixture of 2,4,6-trichloro-1-naphthoic acid and 2,4,7-trichloro-1-naphthoic acid (3.63 g, 13.2 mmol) was dissolved in ether and cooled to 5 °C. Diazomethane, in ether (generated from 3.40 g of *N*-nitroso-*N*-methylurea and base in 50 mL of ether at 5 °C), was added dropwise to maintain the internal temperature below 5 °C. An excess was noted by the persistence of a yellow color. The reaction mixture was stirred a few minutes and the excess diazomethane was blown off with nitrogen, and the solvent was evaporated in vacuo to leave 3.7 g of the product mixture.

The two isomers were separated by preperative HPLC (Waters 500) using 5% methylene chloride in hexane. The solvent from the first isomer to emerge from the column was evaporated in vacuo to leave 1.4 g of methyl 2,4,7-trichloro-1-naphthoate (27a).[18] mp: 113–115 °C. ^{1}H NMR: δ 4.09 (3 H, s), 7.25–8.25 (4 H, m). Anal. ($C_{12}H_7Cl_3O_2$): C, H.

The solvent containing the second isomer from the column was evaporated in vacuo to leave 1.1 g of methyl 2,4,6-trichloro-1-naphthoate (27b).[18] mp: 110–112 °C. ^{1}H NMR: δ 4.07 (3 H, s), 7.25–8.3 (4 H, m). Anal. ($C_{12}H_7Cl_3O_2$): C, H.

(2,4,7-Trichloronaphthalen-1-yl)methanol (28a). A solution of methyl 2,4,7-trichloro-1-naphthoate (1.3 g, 4.5 mmol) in ether (50 mL) was added dropwise (15 min) to a well-stirred suspension of lithium aluminum hydride (0.25 g, 6 mmol) in ether (25 mL). After stirring at room temperature for 17 h, the reaction mixture was treated with an additional 0.25 g of lithium aluminum hydride. The mixture was stirred for 3 h, cooled in an ice bath, and treated dropwise with 0.5 mL of water, 1.5 mL of 20% (w/v) of aqueous NaOH solution, and 0.5 mL of water. After filtration, the solid was extracted with ether. The combined ether solutions were dried ($MgSO_4$), filtered, and concentrated in vacuo to give 1.0 g of the product. mp: 107–112 °C. ^{1}H NMR: δ 5.23 (2 H, d), 7.55–8.28 (4 H, m).

1-(Chloromethyl)-2,4,7-trichloronaphthalene (29a). (2,4,7-Trichloronaphthalen-1-yl)methanol (1.0 g, 3.8 mmol) was added portionwise to thionyl chloride (10 mL) with cooling (ice bath). The reaction mixture was stirred at room temperature for 30 min at a reflux for 2 h and then concentrated to dryness in vacuo. The oily residue was taken up in methylene chloride and the solution was dried over $MgSO_4$. The solution was filtered and concentrated in vacuo to give 1.0 g of the product. ^{1}H NMR: δ 5.12 (2 H, s), 7.58–8.27 (4 H, m).

3-(2,4,7-Trichloronaphthalen-1-yl)propanal (31a). A solution of *n*-butyllithium in hexane (3.2 mL, 4.3 mmol) was added dropwise (3 min) to a solution of diisopropylamine (0.45 g, 4.5 mmol) in dry tetrahydrofuran (10 mL) with cooling (ice bath). After stirring under nitrogen for 15 min, ethylidenecyclohexylamine (0.55 g, 4.3 mmol) was added dropwise (5 min) at 0 °C. The mixture was stirred for 15 min and then the ice bath was replaced by a dry ice–acetone bath. A solution of 1-(chloromethyl)-2,4,7-trichloronaphthalene (1.0 g, 3.8 mmol) in tetrahydrofuran (15 mL) was added (5 min) at −75 °C. The reaction mixture was stirred at −70 °C for 30 min and at room temperature overnight (20 h) and then concentrated to dryness in vacuo. The residual oil was taken up in ether (100 mL) and 5% aqueous oxalic acid (100 mL) and the mixture was stirred at room temperature for 3.5 h. The layers were separated, and the aqueous phase was extracted (2×) with ether. The ether extracts were combined, washed with cold water and brine, and dried over $MgSO_4$. The solution was filtered and concentrated in vacuo to give a red-brown oil (1.1 g). This material was chromatographed with a 50-mm flash column containing 150 g of silica gel (230–400 mesh) eluting with 30% methylene chloride in hexane (v/v) to give 0.21 g of the product as a pale yellow solid. ^{1}H NMR: δ 2.81 (2 H, m),

(18) One of the compounds gave a 3% NOE of the proton in the 8-position when the methyl protons of the ester was irradiated. This compound was assigned structure 27a because it has no adjacent hydrogen for relaxation of the NOE. The other compound did not show an NOE. Further, when ester 27a is reduced to hydroxy methylene, the proton in the 8-position exhibits a 10% NOE when the methylene hydrogens are irradiated.

3.48 (2 H, m), 7.53–8.27 (4 H, m), 9.92 (1 H, b s).

3-(2,4,6-Trichloronaphthalen-1-yl)propanol (31b). With essentially the same chemistry with the other isomeric methyl 2,4,6-trichloro-1-naphthoate, there was obtained via essentially the same three steps the isomeric propanol (31b). ^{1}H NMR: δ 2.81 (2 H, t), 3.50 (2 H, t), 7.57 (1 H, dd), 7.63 (1 H, s), 7.93 (1 H, d), 8.28 (1 H, d), 9.91 (1 H, s). Anal. ($C_{13}H_9Cl_3O$): C, H.

3-(4,6-Dichloronaphthalen-1-yl)propanal (33). A small amount of a second product isolated by the chromatographic purification of 3-(2,4,6-trichloronaphthalen-1-yl)propanal was identified as 3-(4,6-dichloronaphthalen-1-yl)propanal by its ^{1}H NMR and by its conversion to 9. ^{1}H NMR: δ 2.78 (2 H, t), 3.34 (2 H, t), 7.14–8.2 (5 H, m), 9.5 (1 H, s). This reduction probably took place at the reduction of the ester methyl 2,4,6-trichloro-1-naphthoate via a six-membered intramolecular hydride transfer from an intermediate oxyaluminum hydride complex and was carried through the reaction sequence.

3-(2,4-Dimethylphenyl)propyl Benzoate (35). Benzoyl chloride (21.0 g, 0.15 mol) dissolved in dry pyridine (10 mL) was added slowly dropwise (15 min) to a well-stirred solution of 3-(2,4-dimethylphenyl)propanol (21.7 g, 0.132 mol) in dry pyridine (40 mL) with cooling in an ice-water bath. The reaction was then stirred overnight and then poured into ice–water (300 mL) and the excess pyridine was removed by azeotropic evaporation of solvent in vacuo. The remainder was partitioned between ether and water. The ether layer was washed successively with water, aqueous $NaHCO_3$, and brine, and then dried ($MgSO_4$) and filtered, and the solvent was evaporated in vacuo to leave 39 g of crude product, which was distilled in vacuo to give 35.1 g of pure product. bp1.5 mm: 176–182 °C. Anal. ($C_{18}H_{20}O_2$): C, H.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-4-oxo-2-methylbutyric Acid (37). Aluminum chloride (4.6 g, 34 mmol) was added in divided portions (5 min) to a well-stirred solution of 3-(2,4-dimethylphenyl)propyl benzoate (2.7 g, 10 mmol) and methylsuccinic anhydride (1.2 g, 10.5 mmol) in anhydrous nitroethane (15 mL), which was cooled in an ice-water bath. After the addition was complete, the ice bath was removed and the reaction stirred at ambient temperature for 2 h and then poured into ice–water (150 mL) containing 2 mL of concentrated HCl. The product was extracted (2×) with ether, and the combined ether extracts were washed with cold water and then brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 3.8 g of crude product, which is pure enough for the next step but may be purified with silica gel flash chromatography (60 × 150 mm), eluting with methylene chloride and then a mixture of acetic acid (0.5%), acetone (4.5%), and methylene chloride (95%). After evaporation of the fractions containing the product, there remained 3.1 g of product as an oil. Anal. ($C_{23}H_{26}O_5$): C, H.

Activated Zinc Dust. Zinc dust (24 g) was stirred with 2% aqueous HCl (150 mL) for 5 min, filtered by suction, and washed with water until the washings were neutral. The zinc was then washed successively with ethanol (75 mL), acetone (150 mL), and ether and then dried in a vacuum oven at 90 °C for 15 min and then used promptly in the following reaction.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-2-methylbutyric Acid (38). Dry gaseous HCl was bubbled vigorously into a solution of 4-[2,4-dimethyl-5-[3-(benzoyloxy)-propyl]phenyl]-4-oxo-2-methylbutyric acid (8.0 g, 20 mmol) in dry ether (360 mL) for 15 min while being cooled in an ice-water cooling bath. Activated zinc dust was added in small portions with cooling in an ice-water bath so as to keep the internal temperature below 80 °C. After the addition, the reaction was cooled with an ice–water bath and stirred for 1 h. The reaction mixture was diluted with ether and then passed onto ice-water (350 mL) containing a little HCl (2 mL) and extracted with ether (2×). The combined ether extracts were washed with water and brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 7.3 g of crude oily product, which was pure enough for the next step. A 0.2-g sample was purified by silica gel flash chromatography on a 20 × 150 mm Still column after eluting with methylene chloride and then with a mixture of 0.5% acetic acid, 4.5% acetone, and 95% methylene chloride. The fractions containing the product were combined, and the solvent was evaporated in vacuo to give 0.11 g of pure product as an oil. Anal. ($C_{23}H_{28}O_4$): C, H.

3-(5,6,7,8-Tetrahydro-2,4,7-trimethyl-8-oxonaphthalen-1-yl)propyl Benzoate (39). A solution of 4-[2,4-dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-2-methylbutyric acid (7.4 g, 20 mmol) in methylene chloride (20 mL) was added dropwise in 10 min to oxalyl chloride (20 mL) with stirring and cooling in an ice-water bath. After the addition, the reaction was stirred at room temperature for 30 min and then warmed slowly to a bath temperature of 65 °C when the reaction refluxed. The refluxing was continued with stirring for 2 h. The reaction was then cooled, and the excess oxalyl chloride and solvent were evaporated in vacuo to leave 4-[2,4-dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-2-methylbutyryl chloride as an oil which was dissolved in dry methylene chloride (20 mL) and cooled in an ice-water bath. To this was added a solution of stannic chloride (20 mL) in dry methylene chloride (20 mL) at a rapid drip (10 min). The reaction was stirred at room temperature for 30 min and poured into ice–water (300 mL), containing concentrated HCl (20 mL). The mixture was extracted with ether (3×). The combined ether extracts were washed successively with water twice, aqueous sodium bicarbonate, water, and brine, dried ($MgSO_4$), and filtered and the solvent was evaporated to leave 7.2 g of crude product, which was purified by silica gel flash chromatography using an 80 × 160 mm Still column eluting with methylene chloride for 35 × 125 mL fractions and then 2% acetone in methylene chloride for 20 × 125 mL fractions. The fractions containing the product were combined, and the solvent was evaporated in vacuo to leave 3.3 g of oil product. ^{1}H NMR: δ 1.22 (3 H, d), 1.82 (1 H, m), 2.00 (1 H, m) 2.1–2.3 (2 H, m), 2.23 (3 H, s), 2.34 (3 H, s), 2.66 (1 H, m), 2.80 (1 H, m), 2.90 (1 H, m), 3.04 (2 H, t), 4.47 (2 H, t), 7.14 (1 H, s), 7.45 (2 H, t), 7.56 (1 H, t), 8.10 (2 H, d). Anal. ($C_{23}H_{26}O_3$): C, H.

***cis*- and *trans*-3-(5,6,7,8-Tetrahydro-8-hydroxy-2,4,7-trimethylnaphthalenyl)propyl Benzoate (40).** Sodium borohydride (0.50 g, 13 mmol) was added in divided portions to a stirred solution of 3-(5,6,7,8-tetrahydro-2,4,7-trimethyl-8-oxonaphthalen-1-yl)propyl benzoate (2.65 g, 7.5 mmol) in ethanol (40 mL) and then stirred at room temperature for 7 h (reaction complete by TLC; 1% acetone in methylene chloride–silica gel). The clear reaction was poured into ice water, acidified with dilute HCl, and extracted with ether (3×). The combined ether extracts were washed successively with cold water and brine, dried ($MgSO_4$), filtered, and the solvent was evaporated in vacuo to leave 2.7 g of the product.

3-(5,6-Dihydro-2,4,7-trimethylnaphthalen-1-yl)propyl Benzoate (41). *cis*- and *trans*-3-(5,6,7,8-tetrahydro-8-hydroxy-2,4,7-trimethylnaphthalen-1-yl)propyl benzoate (2.7 g, 7.7 mmol) were dissolved in dry ether (200 mL), to this was added powdered phosphorus pentoxide (5 g), and the sealed reaction mixture was stirred overnight. The addition of phosphorus pentoxide and stirring overnight was repeated once. When TLC (1% acetone in methylene chloride/silica gel) showed the reaction to be complete. The ether was decanted and the residue was washed with ether by decantation. The phosphorus residue was treated with ice–water and extracted with ether. The combined ether decantations and washings were washed successively with water, aqueous sodium bicarbonate, and brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave 2.8 g of crude product. This product was purified by flash chromatography on a 50 × 160 mm Still column eluting with 50% hexane in methylene chloride. The fractions containing the product were combined, and the solvent was evaporated in vacuo to give 1.6 g of oil product. ^{1}H NMR: δ 1.66–2.2 (2 H, m), 1.9 (3 H, s), 2.2 (3 H, s), 2.25 (3 H, s), 2.6–3.0 (6 H, m), 4.2 (2 H, s), 6.45 (1 H, s), 6.8 (1 (H, s), 7.2–7.6 (3 H, m), 7.9–8.1 (2 H, m).

3-(2,4,7-Trimethylnaphthalen-1-yl)propyl Benzoate (42). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); 0.95 g, 42 mmol) was added to a solution of 3-(5,6-dihydro-2,4,7-trimethylnaphthalen-1-yl)propyl benzoate (1.25 g, 37 mmol) in toluene (60 mL) and stirred at room temperature for 1 h. The reaction mixture was filtered and the solvent was evaporated in vacuo to leave crude product. This product was purified by flash chromatography on a 50 × 150 mm Still silica column eluting with 50% hexane in methylene chloride. The fractions containing the product were combined, and the solvent was evaporated in vacuo to leave 0.78 g of oil product. ^{1}H NMR: δ 2.12 (2 H, m), 2.47 (3 H, s), 2.49 (3 H, s), 2.62 (3 H, s), 3.22 (2 H, t), 4.52 (2 H, 5), 7.19

(1 H, s), 7.29 (1 H, d), 7.48 (2 H, t), 7.59 (1 H, t), 7.83 (1 H, s), 7.87 (1 H, d), 8.12 (2 H, d). Anal. ($C_{25}H_{24}O_2$): C, H.

3-(2,4,7-Trimethylnaphthalen-1-yl)propanol (43). A solution of 3-(2,4,7-trimethylnaphthalen-1-yl)propyl benzoate (0.75 g, 2.3 mmol) was added to a solution of potassium hydroxide (0.5 g, 7 mmol) in ethanol (50 mL) and stirred at room temperature for 4 h. Most of the ethanol was evaporated in vacuo and the residue was partitioned between ether and water. The ether was washed with water twice, dried ($MgSO_4$), and filtered, and the solvent was evaporated in vacuo to leave 0.53 g of product.

3-(2,4,7-Trimethylnaphthalen-1-yl)propanol (44). 3-(2,4,7-Trimethylnaphthalen-1-yl)propanol (0.68 g, 3 mmol) was added to a suspension of pyridinium chlorochromate (1.28 g, 6 mmol) in methylene chloride (20 mL). The reaction mixture was stirred at room temperature for 2 h and then diluted with ether (10 mL) and the solvent was decanted. The black solids were washed with ether by decantation twice. The combined organic extracts were filtered through a pad of Florisil, and the solvent was evaporated in acid to leave 0.53 g of product. Recrystallization from petroleum ether gave a white crystalline solid. mp: 79–83 °C. ^{1}H NMR: δ 2.44 (3 H, s), 2.54 (3 H, s), 2.62 (3 H, s), 2.77 (2 H, t), 3.36 (2 H, t), 7.09 (1 H, s), 7.31 (1 H, d), 7.69 (1 H, s), 7.88 (1 H, d), 9.93 (1 H, t). Anal. ($C_{16}H_{18}O$): C, H.

3-(5,6,7,8-Tetrahydro-2,4-dimethyl-8-oxonaphthalen-1-yl)propyl Benzoate. Following the experimental method of the 2,4,7-trimethyl analogue but substituting succinic anhydride for 3-methyl succinic anhydride, there was obtained in succession the following.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]-4-oxobutyric acid (45) as an oil (3.68 g, 78%). Anal. ($C_{22}H_{24}O_5$): C, H.

4-[2,4-Dimethyl-5-[3-(benzoyloxy)propyl]phenyl]butyric Acid (46).

3-(5,6,7,8-Tetrahydro-2,4-dimethyl-8-oxonaphthalen-1-yl)propyl Benzoate (47). TLC: R_f = 0.33, 1% acetone/CH_2Cl_2. ^{1}H NMR: δ 1.75–2.42 (4 H, m), 2.42 (3 H, s), 2.50 (3 H, s), 2.50–2.95 (4 H, m), 2.95–3.32 (2 H, m), 4.72 (2 H, t), 7.15 (1 H, s), 7.3–7.55 (3 H, m), 7.92–8.15 (2 H, m).

7-Chloro-2,4-dimethyl-1-(3-hydroxypropyl)naphthalene. 1-[3-(Benzoyloxy)propyl]-7-chloro-2,4-dimethylnaphthalene (48) (2.60 g, 7.37 mmol) was suspended in ethanol (30 mL) and potassium hydroxide (1.65 g, 29.5 mmol) added and stirred at room temperature for 2 h then at 60–65 °C bath temperature for 1 h. The reaction mixture was cooled in an ice bath, filtered from sodium benzoate, and washed thoroughly with ethanol. The combined filtrates were dissolved in ether and extracted with water. The water was extracted with ether three times. The combined ether extracts were washed with water three times and then with brine, dried ($MgSO_4$) and filtered, and the solvent was evaporated to leave 1.80 g (98%) of the product. A sublimed sample [100 °C bath temp (0.1 mm)] had mp 104–105 °C. Exact mass calcd for $C_{15}H_{17}ClO$: 248.0968. Found: 248.0968. ^{1}H NMR: δ 1.60–2.20 (4 H, m), 2.44 (3 H, s), 2.56 (3 H, s), 3.05 (2 H, q), 3.74 (2 H, t), 7.0–7.95 (4 H, m).

3-(7-Chloro-2,4-dimethylnaphthalen-1-yl)propanal (49). Pyridinium chlorochromate (3.12 g, 14.47 mmol) and powdered 3-Å molecular sieves (3.6 g) were suspended in methylene chloride (25 mL), and 7-chloro-2,4-dimethyl-1-(3-hydroxypropyl)-naphthalene (1.70 g, 6.83 mmol) dissolved in methylene chloride (25 mL) was added all at once and stirred for 2 h. The reaction mixture was worked up by diluting with ether (50 mL) and filtering through a silica gel pad. The pad was washed with ether and the solvent was evaporated in vacuo to give 1.24 g (73%) of product. Exact mass calcd for $C_{15}H_{15}ClO$: 246.0811. found: 246.0813. ^{1}H NMR: δ 2.3–2.95 (2 H, m), 2.40 (3 H, s), 2.55 (3 H, s), 3.25 (2 H, t), 7.02–8.0 (4 H, m). TLC: R_f = 0.36 (50% CH_2Cl_2–hexane/silica gel).

Ethyl 3-(4-Chloro-2-methylphenyl)propionate (51b). Boron trifluoride etherate (1.5 mL, 0.012 mol) was added dropwise to a solution of 3-(4-chloro-2-methylphenyl)propionic acid (1.99 g, 0.01 mol) in absolute ethanol (14 mL). The reaction mixture was heated at reflux for 6.5 h, cooled, and concentrated in vacuo to remove the solvent, and the residual oil was taken up in ether. The ether solution was washed with aqueous Na_2CO_3 and cold water, dried, and evaporated to give an orange oil, which was distilled at about 1.5 mm to give the product as an oil (1.5 g, 66%). bp: 126–131 °C. Anal. ($C_{12}H_{13}ClO_2$): C, H.

4-[2′-Chloro-4′-methyl-5′-[2-(ethoxycarbonyl)ethyl]-phenyl]-4-oxobutyric Acid (52b). Aluminum chloride (5.87 g, 0.044 mol) was added portionwise (5 min) to a mixture of succinic anhydride (1.1 g, 0.011 mol) and ethyl 3-(4-chloro-2-methylphenyl)propionate (2.27 g, 0.01 mol) in CH_2Cl_2 (20 mL) with cooling (ice bath). The reaction mixture was stirred at room temperature for 24 h, poured into ice and 10 mL of concentrated HCl, and extracted with ether. The ether solution was dried and evaporated to give a yellow brown oil, which was purified by flash column chromatography (silica gel and 2% HOAc–10% acetone–90% CH_2Cl_2) to give the product as a yellow oil (3.0 g, 92% yield). Anal. ($C_{16}H_{19}ClO_5$): C, H.

4-[2′-Chloro-4′-methyl-5′-[2-(ethoxycarbonyl)ethyl]-phenyl]butyric Acid (53b). Gaseous HCl was bubbled into a well-stirred solution of 4-[2′-chloro-4′-methyl-5′-[(ethoxycarbonyl)ethyl]phenyl]-4-oxobutyric acid (3.27 g, 0.01 mol) in acetic anhydride (60 mL) for 20 min with cooling (ice–acetone bath). Activated zinc dust (13.11 g 6.2 mol) was added portionwise (15 min) to keep the temperature below 0 °C. The reaction mixture was stirred at about 0 °C for 7 h, filtered (glass wool) into ice and water and extracted with ether. The ether solution was dried and evaporated to give a brown oil, which was purified by flash column chromatography (silica gel and 0.5% HOAc–4.5% acetone–95% CH_2Cl_2) to yield the product as a viscous yellow oil (2.17 g, 69%).

Ethyl 3-(4-Chloro-2-methyl-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)propanoate (54b). Oxalyl chloride (23.5 mL) was added dropwise to a well-stirred solution of 4-[2′-chloro-4′-methyl-5′-[(ethoxycarbonyl)ethyl]phenyl]butyric acid (10.38 g, 0.033 mol) in toluene (50 mL). The reaction mixture was stirred at room temperature for 18 h, heated at reflux for 4 h, cooled, and concentrated to dryness, and the residual oil was taken up in CH_2Cl_2 (50 mL). After addition of stannic chloride (31.5 mL) with cooling (ice bath), the reaction mixture was stirred at room temperature for 5 days and then poured into ice and concentrated HCl (20 mL) and extracted with ether. The ether solution was dried and evaporated to give a viscous brown oil, which after silica gel chromatography eluting with 15% ethyl acetate in hexane gave the product as a gum. Exact mass calcd for $C_{16}H_{19}ClO_3$: 294.1021. Found: 294.1019. ^{1}H NMR: δ 1.27 (3 H, t, CH_2CH_3), 2.10 (2 H, p, CH_2), 2.34 (3 H, s, $ArCH_3$), 2.57 (2 H, t, CH_2), 2.65 (2 H, t, CH_2), 2.99 (2 H, t, CH_2), 3.21 (2 H, t, CH_2), 4.16 (2 H, q, CH_2CH_3), 7.36 (1 H, s, Ar).

3-(4,7-Dichloro-2-methylnaphthalen-1-yl)propanal (56b). Ethyl 3-(4,7-dichloro-2-methylnaphthalen-1-yl)propanoate (1.583 g, 5.087 mmol) was dissolved in dry toluene (25 mL) under nitrogen with syringe cap attached to flask. The solution was cooled to −78 °C in dry ice–acetone bath and diisobutylaluminum hydride (3.62 mL of a 1.5 M solution in toluene, 5.443 mmol) was added dropwise slowly by syringe. Stirring was continued at −78 °C for 1 h. Then while still at −78 °C, the reaction was poured quickly into an aqueous NH_4Cl solution with stirring. This mixture was extracted two times with ether. The combined ether extracts were extracted successively with NH_4Cl solution, water, and brine, and then dried ($MgSO_4$), and filtered, and the solvent was evaporated to leave a solid. This solid was triturated with a little ether in hexane to give 0.701 g of pure solid product (mp: 104–106 °C). The solvent was stripped from the mother liquor to give 0.681 g of impure product. This impure product was flash chromatographed on a 20 × 200 mm silical column eluting with 70% CH_2Cl_2 in hexane to give 0.45 g of pure solid product. mp: 103–105 °C. Combining the two samples of pure solid product gave 1.15 g of pure product (mp: 104–106 °C) after drying. ^{1}H NMR: δ 2.47 (3 H, s, CH_3), 2.76 (2 H, t, CH_2), 3.28 (2 H, t, CH_2), 7.42 (1 H, s, Ar), 7.49 (1 H, dd, Ar), 7.90 (1 H, d, Ar), 8.22 (1 H, d, Ar), 9.92 (1 H, s, *CHO*). Anal. ($C_{14}H_{12}Cl_2O$) C, H.

With the above experimental procedures but substituting 3-(2-chloro-4-methylphenyl)propionic acid for 3-(4-chloro-2-methylphenyl)propionic acid there was obtained in succession the following.

Etbyl 3-(2-Chloro-4-methylphenyl)propanoate (51a). Bp: 104–107 °C. Anal. ($C_{12}H_{15}ClO_2$): C, H.

4-[4′-Chloro-2-methyl-5-[2-(ethoxycarbonyl)etbyl]-phenyl]-4-oxobutyric Acid (52a). Mp: 72–74 °C. Anal. ($C_{16}H_{19}ClO_5$): C, H.

4-[4-Chloro-2-methyl-5-[2-(ethoxycarbonyl)ethyl]-phenyl]butyric Acid (53a). Mp: 50–52 °C. Anal. ($C_{16}H_{21}ClO_4$): C, H.

Ethyl 3-(2-Chloro-4-methyl-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl) (54a). Mp: 63–65 °C. Anal. ($C_{16}H_{19}ClO_3$): C, H.

3-(2,7-Dichloro-4-methylnapbthalen-1-yl)propanal (56a). mp: 103–105 °C. ^{1}H NMR: δ 2.66 (3 H, s), 2.82 (2 H, t), 3.49 (2 H, t), 7.34 (1 H, s), 7.52 (1 H, d), 7.95 (2 H, m), 9.95 (1 H, s). Anal. ($C_{14}H_{12}Cl_2O$): C, H.

Registry No. 2, 124243-86-3; 2-Na, 124244-18-4; 3, 124243-87-4; 3-Na, 124244-19-5; 4, 124243-88-5; 4-Na, 124244-20-8; 5, 124243-89-6; 5-Na, 124244-21-9; 6, 124243-90-9; 6-Na, 124244-22-0; 7, 124243-91-0; 7-Na, 124244-23-1; 8, 124243-92-1; 8-Na, 124244-24-2; 9, 124243-93-2; 9-Na, 124244-25-3; 10, 124243-94-3; 10-Na, 124244-26-4; 11, 124243-95-4; 11-Na, 124244-27-5; 12, 108579-26-6; 12-Na, 124244-28-6; 13, 108579-36-8; 13-Na, 124244-29-7; 14, 124243-96-5; 15, 124243-97-6; 16, 124243-98-7; 17, 81945-11-1; 18, 108578-92-3; 19, 108578-93-4; 20, 10578-94-5; 21, 108578-95-6; 22, 108578-96-7; 23, 108578-97-8; 24, 108578-98-9; 25a, 108578-99-0; 25b, 108579-00-6; 26a, 108579-02-8; 26b, 108579-01-7; 27a, 108579-04-0; 27b, 108579-03-9; 28a, 108579-05-1; 28b, 124244-13-9; 29a, 108579-06-2; 29b, 124244-14-0; 31a, 108579-07-3; 31b, 108579-11-9; 33, 124243-99-8; 34, 27650-80-2; 35, 124244-00-4; 37, 124244-01-5; 38, 124244-02-6; 39, 124244-03-7; *cis*-40, 124244-04-8; *trans*-40, 124244-17-3; 41, 124244-05-9; 42, 124244-06-0; 43, 124244-07-1; 44, 124244-08-2; 45, 124266-46-2; 46, 124244-09-3; 47, 124244-10-6; 48, 124244-11-7; 49, 124244-12-8; 50a, 615-65-6; 50b, 95-69-2; 51a, 108579-27-7; 51b, 108579-13-1; 52a, 108579-28-8; 52b, 108579-14-2; 53a, 108579-29-9; 53b, 108579-15-3; 54a, 108579-30-2; 54b, 108579-16-4; 55a, 108579-34-6; 55b, 108579-22-2; 56a, 108579-35-7; 56b, 108579-23-3; Cl_3CCO_2Et, 515-84-4; H_2-CCOC-HCO_2Me, 30568-00-4; 3-hydroxy-3-methylglutaryl-coenzyme A, 1553-55-5; *N*-ethylidenecyclohexylamine, 1193-93-7; methylsuccinic anhydride, 4100-80-5; succinic anhydride, 108-30-5; 7-chloro-2,4-dimethyl-1-(3-hydroxypropyl)naphthalene, 124244-15-1; 3-(4-chloro-2-methylphenyl)propionic acid, 879-75-4; 3-(2-chloro-4-methylphenyl)propionic acid, 124244-16-2.

Lipophilic 1,3-Xylyl-21-crown-6 Macrocyclic Polyether 2-Carboxylic Acids as Biological Mimics of the Ionophore Antibiotics

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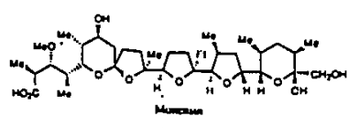
Twelve lipophilic 1,3-xylyl-21-crown-6 macrocyclic polyether 2-carboxylic acids (9a–9l), two lariat ether 1,3-xylyl-21-crown-6 macrocyclic polyether 2-carboxylic acids (21 and 22), and two 1,3-xylyl-28-crown-8 macrocyclic polyether 2-carboxylic acids (10a and 10b) were synthesized and tested for in vitro antibacterial activity, in vitro stimulation of rumen propionic acid production, and in vivo anticoccidial activity in chickens. These are biological screens relevant to animal health areas where the ionophore antibiotics such as monensin have found application. While the parent structure 1 without lipophilic substituents was biologically inactive, the lipophilic macrocycles were active in the two in vitro tests but not against chicken coccidiosis. One compound (9f) was tested in cattle and was found to increase levels of propionic acid in the rumen fermentation. This effect is considered an important factor for increasing the efficiency of feed utilization in cattle exhibited by the ionophore antibiotic monensin. The alkali ion salts of these lipophilic macrocyclic polyether carboxylic acids are very soluble in organic solvents and insoluble in water. These compounds are proposed to act as ion-transport agents and functional mimics of the ionophore antibiotics in the biological systems described above.

The ionophore antibiotics with their fascinating array of complex structures have provided a continuing challenge to organic chemists.[1] These compounds exhibit unique activity in many biological systems via a mechanism of action which is deceptively simple: the exchange of alkali ions for protons across biological membranes.[2] Synthetic molecules which try to mimic the physical properties of the natural antibiotics have been described,[3] but only marginal success was achieved in demonstrating biological activity and no in vivo activity in either animal health area where the ionophores have made a major impact, coccidiosis control in chickens or cattle performance enhancement, has been reported. In this paper, we describe our efforts in the synthesis of polyether mimics of natural ionophores with in vivo activity in cattle and in vitro antibacterial activity.

In 1967, monensin was the first polyether antibiotic to have its structure[4] and potent biological activities,[5] such as inhibition of alkali metal cation transport in mitochondria and broad-spectrum anticoccidial activity, disclosed. It was approved for commercial use as a poultry anticoccidial in 1971 and as a cattle performance enhancer in 1975. The structure of the silver salt of monensin,[4] which is typical for the entire class, has a lipophilic exterior and a hydrophilic central cavity lined with oxygen atoms which serve as ligands for encapsulated alkali ions; the molecule as a whole is therefore neutral and lipophilic. When the carboxylate is protonated, at an interface, either biological or in solvent, the complexation of the ion, while still possible in dry, organic solvents, is weaker by several orders of magnitude[6] and the alkali ion is readily given up to the acidic aqueous layer. It is this large difference in complexation constant for alkali ions between the carboxylic acid and the carboxylate forms of the ionophore



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The solution was washed with saturated $NaHCO_3$ and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–MeOH) to give the title compound (387 mg, 24%) after crystallization from petroleum ether: ^1H NMR ($CDCl_3$) δ 0.06 (s, 6 H, Me_2Si), 0.89 (s, 9 H, Me_3C), 3.66, 3.77 (A_2B_2, 4 H, $SiOCH_2CH_2O$), 5.25 (s, 2 H, NCH_2O), 5.27 [dd, J = 10.9, 1.1 Hz, 1 H, CH═C*H*(*Z*)H(*E*)], 5.98 [dd, J = 17.6, 1.1 Hz, 1 H, CH═CH(*Z*)*H*(*E*)], 6.42 (dd, J = 17.6, 10.9 Hz, 1 H, *CH*═CH_2), 7.41 (s, 1 H, 6-H), 9.59 (br, 1 H, NH).

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-6-(phenylthio)-5-vinyluracil. Following the general procedure for the preparation of 17–19, the title compound was prepared from the above compound with diphenyl disulfide as an electrophile: yield 46%; ^1H NMR ($CDCl_3$) δ 0.01 (s, 6 H, Me_2Si), 0.84 (s, 9 H, Me_3C), 3.63 (s, 4 H, $SiOCH_2CH_2O$), 5.33 [dd, J = 11.8, 2.0 Hz, 1 H, CH═C*H*(*Z*)H(*E*)], 5.61 (s, 2 H, NCH_2O), 6.33 [dd, J = 16.8, 2.0 Hz, 1 H, CH═CH(*Z*)*H*(*E*)], 6.71 (dd, J = 16.8, 11.8 Hz, 1 H, *CH*═CH_2), 7.15–7.30 (m, 5 H, SPh), 10.15 (br, 1 H, NH).

Following method A, 55 was prepared from the above compound.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-5-vinyluracil (55): yield 41%; mp 100–103 °C (EtOAc–petroleum ether); UV (MeOH) λ_{max} 306 (ϵ 7600), 243 nm (ϵ 14 000); MS m/z 320 (M^+); ^1H NMR (Me_2SO-d_6) δ 3.35–3.52 (m, 4 H, $HOCH_2CH_2O$), 4.62 (t, J = 5.4 Hz, 1 H, OH), 5.22 [dd, J = 11.3, 2.2 Hz, 1 H, CH═C*H*(*Z*)H(*E*)], 5.4[illegible], 2 H, NCH_2O), 6.21 [dd, J = 16.4, 2.2 Hz, 1 H, CH═CH(*Z*)*H*(*E*)], 6.63 (dd, J = 16.4, 11.3 Hz, 1 H, *CH*═CH_2), 7.23–7.40 (m, 5 H, SPh), 11.75 (br, 1 H, NH). Anal. ($C_{15}H_{16}N_2O_4S$·$^1/_2H_2O$) C, H, N.

Antiviral Assay Procedures. The anti-HIV assays were based on the inhibition of the virus-induced cytopathic effect in MT-4 cells as previously described.[33] Briefly, MT-4 cells were suspended in culture medium at 2.5×10^5 cells/mL and infected with 1000 $CCID_{50}$ (50% cell culture infective dose) of HIV. Immediately after virus infection, 100 μL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4 (Table II) or 5 (Table I) day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[33] Cytotoxicity of the compounds was assessed in parallel with their antiviral activity. It was based on the viability of mock-infected host cells as determined by the MTT method.[33]

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Inhibitors of Cholesterol Biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-one Inhibitors of HMG-CoA Reductase. 2. Effects of Introducing Substituents at Positions Three and Four of the Pyrrole Nucleus

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A series of *trans*-tetrahydro-4-hydroxy-6-[2-(2,3,4,5-substituted-1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-ones and their dihydroxy acids were prepared and tested for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Inhibitory potency was found to increase substantially when substituents were introduced into positions three and four of the pyrrole ring. A systematic exploration of structure–activity relationships at these two positions led to the identification of a compound ((+)-33, (+)-(4*R*)-trans-2-(4-fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)ethyl]-1*H*-pyrrole-4-carboxamide) with five times the inhibitory potency of the fungal metabolite compactin.

Inhibition of HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, has proven to be an effective means for lowering total and low-density lipoprotein (LDL) cholesterol in animal models and man.[1,2] The early reports describing the activity of the fungal metabolites compactin (mevastatin)[3] and mevinolin (lovastatin)[4] have been followed by a host of publications describing a large variety of natural[5] and synthetic inhibitors.[6] Previously, we disclosed a series of 1,2,5-trisubstituted-pyrrol-1-ylethylmevalonolactones which were found to be moderately potent inhibitors of HMGR in vitro.[7] By systematically altering the 2 and 5 substituents, maximal potency was obtained with the 2-(4-fluorophenyl)-5-isopropyl analogue (1). On the basis of those results, a molecular-modeling analysis led to the description of a pharmacophore model which characterized the size of the substituents at positions 2 and 5 and the conformation of the side chain. We have now discovered

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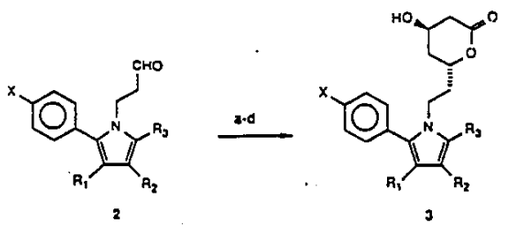
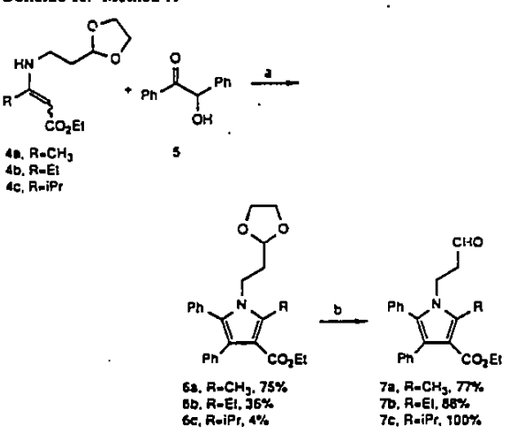
Scheme I[a]



[a] (a) $\overline{CH_2}C\overline{O}\overline{CH}CO_2ET$, THF, −78 °C; (b) n-Bu_3B/$NaBH_4$, −78 °C; (c) H_2O_2, NaOH; (d) toluene, reflux.

Scheme II. Method A[a]



4a, R=CH_3
4b, R=Et
4c, R=iPr

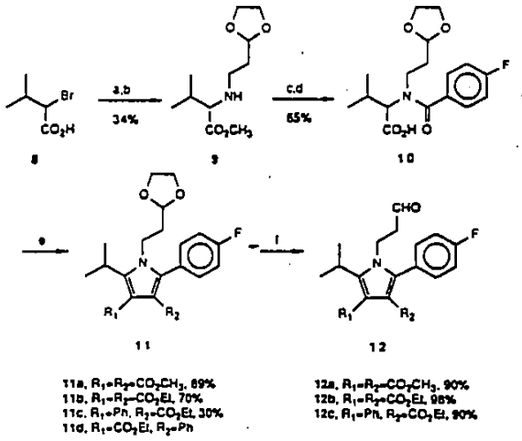
6a, R=CH_3, 75%
6b, R=Et, 36%
6c, R=iPr, 4%

7a, R=CH_3, 77%
7b, R=Et, 88%
7c, R=iPr, 100%

[a] (a) $ZnCl_2$, EtOH, reflux; (b) p-TSA, acetone–water, reflux.

that the introduction of substituents into the 3 and 4 positions of the pyrrole ring results in significant improvements in potency at inhibiting HMGR in vitro. The results of these studies are described in this report.

Scheme III. Method B[a]

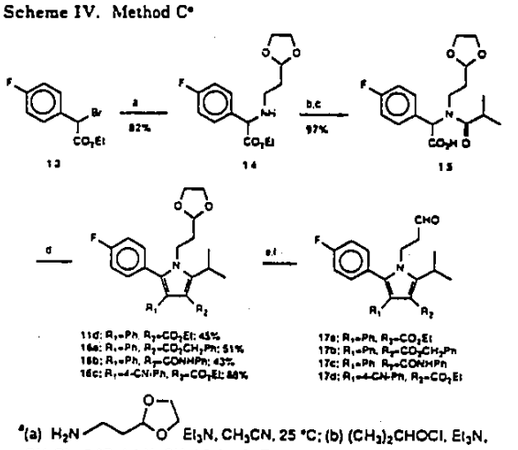


11a, R_1=R_2=CO_2CH_3, 89%
11b, R_1=R_2=CO_2Et, 70%
11c, R_1=Ph, R_2=CO_2Et, 30%
11d, R_1=CO_2Et, R_2=Ph

12a, R_1=R_2=CO_2CH_3, 90%
12b, R_1=R_2=CO_2Et, 98%
12c, R_1=Ph, R_2=CO_2Et, 90%

[a] (a) CH_3OH,DCC, DMAP; (b) H_2N–(dioxolane) Et_3N, CH_3CN, reflux; (c) 4-F-Ph-COCl, Et_3N; (d) NaOH; (e) R_1—≡—R_2, Ac_2O, 90 °C; (f) p-TSA, acetone–water, reflux.

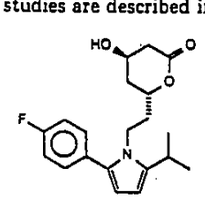
Scheme IV. Method C[a]



11d: R_1=Ph, R_2=CO_2Et; 45%
16a: R_1=Ph, R_2=CO_2CH_2Ph; 51%
16b: R_1=Ph, R_2=CONHPh; 43%
16c: R_1=4-CN-Ph, R_2=CO_2Et; 88%

17a: R_1=Ph, R_2=CO_2Et
17b: R_1=Ph, R_2=CO_2CH_2Ph
17c: R_1=Ph, R_2=CONHPh
17d: R_1=4-CN-Ph, R_2=CO_2Et

[a] (a) H_2N–(dioxolane) Et_3N, CH_3CN, 25 °C; (b) $(CH_3)_2CHOCl$, Et_3N, CH_2Cl_2, 0 °C; (c) NaOH; (d) Ac_2O, R_1—≡—R_2, 90 °C; (e) HCl, EtOH, reflux; (f) p-TSA, acetone–water, reflux.



1

Chemistry

The general synthetic strategy employed was identical with that employed previously.[7] Thus, the pyrrole-3-propionaldehydes 2 were converted to the racemic, trans

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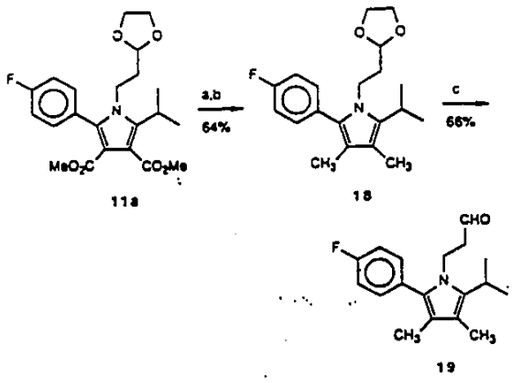
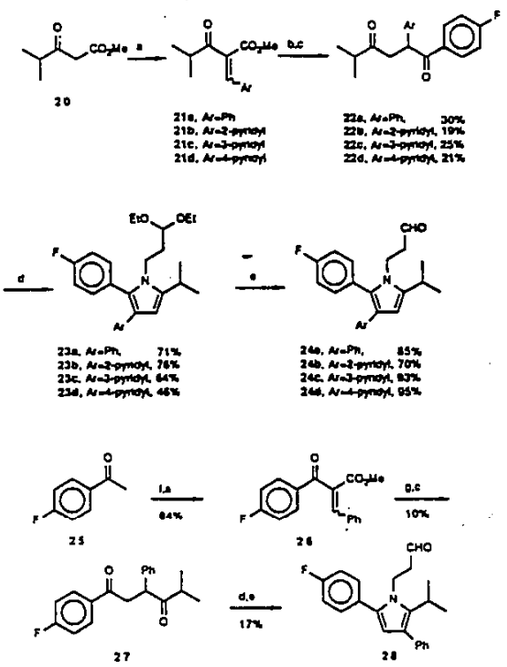
Scheme V. Method D[a]



[a] (a) $LiAlH_4$, ether–dichloromethane, reflux; (b) Et_3SiH, TFA–CH_2Cl_2, 0 °C; (c) *p*-TSA, acetone–water.

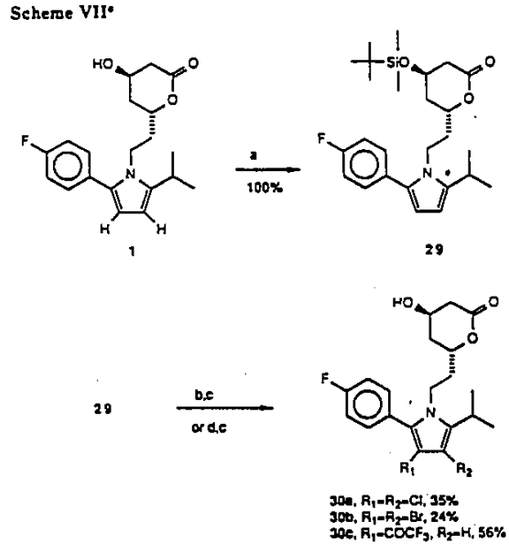
lactone stereoisomers 3 by (1) Weiler dianion condensation with ethyl acetoacetate, (2) stereoselective reduction to the syn-1,3-diol with tributylborane and sodium borohydride, (3) base hydrolysis, and (4) lactonization by refluxing in toluene with azeotropic removal of water (Scheme I). The requisite propionaldehydes 2 were prepared by several different synthetic routes. The less sterically hindered pentasubstituted pyrrole-3-propionaldehydes (7a, R = CH_3; 7b, R = Et, Scheme II) could be prepared by $ZnCl_2$-catalyzed condensation of enamines 4a and 4b (prepared from 2-(2-aminoethyl)-1,3-dioxolane[8] and the requisite β-keto ester) with benzoin 5 (method A).[9] This reaction proved ineffective for the more sterically hindered pyrrole 7c, containing the preferred 5-isopropyl substituent. The 5-isopropylpyrroles could be prepared in good yields, however, by the regioselective [3 + 2] cycloaddition of acetylenes with the amido acids 10 or 15 (Schemes III and IV).[10] Thus, reaction of ethyl phenylpropiolate with amido acid 10 in hot acetic anhydride afforded a 4:1 mixture of 11c and 11d (Scheme III, method B) from which 11c crystallized in 30% yield. The reaction of 15 under identical conditions was regiospecific, producing 11d as the sole product (Scheme IV, method C). The regiochemistry of compounds 11c and 11d were determined by comparison of their proton NMRs with that of the closely related 6c ($(CH_3)_2CH$, occurs at δ 3.50 ppm in both 6c and 11d, but at δ 3.00 ppm in 11c). As expected, the yield in this cycloaddition reaction was improved when more electron-deficient acetylenes were employed (compare 11a, 11b, and 16c vs 11d Scheme IV). The 3,4-dimethylpyrrole analogue 19 was prepared by reduction of diester 11a to the corresponding diol with lithium aluminum hydride, followed by deoxygenation with triethylsilane and trifluoroacetic acid (Scheme V, method D).[11] The regioisomeric 3- and 4-arylpyrrole-3-propionaldehyde isomers 24a–d and 28 were prepared by a Stetter reaction[12] of the appropriate aldehydes with the complementary α-benzylidene-β-keto esters (4-fluorobenzaldehyde with 21 and isobutyraldehyde with 26, Scheme VI), followed by Paal–Knorr cyclization[13] with 3,3-diethoxy-1-amino-

Scheme VI. Met. E[a]



[a] (a) ArCHO, *p*-TSA, toluene, reflux; (b) 4-F-Ph-CHO, Et_3N, 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride; (c) NaOH, CH_3OH, 25 °C; (d) $H_2NCH_2CH_2CH(OEt)_2$, *p*-TSA, toluene, reflux; (e) H_3O^+, (f) NaH, $(CH_3O)_2CO$; (g) $(CH_3)_2CHCHO$, Et_3N, 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride.

Scheme VII[a]



[a] (a) *t*-$BuMe_2SiCl$, imidazole, DMF, 25 °C, 18 h; (b) 2 equiv *N*-halosuccinimide, DMF, 0 °C; (c) *n*-Bu_4NF, HOAc, THF, 25 °C; (d) $(CF_3CO)_2O$, DMF, 0 °C.

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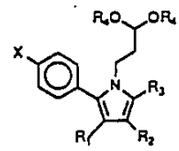
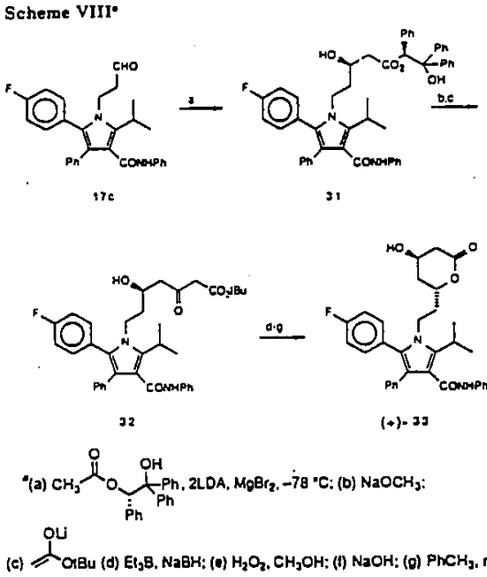
Table I



no.	X	R_1	R_2	R_3	R_4	% yield (method)	mp,[a,b] °C
6a	H	Ph	CO_2Et	CH_3	$-CH_2CH_2-$	75 (A)	oil[c]
6b	H	Ph	CO_2Et	Et	$-CH_2CH_2-$	36 (A)	oil[c]
6c	H	Ph	CO_2Et	*i*-Pr	$-CH_2CH_2-$	4 (A)	oil[c]
11a	F	CO_2CH_3	CO_2CH_3	*i*-Pr	$-CH_2CH_2-$	65 (B)	143–6
11b	F	CO_2Et	CO_2Et	*i*-Pr	$-CH_2CH_2-$	70 (B)	oil[c]
11c	F	CO_2Et	Ph	*i*-Pr	$-CH_2CH_2-$	30 (B)	146–8
16a	F	Ph	CO_2Et	*i*-Pr	$-CH_2CH_2-$	45 (C)	158–9
16b	F	Ph	CO_2CH_2Ph	*i*-Pr	$-CH_2CH_2-$	51 (C)	oil[c]
16c	F	Ph	CONHPh	*i*-Pr	$-CH_2CH_2-$	43 (C)	161–3
16d	F	4-CNPh	CO_2Et	*i*-Pr	$-CH_2CH_2-$	88 (C)	oil[c]
18	F	CH_3	CH_3	*i*-Pr	$-CH_2CH_2-$	64 (D)	oil[c]
23a	F	Ph	H	*i*-Pr	Et	71 (E)	84–7
23b	F	2-pyridyl	H	*i*-Pr	Et	76 (E)	84–6
23c	F	3-pyridyl	H	*i*-Pr	Et	64 (E)	96–8
23d	F	4-pyridyl	H	*i*-Pr	Et	46 (E)	123–5

[a] All compounds possess ^1H NMR spectra consistent with assigned structure. [b] Combustion analyses within ±0.4% of theoretical unless otherwise noted. [c] This compound was purified, but not analyzed before use in the next step.

Scheme VIII[a]

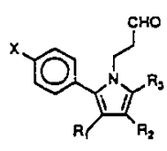


[a] (a) CH_3, 2LDA, $MgBr_2$, −78 °C; (b) $NaOCH_3$; (c) OTBu (d) Et_2B, $NaBH_4$; (e) H_2O_2, CH_3OH; (f) NaOH; (g) $PhCH_3$, reflux.

propane[14] and deprotection (Scheme VI, method E). Finally, the 3,4-dichloro, 3,4-dibromo, and 3-trifluoroacetyl analogues (30a–c) were prepared from 1 by protection of the 4′-hydroxyl as the *tert*-butyldimethylsilyl ether, followed by electrophilic substitution on the pyrrole ring[15] and deprotection with *n*-Bu_4NF buffered with acetic acid (Scheme VII). The assignment of the regiochemistry of 30c was made in a manner analogous to 11c and 11d.

Chiral lactone (+)-33 was prepared by application of the asymmetric aldol procedure developed by Braun (Scheme VIII).[16] Thus, reaction of aldehyde 17c with the magnesium enolate of (*S*)-(+)-2-acetoxy-1,1,2-triphenylethanol afforded alcohol 31 in 60% yield and 97% ee. Transesterification ($NaOCH_3$, CH_3OH) followed by Claisen condensation with excess lithio *tert*-butylacetate produced δ-hydroxy-β-keto ester 32 in 75% yield. After reduction with Et_2B and $NaBH_4$, base hydrolysis, and lactonization, (+)-33 was isolated as a 98:2 mixture of stereoisomers. Fortuitously, the *d,l* pair selectively crystallized from ethyl acetate–hexanes and pure (+)-33 ($[\alpha]^{23}_D$ = +24.53°, 0.53% in $CHCl_3$) could then be isolated from the mother liquors as a foamy solid.[17]

Table II



no.	X	R_1	R_2	R_3	% yield	mp,[a,b] °C
7a	H	Ph	CO_2Et	CH_3	77	100–1
7b	H	Ph	CO_2Et	Et	88	oil[c]
7c	H	Ph	CO_2Et	*i*-Pr	100	oil[c]
12a	F	CO_2CH_3	CO_2CH_3	*i*-Pr	90	oil[c]
12b	F	CO_2Et	CO_2Et	*i*-Pr	95	oil[c]
12c	F	CO_2Et	Ph	*i*-Pr	90	oil[c]
17a	F	Ph	CO_2Et	*i*-Pr	81	127–8
17b	F	Ph	CO_2CH_2Ph	*i*-Pr	60	oil[c]
17c	F	Ph	CONHPh	*i*-Pr	86	164–5
17d	F	4-CNPh	CO_2Et	*i*-Pr	75	oil[c]
19	F	CH_3	CH_3	*i*-Pr	66	oil[c]
24a	F	Ph	H	*i*-Pr	85	oil[c]
24b	F	2-pyridyl	H	*i*-Pr	70	120–2
24c	F	3-pyridyl	H	*i*-Pr	93	oil[c]
24d	F	4-pyridyl	H	*i*-Pr	95	oil[c]
28	F	H	Ph	*i*-Pr	90	oil[c]

[a] All compounds possessed ^1H NMR and IR spectra consistent with assigned structure. [b] Combustion analyses within ±0.4% of theoretical unless otherwise noted. [c] This compound was purified by chromatography, but not analyzed before use in the next step.

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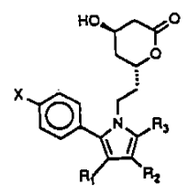
Table III



no.	X	R_1	R_2	R_3	mp, °C	formula[a]	IC_{50},[b] μM	relative potency[c]
1	F	H	H	*i*-Pr	105–6	$C_{30}H_{24}FNO_3$	0.23	10.9
3a	H	Ph	CO_2Et	CH_3	oil	$C_{27}H_{29}NO_5$	4.0	0.6
3b	H	Ph	CO_2Et	Et	65–8	$C_{28}H_{31}NO_5$	0.89	6.3
3c	H	Ph	CO_2Et	*i*-Pr	157–9	$C_{29}H_{33}NO_5$	0.17	23.5
3d	F	CO_2CH_3	CO_2CH_3	*i*-Pr	169–170	$C_{24}H_{28}FNO_7$	0.180	14.3
3e	F	CO_2Et	CO_2Et	*i*-Pr	121–3	$C_{26}H_{32}FNO_7$	0.35	2.8
3f	F	CO_2Et	Ph	*i*-Pr	158–9	$C_{29}H_{32}FNO_5$	0.050	100
3g	F	Ph	CO_2Et	*i*-Pr	159–160	$C_{29}H_{32}FNO_5$	0.20	35.5
3h	F	Ph	CO_2CH_2Ph	*i*-Pr	174–5	$C_{34}H_{34}FNO_5$	0.040	24.0
(±)-3i	F	Ph	CONHPh	*i*-Pr	104–110	$C_{33}H_{33}FN_2O_4$	0.025	81.4
3j	F	4-CN-Ph	CO_2Et	*i*-Pr	oil	$C_{30}H_{31}FN_2O_5$	0.280	16.2
3k	F	CH_3	CH_3	*i*-Pr	oil	$C_{24}H_{28}FNO_3$	0.140	16.0
3l	F	Ph	H	*i*-Pr	oil	$C_{26}H_{25}FNO_3$	0.347	12.5
3m	F	2-pyridyl	H	*i*-Pr	166–7	$C_{25}H_{27}FN_2O_3$	0.046	76
3n	F	3-pyridyl	H	*i*-Pr	70–4	$C_{25}H_{27}FN_2O_3$	0.071	9.4
3o	F	4-pyridyl	H	*i*-Pr	174–6	$C_{25}H_{27}FN_2O_3$	0.310	2.1
3p	F	H	Ph	*i*-Pr	135–6	$C_{26}H_{28}FNO_3$	0.120	36.3
30a	F	Cl	Cl	*i*-Pr	129–131	$C_{20}H_{22}Cl_2FNO_3$	0.028	78.6
30b	F	Br	Br	*i*-Pr	141.2	$C_{20}H_{22}Br_2FNO_3$	0.028	78.6
30c	F	$COCF_3$	H	*i*-Pr	oil	$C_{22}H_{23}F_4NO_4$	0.800	8.8
(+)-33	F	Ph	CONHPh	*i*-Pr	foam	$C_{33}H_{33}FN_2O_4$	0.007	500
(−)-33	F	Ph	CONHPh	*i*-Pr	foam	$C_{33}H_{33}FN_2O_4$	0.440	13.9
		compactin					0.030	100

[a] Analytical results are within ±0.4% of theoretical values except where otherwise noted. [b] CoA reductase inhibition (COR) screen: a measure of the direct conversion of D,L-[^{14}C]HMG-CoA to mevalonic acid. Assays of each inhibitor were performed at four concentrations in triplicate. The precision for compactin was 37%. See ref 7 for experimental details. [c] Calculated as follows: (IC_{50} of compactin/IC_{50} of test compound determined simultaneously) × 100. Compactin arbitrarily assigned a value of 100.

Alternatively, relatively pure (+)- and (−)-33 could be obtained by preparation of the corresponding diastereomeric (*R*)-α-methylbenzylamides, separation by preparative HPLC, hydrolysis, and relactonization.[6b] This process afforded 94.6% pure (+)-33 ($[\alpha]^{23}_D$ = +25.5°, 0.51% in $CHCl_3$) and 97.8% pure (−)-33 ($[\alpha]^{23}_D$ = −24.8°, 0.51% in $CHCl_3$).

Biological Results and Discussion

The compounds listed in Table III were all hydrolyzed to the corresponding dihydroxy acid sodium salts and evaluated for their ability to inhibit a partially purified preparation of rat liver HMG-CoA reductase.[3] Two conclusions were readily apparent. The first was the confirmation of the 5-isopropyl as the preferred substituent (compare 3c with 3a and 3b). The second was the significant increase in in vitro potency found with the introduction of certain lipophilic electron-withdrawing groups into the 3 and 4 positions of the pyrrole ring (e.g., Cl or Br, compare 1 with 30a and 30b), such that, these compounds displayed potency equivalent to compactin. This effect did not hold for the esters or ketones (CO_2Me, CO_2Et, $COCF_3$, compounds 3d, 3e, 30c), except when combined with a phenyl (compounds 3f, 3h, and 3i). There also appeared to be a positional effect, since the 3-carbethoxy-4-phenyl analogue (3f) was 4 times more potent than the 3-phenyl-4-carbethoxy analogue (3g). In vitro activity for the 3-phenyl analogues were improved significantly by increasing the size of the 4-substituent (compare 3h, 3i, and 3g with 3l). Potency was also increased when the 3-phenyl was replaced with a 3-(2-pyridyl) moiety (compound 3m). The 3-(3- and 4-pyridyl) isomers (3n and 3o) were equipotent to phenyl (3l). Introduction of the electron-withdrawing cyano group into the 4-position of the 3-phenyl (3j) led to a slight reduction in potency. Finally, as others have reported, in the case of 3i essentially all of the biological activity was contained in the dextrorotatory stereoisomer ((+)-33 vs 3i).[6b] We speculate that the activity found in (−)-33 (97.8% pure) is derived from the 2% contamination with (+)-33.

An attempt was made to confirm these observations with a quantitative structure–reactivity relationship (QSAR) analysis. In the early stages of the development of the series, there was an indication that size, as parameterized by MR of the combined 3- and 4-substituents, as well as electronic-withdrawing character might be possible contributors to activity and this preliminary analysis partially guided further synthesis. Synthetic constraints precluded the preparation of an optimally designed set, however, and the set of compounds described in this paper did not ultimately support the derivation of a significant Hansch equation including these parameters. Furthermore, available parameters for electronic and lipophilic effects of these highly hindered functional groups are likely to be seriously inaccurate. Nevertheless, the trends observed from plots and single parameter correlations supported the observation that a size benefit exists, but derives mainly from the 4-substituent, as opposed to the 3-substituent. Polar functionality can be tolerated in this region, although there is a suggestion that lipophilicity may ultimately play

(17) A similar sequence was employed by Lynch et al.: Lynch, J. E.; Volante, R. P.; Wattley, R. V.; Shinkai, I. *Tetrahedron Lett.* 1987, 1385–8.

the dominant role among the simple parameterized effects, since $Pi_{3,4}$ has one of the best single parameter correlations with activity ($r = 0.46$). Clearly, other factors not readily parameterized have equal or larger influence on relative activity in this series. The activity of polar-substituted analogues is enhanced when the polar group is "insulated" from the enzyme as in 3m vs 3n and 3o. Similarly, the better activity of 3f over 3g may derive from the better shielding of the polar ester group in the former compound by the flanking phenyl groups as opposed to a phenyl and isopropyl group in the latter. The activity of the halogenated analogues 30a and 30b is better accommodated by a lipophilicity effect, rather that a size or dispersion effect reflected in MR. Other QSAR analyses of synthetic HMG-CoA reductase inhibitors have reached similar conclusions about structural variations in this region of related molecules.[18,19]

In conclusion, although it is still most critical in this type to have the optimal substituents flanking the dihydroxyglutarate side chain, i.e., 4-fluorophenyl and isopropyl,[7] this work shows that further modulation and improvement in potency at inhibiting HMG-CoA reductase may be obtained with a variety of additional substituents capable of interacting with an apparently fairly spacious hydrophobic region distal from the side-chain location. The importance of this interaction is further supported by the potent inhibition evidenced by other inhibitors which possess substituents in this region.[1] Preparation of the optically pure *R,R*-isomer ((+)-33) of the most potent compound in this series (3i) resulted in a compound which was 5 times more potent than the fungal metabolite compactin in vitro. Further in vivo studies with (+)-33 will be described in subsequent papers from this laboratory.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. THF was distilled from sodium and benzophenone. All organic extracts were dried over $MgSO_4$ except when otherwise noted. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. NMR spectra were determined on either a Varian EM-390 spectrometer, or a Varian XL-200 or Bruker 250 MHz instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses for carbon, hydrogen, and nitrogen were determined on a Perkin-Elmer Model 240C elemental analyzer and are within 0.4% of theory unless noted otherwise. Optical rotations were determined with use of a Perkin-Elmer 241 polarimeter. Routine HPLC analyses were performed with use of a Varian 5500 unit equipped with a Reodyne 7126 loop injector, a Dupont variable wavelength detector, and an octadecylsilane (Alltech C18 600RP, CH_3CN-H_2O eluant, 60:40, v/v) or silica gel column (Beckman Altex Ultrasphere 5 μm) interfaced to Varian 402 data system for computation of peak areas. Chiral HPLC analyses were performed with use of a Chiracel of 10-μm column (Diacel Chem. Ind., LTD).

Method A. Ethyl 3-[2-(1,3-Dioxolan-2-yl)ethyl]amino-2-pentenoate (4b). A solution of methyl propionylacetate (12.55 mL, 100 mmol), 2-(2-aminoethyl)-1,3-dioxolane[9] (12.3 g, 105 mmol) and one drop of glacial acetic acid was stirred and heated in refluxing toluene (200 mL) for 2 h with azeotropic removal of water. The cooled solution was concentrated to provide 24 g of pure 4b, which was used without further purification.

Ethyl 2-Ethyl-1-[2-(1,3-dioxolan-2-yl)ethyl]-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (6b). A mixture of benzoin (4.25 g, 20 mmol), 4b (5.44 g, 22 mmol), and $ZnCl_2$ (6 g, 44 mmol) in 50 mL of absolute ethanol was stirred and heated at reflux for 48 h. The cooled solution was diluted with ether (500 mL), washed with water (50 mL), 2 *M* HCl (2 × 50 mL), saturated aqueous bicarbonate (50 mL), and brine (50 mL), and dried. Flash chromatography (silica gel, 10:1 v/v hexane–ethyl acetate) provided 3 g (36%) of 6b: 90-MHz NMR ($CDCl_3$) δ 0.98 (t, 3 H, J = 7 Hz), 1.34 (t, 3 H, J = 7 Hz), 1.85 (m, 2 H), 3.08 (q, 2 H, J = 7 Hz), 3.7–4.1 (m, 8 H), 4.60 (t, 1 H, J = 4 Hz), 7.1 (s, 5 H), 7.22 (s, 5 H) ppm.

Ethyl 2-Ethyl-1-[1-(3-oxopropyl)]-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (7b). A solution of 6b (2.4 g, 5.7 mmol) in 100 mL of absolute ethanol containing 1 drop of concentrated HCl was stirred and heated at reflux for 24 h. The cooled solution was concentrated and dissolved in 125 mL of 4:1 acetone–water, and 1 g of *p*-TSA–H_2O was added. The resulting solution was stirred and heated at reflux for 24 h. The cooled solution was concentrated and partitioned between ether and water. The ether layer was then washed with saturated aqueous bicarbonate and brine and dried. Filtration and concentration afforded 1.9 g of 7b (88%): 90-MHz NMR ($CDCl_3$) δ 1.0 (t, 3 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz), 2.58 (m, 2 H), 3.10 (q 2 H, J = 7 Hz), 4.05 (q, 2 H, J = 7 Hz), 4.2 (m, 2 H), 7.05 (s, 5 H), 7.1–7.4 (m, 5 H), 9.50 (s, 1 H) ppm.

Ethyl 3-[[2-(1,3-Dioxolan-2-yl)ethyl]amino]-4-methyl-2-pentanoate (4c). A solution of ethyl isobutyrylacetate (6 g, 42 mmol) and 2-(2-aminoethyl)-1,3-dioxolane (5.4 g, 46.7 mmol) in toluene (50 mL) containing 2 drops of glacial acetic acid was stirred and heated at reflux with azeotropic removal of water for 2 h. Concentration provided crude 4c which was used without further purification.

Ethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(1-methylethyl)-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (6c). A mixture of 4c (17 g, 80 mmol), benzoin acetate (75 mmol, 19 g), and $ZnCl_2$ (20 g, 147 mmol) in 100 mL of ethanol was stirred and heated at reflux for 2 days. The mixture was cooled to room temperature, poured into ether (1 L), washed with water (200 mL), 2 M HCl (100 mL), H_2O (100 mL), and brine, and dried. Flash chromatography (silica gel, 10:1 v/v hexane–ethyl acetate) provided 1.2 g of 6c: 90-MHz NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz); 1.45 (d, 6 H, J = 7 Hz), 1.90 (m, 2 H), 3.45 (septet, 1 H, J = 7 Hz), 3.8–4.1 (m, 8 H), 4.60 (t, 1 H, J = 4 Hz), 7.0 (s, 5 H), 7.0–7.3 (m, 5 H) ppm.

Ethyl 1-(3-Oxopropyl)-5-(1-methylethyl)-4,5-diphenyl-1*H*-pyrrole-3-carboxylate (7c). A solution of 6c (1.3 g, 3 mmol) and *p*-TSA–H_2O (0.6 g, 3 mmol) in 50 mL of 4:1 acetone–water was stirred and heated at reflux overnight. The cooled mixture was poured into ether (200 mL), washed with saturated aqueous bicarbonate (2 × 50 mL), water (50 mL), and brine (50 mL), and dried. Filtration and concentration provided 1.0 g (100%) of pure 7c which was used without further purification: 90-MHz NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz), 1.40 (d, 6 H, J = 7 Hz), 2.55 (m, 2 H), 3.44 (septet, 1 H, J = 7 Hz), 3.95 (q, 2 H, J = 7 Hz), 4.15 (m, 2 H), 7.0 (s, 5 H), 7–7.3 (m, 5 H), 9.43 (s, 1 H) ppm.

Method B. *N*-[2-(1,3-Dioxolan-2-yl)ethyl]-DL-valine, Methyl Ester (9). A solution of the methyl 2-bromo-3-methylbutyrate (4.6 g, 23.6 mmol), 2-(2-aminoethyl)-1,3-dioxolane (2.9 g, 25 mmol), and triethylamine (3.5 mL, 25 mmol) in 25 mL of acetonitrile was stirred and heated at reflux for 20 h. The cooled solution was poured into ether (500 mL) and extracted with 2 M HCl (2 × 50 mL). The aqueous layer was made alkaline with 25% aqueous NaOH and extracted with ethyl acetate (2 × 100 mL). The combined ethyl acetate extracts were washed with brine and dried. Filtration and concentration provided 3 g (55%) of 9 as a yellow oil: 90-MHz NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 6H), 1.70 (br s, 1 H, 4NH), 1.86 (m, 2 H), 2.60 (m, 3 H), 2.94 (d, J = 6 Hz, 1 H), 3.68 (s, 3 H), 3.85 (m, 4 H), 4.89 (t, J = 4 Hz, 1 H) ppm.

***N*-[2-(1,3-Dioxolan-2-yl)ethyl]-*N*-(4-fluorobenzoyl)-DL-valine (10).** To a stirred solution of 9 (3 g, 13 mmol) and triethylamine (3.6 mL, 26 mmol) in 20 mL of CH_2Cl_2, cooled to 0 °C, was added a solution of 4-fluorobenzoyl chloride (1.65 mL, 14 mmol) in 10 mL of CH_2Cl_2. The solution was stirred 50 min at 0 °C and 60 min at room temperature. It was then poured into ether (200 mL), washed with water (2 × 50 mL), saturated aqueous bicarbonate (50 mL), and brine (50 mL), and dried. Flash chromatography (silica gel, 1:1 v/v hexane–ethyl acetate) provided 3 g (65%) of crude (±)-methyl *N*-(4-fluorobenzoyl)-*N*-[2-(2-ethyl)-1,3-dioxolanyl]valine: 90-MHz NMR ($CDCl_3$) δ 0.90, (br

(18) Aggarwal, D.; Saha, R. N.; Gupta, J. K.; Gupta, S. P. *J. Pharmacobio-Dyn.* 1988, *11*, 591.
(19) Prabhakar, Y. S.; Saxena, A. K.; Doss, M. J. *Drug Des. Deliv.* 1989, *4*, 97.

d, J = 7 Hz, 6 H), 1.8–2.5 (m, 3 H), ...5 (br dd, J = 6, 8 Hz, 1 H), 3.72 (s, 3 H), 3.80 (m, 6 H), 4.80 (m, 1 H), 6.9–7.5 (m, 4 H) ppm.

A solution of this methyl ester (1 g, 2.83 mmol) and NaOH (0.4 g, 10 mmol) in 10 mL of 4:1 methanol–water was stirred and heated at reflux for 3 h. The cooled solution was diluted with water and extracted with ether. The aqueous layer was acidified with 6 M HCl and extracted with ethyl acetate (2×). The combined ethyl acetate extracts were washed with brine and dried. Filtration and concentration provided 0.96 g (2.8 mmol) of 10 as a gum: 90-MHz NMR ($CDCl_3$) δ 0.85 (m, 6 H), 1.8 (m, 2 H), 2.5 (m, 1 H), 3.3–3.9 (m, 7 H), 4.6 (m, 1 H), 6.8–7.4 (m, 4 H) ppm.

Dimethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(4-fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrole-3,4-dicarboxylate (11a). Dimethyl acetylenedicarboxylate (1.3 mL, 10.6 mmol) was added to a solution of 10 (1.8 g, 5.28 mmol) in 10 mL of acetic anhydride at room temperature. Carbon dioxide evolution began immediately. The solution was stirred a further 2 h, concentrated to remove excess dimethyl acetylenedicarboxylate and solvent, and then filtered through silica gel. This provided 2 g (89%) of 11a as a colorless solid. Recrystallization from isopropyl ether–hexane afforded colorless crystals: mp 143–146 °C; IR (KBr) 1719, 1449, 1241, 1209, 1178, 945 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.35 (d, J = 7 Hz, 6 H), 1.80 (m, 2 H), 3.18 (septet, J = 7 Hz, 1 H), 3.56 (s, 3 H), 3.7 to 4.0 (m, 6 H), 3.83 (s, 3 H), 4.64 (t, J = 4 Hz, 1 H), 7–7.3 (m, 4 H) ppm. Anal. C, H, N.

Dimethyl 2-(4-Fluorophenyl)-5-(1-methylethyl)-1-(3-oxopropyl)-1*H*-pyrrole-3,4-dicarboxylate (12a). A solution of 11a (0.5 g, 1.18 mmol) and *p*-TSA–H_2O (0.23 g, 1.2 mmol) in 12 mL of 5:1 acetone–water was stirred and heated at reflux for 48 h. The cooled solution was concentrated, diluted with ether (200 mL), washed with saturated aqueous bicarbonate (2 × 50 mL) and brine (50 mL), and dried. Flash chromatography on silica gel (4:1 v/v hexane–ethyl acetate) provided 0.4 g (90%) of pure 12a: 90-MHz NMR ($CDCl_3$) δ 1.35 (d, J = 7 Hz, 6 H), 2.61 (t, J = 7 Hz, 2 H), 3.18 (septet, J = 7 Hz, 1 H), 3.53 (s, 3 H), 3.81 (s, 3 H), 4.03 (t, J = 7 Hz, 2 H), 6.9–7.3 (m, 4 H), 9.45 (s, 1 H) ppm.

Ethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(4-fluorophenyl)-5-(1-methylethyl)-4-phenyl-1*H*-pyrrole-3-carboxylate (11c). A mixture of 10 (3.0 g, 8.8 mmol), acetic anhydride (15 mL), and ethyl phenylpropiolate (3.0 g, 17.6 mmol) was stirred at 110 °C for 5 h. The solution was then cooled and the excess acetic anhydride removed under vacuum. The residual dark oil was purified by flash chromatography on silica gel (4:1 v/v ethyl acetate–hexane). The product solidified on standing and was recrystallized from ether–hexane. The first crop gave 2.2 g (30%) of pure 11c: 90-MHz NMR ($CDCl_3$) δ 0.65 (t, 3 H, J = 7 Hz), 1.10 (d, 6 H, J = 7 Hz), 1.7–2.0 (m, 2 H), 3.00 (septet, 1 H, J = 7 Hz), 3.6–4.0 (m, 8 H), 4.60 (t, 1 H, J = 4 Hz), 6.9–7.4 (m, 9 H) ppm.

Method C. Ethyl α-[[2-(1,3-Dioxolan-2-yl)ethyl]amino]-4-fluorobenzeneacetate (14). A solution of 26 g (220 mmol) of 2-(2-aminoethyl)-1,3-dioxolane in 50 mL of acetonitrile was added at room temperature with stirring to a solution of 52 g (200 mmol) of ethyl α-bromo-4-fluorobenzeneacetate[20] and 42 mL (300 mmol) of triethylamine in 350 mL of acetonitrile. The resulting mixture was stirred at room temperature overnight and then poured into ether (500 mL). The suspension which resulted was washed with water (300 mL) and 2 M HCl (2 × 300 mL). The combined acidic extracts were made alkaline with 25% aqueous NaOH and extracted with ethyl acetate (2 × 500 mL). The ethyl acetate extracts were combined, washed successively with water and brine, and dried. Filtration and concentration yielded 49.5 g (82.5%) of 14 as an oil: 90-MHz NMR ($CDCl_3$) δ 1.18 (t, 3 H, J = 7 Hz), 1.85 (m, 2 H), 2.20 (br s, 1 H), 2.6 (m, 2 H), 3.85 (m, 4 H), 4.1 (q, 2 H, J = 7 Hz), 4.22 (s, 1 H), 4.83 (t, 1 H, J = 4.5 Hz), 6.8–7.3 (m, 4 H) ppm.

α-[[2-(1,3-Dioxolan-2-yl)ethyl](2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic Acid (15). 14 (30 g, 100 mmol) was dissolved in 200 mL of CH_2Cl_2 with 28.6 mL (205 mmol) of triethylamine. The resulting mixture was cooled to 0 °C under dry nitrogen. A solution of 11 mL (105 mmol) of isobutyryl chloride in 50 mL of CH_2Cl_2 was slowly added with stirring. After addition was complete, the mixture was stirred for an additional 1 h and then poured into 100 mL of ether. The ether solution was washed successively with water (25 mL), 2 M HCl (25 mL), saturated aqueous bicarbonate (25 mL), and brine (25 mL), and dried. Filtration and evaporation of the solvents yielded 35 g of α-[[2-(1,3-dioxolan-2-yl)ethyl](2-methyl-1-oxopropyl)amino]-4-fluorobenzeneacetic acid, ethyl ester: 90-MHz NMR ($CDCl_3$) δ 1.2 (m, 9 H), 1.7 (m, 2 H), 2.85 (m, 1 H), 3.35 (m, 2 H), 3.80 (m, 4 H), 4.20 (q, 2 H, J = 7 Hz), 4.60 (t, 1 H, J = 4.5 Hz), 5.81 (s, 1 H), 6.8–7.3 (m, 4 H) ppm.

A solution of this ester (35 g) and 12 g (300 mmol) of NaOH in 480 mL of 5:1 methanol–water was stirred and heated at reflux for 2 h. The solution was cooled to room temperature, concentrated, and diluted with 500 mL of water. The resulting solution was extracted with ether. The aqueous layer was then acidified with ice-cold 6 M HCl and extracted with ethyl acetate (2 × 300 mL).

The combined ethyl acetate extracts were washed with brine, dried, filtered, and evaporated to yield 30 g of crude 15 as a gum which was used without further purification: 90-MHz NMR ($CDCl_3$) δ 1.11 (d, 6 H, J = 7 Hz), 1.4–1.9 (m, 2 H), 2.85 (m, 1 H), 3.32 (m, 2 H), 3.75 (m, 4 H), 4.52 (t, 1 H, J = 4.5 Hz), 5.73 (s, 1 H), 6.8–7.3 (m, 4 H) ppm.

1-[2-(1,3-Dioxolan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (16b). A solution of 95 g (280 mmol) of 15 and 98 g (439 mmol) of *N*,3-diphenylpropynamide[21] in acetic anhydride (200 mL) was heated at 90 °C with stirring for 4 h (vigorous gas evolution). The mixture was then cooled to room temperature, concentrated, and chromatographed twice on silica gel (4:1 v/v hexane–ethyl acetate) to separate the product (R_f = 0.35, 4:1 hexane–ethyl acetate) from the *N*,3-diphenylpropynamide (R_f = 0.5). Recrystallization of the product from isopropyl ether provided 59.5 g (119 mmol) of 16b as colorless crystals: mp 159–162 °C; 200-MHz NMR ($CDCl_3$) δ 1.54 (d, 6 H, J = 7 Hz), 1.91 (m, 2 H), 3.60 (septet, 1 H, J = 7 Hz), 3.7–4.1 (m, 6 H), 4.74 (t, 1 H, J = 4.3 Hz), 7.0–7.3 (m, 15 H); IR (KBr) 3400, 1658, 1596, 1530 cm^{-1}. Anal. C, H, N.

5-(4-Fluorophenyl)-2-(1-methylethyl)-1-(3-oxopropyl)-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (17c). A solution of 59 g (118 mmol) of 16c and 0.4 mL of concentrated HCl in 1200 mL of absolute ethanol was heated under reflux with stirring for 24 h. The mixture was cooled to room temperature and concentrated and the residue taken up in 3:1 acetone–water (1200 mL). *p*-TSA–H_2O (5 g) was added. This mixture was heated under reflux with stirring for 2 days, cooled to room temperature, and partitioned between ether (1000 mL) and brine (200 mL). The organic layer was separated, washed successively with saturated aqueous bicarbonate (2 × 200 mL) and brine (100 mL), dried, filtered, and concentrated. The resulting oil was dissolved in the minimum amount of hot isopropyl ether, and the crystals which formed upon cooling were collected by filtration to yield 36.8 g (81 mmol) of 17c, mp 164–5 °C. A further crop of 9.8 g was obtained from the mother liquor: 200-MHz NMR ($CDCl_3$) δ 1.52 (d, 6 H, J = 7 Hz), 2.68 (br t, 2 H, J = 4 Hz), 3.63 (septet, 1 H, J = 7 Hz), 4.27 (br t, 2 H, J = 4 Hz), 6.86 (br s, 1 H), 7.0–7.2 (m, 14 H), 9.60 (s, 1 H); IR (KBr) 3400, 2966, 1720, 1673, 1596, 1511 cm^{-1}. Anal. C, H, N.

Methyl 7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-5-oxo-1-heptanoate. A solution of methyl acetoacetate (26.4 mL, 243 mmol) in 250 mL of anhydrous THF was added dropwise to a stirred suspension of hexane-washed sodium hydride (6.4 g, 267 mmol) in 200 mL of THF at 0 °C. When gas evolution was complete, 97.2 mL of a 2.5 M solution of *n*-butyllithium in hexanes was added dropwise over 1 h.

The resulting solution was stirred for 30 min at 0 °C and cooled to −78 °C, and a solution of 36.8 g (81 mmol) of 17c in 100 mL of THF was added over a period of 30 min. The resulting solution was stirred for 30 min at −78 °C, then warmed to 0 °C, and held for an additional 1 h.

(20) Epstein, J. W.; Brabander, H. J.; Fanshawe, W. J.; Hofmann, C. M.; McKenzie, T. C.; Safir, S. R.; Osterberg, A. C.; Cosulich, D. B.; Lovell, F. M. *J. Med. Chem.* 1981, *24*, 481–90.

(21) Cabre, J.; Palomo, A. L. *Synthesis* 1984, 413–7.

The mixture was then acidified by the dropwise addition of 300 mL of ice-cold 3 M HCl, diluted with ether, washed with water and brine, dried, filtered, and evaporated. Flash chromatography on silica gel (3:1 v/v hexane–ethyl acetate) yielded 37.9 g of methyl 7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxo-1-heptanoate: 90-MHz NMR ($CDCl_3$) δ 1.50 (d, 6 H, $J \approx 7$ Hz), 1.8 (m, 2 H), 2.45 (d, 2 H, $J = 7$ Hz), 2.8 (br s, 1 H), 3.33 (s, 2 H), 3.5 (m, 1 H), 3.67 (s, 3 H), 3.8–4.0 (m, 2 H), 6.8–7.3 (m, 14 H) ppm.

(±)-*trans*-5-(4-Fluorophenyl)-2-(1-methylethyl)-*N*,4-diphenyl-1-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-3-carboxamide (3i). Air (60 mL) was bubbled via a syringe through a solution of methyl 7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxo-1-heptanoate (48 g, 84 mmol) and 92.5 mL of a 1 M THF solution of tributylborane in 100 mL of anhydrous THF. The mixture was stirred overnight at room temperature and then cooled to −78 °C. Sodium borohydride (3.85 g, 102 mmol) was added to the cooled mixture in one portion. The vigorously stirred suspension was allowed to warm slowly to 0 °C over 3 h (vigorous gas evolution ensued).

The dry ice–acetone bath cooling the reaction vessel was replaced by an ice bath and 18.3 mL of glacial acetic acid was added dropwise, followed by 204 mL of 3 N NaOH and 30.5 mL of 30% aqueous H_2O_2.

The mixture was vigorously stirred and allowed to warm to room temperature overnight. The mixture was partitioned between ether and water. The aqueous layer was separated, acidified, and extracted with ethyl acetate (2×).

The ethyl acetate extracts were washed with brine, dried, and evaporated to yield crude (*R**,*R**)-3,5-dihydroxy-7-[(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)-carbonyl]-1*H*-pyrrol-1-yl]-1-heptanoic acid which was used without further purification.

The crude acid was taken up in toluene and heated at reflux for 6 h with azeotropic removal of water. Chromatography (silica gel, 1:1 v/v hexane–ethyl acetate) provided 30 g of 3i as a foamy solid, mp 90–97 °C.

This material was found by HPLC analysis to be a 9:1 mixture cis and trans isomers. Recrystallization from toluene–ethyl acetate yielded essentially pure trans 3i: mp 148–9 °C; 200-MHz NMR ($CDCl_3$) δ 1.52 (m, 6 H), 1.6–2.0 (m, 4 H), 2.48 (br s, 1 H), 2.51 (m, 2 H), 3.55 (septet, 1 H, $J = 7$ Hz), 4.0–4.2 (m, 2 H), 4.29 (m, 1 H), 4.52 (m, 1 H), 6.90 (br s, 1 H), 7.0–7.3 (m, 14 H) ppm; IR (KBr) 3400, 1734, 1654, 1597, 1511 cm^{-1}. Anal. C, H, N.

Phenylmethyl 1-[2-(1,3-Dioxolan-2-yl)ethyl]-5-(4-fluorophenyl)-2-(1-methylethyl)-4-phenyl-1*H*-pyrrole-3-carboxylate (16a). A solution of 15 (10 g, 29 mmol) and benzyl phenylpropiolate (7.7 g, 44 mmol) was stirred and heated in 30 mL of acetic anhydride at 90 °C for 6 h. After cooling to room temperature, the solution was concentrated, diluted with ether, washed with water, saturated aqueous bicarbonate, and brine, and dried. Flash chromatography on silica gel (10:1 v/v hexane–ethyl acetate) provided 5.9 g (45%) of crude 16a. Recrystallization from isopropyl ether provided 4.8 g of colorless 16a: mp 158–9 °C; IR (KBr) 1683 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 0.93 (t, 3 H, $J = 7$ Hz), 1.48 (d, 6 H, $J = 7$ Hz), 1.93 (m, 2 H), 3.50 (septet, 1 H, $J = 7$ Hz), 3.7–4.1 (m, 8 H), 4.71 (t, 1 H, $J = 4.4$ Hz), 6.95–7.2 (m, 9 H) ppm. Anal. C, H, N.

Method D. 1-[2-(1,3-Dioxolan-2-yl)ethyl]-2-(4-fluorophenyl)-3,4-dimethyl-5-(1-methylethyl)-1*H*-pyrrole (18). A solution of 11a (1.0 g, 2.37 mmol) in 5 mL of CH_2Cl_2 was added dropwise to a stirred suspension of lithium aluminum hydride (0.3 g, 7.4 mmol) in 20 mL of ether at room temperature. When addition was complete, the mixture was heated to reflux for 30 min, cooled to room temperature, and quenched by dropwise addition of water (0.3 mL), 25% aqueous NaOH (0.2 mL), and water (0.9 mL). After stirring vigorously for 30 min, the mixture was filtered and washed well with CH_2Cl_2. The filtrated was dried, filtered, and concentrated, providing 0.78 g (90%) of pure diol.

Trifluoroacetic acid (5.2 mL, 67 mmol) was added to a stirred solution of the diol (1.23 g, 3.4 mmol) and triethylsilane (1.2 mL, 7.5 mmol) in 10 mL of CH_2Cl_2 cooled to 0 °C under dry nitrogen. The solution was stirred for 2 h at 0 °C before warming to room temperature for 1 h. It was then poured into 300 mL of 50:50 ether–hexane and washed with saturated aqueous bicarbonate (3 × 50 mL) and brine (50 mL), and dried. Flash chromatography on silica gel (10:1 v/v hexane–ethyl acetate) provided 0.80 g (71%) of 18 as an oil: 90-MHz NMR ($CDCl_3$) δ 1.32 (d, 6 H, $J = 7$ Hz), 1.7–1.9 (m, 2 H), 1.86 (s, 3 H), 2.07 (s, 3 H), 3.10 (septet, 1 H, $J = 7$ Hz), 3.7–4.0 (m, 6 H), 4.58 (t, 1 H, $J = 4$ Hz), 6.9–7.3 (m, 4 H) ppm.

Method E. Methyl 4-Methyl-3-oxo-2-(phenylmethylene)pentanoate (21a). A mixture of methyl isobutyrylacetate (144 g, 1 mol), benzaldehyde (116 g, 1.1 mol), piperidine (4 mL), and HOAc (12 mL) in 200 mL of toluene was stirred and heated at reflux with azeotropic removal of water for 3 h. The solution was cooled, poured into ether (1 L), washed with 1 M HCl (200 mL), saturated aqueous bicarbonate (200 mL), and brine, and dried. Concentration and distillation (bp 127–130 °C/1 mmHg) provided 186.6 g (80%) of 21a as a mixture of diastereomers (isomer 1, major ~70%): 90-MHz NMR ($CDCl_3$) δ 0.98 (d, 6 H, $J = 7$ Hz), 2.58 (septet, 1 H, $J = 7$ Hz), 3.70 (s, 3 H), 7.28 (s, 5 H), 7.68 (s, 1 H) ppm. Isomer 2: 90-MHz NMR ($CDCl_3$) δ 1.14 (d, 6 H, $J = 7$ Hz), 3.14 (septet, 1 H, $J = 7$ Hz), 3.70 (s, 3 H), 7.80 (s, 5 H), 7.48 (s, 1 H) ppm.

1-(4-Fluorophenyl)-5-methyl-2-phenyl-1,4-hexanedione (22a). To a solution of 21a (376 g, 1.62 mol), 4-fluorobenzaldehyde (201 g, 1.62 mol), and Et_3N (158 mL) in a 3-L three-neck round-bottom flask with an air-driven stirrer was added 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride (65.5 g, 243 mmol). The mixture was stirred and heated at 70 °C for 24 h. After cooling to room temperature, the mixture was diluted with ether (3 L), washed with water, dilute HCl, saturated aqueous bicarbonate, and brine, and dried. The crude oil which remained after filtration and concentration was dissolved in THF (1500 mL) and added to a solution of NaOH (130 g) in 750 mL of water. The mixture was vigorously stirred overnight, acidified (pH 5) with 6 N HCl, and extracted with ether. The ether layer was washed several times with 3 N NaOH and water (to remove a low R_f, base soluble material) and brine and dried. The crude material was filtered through silica gel (100 g) and concentrated. It was then Kugelrohr distilled in two portions to afford 314 g (66%) of 22a: bp 145 °C (0.3 mmHg) IR (film) 1711, 1684, 1600 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.08 (d, 3 H, $J = 7$ Hz), 1.13 (d, 3 H, $J = 7$ Hz), 2.65 (septet, 1 H, $J = 7$ Hz), 2.77 (dd, 1 H, $J = 18$, 4 Hz), 3.63 (dd, 1 H, $J = 18$, 10 Hz), 5.07 (dd, 1 H, $J = 10$, 4 Hz), 7.10 (m, 2 H), 7.27 (m, 5 H), 7.98 (m, 2 H) ppm.

1-(3,3-Diethoxypropyl)-2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-1*H*-pyrrole (23a). To a solution of 22a (230 g, 0.77 mol) in 1 L of toluene was added 3,3-diethoxy-1-aminopropane[19] (176 g, 1.2 mol) at room temperature. The mixture solidified, but dissolution occurred on adding *p*-TSA–H_2O and heating to reflux (Dean–Stark) for 24 h. To the cooled solution was added 100 mL of absolute ethanol and the mixture concentrated and filtered through silica gel. The residue on concentration was dissolved in the minimum amount of isopropyl ether and allowed to crystallize. A first crop of 89 g (mp 84–7 °C) was isolated. A further 145 g were isolated as an oil: IR (KBr) 2973, 1603, 1511 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.11 (t, 3 H, $J = 7$ Hz), 1.35 (d, 6 H, $J = 7$ Hz), 1.75 (m, 2 H), 3.04 (septet, 1 H, $J = 7$ Hz), 3.2–3.6 (m, 4 H), 3.91 (m, 2 H), 4.27 (t, 1 H, $J = 4.4$ Hz), 6.20 (s, 1 H), 7.0–7.4 (m, 9 H) ppm. Anal. C, H, N.

Methyl 3-(4-Fluorophenyl)-3-oxopropanoate. To a suspension of dimethyl carbonate (195 g, 2.17 mmol) and hexane-washed NaH (72g, 3.0 mol) in dry THF (600 mL) at 60 °C was added 164 g (1.2 mol) of *p*-fluoroacetophenone dropwise. The reaction was maintained at gentle reflux by adjusting the temperature and addition rate (exothermic). After the addition was complete, the reaction was heated at reflux for 4 h, then cooled to room temperature.

The reaction was poured carefully into ice cold acetic acid (183 mL, 3.2 mol) and water (400 mL). The product was extracted with ether (2×), and the combined ether layers were washed with saturated aqueous bicarbonate, brine and dried. Distillation provided 204 g (96%) of desired product (bp 91 °C/0.5 mmHg): 90-MHz NMR ($CDCl_3$) δ 3.65 (s, 3 H), 3.92 (s, 2 H), 6.82–7.20 (m, 2 H), 7.57–8.01 (m, 2 H), 12.45 (singlet, 1 H) ppm.

Methyl 3-(4-Fluorophenyl)-3-oxo-2-(phenylmethylene)-propanoate (26). A mixture of methyl, 3-(4-fluorophenyl)-3-oxopropionate (100 g, 510 mmol), benzaldehyde (59.5 g, 561 mmol), piperidine (2 mL), and acetic acid (6 mL) in toluene (100 mL)

was stirred and heated at reflux with azeotropic removal of water for 4 h. The solution was cooled and filtered through silica gel (600 g) with toluene as eluant. Concentration afforded 127.2 g (88%) of 26 as a mixture of *E*- and *Z*-isomers: 90-MHz NMR ($CDCl_3$) δ 2.22 (s, 3 H, isomer 1), 3.62 (s, 3 H, isomer 2), 6.80–8.11 (m, 10 H) ppm.

1-(4-Fluorophenyl)-3-phenyl-5-methylhexane-1,4-dione (27). A mixture of 26 (130 g, 454 mmol), isobutyraldehyde (41 mL, 454 mmol), Et_3N (33 mL), and 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride (24 g, 91 mmol) was stirred and heated at 70 °C for 18 h. Additional isobutyraldehyde (6 g) was added and stirring continued for a further 6 h. After cooling to room temperature, the mixture was diluted with ether, washed with 2 M HCl (2×), saturated aqueous bicarbonate, and brine, and dried. The crude product was used without further purification.

To a solution of the crude diketo ester (31 g, 86.9 mmol) in 5:1 THF–H_2O (500 mL) was added NaOH (8 g, 200 mmol) in one portion. A small amount of methanol was added to ensure homogeneity. The reaction was stirred overnight at room temperature. The solvent was removed on the rotary evaporator, and the residue was dissolved in ether. This was then washed with 2 M HCl and brine and dried. Purification by flash chromatography (9:1 v/v ethyl acetate–hexane) gave 9.0 g (35%) of 27 as an oil: 90-MHz NMR ($CDCl_3$) δ 0.8 (d, 3 H, J = 7 Hz), 1.2 (d, 3 H, J = 7 Hz), 2.4–3.0 (m, 1 H), 3.6–4.0 (m, 1 H), 4.4–4.55 (m, 1 H), 6.8–7.3 (m, 7 H), 7.7–7.9 (m, 2 H) ppm.

5-(4-Fluorophenyl)-2-(1-methylethyl)-3-phenyl-1*H*-pyrrole-1-propanal (28). To a solution of 17 (9.0 g, 30.2 mmol) and 3,3-diethoxy-1-aminopropane (6.6 g, 45.3 mmol) in toluene (150 mL) was added a catalytic amount of *p*-TSA–H_2O. The resulting mixture was heated to reflux with azeotropic removal of water (Dean–Stark) overnight.

The solution was cooled and concentrated, and the residue was purified by flash chromatography on silica gel (10:1 v/v ethyl acetate–hexane). This provided 2.4 g (19%) of the pyrrole acetal as an oil and 7.1 g of recovered 27. The pyrrole acetal was taken up in 5:1 acetone–water. Camphorsulfonic acid (0.2 g) was added and the solution refluxed for 18 h. The cooled solution was concentrated, diluted with ether, washed with water, bicarbonate, and brine, and dried. Flash chromatography on silica gel (9:1 v/v hexane–ethyl acetate) afforded 1.9 g of 28 as an oil: 90-MHz NMR ($CDCl_3$) δ 1.3 (d, 6 H, J = 7 Hz), 2.56 (m, 2 H), 3.22 (septet, 1 H, J = 7 Hz), 4.37 (m, 2 H), 6.1 (s, 1 H), 6.9–7.5 (m, 9 H), 9.5 (s, 1 H) ppm.

(2*R*)-*trans*-4-[[(1,1-Dimethylethyl)silyl]oxy]-6-[2-[2-(4-fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]ethyl]-tetrahydro-2*H*-pyran-2-one (29). To a solution of 1 (0.52 g, 1.5 mmol) and *tert*-butyldimethylchlorosilane (0.27 g, 1.8 mmol) in 5 mL of dry DMF was added imidazole (0.31 g, 4.5 mmol) in one portion. The solution was stirred overnight at room temperature before partitioning between hexane (100 mL) and water (50 mL). The aqueous layer was extracted with two 50-mL portions of hexane. The combined hexane extracts were washed with water (2 × 25 mL) and brine (25 mL) and dried. Filtration through silica gel and concentration provided 0.7 g (100%) of 29 as a colorless oil: 90-MHz NMR ($CDCl_3$) δ 0.10 (s, 6 H), 0.90 (s, 9 H), 1.30 (d, J = Hz, 6 H), 1.4–1.8 (m, 4 H), 2.48 (m, 2 H), 2.95 (m, 1 H), 3.9–4.3 (m, 3 H), 5.85 (d, J = 2 Hz, 1 H), 6.02 (d, J = 2 Hz, 1 H), 6.8–7.3 (m, 4 H).

(2*R*)-*trans*-6-[2-[3,4-Dichloro-2-(4-fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (30a). *N*-Chlorosuccinimide (6.48 mmol, 0.87 g) was added in one portion to a stirred solution of 29 (1.49 g, 3.24 mmol) in dry DMF (10 mL) cooled to 0 °C under dry nitrogen. The solution was stirred for 1 h at 0 °C then warmed to room temperature over 3 h. This was then diluted with water (50 mL) and extracted with ether (2 × 100 mL). The ether extracts were diluted with 100 mL of hexane, washed with water (50 mL), saturated aqueous bicarbonate (50 mL), 10% aqueous $NaHSO_3$ (50 mL), and brine (50 mL), and dried. After filtration and concentration, the crude product was dissolved in THF (15 mL) and treated with glacial acetic acid (0.75 mL, 13 mmol) and *n*-Bu_4F (9.72 mL of 1 M THF solution). The solution was stirred for 5 h, diluted with ethyl acetate (100 mL), washed with saturated aqueous bicarbonate (2 × 50 mL) and brine (25 mL), and dried. The residue which remained after filtration and concentration was flash chromatographed on silica gel (2:1 v/v hexane–ethyl acetate). This provided 0.50 g (35%) of 30a as a colorless solid. Recrystallization from ether–hexane provided colorless crystals: mp 129–131 °C; IR (KBr) ν 3550, 2990, 1711, 1518, 1225, 1160, 1055, 851, 816 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.44 (d, J = 7 Hz, 6 H), 1.8 (m, 4 H), 2.12 (d, J = 3 Hz, 1 H, OH), 2.55 (m, 2 H), 3.10 (m, 1 H), 4.0 (m, 2 H), 4.30 (m, 1 H), 4.45 (m, 1 H), 7.0–7.4 (m, 4 H) ppm. Anal. C, H, N.

(2*R*)-*trans*-6-[2-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-(trifluoroacetyl)-1*H*-pyrrol-1-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (30c). Trifluoroacetic anhydride (0.17 mL, 1.2 mmol) was added dropwise to a stirred solution of 29 (0.50 g, 1.09 mmol) in 2 mL of DMF cooled to 0 °C under nitrogen. The light yellow solution was stirred for 1 h at 0 °C, diluted with 150 mL of 50:50 ether–hexane, washed with saturated aqueous bicarbonate (3 × 50 mL), and brine, and dried. Filtration and concentration provided a single product which was dissolved in 5 mL of anhydrous THF and stirred overnight at room temperature with 4 equiv of glacial acetic acid and 3 equiv of *n*-Bu_4NF. The mixture was then diluted with ether, washed with 2 M HCl and brine, and dried. Flash chromatography on silica gel (2:1 v/v hexane–ethyl acetate) provided 0.25 g of 30c as an oil: IR (KBr) 3450, 1687, 1609 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.31 (d, 8 H, J = 7 Hz), 1.4–2.0 (m, 5 H), 2.6 (m, 2 H), 3.00 (septet, 1 H, J = 7 Hz), 3.9–4.1 (m, 2 H), 4.33 (m, 1 H), 4.49 (m, 1 H), 6.48 (q, 1 H, J = 2.1 Hz), 7.0–7.4 (m, 4 H) ppm. Anal. C, H, N.

[*S*-(*R,*S**)]-5-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-1-pentanoic Acid, 2-Hydroxy-1,2,2-triphenylethyl Ester (31).** *n*-Butyllithium in hexane (285 mL, 2.2 M) was added dropwise with stirring to diisopropylamine (92 mL) in THF (300 mL) at −50 to −60 °C in a 1000-mL one-neck flask via a dropping funnel under nitrogen. The yellow solution was allowed to warm to approximately −20 °C, then cannulated into a suspension of 99 g of (*S*)-(+)-2-acetoxy-1,1,2-triphenylethanol[16] in 500 mL of anhydrous THF at −70 °C. When addition was complete, the reaction mixture was allowed to warm to −10 °C over a period of 2 h. Meanwhile, a suspension of 0.63 mol of $MgBr_2$ was prepared by addition of 564 mL (0.63 mol) of bromine dropwise into a suspension of 15.3 g of magnesium (0.63 mol) in 500 mL of THF in a 3-L flask equipped with reflux condenser and mechanical stirrer. The $MgBr_2$ suspension was cooled to −78 °C and the enolate solution cannulated into the suspension over 30 min. Stirring was continued for 1 h at −73 °C. 17c (150 g) in 800 mL of THF was then added dropwise over 30 min. The solution was stirred for 1.5 at −78 °C and then quenched with 200 mL of glacial acetic acid at −78 °C. After warming to 0 °C, 500 mL of water were added and the mixture concentrated in vacuo at 40–50 °C. 1:1 ethyl acetate–heptane (500 mL) was added to the yellow slurry, which was then filtered. The filtrate was washed extensively with 0.5 N HCl, then several times with water, and finally with cold (−20 °C) ethyl acetate–heptane (3:1). The light brown crystalline product was dried in vacuo at 40 °C, affording 194 g of crude aldol product. Recrystallization from ethyl acetate at −10 °C yielded 100 g of 31 (mp 229–230 °C) which analyzed as a 97.4:2.2 mixture of the *R,S*-:*S,S*-isomers by HPLC: IR (KBr) 3400, 2961, 1716, 1663, 1595, 1511, 701 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.44 (d, 6 H, J = 7 Hz), 1.5 (m, 2 H), 2.12 (m, 2 H), 2.39 (br s, 1 H) 3.40 (septet, 1 H, J = 7 Hz), 3.62 (m, 1 H), 3.81 (m, 1 H), 4.07 (m, 1 H), 6.63 (s, 1 H), 6.8–7.5 (m, 29 H) ppm. Anal. C, H, N.

Methyl (*R*)-(+)-5-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-1-pentanoate. To a suspension of 162 g (0.206 M) of the triphenylethanediol ester prepared above in 800 mL methanol–THF (5:3) cooled to 0 °C was added 11.7 g of sodium methoxide. The mixture was stirred until dissolution occurred and then put in the freezer overnight. The reaction mixture was then allowed to warm to room temperature, quenched with 15 mL of glacial acetic acid and concentrated in vacuo at 40 °C to obtain an oil, which was partitioned between water (500 mL) and ethyl acetate (2 × 300 mL). The combined organic extracts were washed with saturated aqueous bicarbonate and brine, dried, and filtered and the solvent evaporated. The residue was chromatographed on silica gel (1:4 v/v, ethyl acetate–heptane) to yield 109 g of the methyl ester as a colorless oil which solidified on

standing. Recrystallization from e. -heptane yielded 73.9 g of colorless crystals: mp 125–6 °C; $[\alpha]^{20}_D$ = 4.23° (1.17 M, CH_3OH); IR (KBr) 3400, 2960, 1720, 1646, 1511, 1160, 755 cm^{-1}; 250-MHz NMR ($CDCl_3$) δ 1.53 (d, 6 H, J = 7 Hz) 1.6–1.7 (m, 2 H), 2.30 (d, 2 H, J = 6 Hz), 2.88 (br s, 1 H), 3.57 (septet, 1 H, J = 7 Hz), 3.67 (s, 3 H), 3.85 (m, 1 H), 3.97 (m, 1 H), 4.15 (m, 1 H), 6.85 (s, 1 H), 6.95–7.25 (m, 14 H) ppm. Anal. C, H, N.

1,1-Dimethylethyl (*R*)-7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxo-1-heptanoate (32). Diisopropylamine (75 mL, 550 mmol) was dissolved in THF (250 mL) in a 2000-mL three-neck flask equipped with thermometer and dropping funnel under nitrogen. The mixture was cooled to −42 °C and then 200 mL of 2.2 M *n*-butyllithium in hexane was added dropwise over 20 min. After stirring for 20 min, 62 mL (460 mmol) of *tert*-butyl acetate dissolved in THF (200 mL) was added over 30 min. This mixture was stirred for 30 min at −40 °C, then a further 140 mL of 2.2 M *n*-butyllithium was added over 20 min. When addition was complete, 81 g (153 mmol) of methyl (*R*)-(+)-5-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrol-1-yl]-3-hydroxy-1-pentanoate in anhydrous THF (500 mL) was added as quickly as possible without allowing the temperature to rise above −40 °C. Stirring was continued for 4 h at −70 °C. The reaction mixture was quenched with glacial acetic acid (69 mL) and allowed to warm to room temperature. It was then concentrated in vacuo and the residue taken up in ethyl acetate, washed extensively with water, saturated aqueous NH_4Cl, saturated aqueous bicarbonate, and brine. The organic layer was dried and filtered and the solvent evaporated to produce 73 g of 32: IR (KBr) 3400, 2933, 1700, 1665, 1511, 1151 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.45 (s, 9 H), 1.53 (dd, 6 H, J = 7.1 Hz), 1.6 (m, 2 H), 2.51 (s, 1 H), 2.53 (d, 1 H, J = 2 Hz), 2.80 (d, 1 H, J = 2 Hz, O*H*), 3.31 (s, 2 H), 3.60 (septet, 1 H, J = 7 Hz), 3.9–4.0 (m, 2 H), 4.09–4.22 (m, 1 H), 6.85 (s, 1 H), 6.95–7.2 (m, 14 H) ppm. Anal. C, H, N.

(+)-(4*R*)-*trans*-2-(4-Fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-2-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-4-carboxamide ((+)-33). To a solution of 73 g (119 mmol) of 32 in THF (500 mL) was added triethylborane (120 mL of a 1 M THF solution) and pivalic acid (0.7 g). The mixture was stirred for 10 min and cooled to −78 °C and methanol (70 mL) was added, followed by $NaBH_4$ (4.5 g, 119 mmol). The mixture was stirred at −78 °C for 6 h, then poured slowly into a 4:1:1 mixture of ice–30% aqueous H_2O_2–water. This mixture was stirred overnight and then allowed to warm to room temperature. Chloroform (400 mL) was added and the mixture partitioned between chloroform and water. The aqueous layer was further extracted with chloroform. The organic extracts were combined and washed extensively with water until a test for peroxide was negative. The organic layer was dried, filtered, and evaporated. The residue was flash chromatographed on silica gel (1:3 v/v ethyl acetate–hexane) to yield 51 g of crude dihydroxy ester which was dissolved in THF–methanol and 1 N NaOH (100 mL) was added with stirring at room temperature. After 4 h, the solution was concentrated, water (100 mL) was added, and it was extracted with ether (2 × 100 mL). The aqueous layer was acidified with 1 N HCl and extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with water. The organic layer was dried, filtered and evaporated. The residue was taken up in toluene (2 L) and heated to reflux (Dean–Stark) for 20 min. After cooling, the procedure above was repeated. The reaction was left at room temperature for 10 days and then concentrated to yield 51 g of crude (+)-33 as a colorless foam. This was dissolved in the minimum amount of chloroform and chromatographed on silica gel (1:1 v/v ethyl acetate–heptane) to yield 23 g of impure (+)-33. Further chromatography on silica gel (98.5:1.5 v/v chloroform–propanol) yielded 13.2 g of (+)-33 as a crude solid.

Recrystallization from ethyl acetate–hexane produced 8.2 g of crystals shown to be a mixture of isomers by HPLC. Concentration of the mother liquors yielded 4.6 g of an oil which was shown to be 100% of pure (+)-33 by HPLC. Chromatography (silica gel, 98:2 v/v chloroform–2-propanol) afforded 4.18 g of (+)-33 as colorless foam, $[\alpha]^{23}_D$ = +24.53° (0.53% in $CHCl_3$).

α-Methylbenzeneacetamides. A solution of 3i (30 g, 55.5 mmoL) in (*R*)-(+)-α-methylbenzylamine (575 mL, 4.45 mol, 98% Aldrich) was stirred overnight at room temperature. The resulting solution was diluted with ether (2 L) and washed exhaustively with 2 M HCl (4 × 500 mL), water (2 × 500 mL), and brine (2 × 500 mL). The organic extract was dried, filtered, and concentrated in vacuo to yield 28.2 g of the diastereomeric α-methylbenzylamides as a white solid, mp 174–7 °C. The α-methylbenzylamides were separated by dissolving 1.5 g of the mixture in 1.5 mL of 98:1.9:0.1 chloroform–methanol–NH_4OH and injecting onto a preparative HPLC column (silica gel, 300 mm × 41.4 mm i.d.) by a gas-tight syringe and eluting with the above solvent mixture. Diastereomer 1 eluted at 41 min. Diastereomer 2 eluted at 49 min. Center cut fractions were collected. This procedure was repeated 3 times and the like fractions combined and concentrated. Examination of each by analytical HPLC indicated that diastereomer 1 was 99.84% pure and diastereomer 2 was 96.53% pure. Each isomer was taken on separately.

(+)-(4*R*)-*trans*-2-(4-Fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-2-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-4-carboxamide ((+)-33). To an ethanolic solution (50 mL) of diastereomer 1, [3*R*-[3*R**,5*R**]]-7-[2-(4-fluorophenyl)-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)-carbonyl]-1*H*-pyrrol-1-yl]-3,5-dihydroxy-*N*-[(*R*)-1-phenylethyl]-1-heptanamide, (1 g, 1.5 mmol) was added 1 N NaOH (3.0 mL, 3 mmol). The resulting solution was heated to reflux for 48 h.

The solution was cooled to room temperature and concentrated in vacuo. The residue was resuspended in water and carefully acidified with 6 N HCl. The resulting acidic solution was extracted with ethyl acetate. The organic extract was washed with water and brine, dried, filtered, and concentrated in vacuo. This residue was redissolved in toluene (100 mL) and heated to reflux with azeotropic removal of water for 3 h. This was cooled to room temperature and concentrated in vacuo to yield 1.2 g of a yellow semisolid. Flash chromatography on silica gel (2:3 v/v ethyl acetate–hexane) afforded 0.42 g of a white solid which still contained some impurities. This was rechromatographed (same system) to produce 0.1 g of essentially pure (+)-33, as a white foam. HPLC showed this material to be 94.6% chemically pure ($[\alpha]^{22}_D$ = +25.5° (0.51% in $CHCl_3$). The peak with a retention time of 53.46 min was tentatively assigned to an unknown diastereomer resulting from the 2% (*S*)-(−)-α-methylbenzylamine present in the Aldrich α-methylbenzylamine.

Preparation of (−)-(4*S*)-*trans*-2-(4-Fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-2-oxo-2*H*-pyran-6-yl)ethyl]-1*H*-pyrrole-4-carboxamide ((−)-33). Carrying out the procedure described above on diastereomer 2 afforded 0.6 g of a foamy solid which was flash chromatographed on silica gel (1:1 v/v ethyl acetate–hexane) to afford 0.46 g of essentially pure (−)-33, as a white foam. HPLC showed this material to be 97.83% chemically pure, $[\alpha]^{22}_D$ = −24.8% (0.51% in $CHCl_3$).

Acknowledgment. We are indebted to Dr. S. Brennan, Mr. T. Hurley, and Mr. D. Sherwood for HPLC analyses and the separation of the α-methylbenzylamides derived from 3i, Dr. G. McClusky, Ms. S. Uhlendorf, and staff for spectral and analytical data, and Ms. P. Elka for manuscript preparation.

Inhibitors of Cholesterol Biosynthesis. 4. *trans*-6-[2-(Substituted-quinolinyl)ethenyl/ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors[1]

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A series of substituted quinoline mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase both in vitro and (cholesterol biosynthesis) in vivo. Since previous studies suggested that the 4-(4-fluorophenyl) and 2-(1-methylethyl) substituents afforded optimum potency, attention was focused on variations at position 6 of the quinoline ring. Biological evaluation of a small number of analogues bearing a variety of 6-substituents showed that modification at this position had little effect on potency. Several compounds (8b, 8e, and 11) were identified that showed comparable potency to compactin and mevinolin in both the in vitro and in vivo assays.

We have previously described two series of novel HMG-CoA reductase inhibitors. In each series the structurally complex hexahydronaphthalene ring system common to the naturally occurring fungal metabolites compactin and mevinolin was replaced by a five-membered monocyclic heteroaromatic system, such as the nonbasic pyrrole[2] and pyrazole[3] ring systems. Inhibitors containing basic six-membered monocyclic heteroaromatic[4] and nonbasic[5,6] heteroaromatic ring systems have been reported.

This report describes the synthesis and biological activity of a series of quinoline mevalonolactones, the first HMG-CoA reductase inhibitors to contain a basic bicyclic heteroaromatic ring system.

In addition, many of the compounds described herein exhibit improved in vitro potency when compared to both the pyrrole and pyrazole mevalonolactones previously reported.

Chemistry

Most potent inhibitors of HMG-CoA reductase have the 4-hydroxy-2*H*-pyran-2-one moiety flanked by a bulky lipophilic group and an alkyl group, where both of these groups are anchored in the correct spatial arrangement by various carbocyclic and heterocyclic structures.[7]

We initially investigated the synthesis of quinoline-containing mevalonolactones in which the lactone moiety was connected to position 3 of the quinoline nucleus via a two-carbon spacer and was flanked at positions 2 and 4 by an alkyl group and a 4-fluorophenyl group, respectively.[2,3] By attaching the lactone moiety at position 4 of the quinoline nucleus and employing an alkyl flanking group at position 3 we were able to investigate whether the "benzenoid" ring of the quinoline nucleus could replace the 4-fluorophenyl flanking group and give a compound which retained biological activity. Our general synthetic strategy to the quinolin-3-ylmevalonolactones employed the Friedlander reaction between a suitably substituted benzophenone derivative and an active methylene compound to construct the target quinoline nucleus (Scheme I).

Acid-catalyzed condensation of the requisite 2-aminobenzophenones[8] with various β-keto esters produced esters 1a–e. Reduction to alcohols 2a–e followed by Swern oxidation afforded the corresponding aldehydes 3a–e, which were converted, with >95% *E* selectivity, to α,β-unsaturated esters 4a–e by reaction with carbomethoxymethylenetriphenylphosphorane. DIBAL-H reduction afforded alcohols 5a–e, which were oxidized to aldehydes 6a–e by employing either MnO_2 or the Swern procedure. Condensation with the dianion of ethyl acetoacetate[9] then gave δ-hydroxy-β-keto esters 7a–e. Stereoselective reduction employing the boron-chelation method of Narasaka and Pai[10] gave, after hydrolysis, a mixture of *erythro*- and *threo*-1,3-dihydroxy acids (>12:1) which were lactonized by refluxing in toluene with azeotropic removal of water. Generally, the lactones were crystalline, such that the small amount of the cis stereoisomer present was easily removed by recrystallization, providing almost exclusively the racemic trans stereoisomers 8a–e.

Compounds containing a saturated bridging unit were readily available from 4 via catalytic hydrogenation to give 9. The same sequence of steps utilized for the synthesis of lactones 8a–e was then employed to convert 9 to lactone 10.

Treatment of lactone 8d with *m*-chloroperbenzoic acid in refluxing dichloromethane produced *N*-oxide 11, which was expected to exhibit very different physicochemical properties than the parent quinoline (vide supra).

Lactone 8d was also synthesized as the pure, biologically active 3*R*,5*S* stereoisomer employing Heathcock's β-ketophosphonate lactone synthon[11] (Scheme II). Thus, β-ketophosphonates 12 and 13 (prepared as an 8:1 mixture of diastereomers employing the literature procedure[12]) were

(1) A preliminary report of this work was presented at the 198th Meeting of the ACS, Miami, FL, September 10–15, 1989, MEDI 73. Following this report workers at Bayer AG presented data on a similar series of compounds at the 10th International Symposium on Drugs Affecting Lipid Metabolism, Houston, TX, November 8–11, 1989, Abstracts 510, 511.
(2) Roth, B. D.; Ortwine, D. F.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. *J. Med. Chem.* 1990, *33*, 21 and references contained therein.
(3) Sliskovic, D. R.; Roth, B. D.; Wilson, M. W.; Hoefle, M. L.; Newton, R. S. *J. Med. Chem.* 1990, *33*, 31.
(4) Beck, G.; Kesseler, K.; Baader, E.; Bartmann, W.; Bergmann, E.; Granzer, H.; Jendralla, B.; v. Kerekjarto, B.; Krause, R.; Paulus, E.; Schubert, W.; Wess, G. *J. Med. Chem.* 1990, *33*, 52.
(5) Coppola, G. M.; Scallen, T. J.; DelPrete, A.; Montano, R. *Heterocycles* 1989, *29*, 1497.
(6) Kathawala, F. G.; Scallen, T. J.; Engstom, R. G.; Weinstein, D. B.; Schuster, H.; Stabler, R. *Abstracts of Papers*, 194th National Meeting of the American Chemical Society, New Orleans, August 30–September 4, 1987; American Chemical Society: Washington, DC, 1987; MEDI 79.
(7) Roth, B. D.; Sliskovic, D. R.; Trivedi, B. K. *Annu. Rep. Med. Chem.* 1989, *24*, 147.
(8) Walsh, D. A. *Synthesis* 1980, 677 and references contained therein.
(9) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* 1981, *96*, 1082.
(10) (a) Narasaka, K.; Pai, H. C. *Chem. Lett.* 1980, 1415. (b) Ibid. *Tetrahedron* 1984, *40*, 2233.
(11) Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. *J. Med. Chem.* 1987, *30*, 1858.

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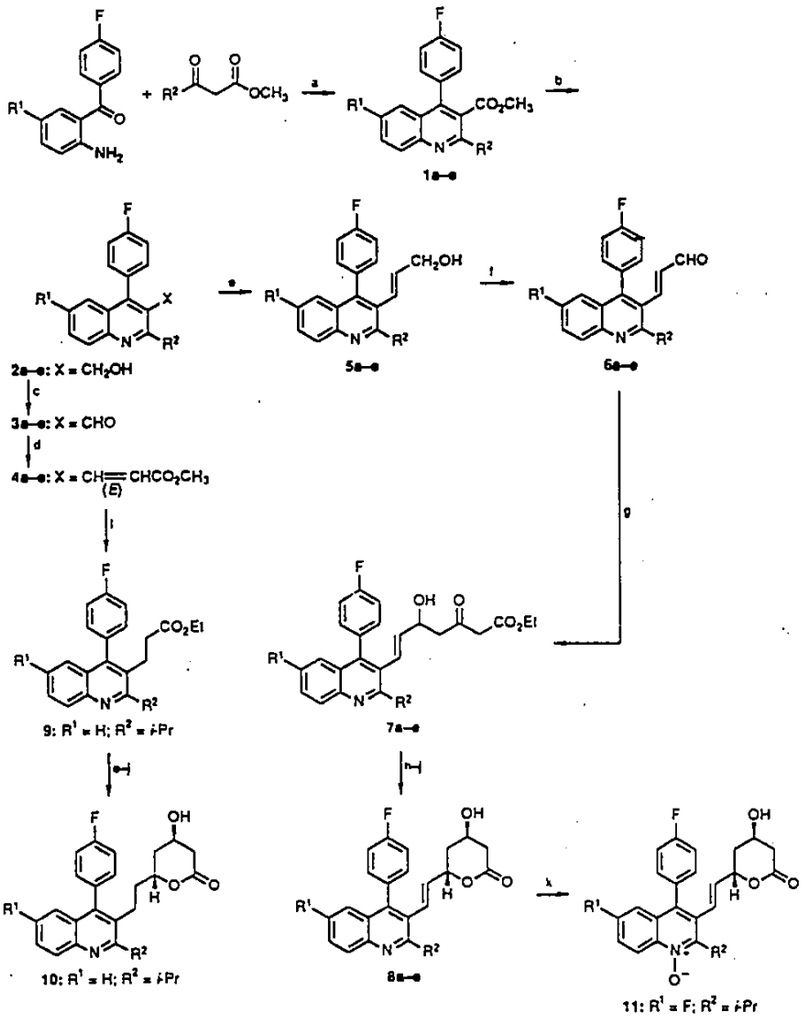
Scheme I*



*(a) pTSA, toluene, Δ; (b) DIBAL-H, CH_2Cl_2, −78 °C; (c) $(COCl)_2$, DMSO, TEA, −78 °C; (d) $Ph_3P{=}CHCO_2CH_3$; (e) DIBAL-H, CH_2Cl_2, −78 °C; (f) Swern or MnO_2, toluene, Δ; (g) $^-CH_2CO^-CHCO_2Et$; (h) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (i) NaOH then HCl; (j) toluene, Δ; (k) *m*CPBA, CH_2Cl_2, Δ; (l) 10% Pd/C, H_2, MeOH.

coupled with aldehyde 3, employing the conditions developed by Roush and Masamune[13] (LiCl, DBU, CH_2Cl_2), in 64% yield. This yield represents the best achieved.[14] The resulting enones (14 and 15) were deprotected and stereoselectively reduced (Et_3B, $NaBH_4$) to give a mixture of *erythro*- (16) and *threo*-1,3-dihydroxy esters. Saponification followed by lactonization and chromatography gave predominantly *trans*-lactone (+)-17 (trans:cis = 26:1). HPLC analysis of the corresponding (*R*)-(+)-α-methylbenzamide derivatives indicated an enantiomeric purity of 89% ee.

In an attempt to increase the aqueous solubility of these compounds (and thereby improve absorption in vivo), a dimethylamino group was incorporated into position 2 of the quinoline ring in place of the isopropyl group (Scheme III).

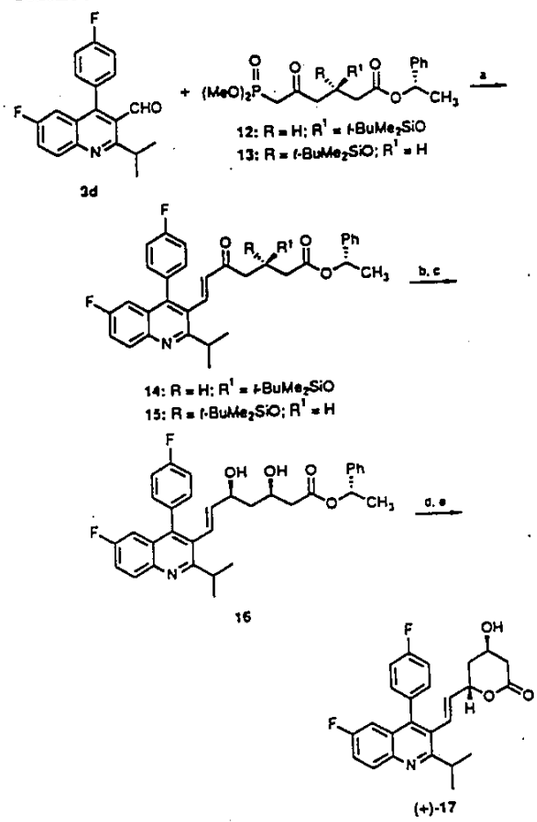
Treatment of benzophenone 18 with ethyl malonyl chloride and silica gel gave 1,2-dihydroquinoline 19 in 88% yield. Chlorination using phosphoryl chloride gave ester 20, which was then reduced and reoxidized to aldehyde 21. Nucleophilic substitution of the chloride with dimethylamine gas in toluene at 130 °C (autoclave) gave dimethylamino aldehyde 22. Aldehyde 22 was then con-

(12) This ratio of diastereomers may be improved to 22:1 by employing (*R*)-1-(1′-naphthyl)ethanol as chiral auxilliary; see: Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* 1988, *53*, 2374.

(13) Blanchette, M. A.; Choy, C. O.; Davis, J. T.; Essenfield, A. P.; Masamune, S.; Roush, W. R. *Tetrahedron Lett.* 1984, *25*, 2183.

(14) A variety of other conditions were examined, e.g., K_2CO_3/18-crown-6/toluene, $(NH_4)_2CO_3$/toluene, and NaH/THF, however, all of these led to β-elimination products derived from both the starting materials (12 and 13) and products (14 and 15). See: Rosen, T.; Heathcock, C. H. *J. Am. Chem. Soc.* 1985, *107*, 3731.

Scheme II[a]



[a] (a) LiCl, DBU, CH_2Cl_2, −10 °C; (b) HF, CH_3CN; (c) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (d) NaOH then HCl; (e) toluene, Δ.

verted to the desired lactone 26 by employing the chemistry described previously.

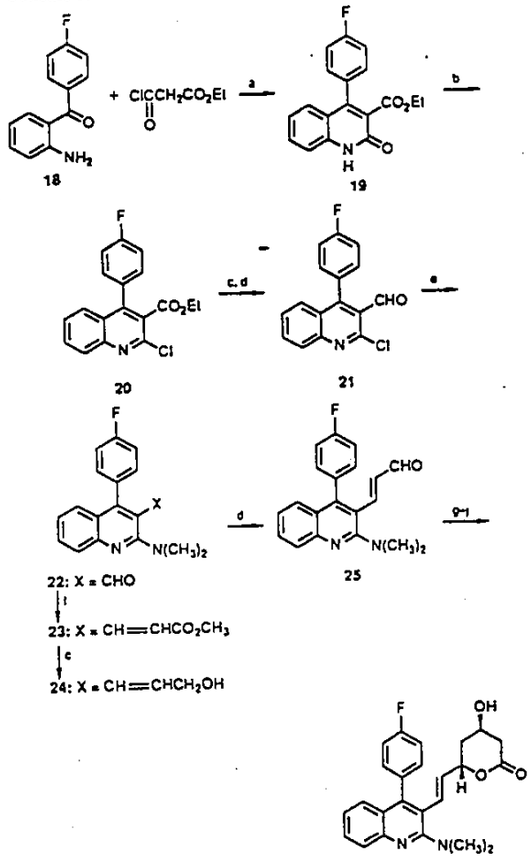
Quinolin-4-ylmevalonolactone 34 was synthesized as shown in Scheme IV. Methyl 3-methyl-4-quinolinecarboxylate[15] (27) was reduced to alcohol 28 and then oxidized under Swern conditions to aldehyde 29. α,β-Unsaturated aldehyde 32 was constructed in an entirely analogous manner to that depicted in Scheme I and was subsequently treated with the dianion of ethyl acetoacetate to yield 33, which was converted to the target lactone 34 (trans:cis = 23:1).

Biological Results

The lactones listed in Table I were saponified to the 3,5-dihydroxy acids and tested for their ability to inhibit the enzyme HMG-CoA reductase, employing two protocols.[2] Method I (cholesterol synthesis inhibition screen or CSI) measured the rate of conversion of [^{14}C]acetate to cholesterol by employing a crude liver homogenate derived from rats fed a chow diet containing 5% cholestyramine. Method II (HMG-CoA reductase inhibition screen or COR) was a more specific screen employing a partially purified microsomal enzyme preparation to measure the direct conversion of [^{14}C]HMG-CoA to mevalonic acid. The

(15) Lindberg, U. H.; Ulff, B.; Yeoman, G. *Acta. Pharm. Suec.* 1968, *5*, 441.

Scheme III[a]



[a] (a) CH_2Cl_2 then SiO_2; (b) $POCl_3$, Δ; (c) DIBAL-H, CH_2Cl_2, −78 °C; (d) $(COCl)_2$, DMSO, TEA, −78 °C; (e) $HN(CH_3)_2$, toluene, autoclave, 130 °C; (f) $Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2; (g) $^-CH_2CO$-$CHCO_2Et$; (h) $B(Et)_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (i) NaOH then HCl; (j) toluene, Δ.

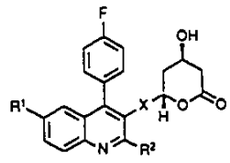
biological activities are displayed in Table I as an IC_{50} (i.e., the concentration needed to inhibit enzyme activity by 50%). Compactin was employed as the internal standard in each testing protocol. The compounds were also evaluated for their ability to inhibit cholesterol biosynthesis in male rats, as determined by the inhibition of the incorporation of sodium [1-^{14}C]acetate into plasma [^{14}C]-cholesterol after po administration of the test substance.[16] This screen was designated the AICS (acute inhibition of cholesterol synthesis) screen.

Most of the compounds tested were more potent than compactin in the in vivo screen and 8b–e exhibited both in vitro and in vivo potencies comparable to those of mevinolin.

As expected, an isopropyl group at position 2 of the quinolinyl-3-mevalonolactones produced a compound, 8b,

(16) Alberts, A. W.; Chen, J.; Kuron, J.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*, 3997.

Table I. Physical Properties and in Vitro and in Vivo HMG-CoA Reductase Inhibitory Activities of Quinoline Mevalonolactones I

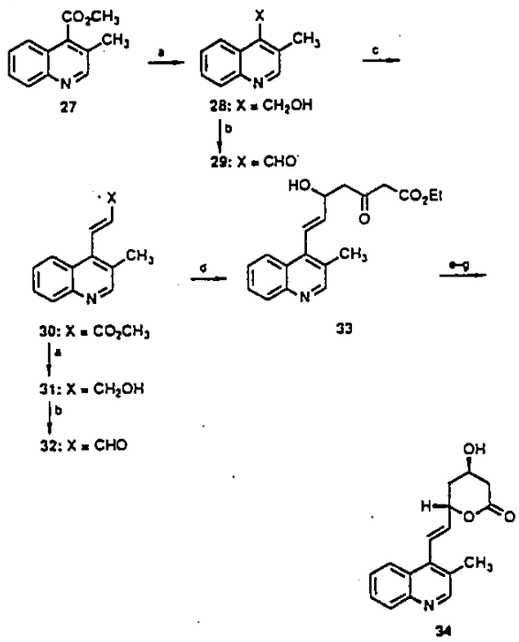


I

no.	R^1	R^2	X	mp, °C	formula[a]	CSI[b,c] IC_{50}, μM	rel (CSI)[d] potency	COR[c,e] IC_{50}, μM	AICS[f] (% inhibn)
compactin						0.030		0.025	36
mevinolin						0.025	118	0.028	72
8a	Cl	CH_3	-CH=CH-	188–190	$C_{23}H_{19}ClFNO_3$	0.4	6.3	0.72	18 (1.5)
8b	Cl	$CH(CH_3)_2$	-CH=CH-	173–175	$C_{25}H_{25}ClFNO_3$	0.032	100	0.025	61 (1.5)
8c	H	$CH(CH_3)_2$	-CH=CH-	168–170	$C_{25}H_{26}FNO_3$	0.042	75.8	0.032	70
10	H	$CH(CH_3)_2$	$-CH_2CH_2-$	199–202	$C_{25}H_{28}FNO_3$	>1.0	<1	-	-
8d	F	$CH(CH_3)_2$	-CH=CH-	174–176	$C_{25}H_{25}F_2NO_3$	0.05	77.6	0.20	68
17	F	$CH(CH_3)_2$	-CH=CH-	foam	$C_{25}H_{25}F_2NO_3 \cdot 0.25C_4H_8O_2$	ND[g]	ND[g]	-	69
11 (*N*-oxide)	F	$CH(CH_3)_2$	-CH=CH-	235–238	$C_{25}H_{25}F_2NO_4$	0.018	112	0.079	47
8e	OCH_3	$CH(CH_3)_2$	-CH=CH-	foam	$C_{26}H_{26}FNO_4$[h]	0.013	100	0.053	60
26	H	$N(CH_3)_2$	-CH=CH-	150–152	$C_{24}H_{23}FN_2O_3 \cdot 0.5C_4H_8O_2$	0.047	13.2	0.35	52
34				198–200	$C_{17}H_{17}NO_3 \cdot 0.25C_4H_8O_2$	>1.0	<1	-	42

[a] Analytical results are within ±0.4% of the theoretical values unless otherwise noted. [b] Cholesterol synthesis inhibition (CSI). Assays of each inhibitor concentration were performed in triplicate, and the precision for compactin was 37%. [c] All compounds tested had a diastereomeric purity of >95% of the trans diastereomer as determined by HPLC and/or 200-MHz NMR. [d] Potency of compactin arbitrarily assigned a value of 100 and the IC_{50} value of the test compound was compared with that of compactin determined simultaneously. [e] All compounds were dosed in DMA/PEG solution of 1.0 mg/kg unless otherwise indicated in parentheses. [f] Anal. Calcd: C, 71.70. Found: C, 70.67. >98% pure by HPLC. [g] Not determined. [h] HMG-CoA reductase inhibition (COR). Assays of each inhibitor concentration were performed in triplicate, and the precision for compactin was 37%.

Scheme IV[a]



[a] (a) DIBAL-H, CH_2Cl_2, −78 °C; (b) $(COCl)_2$, DMSO, TEA, −78 °C; (c) $Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2; (d) $^-CH_2CO^-CHCO_2Et$; (e) B-(Et)$_3$, $NaBH_4$, $(CH_3)_3CCO_2H$ then H_2O_2; (f) $NaOH^-$ then HCl; (g) toluene, Δ.

significantly more potent both in vitro and in vivo than the corresponding 2-methyl compound 8a. Compound 10, which has a saturated two-carbon bridging unit between the quinoline moiety and the mevalonolactone, was considerably less potent than the corresponding unsaturated bridge containing compound 8c.

As previous studies suggested that the 4-(4-fluorophenyl) and 2-(1-methylethyl) substitution afforded optimum potency, attention was focused on variations at position 6 of the quinoline ring. From the limited number of compounds prepared (i.e., 8b–e), it can be seen that varying the substitution at position 6 did not significantly effect either in vivo or in vitro potencies. The dimethylamino-containing compound 26 retained in vivo potency when compared to the corresponding isopropyl-containing compound 8c, but was somewhat less potent in vitro.

N-Oxide 11 was as potent in vitro as compactin and mevinolin and more potent than the corresponding free base but was slightly less potent in vivo.

Quinolin-4-ylmevalonolactone 34 was considerably less potent than either compactin or mevinolin in vitro, however it was comparable to compactin when tested in vivo. The source of the in vivo activity for 34, despite its lack of in vitro activity, is unclear.

Conclusion

A series of quinoline mevalonolactones was prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro and cholesterol biosynthesis in vivo. By focusing on compounds possessing the 4-(4-fluorophenyl) and 2-(1-methylethyl) substituents found to be optimum in previous studies, several compounds, i.e., 8b, 8e, and 11, were identified that were of comparable potency to compactin and mevinolin both in vitro and in vivo. Modifications at position 6 of the quinoline ring had little effect on potency.

In conclusion it has been shown that the quinoline nucleus can be used as a suitable replacement for the hexahydronaphthalene ring present in the fungal metabolites compactin and mevinolin. Compounds have been described which are equipotent to both naturally occurring HMG-CoA reductase inhibitors under the conditions studied.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. All organic extracts were dried over $MgSO_4$ except where otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrometer. Nuclear magnetic resonance spectra were determined on either a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses were determined on a Perkin-Elmer 240C elemental analyzer. HPLC analyses were performed on a Varian 5500 HPLC with a UV 200 detector (wavelength was 251 nm) and an octadecylsilane column [Alltech Econisil C18; mobile phase, 50:50 0.05 M citric acid (pH = 4.0)–CH_3CN]. Optical rotations were performed on a Perkin-Elmer 241 polarimeter. The detailed protocols of the in vitro biological assays are described in ref 2.

Methyl 4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinecarboxylate (1c). A solution of methyl 4-methyl-3-oxopentanoate (14.7 g, 0.102 mol), (2-aminophenyl)-4-(fluorophenyl)methanone[15] (18.34 g, 0.085 mol), and a small amount of *p*-TSA in toluene (400 mL) was heated under reflux with azeotropic removal of water for 5 h. The solution was then cooled and concentrated in vacuo. Flash chromatography of the residue, eluting with 10% ethyl acetate–hexane, gave 1c (7.66 g, 28%): ^1H NMR ($CDCl_3$) δ 3.05 (d, 1 H), 7.72–6.95 (m, 7 H), 3.52 (s, 3 H), 3.16 (heptet, 1 H), 1.40 (d, 6 H) ppm. Anal. ($C_{20}H_{18}FNO_2$) C, H, N.

4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinemethanol (2c). To a solution of 1c (7.66 g, 0.024 mol) in dichloromethane (100 mL) at −78 °C under an atmosphere of nitrogen was added 55 mL of a 1.0 M solution of DIBAL-H. The resulting solution was stirred for 3 h before quenching with saturated aqueous sodium sulfate (20 mL). After warming to room temperature, the solution was filtered through Celite and the resulting filtrate dried and concentrated in vacuo to yield 6.61 g (94%) of 2c: ^1H NMR ($CDCl_3$) δ 7.97 (d, 1 H), 7.57–6.93 (m, 7 H), 4.52 (bs, 2 H), 3.62 (heptet, 1 H), 1.9 (bs, 1 H), 1.43 (d, 6 H) ppm. Anal. ($C_{19}H_{18}FN_2O$) C, H, N.

4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinecarboxaldehyde (3c). To a solution of oxalyl chloride (2.3 mL, 0.027 mol) in anhydrous dichloromethane (50 mL), at −78 °C under an atmosphere of nitrogen, was added dimethyl sulfoxide (3.8 mL, 0.053 mol). After complete addition the resulting solution was stirred for 15 min at −78 °C and then a solution of 2c (6.05 g, 0.02 mol) in dichloromethane (50 mL) was added dropwise. This was stirred for a further 1 h at −78 °C and then quenched by the addition of triethylamine (14.3 mL, 0.103 mol) and saturated aqueous ammonium chloride solution (15 mL). The organic layer was separated and the aqueous layer was extracted with additional dichloromethane. The combined organic layers were dried, filtered, and concentrated in vacuo to yield 3c (6.38 g, quant.) as a pale yellow solid: mp 119–121 °C; ^1H NMR ($CDCl_3$) δ 9.92 (s, 1 H), 8.02 (d, 1 H), 7.72–7.52 (m, 1 H), 7.37–6.98 (m, 6 H), 3.94 (heptet, 1 H), 1.38 (d, 6 H) ppm. Anal. ($C_{19}H_{16}FNO$) C, H, N.

Methyl (*E*)-3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-2-propenoate (4c). Methyl (triphenylphosphoranylidene)acetate (7.5 g, 0.024 mol) and 3c (6.38 g, 0.021 mol) in dichloromethane (100 mL) were stirred at room temperature under nitrogen for 72 h. The solution was then concentrated in vacuo. Flash chromatography on silica gel, eluting with hexanes–ethyl acetate, gave 4c (5.62 g, 74%) as a pale orange solid: mp 147–149 °C; ^1H NMR ($CDCl_3$) δ 7.96 (d, 1 H), 7.72–7.04 (m, 8 H), 5.58 (d, 1 H), 3.63 (s, 3 H), 3.38 (heptet, 1 H), 1.35 (d, 6 H) ppm. Anal. ($C_{22}H_{20}FNO_2$) C, H, N.

(*E*)-3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-2-propen-1-ol (5c). To a solution of 4c (5.62 g, 0.016 mol) in dichloromethane (100 mL) at −78 °C under an atmosphere of nitrogen was added 37.7 mL of a 1.0 M solution of DIBAL-H. The resulting solution was stirred for 2 h at −78 °C and then quenched by addition of saturated aqueous sodium sulfate (15 mL). After warming to room temperature, the solution was filtered through Celite. The resulting filtrate was dried and concentrated in vacuo. The residue was flash chromatographed, eluting with 10% ethyl acetate–hexanes, to yield 5c (4.7 g, 91%) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 7.99 (d, 1 H), 7.60–6.97 (m, 7 H), 6.48 (d, 1 H), 5.45 (dt, 1 H), 4.00 (bs, 2 H), 3.48 (heptet, 1 H), 2.05 (bs, 1 H), 1.38 (d, 6 H) ppm.

(*E*)-3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-2-propenal (6c). To a solution of oxalyl chloride (1.66 mL, 0.019 mol) in anhydrous dichloromethane (25 mL), at −78 °C under an atmosphere of nitrogen, was added dimethyl sulfoxide (2.75 mL, 0.038 mol) in dichloromethane (25 mL). The resulting solution was stirred for 15 min at −78 °C and then a solution of 5c (4.7 g, 0.015 mol) in dichloromethane (50 mL) was added dropwise. This was stirred for 1 h and then quenched by the addition of triethylamine (10.2 mL, 0.073 mol) and saturated aqueous ammonium chloride solution (15 mL). The organic layer was separated and the aqueous layer was extracted with additional dichloromethane. The combined organic layers were dried, filtered, and concentrated in vacuo to yield 6c (4.37 g, 94%): ^1H NMR ($CDCl_3$) δ 9.36 (d, 1 H), 7.96 (d, 1 H), 7.63–7.00 (m, 8 H), 5.90 (dd, 1 H), 3.4 (heptet, 1 H), 1.4 (d, 6 H) ppm.

Ethyl (*E*)-7-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-5-hydroxy-3-oxo-6-heptenoate (7c). Ethyl acetoacetate (2.25 g, 0.017 mol) in anhydrous THF (25 mL) was added dropwise to a stirred suspension of sodium hydride (60% oil suspension, 0.74 g, 0.018 mol) in anhydrous THF (25 mL) at 0 °C under a nitrogen atmosphere. When gas evolution was complete, a 2.4 M solution (7.2 mL, 0.017 mol) of *n*-butyllithium in hexanes was added over 30 min. This was then treated with a solution of 6c (3.68 g, 0.011 mol) in anhydrous THF added dropwise over 30 min. The resulting solution was stirred for 1 h at −78 °C and then quenched by the addition of glacial acetic acid (15 mL) with vigorous stirring. The resulting mixture was then partitioned between diethyl ether and water. After separation of the phases, the aqueous layer was reextracted with diethyl ether, and the combined organic extracts were washed with saturated aqueous sodium bicarbonate and dried. The solvents were removed in vacuo, and the residue was flash chromatographed with hexanes–ethyl acetate as eluant to yield 5.1 g (95%) of the title compound 7c as an orange oil: ^1H NMR ($CDCl_3$) δ 8.07 (d, 1 H), 7.64–7.17 (m, 7 H), 6.62 (d, 1 H), 5.34 (dd, 1 H), 4.59 (m, 1 H), 4.21 (q, 2 H), 3.48 (heptet, 1 H), 3.41 (s, 2 H), 2.44 (d, 2 H), 1.38 (d, 6 H), 1.29 (t, 3 H) ppm.

[4α,6β(*E*)]-6-[2-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (8c). To a room temperature solution of triethylborane (7.2 mL of a 1 M THF solution; 0.007 mol) under a dry-air atmosphere was added, with stirring, a catalytic amount of pivalic acid (0.7 g, 0.0007 mol). The resulting solution was stirred at room temperature for 10 min before a THF (25 mL) solution of 7c (3.0 g, 0.007 mol) was added dropwise. The resulting solution was stirred at room temperature for a further 15 min before cooling to −78 °C. Methanol (5 mL) was added followed by the addition of sodium borohydride (0.28 g, 0.007 mol) in one portion. Vigorous effervescence ensued. This mixture was stirred at −78 °C for 6 h. It was then quenched by pouring into ice-cold 30% hydrogen peroxide (10 mL). The mixture was allowed to warm slowly to room temperature and then was partitioned between chloroform and water. The organic layer was washed extensively with water, dried, and concentrated in vacuo to yield 3.07 g of the corresponding 1,3-diols as a mixture of erythro and threo diastereomers which were used without any further purification.

This residue was then redissolved in THF (50 mL) and methanol (5 mL) and treated with 1 N aqueous sodium hydroxide (6.7 mL). The resulting solution was stirred at room temperature for 2 h and then concentrated to dryness. The residue was then partitioned between water and ether. The ether layer was extracted with 1 N aqueous NaOH. The aqueous layers were combined, acidified with concentrated HCl, and extracted with ethyl acetate. The ethyl acetate extracts were combined, washed with water, and dried. Removal of the solvents in vacuo yielded a yellow foam which was dissolved in toluene (100 mL) and heated for 3 h rt reflux with azeotropic removal of water. The cooled solution was concentrated and the residue flash chromatographed on silica gel, eluting with 50% hexanes–ethyl acetate to yield 8c (1.26 g, 56%) as a white solid, which was shown to be a 97:3 mixture of trans and cis diastereomers by HPLC: mp 168–170

°C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1 H), 7.11 (dt, 1 H), 7.51–7.28 (m, 6 H), 6.69 (d, 1 H), 5.48 (dd, 1 H), 5.24 (bs, 1 H), 5.10–5.00 (m, 1 H), 4.0 (bs, 1 H), 3.48 (heptet, 1 H), 2.67–2.31 (m, 2 H), 1.57–1.42 (m, 2 H), 1.33 (d, 6 H) ppm; IR (KBr) 3430, 2967, 1715, 1514, 1256, 1224, 1160, 1067, 974 cm^{-1}. Anal. ($C_{25}H_{24}FNO_3$) C, H, N.

Compounds 8a–e were synthesized by the general method outlined in Scheme I and exemplified for compound 8c; their physical and biological properties are listed in Table I.

[4α,6β(*E*)]-6-[2-[6-Fluoro-4-(4-fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one, *N*-Oxide (11). A dichloromethane solution (100 mL) of 8d and *m*-CPBA was heated under reflux for 6 h under an atmosphere of nitrogen. The solution was then cooled and washed with saturated aqueous sodium bicarbonate solution. The organic layer was then dried, filtered, and concentrated in vacuo to yield an orange foam (1.24 g), which was flash chromatographed (eluant, 30% ethyl acetate–hexanes) to yield 11 (0.77 g, 74%) as a white solid: mp 235–238 °C; ^{1}H NMR ($CDCl_3$) δ 8.81 (dd, 1 H), 7.49–7.41 (m, 1 H), 7.20 (d, 4 H), 7.01 (dd, 1 H), 6.53 (d, 1 H), 5.44 (dd, 1 H), 5.18–5.13 (m, 1 H), 5.02 (bs, 1 H), 4.15–4.09 (m, 1 H), 3.74 (m, 1 H), 2.79 (bs, 2 H), 2.60 (d, 2 H), 1.55 (d, 6 H) ppm; IR (KBr) 3430, 3260, 1730, 1624, 1513, 1303, 1248, 1218, 1049, 831 cm^{-1}. Anal. ($C_{25}H_{23}F_2NO_4$) C, H, N.

The compounds bearing a saturated two-carbon spacer between the quinoline nucleus and the lactone moiety can be synthesized in an entirely similar manner to that of lactones 8a–e. The experimental details for the key reduction of the α,β-unsaturated esters 4 is exemplified below for the preparation of compound 9.

Methyl 3-[4-(4-Fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]propanoate (9). Compound 4c (10.0 g, 0.029 mol) and 10% Pd/C (0.75 g) were stirred in methanol (250 mL) at room temperature under 50 psi of hydrogen gas. After 5 h, the suspension was filtered and the filtrate concentrated in vacuo to yield 10.14 g of an orange oil. Trituration with hexanes afforded 6.06 g (60%) of 9 as an off-white solid: mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1 H), 7.62 (t, 1 H), 7.33 (t, 1 H), 7.29–7.16 (m, 5 H), 3.64 (s, 3 H), 3.44 (heptet, 1 H), 2.96 (t, 2 H), 2.39 (t, 2 H), 1.44 (d, 6 H) ppm. Anal. ($C_{22}H_{22}FNO_2$) C, H, N.

[*R*-(*R,*R**)]-1-Phenylethyl 3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-7-[6-fluoro-4-(4-fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]-5-oxo-6-heptenoate (14).** To a solution of 3d (0.6 g, 0.002 mol) and β-ketophosphonates (12–13, 8:1 mixture of diastereomers) (1.35 g, 0.003 mol) in dichloromethane (10 mL) at −10 °C under a nitrogen atmosphere was added a small amount of LiCl and DBU (2.85 mL, 0.019 mol). The resulting orange solution was stirred at −10 °C for 1.5 h and then quenched by addition of ice-cold phosphoric acid (0.5 M). The organic layer was separated, washed with water, dried, filtered, and concentrated in vacuo to yield a yellow oil (1.65 g). Flash chromatography on silica gel, eluting with 10% ethyl acetate–hexanes gave recovered aldehyde 3d (0.29 g, 0.0009 mol, 48%), 14–15 (0.42 g, 0.0006 mol, 33%), and recovered β-ketophosphonate 12–13: ^{1}H NMR ($CDCl_3$) δ 7.98 (dd, 1 H), 7.51 (d, 1 H), 7.33–6.84 (m, 11 H), 5.89 (d, 1 H), 5.77 (q, 1 H), 4.45 (m, 1 H), 3.34 (heptet, 1 H), 2.59 (d, 2 H), 2.40 (d, 2 H), 1.48 (d, 3 H), 1.33 (d, 6 H), 0.78 (s, 9 H), 0.01 (s, 6 H) ppm.

[4*R*-[4α,6β(*E*)]]-6-[2-[6-Fluoro-4-(4-fluorophenyl)-2-(1-methylethyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (17). A solution of 48% aqueous HF (0.36 mL, 0.0007 mol) in acetonitrile (3 mL) was added to a solution of 14–15 (0.42 g, 0.0006 mol) in acetonitrile (3 mL). The resulting solution was stirred at room temperature for 1.5 h. It was then diluted with diethyl ether (20 mL) and washed with saturated aqueous sodium bicarbonate solution. The organic layer was dried and concentrated in vacuo to give the desilylated compound (0.31 g, 0.0006 mol, 89%) as a colorless oil, which was used in the next step without any further purification: ^{1}H NMR ($CDCl_3$) δ 8.02 (dd, 1 H), 7.58 (d, 1 H), 7.39–6.83 (m, 11 H), 5.93 (d, 1 H), 5.85 (q, 1 H), 4.34 (m, 1 H), 3.34 (heptet, 1 H), 2.59 (d, 2 H), 2.48 (d, 2 H), 1.52 (d, 3 H), 1.37 (d, 6 H) ppm.

The alcohols were then dissolved in anhydrous THF (5 mL) containing pivalic acid (0.006 g, 0.00006 mol) under a dry-air atmosphere at room temperature. To this solution was added triethylborane (0.63 mL of a 1 M THF solution; 0.0006 mol). The resulting solution was stirred at room temperature for 10 min before cooling to −78 °C. Methanol (1 mL) was added, followed by sodium borohydride (0.024 g, 0.0006 mol) in one portion. Vigorous effervescence ensued. This mixture was stirred at −78 °C for 6 h and then quenched by pouring into ice-cold 30% hydrogen peroxide (1 mL). The mixture was allowed to warm slowly to room temperature and then partitioned between chloroform and water. The organic layer was washed extensively with water, dried, and concentrated in vacuo to yield a foam (0.25 g) which contained compound 16 as its major component.

The crude product was then dissolved in THF (5 mL) and methanol (0.5 mL) and treated with 1 N aqueous sodium hydroxide (0.46 mL). This solution was stirred at room temperature for 3 h, and then all solvents were removed in vacuo. The residue was partitioned between diethyl ether and water. The aqueous layer was acidified with 1 N hydrochloric acid, extracted with ethyl acetate, dried, filtered, and concentrated in vacuo to yield a yellow foam, which was redissolved in toluene (60 mL) and heated for 6 h at reflux with azeotropic removal of water. The cooled solution was concentrated and the residue flash chromatographed on silica gel, eluting with 30% ethyl acetate–hexanes, to give 17 (0.035 g, 18%) as a white foam: $[\alpha]_D$ = +3.4° (*c* = 0.235, $CHCl_3$); HPLC analysis of the corresponding (*R*)-(+)-α-methylbenzylamide derivative indicated an enantiomeric purity of 89% ee; ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, 1 H), 7.47–7.37 (m, 1 H), 7.27–7.18 (m, 4 H), 6.99 (dd, 1 H), 6.68 (d, 1 H), 5.38 (dd, 1 H), 5.20–5.10 (m, 1 H), 4.25–4.19 (m, 1 H), 3.46 (heptet, 1 H), 2.77–2.52 (m, 2 H), 1.83–1.26 (m, 9 H) ppm. Anal. ($C_{25}H_{23}F_2NO_3 \cdot 0.25C_4H_8O_2$) C, H, N.

Ethyl 4-(4-Fluorophenyl)-1,2-dihydro-2-oxo-3-quinolinecarboxylate (19). Ethyl malonyl chloride (125 g, 0.84 mol) was added in portions to a solution of 18[17] in dichloromethane (1 L) at 0 °C under an atmosphere of nitrogen. The reaction mixture was warmed slowly (~1 h) to room temperature, dried, and concentrated to an approximate volume of 600 mL. Silica gel (50 g) was then added. The resulting suspension was stirred overnight at room temperature, and filtered, and the silica gel was washed extensively with ethyl acetate. The filtrate was then concentrated and the residue triturated with hexanes to yield 19 (192 g, 88%) as a white solid: mp 204–206 °C; ^{1}H NMR ($CDCl_3$) δ 12.60 (bs, 1 H), 7.60–7.10 (m, 8 H), 4.17 (q, 2 H), 1.04 (t, 3 H) ppm. Anal. ($C_{18}H_{14}FNO_3$) C, H, N.

Ethyl 2-Chloro-4-(4-fluorophenyl)-3-quinolinecarboxylate (20). A solution of 19 (12.8 g, 0.041 mol) in phosphorus oxychloride (40 mL) was heated to reflux under an atmosphere of nitrogen for 1 h. It was then cooled and concentrated in vacuo and the resulting residue neutralized by the careful addition of cold 1 N sodium hydroxide solution. This was then extracted with ethyl acetate; the organic solution was filtered through a small bed of silica gel to yield 20 (13.2 g, 98%) as a white solid: mp 113–114 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1 H), 7.75–7.70 (m, 1 H), 7.52–7.43 (m, 2 H), 7.34–7.28 (m, 2 H), 7.20–7.12 (m, 2 H), 4.14–4.07 (q, 2 H), 1.02 (t, 3 H) ppm. Anal. ($C_{18}H_{13}ClFNO_2$) H, N, Cl, F; C: calcd, 65.56; found, 66.17.

2-Chloro-4-(4-fluorophenyl)-3-quinolinecarboxaldehyde (21). Compound 20 was reduced to the corresponding alcohol, 2-chloro-4-(4-fluorophenyl)-3-quinolinemethanol, in 83% yield in a manner analogous to the reduction of compounds 1a–e to compounds 2a–e in Scheme I: mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 1 H), 7.79–7.70 (m, 1 H), 7.53–7.22 (m, 6 H), 4.67 (d, 2 H), 2.24 (t, 1 H) ppm. Anal. ($C_{16}H_{11}ClFNO$) C, H, N.

This compound was then oxidized to 21 in a manner analogous to the oxidation of compounds 2a–e to compounds 3a–e in Scheme I: mp 168–169.5 °C; yield 90%; ^{1}H NMR ($CDCl_3$) δ 10.25 (s, 1 H), 8.12 (d, 1 H), 7.91–7.83 (m, 1 H), 7.57–7.53 (m, 2 H), 7.36–7.22 (m, 4 H) ppm. Anal. ($C_{16}H_9ClFNO$) C, H, N.

2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinecarboxaldehyde (22). A solution of 21 (5.28 g, 0.019 mol) and dimethylamine (15 mL) in toluene (75 mL) was heated in an autoclave at 123–126 °C for 14 h. It was then cooled and concentrated in vacuo. The residue was partitioned between ethyl acetate and saturated aqueous potassium carbonate solution. The organic layer was dried, filtered, and concentrated in vacuo. The residue was flash chromatographed on silica gel, eluting with 10%

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ethyl acetate–hexanes, to yield 22 (4.2 g, 77%) as an orange solid: ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1 H), 7.78–6.96 (m, 8 H), 3.10 (s, 6 H) ppm. Anal. ($C_{18}H_{15}FN_2O$) C, H, N.

Methyl (*E*)-3-[2-(dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]-2-propenoate (23) was prepared analogously to compounds 4a–e in Scheme I: yield 92%; ^{1}H NMR ($CDCl_3$) δ 7.78–6.87 (m, 9 H), 5.98 (d, 1 H), 3.60 (s, 3 H), 2.95 (s, 6 H) ppm. Anal. ($C_{21}H_{19}FN_2O_2$) C, H, N.

(*E*)-3-[2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]-2-propen-1-ol (24) was prepared analogously to compounds 5a–e in Scheme I: yield 98%; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1 H), 7.50–7.30 (m, 1 H), 7.20–6.98 (m, 6 H), 6.31 (d, 1 H), 5.72 (dt, 1 H), 3.99 (bd, 2 H), 2.96 (s, 6 H), 1.54 (bs, 1 H) ppm. Anal. ($C_{20}H_{19}FN_2O$) H; C: calcd, 74.51; found, 72.52; N: calcd, 8.69; found, 7.84.

(*E*)-3-[2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]-2-propenal (25) was prepared analogously to compounds 6a–e in Scheme I: yield 92%; ^{1}H NMR ($CDCl_3$) δ 9.35 (d, 1 H), 7.75 (d, 1 H), 7.58–6.98 (m, 8 H), 6.32 (dd, 1 H), 2.99 (s, 6 H) ppm. Anal. ($C_{20}H_{17}FN_2O$) H, N; C: calcd, 74.98; found, 72.85.

[4α,6β(*E*)]-6-[2-[2-(Dimethylamino)-4-(4-fluorophenyl)-3-quinolinyl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (26) was prepared in 29% overall yield from compound 25 in an analogous manner to the preparation of lactones 8a–e from aldehydes 6a–e: mp 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1 H), 7.57–7.50 (m, 1 H), 7.26–7.16 (m, 6 H), 6.49 (d, 1 H), 5.66 (dd, 1 H), 5.16–5.06 (m, 1 H), 4.28–4.25 (m, 1 H), 3.01 (s, 6 H), 2.75–2.60 (q, 2 H), 2.07 (bs, 1 H), 1.82–1.51 (m, 1 H) ppm. Anal. ($C_{24}H_{23}FN_2O_3 \cdot 0.5C_4H_8O_2$) C, H, N.

3-Methyl-4-quinolinemethanol (28) was prepared in 73% yield via a DIBAL-H reduction of 27:[15] ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1 H), 8.17–7.90 (m, 2 H), 7.68–7.42 (m, 2 H), 5.05 (s, 2 H), 2.46 (s, 3 H), 2.20 (bs, 1 H) ppm.

3-Methyl-4-quinolinecarboxaldehyde (29) was prepared in 70% yield from 28 via a Swern oxidation: ^{1}H NMR ($CDCl_3$) δ 10.77 (s, 1 H), 8.68 (s, 1 H), 8.52–8.41 (m, 1 H), 8.03–7.87 (m, 1 H), 7.67–7.34 (m, 2 H), 2.67 (s, 3 H) ppm.

Methyl (*E*)-3-(3-methyl-4-quinolinyl)-2-propenoate (30) was prepared in 76% yield via treatment of 29 with methyl (triphenylphosphoranylidene)acetate in an analogous manner to the preparation of compounds 4a–e in Scheme I: ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1 H), 8.10–7.34 (m, 5 H), 6.21 (d, 1 H), 3.80 (s, 3 H), 2.42 (s, 3 H) ppm. Anal. ($C_{14}H_{13}NO_2$) C, H, N.

(*E*)-3-(3-Methyl-4-quinolinyl)-2-propen-1-ol (31) was prepared in 71% yield from 30 via DIBAL-H reduction: ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1 H), 8.10–7.85 (m, 2 H), 7.66–7.33 (m, 2 H), 6.92 (d, 1 H), 6.11 (dt, 1 H), 4.35 (bs, 3 H), 2.46 (s, 3 H) ppm.

(*E*)-3-(3-Methyl-4-quinolinyl)-2-propenal (32) was prepared in 71% yield from 31 via a Swern oxidation. ^{1}H NMR ($CDCl_3$) δ 9.75 (d, 1 H), 8.63 (s, 1 H), 8.02–7.14 (m, 5 H), 6.38 (dd, 1 H), 2.41 (s, 3 H) ppm.

[4α,6β(*E*)]-6-[2-(3-Methyl-4-quinolinyl)ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (34) was prepared in 10% overall yield from aldehyde 32. The low yield is due to inefficient extraction of the dihydroxy acid from the aqueous phase during the acidification procedure: mp 198–200 °C; ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1 H), 7.94–7.87 (m, 2 H), 7.55–7.34 (m, 2 H), 6.87 (d, 1 H), 5.92 (dd, 1 H), 5.46–5.37 (m, 1 H), 4.90 (bs, 1 H), 4.26 (bs, 1 H), 2.62 (d, 2 H), 2.33 (s, 3 H), 2.15–2.03 (m, 1 H), 1.89–1.76 (m, 1 H) ppm.

In Vivo Acute Inhibition of Cholesterol Synthesis Assay (AICS). Male Sprague–Dawley rats (250 g body weight), previously fed 2.5% cholestyramine for 3 days, were randomly divided into groups (N = 5/group) and given a single dose of vehicle (controls) or compound by an oral gavage at the indicated doses. One hour after drug dosing, all rats were injected intraperitoneally with sodium [1-^{14}C]acetate (20.0 μCi/rat in 0.3 mL of saline). After 50 min, blood samples were taken, plasma was obtained by centrifugation, and plasma [^{14}C]cholesterol was measured after saponification and extraction.

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Disubstituted Tetrahydrofurans and Dioxolanes as PAF Antagonists

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A new series of disubstituted tetrahydrofuran and dioxolane derivatives were prepared and evaluated for their PAF antagonist activity in the PAF-induced in vitro platelet-aggregation and in vivo hypotension tests. Several of these compounds exhibited more potent activity than the structurally related 2-[*N*-acetyl-*N*-[[[[2-methoxy-3-[(octadecylcarbamoyl)oxy]propoxy]carbonyl]amino]methyl]-1-ethylpyridinium chloride (CV-6209, 3) in the in vitro assay, whereas all showed less potency in the in vivo test. The role of both the substituent nature and the placement and number of oxygen atoms in the ring are discussed. A qualitative SAR study was carried out on these nuclei.

Platelet activating factor (PAF, 1) is a naturally occurring phospholipid first described in 1972.[1] It is produced by stimulated basophils, neutrophils, platelets, macrophages, endothelial cells, and IgE-sensitized bone marrow cells.[2] PAF is involved in a wide range of biological actions such as stimulation of platelets and leukocytes, bronchoconstriction, hypotension, negative inotropic cardiac effects, and increase in vascular permeability.[3-5] In vivo experiments have demonstrated PAF's role in several pathological conditions,[6] such as asthma,[7] inflammation,[8] anaphylactic shock,[9] gastric ulceration,[10] and

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Phosphorus-Containing Inhibitors of HMG-CoA Reductase. 2.[1] Synthesis and Biological Activities of a Series of Substituted Pyridines Containing a Hydroxyphosphinyl Moiety[2]

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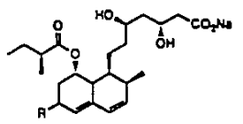
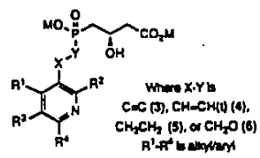
A series of 2,3,4,(5),6-substituted pyridines containing a hydroxyphosphinyl functionality have been prepared and were evaluated for their ability to inhibit the enzyme HMG-CoA reductase. Systematic substitution of both R^1–R^4 and X–Y led to compounds of type 3–6 with in vitro potency greater than that of mevinolin (Na salt).

Introduction

High serum cholesterol levels have been linked to the development of atherosclerosis and coronary heart disease (CHD).[3] A major constituent of serum cholesterol, low density lipoprotein (LDL), is widely believed to be atherogenic upon oxidative modification in vivo,[4] and therefore methods to reduce circulating levels of LDL are highly desirable. Mevinolin (1) and pravastatin (2), two closely



1, R = α-Me (Mevinolin Na)
2, R = β-OH (Pravastatin)

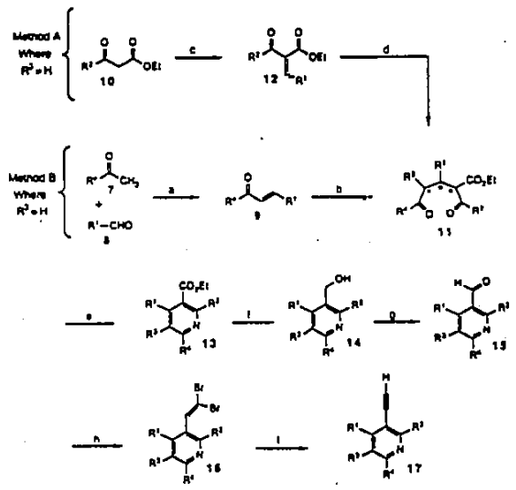


Where X-Y is C=C (3), CH–CH(1) (4), CH_2CH_2 (5), or CH_2O (6); R^1–R^4 is alkyl/aryl and M is Na or Li

related natural products, are currently finding use as therapeutic agents in the treatment of hypercholesterolemia.[5] These compounds act as HMG-CoA reductase (HMGR) inhibitors. Through a complex sequence of regulatory mechanisms, they serve to increase hepatic LDL receptor levels, thereby lowering LDL concentration in the plasma.[6] Inhibition of HMGR, the rate-limiting enzyme in the biosynthesis of cholesterol, is therefore a proven approach to the treatment of hypercholesterolemia.

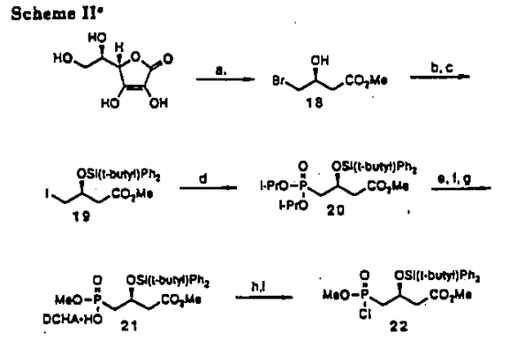
In an attempt to design better, more potent reductase inhibitors, much effort has been expended on replacing the complex decalin portion of the mevinic acids (i.e. 1 or 2) with structurally simpler, achiral aromatic surrogates.[7] In most cases, the 3,5-dihydroxyheptanoic acid portion of the molecule, the pharmacophore that interacts with the 3-

Scheme I*



*(a) EtONa, EtOH, room temperature; (b) 10, EtONa, EtOH, room temperature; (c) 8, piperidine, HOAc, PhH, reflux, $-H_2O$; (d) $R^4COCH_2R^3$, $LiN(TMS)_2$, THF, −78 °C; (e) NH_4OAc, $Cu(OAc)_2$, HOAc, reflux; (f) $LiAlH_4$, THF; (g) Dess–Martin periodinane, *tert*-butyl alcohol, CH_2Cl_2, room temperature, *or* $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C then TEA *or* TPAP, 4-methylmorpholine *N*-oxide, 4A molecular sieves, CH_2Cl_2, room temperature; (h) CBr_4, PPh_3, $CH_2Cl_2(CH_3CN)$; (i) *n*-BuLi (2.2 equiv), THF, −78 °C, then saturated NH_4Cl quench.

Scheme II*



*(a) See ref 12; (b) (*t*-Bu)Ph_2SiCl, DMAP, imidazole, DMF; (c) NaI, MEK, reflux; (d) $(i\text{-PrO})_3P$, 160 °C; (e) TMSBr, BSTFA, CH_2Cl_2; (f) MeOH, DCC, pyridine; (g) dicyclohexylamine, Et_2O; (h) 5% $KHSO_4$, then TMSDEA, CH_2Cl_2; (i) $(CO)_2Cl_2$, catalytic DMF, CH_2Cl_2.

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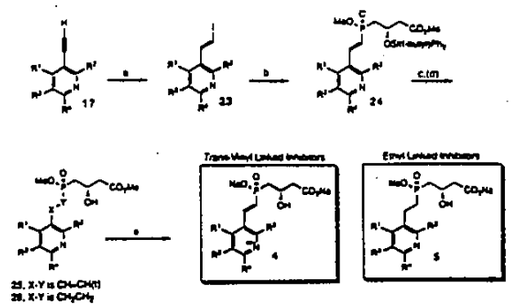
hydroxy-3-methylglutaryl (HMG) binding domain of the enzyme,[8] has been retained. In our previous communication,[1] we described a rationale for the design of a new class of HMGR inhibitors that utilizes a hydroxyphosphinyl functionality in place of the commonly exploited C-5 hydroxy functionality present in the 3,5-dihydroxyheptanoic acid pharmacophore. The hydroxyphosphinyl group was designed to bind to the protonated form of the catalytic group, which serves to activate substrate carbonyl groups toward delivery of a hydride ion in the enzymatic reduction of HMG-CoA to mevalonic acid.

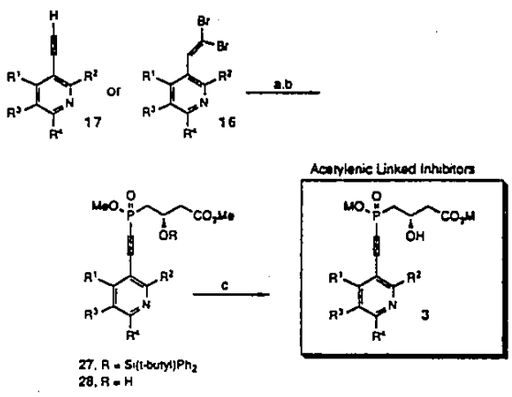
We have prepared hydroxyphosphinyl-containing HMGR inhibitors utilizing a wide variety of aromatic hydrophobic binding domain surrogates. In this paper, we

Scheme III*



* (a) Bu_3SnH, cat. AIBN, 140 °C, then I_2, Et_2O; (b) *t*-BuLi, THF, −78 °C, then 22, THF, −100 °C; (c) TBAF, HOAc, THF; (d) H_2, Pd/C, MeOH; (e) NaOH, H_2O, dioxane, Δ.

Scheme IV*



* (a) *n*-BuLi (1.1 equiv for 17, 2.2 equiv for 16), THF, −78 °C, then 22, THF, −78 °C; (b) TBAF, HOAc, THF, then CH_2N_2, Et_2O; (c) NaOH or LiOH, H_2O, dioxane, 50 °C.

describe the utilization of substituted pyridines[2,9] in the synthesis of hydroxyphosphinyl containing inhibitors 3–6, in which both the "linker" portion (X–Y) of the molecule and the substituents on the pyridine "anchor" have been widely varied.

Chemistry

Methods for the synthesis of the requisite pyridine nuclei are depicted in Scheme I. Claisen–Schmidt condensation of methyl ketone 7 with aldehyde 8 provided *trans*-enone 9. Ethoxide-catalyzed Michael addition of β-keto ester 10 to 9 gave the desired adducts 11, usually as a 1:1 mixture of diastereomers. Method B provides 11

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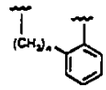
Table I. Pyridyl Alcohols 14

no.	R^1	R^2	R^3	R^4	mp, °C	% yield[a] (method)	formula	anal.[b]
14a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	167–169	82 (B)	$C_{21}H_{20}FNO$	C, H, N
14b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	114–115	65 (B)	$C_{22}H_{22}FNO$	C, H, F, N
14c	4-FC_6H_4	i-C_3H_7	H	2-$(C_6H_5CH_2)C_6H_4$	122–124	40 (B)	$C_{28}H_{26}FNO$	C, H, F, N
14d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	73–75	30 (B)	$C_{25}H_{22}FNO$	C, H, F, N
14e	4-FC_6H_4	i-C_3H_7	H	2,3,5,6-$(F)_4C_6H_1$	130–132	60 (B)	$C_{21}H_{16}F_4NO$	c
14f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	151–153	37 (B)	$C_{19}H_{18}FNOS$	C, H, F, N, S
14g	4-FC_6H_4	i-C_3H_7	H	CH_3	154–155	22 (B)	$C_{16}H_{18}FNO$	C, H, F, N
14h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	88–90	57 (B)	$C_{18}H_{22}FNO$	C, H, F, N
14i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	94–95	24 (B)	$C_{18}H_{20}FNO$	C, H, F, N
14j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	139–140	13 (B)	$C_{28}H_{26}FNO$	C, H, F, N
14k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	112–113	49 (B)	$C_{19}H_{24}FNO$	C, H, F, N
14l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	101–104	40 (B)	$C_{21}H_{26}FNO$	C, H, F, N
14m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	143–145	56 (B)	$C_{22}H_{30}FNO$	C, H, F, N
14n	4-FC_6H_4	i-C_3H_7	H		114–115	42 (B)	$C_{22}H_{20}FNO_3$	C, H, F, N
14o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	182–184	68 (A)	$C_{22}H_{22}FNO$	C, H, F, N
14p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	228–230	53 (A)	$C_{23}H_{24}FNO$	C, H, F, N
14q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	244–246	21 (A)	$C_{24}H_{26}FNO$	C, H, F, N
14r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	169–171	52 (A)	$C_{27}H_{24}FNO$	C, H, F, N
14s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	163–165	8 (A)	$C_{21}H_{19}F_2NO$	c
14t	4-FC_6H_4	i-C_3H_7	$n = 1$		166–167	13 (A)	$C_{22}H_{20}FNO$[d]	C, H, F, N
14u	4-FC_6H_4	i-C_3H_7	$n = 2$		138–139	41 (A)	$C_{23}H_{22}FNO$	C, H, F, N
14v	4-FC_6H_4	i-C_3H_7	$n = 3$		161–162	68 (A)	$C_{24}H_{24}FNO$	C, H, F, N
14w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	oil	20 (B)	$C_{23}H_{24}FNO$	c
14x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	176–177	62 (B)	$C_{21}H_{18}FNO$	C, H, F, N
14y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	140–142	71 (A)	$C_{22}H_{20}FNO$	C, H, F, N
14z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	180–181	61 (A)	$C_{21}H_{20}FNO$	c
14aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	178–180	72 (A)	$C_{20}H_{18}FNO$	C, H, F, N
14bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	172–173	31 (B)	$C_{21}H_{20}FNO$	C, H, F, N
14cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	159–160	60 (B)	$C_{22}H_{22}FNO$	C, H, F, N
14dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	134–135	66 (B)	$C_{22}H_{22}FNO$	C, H, F, N

[a] Represents overall yield from 12 (method A) or from 9 (method B). [b] Analytical results were within ±0.4% of the theoretical value. [c] Microanalysis was not performed. Compound possessed ^1H NMR and MS in accord with assigned structure. [d] Anal. Calcd: C, 79.25. Found: C, 78.74.

in generally good yields in the cases where R^3 = H but was unsatisfactory in cases where R^3 was alkyl or aryl. In these cases, introduction of the R^3 substituent was best carried out utilizing method A. β-Keto α,β-unsaturated ester 12, generated by Knoevenagel condensation of β-keto ester 10 with aldehyde 8, readily underwent Michael addition with lithium enolate $R^4C(OLi)=CHR^3$ to give 11 as a complex mixture of diastereomers. Treatment of 1,5-diketone 11 with NH_4OAc in hot HOAc afforded the intermediate dihydropyridine, which underwent $Cu(OAc)_2$ oxidation[10] in situ, affording pyridyl ester 13. Utilization of either method A or method B allowed for the rapid and convenient generation of tetra- and pentasubstituted pyridines 13, in which the substituents R^1–R^4 could be independently selected from a variety of alkyl or aryl groups. Simple $LiAlH_4$ reduction of 13 gave pyridyl alcohols 14 (Table I). Alcohols 14 provided an entry to phosphonic acid based inhibitors 6 (see Scheme V), but a one-carbon homologation was necessary for generation of the phosphinic acid class of compounds (see Schemes III and IV). Oxidation of 14 could be effected under a variety of conditions to give the corresponding aldehydes 15. Reaction of 15 with CBr_4/PPh_3 provided the vinyl dibromides[11] (Table II) in generally excellent yields. Treatment of 15 with n-BuLi in THF at −78 °C generated the corresponding acetylenic anions in situ. The anions could be utilized in carbon–phosphorus bond formation directly or quenched with a proton source to give acetylenes 17.

The routes we have developed[3] for the synthesis of both the phosphinic and phosphonic acid based inhibitors utilize phosphonochloridate 22 as a synthon for the introduction of the 3-hydroxy-4-(hydroxyphosphinyl)butanoic side chain. The S enantiomer of compound 22 was prepared by a multistep route (outlined in Scheme II) from isoascorbic acid via known[12] bromohydrin ester 18. Silylation of 18 followed by Finkelstein reaction on the silylated bromide provided 19 in 74% overall yield. Arbuzov reaction of 19 was best effected with triisopropyl phosphite to give 20 in 75% yield. Phosphorus deesterification with TMSBr followed by reesterification with MeOH/DCC in pyridine gave the corresponding phosphonic acid monomethyl ester, which was conveniently isolated and stored in stable form as its dicyclohexylamine salt 21. Regeneration of the free acid followed by subsequent treatment with TMSDEA and oxalyl chloride thus provided phos-

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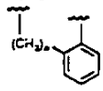
Table II. Pyridyl Vinyl Dibromides 16

no.[a]	R^1	R^2	R^3	R^4	mp, °C	% yield[b] (method)[c]
16a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	oil	88 (C)
16b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	108–110	68 (D)
16c	4-FC_6H_4	i-C_3H_7	H	2-($C_6H_5CH_2$)C_6H_4	foam	62 (D)
16d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	foam	74 (D)
16e	4-FC_6H_4	i-C_3H_7	H	2,3,5,6-$(F)_4C_6H_1$	77	62 (D)
16f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	107–108	86 (D)
16g	4-FC_6H_4	i-C_3H_7	H	CH_3	oil	94 (D)
16h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	52–53	71 (D)
16i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	oil	71 (D)
16j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	141–142	86 (D)
16k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	98–100	68 (D)
16l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	98–100	72 (D)
16m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	176–177	69 (D)
16n	4-FC_6H_4	i-C_3H_7	H		129–131	74 (D)
16o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	169–170	85 (D)
16p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	155–157	82 (D)
16q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	foam	83 (D)
16r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	155–158	88 (D)
16s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	105–107	76 (D)
16t	4-FC_6H_4	i-C_3H_7	n = 1		foam	58 (D)[d]
16u	4-FC_6H_4	i-C_3H_7	n = 2		121–122	76 (E)
16v	4-FC_6H_4	i-C_3H_7	n = 3		173–175	83 (D)
16w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	oil	58 (D)
16x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	170–172	69 (E)
16y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	155–157	77 (E)
16z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	137–138	72 (E)
16aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	141–143	73 (E)
16bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	124–126	84 (C)
16cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	102–104	89 (D)
16dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	128–129	75 (D)

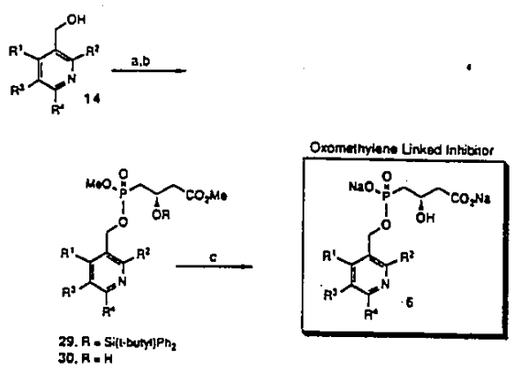
[a] All spectral data were consistent with assigned structures. [b] Represents overall yield from 14. [c] Represents method of oxidation. Method C: Dess–Martin periodinane, *tert*-butyl alcohol, CH_2Cl_2, room temperature. Method D: $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C, then TEA. Method E: TPAP, 4-methylmorpholine *N*-oxide, 4A molecular sieves, CH_2Cl_2, room temperature. [d] CH_3CN used as solvent in the formation of 16w from 15w.

phonochloridate 22. Silylation of the free acid of 21 prior to treatment with oxalyl chloride generates TMSCl rather than HCl as a byproduct of the reaction, allowing the *tert*-butyldiphenylsilyl protecting group to remain intact.

Scheme III outlines the route developed for the synthesis of *trans*-vinyl (X–Y = CH=CH(t)) and ethyl (X–Y = CH_2CH_2) linked inhibitors 4 and 5. Hydrostannylation of acetylene 17 with tributyltin hydride under free-radical conditions[13] followed by treatment of the intermediate *trans*-vinylstannane with iodine stereospecifically provided the *trans*-vinyl iodides 23 in good yields. Metallation of 23 with *tert*-butyllithium generated the corresponding vinyl anion, which was subsequently coupled with phosphonochloridate 22 at −100 °C to give 24 in yields averaging 55%. Higher reaction temperatures led to a substantial diminution in product yield. Desilylation with buffered fluoride provided 25, which was saponified to give *trans*-vinyl-linked inhibitors 4, or, was subjected to catalytic hydrogenation followed by saponification to give ethyl linked inhibitors 5.

Synthesis of ethynyl (X–Y = C≡C) linked inhibitors 3 was, in general, more straight forward (Scheme IV). The lithium anion of 17, generated by the reaction of either 16 or 17 with *n*-butyllithium, smoothly underwent coupling with phosphonochloridate 22 at −78 °C to give 27, usually in 65–80% yields. Desilylation followed by saponification thus provided diacids 3. In the case of the ethynyl-linked compounds, cleavage of the silyl ether of 27 with fluoride ion also led to partial deesterification at the methyl phosphinate ester. Reesterification with diazomethane was

Scheme V[a]



[a] (a) 22, pyridine, 4 °C; (b) TBAF, HOAc, THF; (c) NaOH, H_2O, dioxane, 55 °C.

(13) Tolstikov, G. A.; Miftakhov, M. S.; Danilova, N. A.; Vel'der, Y. L. Regio- and Stereoselective Hydrostannylation of 3-Hydroxy-4-phenoxy-1-butyne: Effective Approach to Intermediates in the Total Synthesis of ω-Aryloxyprostaglandins. *Synthesis* 1986, 496–499.

necessary in order to obtain the desired products, 28, in consistently good yields.

Phosphonic acid based inhibitors 6 were generated as shown in Scheme V. Reaction of pyridyl alcohols 14 with phosphonochloridate 22 in pyridine gave 29, which were subsequently desilylated and saponified to give inhibitors of type 6. Treatment of diesters 30 with base led to a mixture of both 6 and 14, resulting from competing hydrolysis of the methyl and pyridylmethyl phosphonic esters.

Biological Results

Compounds 3–6 were tested for inhibition of the conversion of ^{14}C-HMG-CoA to [^{14}C]mevalonic acid by partially purified HMG-CoA reductase (Table III). Activities are expressed as concentration of drug producing 50% inhibition of the enzyme (I_{50} value). The I_{50}'s of the sodium salts of mevinolin (1) and pravastatin (2) are shown for comparison. Structure–activity relationships were studied by (i) varying the nature of the substituents ortho to the binding domain pharmacophore, (ii) varying the substituents at carbons C-5 and C-6 (R^3 and R^4) on the pyridine ring, (iii) varying the nature of the "linking" group X–Y, and (iv) fusing the C-5 and C-6 positions of the pyridine ring with cycloalkylbenzo substituents.

Workers at Merck had previously shown[7b] in a dihydroxyheptanoic acid based inhibitor series that, for optimal inhibitory potency, an aryl and an alkyl group must flank the HMGR binding domain pharmacophore. Early in our studies, we found that placement of the alkyl substituent (preferably isopropyl) at R^2 and the aryl substituent (preferably 4-fluorophenyl) at R^1 lead to compounds of higher potency relative to their regioisomers (compare 3a and 4a with 3bb and 4bb). Subsequent studies were carried out utilizing this substitution pattern. It is apparent that the enzyme is able to accommodate a wide variety of substituents at C-6 (R^4) of the pyridine nucleus. Very large groups such as naphthyl (3d), 2-benzylphenyl (3c), and adamantyl (3m) are well tolerated. In general, the presence of sterically demanding groups such as diphenylmethyl (3j) and *tert*-butyl (3k) is preferred over smaller substituents such as methyl and isopropyl. A notable exception is seen in the case where R^4 is cyclopropyl (3i). This compound was found to be 20-fold more active than its isopropyl counterpart (3h).

Substitution at C-5 (R^3) of the pyridine nucleus with an alkyl or aryl group dramatically increases intrinsic potency (compare compounds 3o–r with 3a). The effect is greatest with methyl and decreases with increasing steric bulk (i.e. for R^3, methyl > ethyl > isopropyl > phenyl) with R^4 as phenyl. It is believed that this effect is due to a favorable skewing of the R^4 phenyl group out of the plane of the pyridine ring. In order to test this hypothesis, a series of conformationally restricted cycloalkylbenzo-fused pyridines were evaluated (compounds 3t–v). Cyclopentyl- and cyclohexylbenzo-fused pyridines 3t and 3u were essentially equipotent to their nonfused counterpart 3a, whereas cycloheptylbenzo-fused pyridine 3v was 4–5-fold more active. The propylene bridge in 3v necessarily holds the fused phenyl group out of the plane with the pyridine ring.[14] The converse is true with methylene or ethylene bridging units. As proposed above, deviation of planarity of the R^4 phenyl substituent leads to optimal inhibitory potency.

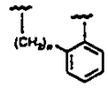
(14) For a study on the conformational analysis of bridged biphenyls and 2,2'-bipyridines, see: Jaime, C.; Font, J. Conformational Analysis of Bridged Biphenyls and 2,2'-Bipyridines Empirical Force Field Calculations (MM2-V4). *J. Org. Chem.* 1990, *55*, 2637–2644.

In order to study the relationship between activity, the linker group X–Y, and the alkyl substituent at R^2, a variety of inhibitors were synthesized in which the R^2 group (R^2 = methyl, ethyl, cyclopropyl, and isopropyl) as well as the linker X–Y (C≡C, CH=CH(t), CH_2CH_2, and CH_2O) were varied. These studies show there is a strong interdependence between R^2 and X–Y. Where R^2 is isopropyl or cyclopropyl (e.g 3–6a,k,o,p,v,y), the general order of activity with respect to X–Y is CH=CH(t) > CH_2O ≥ C≡C > CH_2CH_2. In general, compounds possessing the *trans*-vinyl group are 2–32-fold more active than their acetylenic or methylene ether counterparts and 5–95-fold more potent than their ethyl-linked counterparts. A reversal in activity occurs when R^2 is methyl. In this case (e.g. 3aa, 5aa, and 6aa), the order of activity is CH_2CH_2 ≫ C≡C ≈ CH_2O. As expected, ethyl substitution at R^2 (e.g. 3–6z) exhibits activity that is intermediate between that of isopropyl and methyl substitution (i.e. CH_2CH_2 ≈ C≡C for X–Y). In essentially all cases studied, the *trans*-vinyl group was found to be the superior linking functionality, regardless of the substitution pattern at R^1 and R^2. The SAR of the phosphonic acid based inhibitors 6 (X–Y is CH_2O) more closely parallels that of the inhibitors possessing the acetylenic or *trans*-vinyl linkers, rather than the isosteric ethylene linkers. These data indicate that the alkyl R^2 group must be tailored to the appropriate linker X–Y in order to optimize inhibitory potency. On the basis of these SAR, the most potent compounds possess either an isopropyl or a cyclopropyl group at R^2, a *trans*-vinyl or oxomethylene linker for X–Y, a 4-fluorophenyl group at R^1, and substitution at both R^3 and R^4. Indeed, most of the compounds that possess low to subnanomolar activity against HMGR (i.e. 4o, 4p, 4v, 6v, and 6y) fulfill these criteria.

Since the main site of both LDL synthesis and expression of LDL receptors is in the liver, inhibition of cholesterol biosynthesis in extrahepatic tissue may lead to undesirable side effects. We therefore felt it would be advantageous to develop HMGR inhibitors that would be selective for hepatic cells over extrahepatic cells.[15] Consequently, the phosphorus-based inhibitors were evaluated for their ability to inhibit cholesterol synthesis from [^{14}C]acetate in both hepatic and nonhepatic cells (Table IV). For comparison, mevinolin (1) and pravastatin (2) were also evaluated. One striking difference between pravastatin and mevinolin is exhibited in their ability to inhibit cholesterol synthesis in whole cells. Pravastatin shows inhibition in freshly isolated rat hepatocytes com-

(15) For papers concerning cell and tissue selectivity of HMGR inhibitors, see: (a) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fiji, S. CS-514, A Competitive Inhibitor of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase: Tissue Selective Inhibition of Sterol Synthesis and Hypolipidemic Effect on Various Animal Species. *Biochim. Biophys. Acta* 1986, *877*, 50–60. (b) Reference 7e. (c) Germershausen, J. I.; Hunt, V. M.; Bostedor, R. G.; Bailey, P. J.; Karkas, J. D.; Alberts, A. W. Tissue Selectivity of the Cholesterol-Lowering Agents Lovastatin, Simvastatin, and Pravastatin in Rats in Vivo. *Biochem. Biophys. Res. Commun.* 1989, *158*, 667–675. (d) Roth, B. D.; Bocan, T. M. A.; Blankley, C. J.; Chucholowski, A. W.; Creger, P. L.; Creswell, M. W.; Ferguson, E.; Newton, R. S.; O'Brien, P.; Picard, J. A.; Roark, W. H.; Sekerke, C. S.; Sliskovic, D. R.; Wilson, M. W. Relationship between Tissue Selectivity and Lipophilicity for Inhibitors of HMG-CoA Reductase. *J. Med. Chem.* 1991, *34*, 463–466. (e) Shaw, M. K.; Newton, R. S.; Sliskovic, D. R.; Roth, B. D.; Ferguson, E.; Krause, B. R. HEP-G2 Cells and Primary Rat Hepatocytes Differ In Their Response To Inhibitors Of HMG-CoA Reductase. *Biochem. Biophys. Res. Commun.* 1990, *170*, 726–734.

Table III. Inhibition of HMG-CoA Reductase in Vitro for Compounds 1–6

no.	R^1	R^2	R^3	R^4	X–Y	M	$[\alpha]_D$, deg (c, MeOH)	% yield[a] (method)[b]	formula[c]	I_{50},[d] nM
1[e]	–	–	–	–	–	–	–	–	–	4.0
2	–	–	–	–	–	–	–	–	–	24.0
3a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	C≡C	Li	+4.8 (0.72)	59 (I)	$C_{33}H_{27}FNO_4PLi_2 \cdot 1.5H_2O$	62.0
4a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH=CH(t)	Li	+6.2 (0.56)	36 (F)	$C_{33}H_{29}FNO_4PLi_2 \cdot 1.5H_2O$	1.9
5a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH_2CH_2	Li	–3.3 (0.45)	10[f]	$C_{33}H_{31}FNO_4PLi_2 \cdot 1.5H_2O$	181
6a	4-FC_6H_4	i-C_3H_7	H	C_6H_5	CH_2O	Na	–2.4 (0.47)	34 (J)	$C_{33}H_{29}FNO_4PNa_2 \cdot H_2O$	12.7
3b	4-FC_6H_4	i-C_3H_7	H	2-MeC_6H_4	C≡C	Li	+4.0 (0.59)	15 (H)	$C_{34}H_{29}FNO_4PLi_2 \cdot 1.5H_2O$	32.6
3c	4-FC_6H_4	i-C_3H_7	H	2-($C_6H_5CH_2$)C_6H_4	C≡C	Na	+3.4 (0.53)	55 (H)	$C_{40}H_{33}FNO_4PNa_2 \cdot 1.5H_2O$	62.6
3d	4-FC_6H_4	i-C_3H_7	H	1-naphthyl	C≡C	Na	+4.9 (0.80)	33 (H)	$C_{37}H_{27}FNO_4PNa_2 \cdot 1.5H_2O$	28.7
3e	4-FC_6H_4	i-C_3H_7	H	2,3,4,5-$F_4C_6H_1$	C≡C	Na	+2.7 (0.48)	28 (H)	$C_{33}H_{25}F_5NO_4PNa_2 \cdot 1.5H_2O$	8.9
3f	4-FC_6H_4	i-C_3H_7	H	2-thienyl	C≡C	Na	+7.6 (0.95)	33 (H)	$C_{31}H_{25}FNO_4PS_2Na_2 \cdot 1.5H_2O$	14.6
3g	4-FC_6H_4	i-C_3H_7	H	CH_3	C≡C	Na	+4.7 (0.62)	22 (H)	$C_{28}H_{23}FNO_4PNa_2 \cdot 1.5H_2O$	231
3h	4-FC_6H_4	i-C_3H_7	H	i-C_3H_7	C≡C	Na	+5.6 (0.78)	16 (H)	$C_{31}H_{29}FNO_4PNa_2 \cdot 1.5H_2O$	80.9
3i	4-FC_6H_4	i-C_3H_7	H	c-C_3H_5	C≡C	Na	+5.1 (0.74)	28 (H)	$C_{31}H_{27}FNO_4PNa_2 \cdot 1.5H_2O$	4.2
3j	4-FC_6H_4	i-C_3H_7	H	$(C_6H_5)_2CH$	C≡C	Na	+4.2 (0.38)	17 (I)	$C_{45}H_{33}FNO_4PNa_2 \cdot 1.5H_2O$	14.9
3k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	C≡C	Na	+6.5 (0.77)	33 (H)	$C_{31}H_{29}FNO_4PNa_2 \cdot 1.5H_2O$	6.1
4k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH=CH(t)	Na	+3.3 (0.60)	16 (F)	$C_{31}H_{31}FNO_4PNa_2 \cdot 1.5H_2O$	3.2
5k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH_2CH_2	Na	+0.9 (0.68)	28 (G)	$C_{31}H_{33}FNO_4PNa_2 \cdot 1.5H_2O$	17.2
6k	4-FC_6H_4	i-C_3H_7	H	t-C_4H_9	CH_2O	Na	–1.6 (0.43)	32 (J)	$C_{31}H_{29}FNO_5PNa_2 \cdot 1.5H_2O$	1.4
3l	4-FC_6H_4	i-C_3H_7	H	c-C_6H_{11}	C≡C	Na	+4.4 (0.45)	34 (H)	$C_{34}H_{31}FNO_4PNa_2 \cdot 1.5H_2O$	48.4
3m	4-FC_6H_4	i-C_3H_7	H	1-adamantyl	C≡C	Na	+5.8 (0.72)	15 (H)	$C_{38}H_{37}FNO_4PNa_2 \cdot 1.5H_2O$	43.8
3n	4-FC_6H_4	i-C_3H_7	H		C≡C	Li	+6.9 (0.72)	13 (H)	$C_{27}H_{23}FNO_7PLi_2 \cdot 1.40H_2O$	84.6
3o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	C≡C	Li	+9.4 (0.36)	46 (H)	$C_{27}H_{23}FNO_4PLi_2 \cdot 1.06H_2O$	4.5
4o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH=CH(t)	Na	+10.0 (0.50)	47 (F)	$C_{27}H_{25}FNO_4PNa_2 \cdot 1.5H_2O$	1.2
5o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH_2CH_2	Na	+0.8 (0.49)	65 (G)	$C_{27}H_{27}FNO_4PNa_2 \cdot 1.5H_2O$	9.2
6o	4-FC_6H_4	i-C_3H_7	CH_3	C_6H_5	CH_2O	Na	–2.0 (0.50)	34 (J)	$C_{27}H_{25}FNO_5PNa_2 \cdot 1.5H_2O$	5.1
3p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	C≡C	Na	+11.1 (0.45)	53 (H)	$C_{28}H_{23}FNO_4PNa_2 \cdot 1.5H_2O$	5.6
4p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH=CH(t)	Na	+10.9 (0.52)	36 (F)	$C_{28}H_{25}FNO_4PNa_2 \cdot 1.5H_2O$	0.55
5p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH_2CH_2	Na	+1.0 (0.48)	68 (G)	$C_{28}H_{27}FNO_4PNa_2 \cdot 1.5H_2O$	19.1
6p	4-FC_6H_4	i-C_3H_7	CH_2CH_3	C_6H_5	CH_2O	Na	–0.1 (0.82)	33 (J)	$C_{28}H_{25}FNO_5PNa_2 \cdot H_2O$	2.5
3q	4-FC_6H_4	i-C_3H_7	i-C_3H_7	C_6H_5	C≡C	Na	+11.0 (0.39)	40 (H)	$C_{29}H_{25}FNO_4PNa_2 \cdot 1.5H_2O$	9.8
3r	4-FC_6H_4	i-C_3H_7	C_6H_5	C_6H_5	C≡C	Na	+12.1 (0.52)	49 (H)	$C_{33}H_{25}FNO_4PNa_2 \cdot 1.5H_2O$	15.6
3s	4-FC_6H_4	i-C_3H_7	F	C_6H_5	C≡C	Na	+5.8 (0.48)	40 (H)	$C_{27}H_{22}F_2NO_4PNa_2 \cdot 1.5H_2O$	44.9
3t	4-FC_6H_4	i-C_3H_7	$n = 1$		C≡C	Li	+14.1 (0.46)	18 (H)	$C_{27}H_{23}FNO_4PLi_2 \cdot 1.31H_2O$	75.6
3u	4-FC_6H_4	i-C_3H_7	$n = 2$		C≡C	Na	+11.4 (0.40)	18 (H)	$C_{28}H_{25}FNO_4PNa_2 \cdot 2.25H_2O$	52.5
3v	4-FC_6H_4	i-C_3H_7	$n = 3$		C≡C	Na	+11.2 (0.60)	66 (H)	$C_{29}H_{27}FNO_4PNa_2 \cdot 0.80H_2O$	14.4
4v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH=CH(t)	Na	+12.2 (0.45)	21 (F)	$C_{29}H_{29}FNO_4PNa_2 \cdot 2.50H_2O$	1.3
5v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH_2CH_2	Na	+1.3 (0.38)	80 (G)	$C_{29}H_{31}FNO_4PNa_2 \cdot 2.04H_2O$	18.6
6v	4-FC_6H_4	i-C_3H_7	$n = 3$		CH_2O	Na	–0.3 (0.34)	28 (J)	$C_{29}H_{29}FNO_5PNa_2 \cdot 2.0H_2O$	1.2
3w	4-FC_6H_4	t-C_4H_9	H	C_6H_5	C≡C	Li	+13.1 (0.42)	17 (H)	$C_{27}H_{23}FNO_4PLi_2 \cdot 1.01H_2O$	68.4
3x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	C≡C	Na	+5.6 (0.81)	59 (H)	$C_{29}H_{27}FNO_4PNa_2 \cdot 2.0H_2O$	90.9
6x	4-FC_6H_4	c-C_3H_5	H	C_6H_5	CH_2O	Na	–1.2 (0.50)	35 (J)	$C_{29}H_{27}FNO_5PNa_2 \cdot 2.50H_2O$	29
3y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	C≡C	Na	+8.8 (0.62)	57 (H)	$C_{27}H_{25}FNO_4PNa_2 \cdot 1.25H_2O$	4.6
4y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH=CH(t)	Na	+6.7 (0.50)	30 (F)	$C_{27}H_{27}FNO_4PNa_2 \cdot 1.33H_2O$	2.5
5y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH_2CH_2	Na	–0.3 (0.32)	76 (G)	$C_{27}H_{27}FNO_4PNa_2 \cdot 1.25H_2O$	9.2
6y	4-FC_6H_4	c-C_3H_5	CH_3	C_6H_5	CH_2O	Na	–2.4 (0.41)	32 (J)	$C_{27}H_{25}FNO_5PNa_2 \cdot 1.75H_2O$	1.3
3z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	C≡C	Na	+8.1 (0.35)	21 (H)	$C_{28}H_{25}FNO_4PNa_2 \cdot 2.0H_2O$	109.9
4z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH=CH(t)	Na	+11.8 (0.48)	13 (F)	$C_{28}H_{27}FNO_4PNa_2 \cdot 1.75H_2O$	4.7
5z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH_2CH_2	Na	0 (0.33)	46 (G)	$C_{28}H_{29}FNO_4PNa_2 \cdot 1.25H_2O$	79.8
6z	4-FC_6H_4	C_2H_5	CH_3	C_6H_5	CH_2O	Na	–0.7 (0.45)	22 (J)	$C_{28}H_{27}FNO_5PNa_2 \cdot 3.13H_2O$	19.4
3aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	C≡C	Na	+7.5 (0.4)	19 (H)	$C_{28}H_{25}FNO_4PNa_2 \cdot 2.75H_2O$	1300
5aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	CH_2CH_2	Na	0 (0.44)[j]	44[g]	$C_{28}H_{29}FNO_4PNa_2 \cdot 2.05H_2O$	235
6aa	4-FC_6H_4	CH_3	CH_3	C_6H_5	CH_2O	Na	–0.7 (0.58)	29 (J)	$C_{28}H_{27}FNO_5PNa_2 \cdot 1.84H_2O$	1300
3bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	C≡C	Li	+7.5 (0.85)	41 (I)	$C_{39}H_{29}FNO_4PLi_2 \cdot H_2O$	420
4bb	i-C_3H_7	4-FC_6H_4	H	C_6H_5	CH=CH(t)	Li	+1.8 (0.45)	12 (F)	$C_{39}H_{31}FNO_4PLi_2 \cdot 2.25H_2O$	45.3
3cc	4-F-3-MeC_6H_3	i-C_3H_7	H	C_6H_5	C≡C	Li	+9.5 (0.78)	53 (H)	$C_{27}H_{22}FNO_4PLi_2 \cdot 1.11H_2O$	22.0
3dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	C≡C	Na	+8.8 (0.38)	33 (I)	$C_{27}H_{23}FNO_4PNa_2 \cdot 3.0H_2O$	156
4dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	CH=CH(t)	Na	+1.6 (0.41)	12 (F)	$C_{27}H_{25}FNO_4PNa_2 \cdot 2.2H_2O$	3.9
5dd	4-F-2-MeC_6H_3	i-C_3H_7	H	C_6H_5	CH_2CH_2	Na	–1.9 (0.43)	63 (G)	$C_{27}H_{27}FNO_4PNa_2 \cdot 1.75H_2O$	115

[a] Represents overall yield from vinyl dibromide 16 or acetylene 17. [b] Method F (Scheme III, steps a–c, e). Method G (Scheme III, steps d, e from 25). Method H (Scheme IV, steps a–c from 16). Method I (Scheme IV, steps a–c from 17). Method J (Scheme V, steps a–c). [c] Analyzed for C, H, N, F, and P. Results were within ±0.4% of theory unless otherwise noted. [d] Compounds were assayed against rat microsomal reductase using 100 μM R,S-HMG-CoA and 2.7 mM NADPH. See the Experimental Section for a description of this assay. Reductase enzyme I_{50} values and 95% confidence intervals were calculated from the linear segments of composite log dose versus percent inhibition regressions from 2–5 experiments. The average 95% confidence interval for I_{50} values reported was ±18.4% with a range of 8–40%. [e] Dihydroxy acid form, sodium salt. [f] Anal. Calcd: H, 5.36. Found: H, 5.82. [g] Obtained via hydrogenation of 27 rather than 25. [h] Not analyzed for phosphorus. [i] Anal. Calcd: H, 4.18. Found: H, 3.68. [j] $[\alpha]^{24}_{365} = -3.9°$ (MeOH, $c = 0.44$).

parable to mevinolin but is a much weaker inhibitor in human skin fibroblasts (31-fold). In fact, mevinolin is 7.7-fold more potent in fibroblasts than in hepatocytes. In contrast, our phosphorus-containing inhibitors exhibit a 4–140-fold selectivity for inhibition in hepatocytes versus fibroblasts, with 3o being the most selective. This selectivity is directly related to the presence of the phosphinic acid functionality. The corresponding dihydroxyheptanoic

Table IV. Inhibition of Cholesterol Synthesis from [^{14}C]Acetate in Hepatocytes and Fibroblasts and Inhibition of Cholesterol Biosynthesis from [^{14}C]Acetate in Rats on Intravenous (iv) and Oral (po) Administration[a]

no.	reductase (I_{50}, nM)	hepatocytes (I_{50}, nM)	fibroblasts[b] (I_{50}, nM)	selectivity[c]	in vivo testing (ED_{50}, mpk) iv	po
1[d]	4.0	.146	18.8	0.13	0.033	0.40[e]
2	24.0	100	3080	31	0.053	0.75
3a	59	197	9300	47	0.47	3.9
4a	1.9	77	2000	26	0.22	21.4
3o	4.5	81	11300	140	0.13	3.1
3k	6.1	556	2400	4.3	0.7	3.5
4o	1.2	260	2000	7.7	0.1	0.46
3p	5.6	519	6750	13	ND[f]	4.5
4p	0.55	241	4700	19.5	0.2	>10

[a] The average 95% confidence intervals for the reported reductase, hepatocyte, and fibroblast I_{50} values were ±18.4, 40.9, and 56.9%, respectively. The average 95% confidence intervals for the iv and po ED_{50} values were 33.8 and 37.6%, respectively. All compounds were tested in 2–5 experiments. [b] Human skin fibroblasts. [c] Selectivity is measured as a ratio of I_{50} fibroblasts/I_{50} hepatocytes. [d] Tested as the dihydroxy acid form, sodium salt. [e] Tested po as the corresponding δ-lactone form. [f] Not determined.

acid[16] of 4a (where the P(O)OH group in 4a is replaced by (*S*)-OH) is 69-fold more potent in fibroblast (I_{50} = 2.6 nM) than in hepatocytes (I_{50} = 180 nM). These and other examples[1] indicate that hepatocyte selectivity is a general phenomenon in the phosphinic and phosphonic acid class of reductase inhibitors.

Also listed in Table IV are data obtained for the inhibition of cholesterol biosynthesis from [^{14}C]acetate in rats for a selected number of inhibitors. In general, these phosphinic acids are not as effective as the mevinic acids 1 and 2 upon intravenous (iv) or oral (po) administration. An exception is compound 4o, which shows in vivo activity comparable to that of both 1 and 2. The oral activity of these phosphorus-containing HMGR inhibitors shows no direct correlation with either in vivo reductase inhibitory potency or with in vivo activity after intravenous administration. However, there does appear to be a correlation between iv in vivo activity and activity in isolated rat hepatocytes. For example, despite the fact that 3o and 3k are nearly equipotent against HMGR, 3k is a 7-fold weaker inhibitor of cholesterol biosynthesis in hepatocytes. This is mirrored in a 5-fold loss in potency relative to 3o upon iv administration. However, 3o is still 4-fold less active than mevinolin (1) on iv administration despite equivalent intrinsic potency against reductase. This suggests that the poor in vivo activity of these compounds may be due in part to poor bioavailability to the liver, the target organ. Differences in oral activity (e.g., compare 3o and 4o) are probably due to poor oral absorption. The reasons for the lack of correlation between the in vitro and in vivo potencies of these compounds are currently under investigation.

Conclusion

A potent series of phosphorus-containing reductase inhibitors has been synthesized based on the utilization of highly substituted pyridine nuclei as hydrophobic anchor groups. By proper selection of both the pyridine anchor group and linker X–Y, compounds with enzyme inhibitory activities comparable to or greater than mevinolin (Na salt) have been attained. As determined with rat hepatocytes and human skin fibroblasts, these compounds also show a degree of hepatocyte selectivity not generally exhibited in the dihydroxyheptanoic acid class of inhibitors. In these studies, compound 4o exhibited acute in vivo activity in rats comparable to the clinically proven agents 1 and 2. Inhibitor 4o has been studied for cholesterol-lowering activity in other animal species such as rabbits, dogs, and monkeys. The results of these studies will be presented separately. In addition, an extension of this work to other aromatic and heteroaromatic hydrophobic anchor systems will also be the subject of future disclosures.

Experimental Section

All reactions were carried out under a static atmosphere of argon and stirred magnetically unless otherwise noted. All reagents used were of commercial quality and were obtained from Aldrich Chemical Co. Dry THF and Et_2O were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Dry CH_2Cl_2 was obtained by distillation from CaH_2 under nitrogen. Pyridine and dioxane were obtained from American Burdick and Jackson and were stored over 4A molecular sieves. Boiling points are uncorrected. Melting points were obtained on a Hoover Uni-melt melting point apparatus and are uncorrected. Infrared spectra were recorded on a Mattson Sirius 100-FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-GX270 spectrometer using Me_4Si as an internal standard. Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 polarimeter and c is expressed in g/100 mL. All flash chromatographic separations were performed using E. Merck silica gel (60, particle size, 0.040–0.063 mm). MCI Gel CHP-20P is a highly porous polystyrene–divinylbenzene copolymer resin (75–150 μM) supplied by Mitsubishi Chemical Industries Ltd. Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized with UV light, 5% phosphomolybdic acid in 95% EtOH, or *p*-anisaldehyde in EtOH/H_2SO_4/HOAc.

General Procedure for the Synthesis of 1,5-Diketones 11. Method A. 2-[(4-Fluorophenyl)methylene]-4-methyl-3-oxopentanoic Acid, Ethyl Ester (12, R^1 = 4-FC_6H_4, R^2 = i-C_3H_7). A mixture of 4-fluorobenzaldehyde (3.00 g, 24 mmol), ethyl isobutyrylacetate (3.82 g, 24 mmol), piperidine (240 μL), and HOAc (42 μL) was refluxed in benzene (15 mL) with removal of water (Dean–Stark trap) for 22 h. The cooled mixture was diluted with Et_2O, washed successively with 2% HCl, saturated $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), filtered, and stripped to yield an oil. Distillation of the oil (bp 110–113 °C (0.25 mmHg)) afforded 12 (R^1 = 4-FC_6H_4, R^2 = i-C_3H_7, 5.32 g, 83%) as a pale yellow liquid. The compound was obtained as a 1:1 mixture of *E* and *Z* isomers (a and b): TLC R_f 0.35 (20% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 1.07 (d, *J* = 7.2 Hz, 6 H_a), 1.18 (d, *J* = 7.2 Hz, 6 H_b), 1.25–1.35 (m, 6 $H_{a\&b}$), 2.70 (m, 1 H_a), 3.14 (m, 1 H_b), 4.25–4.37 (m, 4 $H_{a\&b}$), 7.01–7.09 (m, 4 $H_{a\&b}$), 7.34–7.49 (m, 4 $H_{a\&b}$), 7.53 (s, 1 H_b), 7.72 (s, 1 H_a); IR (neat) 1722, 1699, 1605, 1510, 1239 cm^{-1}. Anal. ($C_{15}H_{17}FO_3$) C, H, F. In the same manner, ethyl 3-cyclopropyl-3-oxopropionate[17] (R^2 = c-C_3H_5), methyl propionylacetate (R^2 = CH_2CH_3), and ethyl acetoacetate (R^2 = CH_3) were reacted with 4-fluorobenzaldehyde to give the corresponding Knoevenagel condensation products 12 in 82%, 70%, and 68% yields, respectively.

(16) The corresponding dihydroxyheptanoic acid (Li salt) of 4a was prepared in racemic form from 15a utilizing methods similar to that described in ref 7c.

(17) Jackman, M.; Bergman, A. J.; Archer, S. The Preparation of Some 6-Substituted-2-thiouracils. *J. Am. Chem. Soc.* 1948, *70*, 497–500.

β-(4-Fluorophenyl)-α-(2-methyl-1-oxopropyl)-δ-oxobenzenepentanoic Acid, Ethyl Ester (11o). A −78 °C solution of $LiN(TMS)_2$ (1.0 M in THF, 14.1 mL, 14.1 mmol) in dry THF (15 mL) was treated with a solution of propiophenone (1.900 g, 14.2 mmol) in THF (1.5 mL) over a 5-min period. After 1 h, a solution of compound 12 (R^1 = 4-FC_6H_4, R^2 = i-C_3H_7, 3.717 g, 14.1 mmol) in THF (3 mL) was added dropwise to the above solution. After 1.5 h, the mixture was quenched with saturated NH_4Cl and warmed to room temperature. The mixture was diluted with H_2O and subsequently extracted twice with Et_2O. The combined Et_2O extracts were washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil. Flash chromatography (15% EtOAc in hexane as eluant) afforded Michael adduct 11o (4.755 g, 85%) as a complex mixture of three diastereomers. The mixture was used directly in the next reaction: TLC R_f 0.34–0.31 (20% EtOAc in hexanes); IR ($CHCl_3$) 2974, 1740, 1713, 1682, 1510, 1224 cm^{-1}. In most cases, an excess of ketone $R^4COCH_2R^3$ (1.2 equiv) and $LiN(TMS)_2$ (1.2 equiv) relative to 12 were used for the formation of compound 11. The crude adducts were used directly in the next reaction prior to removal of the volatiles by vacuum distillation (0.2 mmHg at 80 °C).

Method B. 3-(4-Fluoro-3-methylphenyl)-1-phenyl-2-propen-1-one (9, R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5). A mixture of 4-fluoro-3-methylbenzaldehyde 8 (16.000 g, 115.8 mmol) and acetophenone (13.920 g, 115.8 mmol) in absolute EtOH (120 mL) was treated with a solution of EtONa in EtOH (21% wt solution, 4.3 mL, 11.6 mmol). A precipitate soon fell out of solution. After stirring at room temperature for 16 h, the mixture was cooled to −10 °C and the precipitate was collected by filtration. The solid was washed with cold EtOH and dried in vacuo to yield enone 9 (R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5, 23.560 g, 85%) as a pale yellow solid: mp 100–101 °C; TLC R_f 0.42 (20% EtOAc in hexane); ^1H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 7.04 (t, J = 8.8 Hz, 1 H), 7.40–7.62 (m, 6 H), 7.75 (d, J = 15.8 Hz, 1 H), 7.97–8.06 (m, 2 H); IR (KBr) 1659, 1600, 1587, 1501, 1247 cm^{-1}. Anal. ($C_{16}H_{13}FO$) C, H, F.

β-(4-Fluoro-3-methylphenyl)-α-(2-methyl-1-oxopropyl)-δ-oxo-δ-phenylpentanoic Acid, Ethyl Ester (11cc). A slurry of enone 9 (R^1 = 4-F, 3-MeC_6H_3, R^4 = C_6H_5, 23.165 g, 96.5 mmol) and ethyl isobutyrylacetate (22.88 g, 144.6 mmol) in absolute EtOH (400 mL) was treated with a solution of EtONa in EtOH (21% wt solution, 5.4 mL, 14.5 mmol). After being stirred at room temperature for 4.5 h, the solution was concentrated to 200 mL and partitioned between 50% saturated NH_4Cl and EtOAc. The layers were separated, and the EtOAc layer was washed with H_2O (2×) and brine (2×), dried (Na_2SO_4), filtered, and stripped to yield an oil. The oil was taken up in warm hexane and cooled to produce a solid. The solid was boiled in hexanes and cooled to give Michael adduct 11cc (30.815 g, 80%), a 1:1 mixture of diastereomers, as a white amorphous solid: TLC R_f 0.34 and 0.30 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz, integration values are relative) δ 0.70 (d, J = 6.6 Hz, 3 H), 0.94–1.05 (m, 6 H), 1.07–1.13 (m, 6 H), 1.24 (t, J = 7.2 Hz, 3 H), 2.18 (s, 6 H), 2.39 (m, 1 H), 2.76 (m, 1 H), 3.20–3.52 (m, 4 H), 3.93 (q, J = 7.2 Hz, 2 H), 4.06–4.23 (m, 6 H), 6.83 (pseudo t, 2 H), 7.01 (m, 4 H), 7.38–7.57 (m, 6 H), 7.87 (m, 4 H); IR (KBr) 1738, 1711, 1683, 1503, 1245 cm^{-1}. Anal. ($C_{24}H_{27}FO_4$) C, H, F.

General Procedure for the Synthesis of Pyridyl Alcohols 14 (Table I). 4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinecarboxylic Acid, Ethyl Ester (13o). A mixture of 11o (4.730 g, 11.87 mmol), NH_4OAc (2.745 g, 35.6 mmol), and $Cu(OAc)_2$ (5.935 g, 29.7 mmol) in glacial HOAc (30 mL) was gently refluxed for 24 h. The solution was cooled to room temperature and subsequently poured into an ice-cold mixture of concentrated NH_4OH (50 mL) in H_2O (100 mL). The mixture was extracted twice with Et_2O, and the pooled Et_2O extracts were washed with H_2O and brine, dried (Na_2SO_4), filtered, and stripped to yield an oil. The oil was flash chromatographed (20% EtOAc in hexanes as eluant) to give pyridyl ester 13o as an oil (3.916 g, 87%), which slowly solidified on standing: mp 84–88 °C; TLC R_f 0.47 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.00 (t, J = 7.0 Hz, 3 H), 1.33 (d, J = 6.5 Hz, 6 H), 2.04 (s, 3 H), 3.12 (m, 1 H), 4.01 (q, J = 7.0 Hz, 2 H), 7.05–7.59 (m, 9 H); IR (KBr) 1718, 1510, 1270 cm^{-1}. Anal. ($C_{24}H_{24}FNO_2$) C, H, F, N.

4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinemethanol (14o). An ice-cold slurry of $LiAlH_4$ (1.49 g, 39.3 mmol) in dry THF (50 mL) was treated with a solution of ester 13o (4.571 g, [illegible]1 mmol) in dry THF (20 mL). Ten minutes after the addition, the cooling bath was removed and the mixture was stirred at room temperature for 4 h. Additional $LiAlH_4$ (500 mg) was added, and stirring was continued for 2 more h. The solution was recooled to 0 °C and quenched in succession with H_2O (2 mL), 10% NaOH (2.5 mL), and H_2O (6 mL). The solution was filtered, and the salts were washed with EtOAc. The filtrate was washed with H_2O and brine and then dried (Na_2SO_4). Filtration and removal of the solvent afforded a solid. The solid was recrystallized from EtOAc/hexane to provide compound 14o (3.729 g, 92%) as white crystals: mp 182–184 °C; TLC R_f 0.20 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.29 (t, J = 5.3 Hz, 1 H, OH), 1.36 (d, J = 7.0 Hz, 6 H), 1.96 (s, 3 H), 3.50 (m, 1 H), 4.44 (d, J = 5.3 Hz, 2 H), 7.12–7.26 (m, 4 H), 7.33–7.47 (m, 3 H), 7.54–7.60 (m, 2 H); IR (KBr) 3420, 1509, 1218 cm^{-1}. Anal. ($C_{22}H_{22}FNO$) C, H, N, F.

General Procedure for the Synthesis of Pyridyl Vinyl Dibromides 16 (Table II). Oxidation with Dess–Martin Periodinane.[18] 4-(4-Fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinecarboxaldehyde (15a). A slurry of Dess–Martin periodinane (8.60 g, 20.3 mmol) in CH_2Cl_2 (100 mL) was treated with *tert*-butyl alcohol (1.9 mL, 1.49 g, 20.2 mmol), and the mixture was stirred at room temperature for 15 min. A solution of alcohol 14a (5.011 g, 15.6 mmol) in CH_2Cl_2 (85 mL) was then added over a 5-min period. After 30 min, the mixture was diluted with Et_2O and 1 N NaOH and stirred rapidly for 10 min. The organic layer was separated and washed in succession with 1 N NaOH, H_2O, and brine, dried (Na_2SO_4), filtered, and stripped. The solid residue was flash chromatographed (10% EtOAc in hexanes as eluant) to give aldehyde 15a (4.314 g, 87%) as a white solid: mp 105–107 °C (hexane); TLC R_f (0.50 (20% EtOAc in hexanes); ^1H NMR ($CDCl_3$) δ 1.41 (d, J = 6.6 Hz, 6 H), 3.98 (m, 1 H), 7.16 (m, 2 H), 7.33–7.53 (m, 5 H), 7.57 (s, 1 H), 8.17 (m, 2 H), 10.07 (s, 1 H); IR (KBr) 1688, 1573, 1508, 1233 cm^{-1}. Anal. ($C_{21}H_{18}FNO$) C, H, F, N.

Oxidation with TPAP/NMO.[19] 6-(Cyclopropyl)-4-(4-fluorophenyl)-5-methyl-2-(1-methylethyl)-3-pyridinecarboxaldehyde (15y). A solution of 4-methylmorpholine *N*-oxide (4.002 g, 34.2 mmol) in CH_2Cl_2 (130 mL) was dried over $MgSO_4$ for 15 min. The solution was filtered directly into a 500-mL flask, using approximately 30 mL of CH_2Cl_2 to effect the transfer. The flask was then charged with dry 4A molecular sieves (16 g), alcohol 14y (5.686 g, 17.05 mmol), and tetrapropylammonium perruthenate (TPAP, 301 mg, 0.86 mmol). After being stirred at room temperature for 30 min, the black solution was diluted with Et_2O (200 mL), stirred for 5 min, and then filtered through a plug of silica gel (65 × 30 mm), washing with Et_2O. The filtrate was stripped to give a pale yellow solid. The solid was recrystallized from EtOAc/hexane to give aldehyde 15y (3.982 g) as white crystals. Flash chromatography of the mother liquor (20% EtOAc in hexane as eluant) gave additional product, which was recrystallized from hexane (499 mg). Total pooled solids, 4.481 g (79%): mp 137–139 °C; TLC R_f 0.50 (20% EtOAc in hexane); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.00 (m, 2 H), 1.24 (m, 2 H), 2.00 (s, 3 H), 3.16 (m, 1 H), 7.14–7.26 (m, 4 H), 7.39–7.58 (m, 5 H), 9.88 (s, 1 H); IR (KBr) 1686, 1545, 1508, 1223 cm^{-1}. Anal. ($C_{22}H_{18}FNO$) C, H, F, N.

Oxidation with Oxalyl Chloride/DMSO.[20] 4-(4-Fluorophenyl)-6,7-dihydro-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridine-3-carboxaldehyde (15v). A −78 °C solution of oxalyl chloride (630 μL, 917 mg, 7.2 mmol) in CH_2Cl_2 (40 mL) was treated dropwise with a solution of dry DMSO (1.10 mL, 1.21 g, 15.5 mmol) in CH_2Cl_2 (1 mL). After 10 min, a solution

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of alcohol 14v (2.000 g, 5.5 mmol) in THF (5 mL) was added dropwise to the above mixture. Fifteen minutes after the addition, TEA (4.6 mL) was added and the mixture was stirred at −78 °C for 5 min and then warmed to room temperature. The mixture was diluted with Et_2O and washed twice with H_2O and once with brine. The organic layer was dried (Na_2SO_4), filtered, and stripped to give a yellow oil, which produced a solid upon cooling to −78 °C in hexane. The mixture was crystallized from hexane to give aldehyde 15v (1.775 g, 89%) as white needles: mp 132–134 °C; TLC R_f 0.54 (20% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.37 (d, J = 7.0 Hz, 6 H), 2.06 (m, 2 H), 2.18 (m, 2 H), 2.62 (m, 2 H), 3.96 (m, 1 H), 7.11–7.48 (m, 7 H), 7.89 (d, J = 8.0 Hz, 1 H), 9.90 (s, 1 H); IR (KBr) 1693, 1546, 1507, 1223 cm^{-1}. Anal. ($C_{24}H_{22}FNO$) H, F, N; C: calcd 80.20, found 79.56.

3-(2,2-Dibromoethenyl)-4-(4-fluorophenyl)-6,7-dihydro-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridine (16v). A solution of carbon tetrabromide (2.336 g, 7.0 mmol) in CH_2Cl_2 (6 mL) was added over a 7-min period to a cold (0 °C) solution of aldehyde 15v (1.688 g, 4.7 mmol) and triphenylphosphine (3.698 g, 14.1 mmol) in CH_2Cl_2 (20 mL). After the addition was complete, the cooling bath was removed and the mixture was stirred at room temperature for 25 min. The solution was quenched with saturated $NaHCO_3$ and extracted twice with CH_2Cl_2. The organic layers were dried (Na_2SO_4), filtered, and concentrated. The concentrate was flash chromatographed (40% CH_2Cl_2 in hexane as eluant) to give vinyl dibromide 16v as a solid. Recrystallization of the material from EtOAc/hexane provided pure 16v (2.257 g, 93%) as a white solid: mp 173–175 °C; TLC R_f 0.44 (10% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.33 (broad, 6 H), 2.06 (m, 2 H), 2.18 (m, 2 H), 2.61 (m, 2 H), 3.19 (m, 1 H), 7.03–7.43 (m, 8 H), 7.84 (d, J = 8.4 Hz, 1 H); IR (KBr) 2950, 2920, 1603, 1508, 1222 cm^{-1}. Anal. ($C_{25}H_{22}Br_2FN$) C, H, Br, F, N.

(*S*)-4-Iodo-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (19). A solution of bromohydrin 18 (4.00 g, 20.4 mmol), imidazole (6.94 g, 102 mmol), and DMAP (12 mg) in dry DMF (40 mL) was treated with *tert*-butylchlorodiphenylsilane (5.84 mL, 6.17 g, 22.5 mmol), and the homogeneous mixture was stirred at room temperature overnight. The mixture was partitioned between 5% $KHSO_4$ and EtOAc, and the organic phase was washed with H_2O and brine, dried (Na_2SO_4), filtered, and stripped to give 9.32 g (100%) of the crude silyl ether (TLC R_f 0.75 (25% EtOAc in hexanes)). A solution of the silyl ether (9.32 g, 20.1 mmol) in dry methyl ethyl ketone (MEK, 60 mL) was treated with sodium iodide (15.06 g, 100.5 mmol), and the yellow suspension was refluxed for 5 h. The mixture was cooled, diluted with EtOAc, and filtered, and the filtrate was washed with dilute $NaHSO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered, and stripped to give a yellow oil. Flash chromatography (25% CH_2Cl_2 in hexanes as eluant) afforded iodide 19 (7.69 g, 74% from 18) as a colorless oil: TLC R_f 0.75 (25% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.05 (s, 9 H), 2.67 (m, 2 H), 3.20 (m, 2 H), 3.58 (s, 3 H), 3.95 (m, 1 H), 7.28–7.72 (m, 10 H).

(*S*)-4-[Bis(isopropyloxy)phosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (20). Freshly distilled triisopropyl phosphite (113.4 mL, 93.92 gm, 451 mmol) was added in one portion to iodide 19 (21.70 g, 45.1 mmol), and the mixture was heated at 155 °C for 16.5 h. The mixture was cooled to room temperature, and the excess triisopropyl phosphite and volatile reaction products were removed by short path distillation (10 mmHg) followed by Kugelrohr distillation (100 °C, 8 h at 0.5 mmHg). The product was further purified by flash chromatography (6:3:1 hexanes–acetone–toluene as eluant) to afford 20 (17.68 g, 75%) as a clear viscous oil: TLC R_f 0.32 (6:3:1 hexanes–acetone–toluene); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.01 (s, 9 H), 1.12 and 1.19 (2 d, J = 6.3 Hz each, 12 H), 1.87–2.24 (m, 2 H), 2.60 and 2.65 (2 d, J = 7.4 Hz each, 1 H), 2.88 and 2.94 (2 d, J = 3.7 Hz each, 1 H), 3.59 (s, 3 H), 4.44–4.57 (m, 3 H), 7.35–7.45 (m, 6 H), 7.65–7.70 (m, 4 H).

(*S*)-4-(Hydroxymethoxyphosphinyl)-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester, Dicyclohexylamine (1:1) Salt (21). A solution of compound 20 (10.66 g, 30.5 mmol) in dry CH_2Cl_2 (80 mL) was treated dropwise (5 minutes) with bis(trimethylsilyl)trifluoroacetamide (BSTFA, 8.71 mL, 8.44 g, 32.8 mmol), followed by dropwise addition (10 min) of trimethylsilyl bromide (TMSBr, 6.75 mL, 7.84 g, 51.3 mmol). After stirring at room temperature for 20 h, the reaction mixture was quenched with 200 mL of 5% $KHSO_4$ and stirred vigorously for 15 min. The aqueous layer was extract with EtOAc (3×), and the pooled organic layers were washed with brine, dried (Na_2SO_4), filtered, and stripped. The residue was azeotroped twice with 50 mL of toluene. The precipitate that formed was suspended in toluene and removed by filtration. The filtrate was concentrated, and the azeotrope/filter process was repeated to give a viscous, clear oil. The oil was dissolved in pyridine (50 mL) and subsequently treated with dicyclohexylcarbodiimide (DCC, 4.65 g, 22.6 mmol) followed by methanol (1.67 mL, 1.31 g, 41 mmol). After being stirred at room temperature for 20 h, the mixture was filtered through a pad of Celite, which was subsequently washed with EtOAc. The filtrate was stripped, redissolved in EtOAc, and washed with 5% $KHSO_4$ (2×) and brine. The EtOAc solution was dried (Na_2SO_4), filtered, and stripped, and the residue was azeotroped twice with toluene. The residue was suspended in toluene and filtered. The filtrate was again concentrated, taken up in toluene, filtered, stripped, and placed under high vacuum to give the corresponding phosphonate monoester (10.2 g, >100%, TLC R_f 0.50 (7:2:1 *n*-PrOH–NH_4OH–H_2O)) as a clear, viscous oil. The monoester (1.16 g, 2.57 mmol) was dissolved in dry Et_2O (10 mL) and treated with dicyclohexylamine (0.528 mL, 0.481 g, 2.65 mmol). The resulting homogeneous solution was stored at room temperature for 7 h and at −20 °C for 16 h. The solid/liquid suspension was warmed to room temperature and filtered, and the solid was washed with cold Et_2O and dried in vacuo to give 21 (1.25 g, 77% yield) as a white powdery solid: mp 155–156 °C; TLC R_f 0.57 (20% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.00 (s, 9 H), 1.08–1.92 (m, 22 H), 2.56–2.62 (m, 1 H), 2.64–2.77 (m, 2 H), 3.11 (d, J = 11.0 Hz, 3 H), 3.22 and 3.28 (2 m, 1 H), 3.52 (s, 3 H), 4.02 (m, 1 H), 7.32–7.40 (m, 6 H), 7.65–7.71 (m, 4 H); IR (KBr) 1736 cm^{-1}; $[\alpha]_D$ = −16.0° (MeOH, c = 3.57). Anal. ($C_{22}H_{31}O_6PSi \cdot C_{12}H_{23}N$) C, H, N.

General Procedure for the Synthesis of Acetylenic Linked Phosphinic Acids 3. (*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]-ethynyl]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (27v). DCHA salt 21 (3.682 g, 5.83 mmol) was partitioned between EtOAc and 5% $KHSO_4$. The EtOAc layer was washed three times with 5% $KHSO_4$ and then with brine, dried (Na_2SO_4), filtered, and stripped to give a colorless oil (phosphonic acid monomethyl ester). The oil was dissolved in dry CH_2Cl_2 (10 mL) and treated with diethyl(trimethylsilyl)amine (2.10 mL, 1.61 g, 11.1 mmol). After the mixture was stirred at room temperature for 1 h, the solvent was removed in vacuo and the residue was azeotroped with dry toluene (15 mL). The residue was redissolved in dry CH_2Cl_2 (15 mL), cooled to 0 °C, and treated with 2 drops of DMF and oxalyl chloride (620 μL, 902 mg, 7.1 mmol). After 15 min, the solution was warmed to room temperature and stirred for an additional 45 min. The solvent was stripped, and the yellow residue (phosphonochloridate 22) was azeotroped with toluene (15 mL) and dried in vacuo (oil pump) for 1 h.

Meanwhile, a solution of vinyl dibromide 16v (2.000 g, 3.88 mmol) in THF (10 mL) at −78 °C was treated with *n*-BuLi (2.5 M in hexane, 3.3 mL, 8.2 mmol) over a 1-min period, and the resulting clear green solution was stirred at −78 °C for 50 min. The acetylenic anion solution was added dropwise via canula over a 10-min period to a −78 °C solution of the above prepared phosphonochloridate 22 in THF (12 mL). The resulting mixture was stirred at −78 °C for 30 min and then quenched with 50% saturated NH_4Cl. The solution was warmed to 0 °C and poured into saturated $NaHCO_3$. The aqueous phase was extracted once with Et_2O. The Et_2O layer was washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil. The residue was flash chromatographed (40% EtOAc in hexanes as eluant) to afford compound 27v, a mixture of diastereomers, as a colorless foam (2.517 g, 82%): TLC R_f 0.31 (40% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.02 (s, 9 H), 1.31 and 1.35 (2 d, J = 6.6 Hz each, 6 H), 2.00–2.38 (m, 6 H), 2.47–2.81 (m, 4 H), 3.30 and 3.37 (2 d, $J_{H,P}$ = 12.6 Hz each, 3 H), 3.54 (m, 1 H), 3.58 (s, 3 H), 4.51 (m, 1 H), 6.99–7.46 (m, 13 H), 7.56–7.72 (m, 4 H), 7.83 (d, J = 7.2 Hz, 1 H); IR (KBr) 2168, 1740, 1508, 1224, 1036 cm^{-1}. In the case where acetylene 17 is used in the coupling reaction, 1.1 equiv

of *n*-BuLi is added to a solution of the acetylene in 17 in THF at −78 °C. After 20 min, the acetylenic anion solution is then coupled to 22 as described above.

(*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]ethynyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (28v). A mixture of compound 27v (2.487 g, 3.15 mmol) and HOAc (810 μL, 850 mg, 14.1 mmol) in THF (40 mL) was treated with tetra-*n*-butylammonium fluoride (1.0 M in THF, 11.0 mL, 11.0 mmol). After stirring at room temperature for 18 h, the solution was diluted with EtOAc and washed with 5% $KHSO_4$ (3×) and once with brine. The EtOAc layer was dried (Na_2SO_4), filtered, and stripped to afford a yellow oil. The oil was dissolved in Et_2O, cooled to 0 °C, and treated with excess diazomethane for 10 min. The excess diazomethane was destroyed by the addition of HOAc, and the solvent was removed in vacuo. The residue was flash chromatographed (40% acetone in hexanes as eluant) to afford compound 28v (1.534 g, 89%) as a colorless foam: TLC R_f 0.38 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.40 (d, J = 6.6 Hz, 6 H), 1.94–2.15 (m, 4 H), 2.15–2.28 (m, 2 H), 3.53–3.67 (m, 4 H), 3.59 (d, $J_{H,P}$ = 12.6 Hz, 3 H), 3.57–3.70 (m, 2 H, C*H*(CH_3)$_2$ and O*H*), 3.73 (s, 3 H), 4.36 (m, 1 H), 7.12–7.48 (m, 7 H), 7.85 (d, J = 6.6 Hz, 1 H); IR (KBr) 2170, 1737, 1508, 1223, 1035 cm^{-1}.

(*S*)-4-[[[4-(4-Fluorophenyl)-2-(1-methylethyl)benzo[6,7]cyclohepta[1,2-*b*]pyridin-3-yl]ethynyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (3v). A solution of compound 27v (780 mg, 1.42 mmol) in dioxane (7 mL) was treated with 1 N NaOH (5.0 mL, 5.0 mmol), and the mixture was stirred at room temperature for 18 h. The solvent was evaporated, and the residue was chromatographed on CHP-20P (25 mm × 90 mm), eluting in succession with H_2O (200 mL), 50% MeOH in H_2O (200 mL), and MeOH (100 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 3v (744 mg, 90%) as a white solid: TLC R_f 0.17 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 1.36 (d, J = 7.0 Hz, 6 H), 1.55–1.72 (m, 2 H), 2.01–2.20 (m, 4 H), 2.26 (dd, J = 7.8, 15.0 Hz, 1 H), 2.40 (dd, J = 4.2, 15.0 Hz, 1 H), 2.59 (m, 2 H), 3.83 (m, 1 H), 4.19 (m, 1 H), 7.16–7.42 (m, 7 H), 7.72 (m, 1 H); IR (KBr) 2164, 1634, 1508, 1213, 1184, 1058 cm^{-1}. Anal. ($C_{28}H_{27}FNNa_2O_5P\cdot0.80H_2O$) C, H, F, N, P.

General Procedure for the Synthesis of *trans*-Vinyl- and Ethyl-Linked Phosphinic Acids 4 and 5. 3-(1-Ethynyl)-4-(4-fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenylpyridine (17o). To a solution of *n*-BuLi (2.5 M in hexanes, 4.00 mL, 10.0 mmol) in dry THF (8 mL) at −78 °C was added a solution of vinyl dibromide 16o (2.267 g, 4.63 mmol) in dry THF (8 mL) over a 5-min period. After being stirred at −78 °C for 1 h, the pale green solution was quenched with saturated NH_4Cl and warmed to room temperature. The mixture was diluted with H_2O and extracted with Et_2O, and the Et_2O extract was washed with brine, dried (Na_2SO_4), filtered, and stripped to yield a solid. The residue was recrystallized from EtOAc/hexane to afford acetylene 17o (1.420 g, 93%, 2 crops) as a white solid: mp 178.0–178.5 °C; TLC R_f 0.43 (10% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.34 (d, J = 7.0 Hz, 6 H), 2.04 (s, 3 H), 3.18 (s, 1 H), 3.69 (m, 1 H), 7.15 (m, 2 H), 7.27 (m, 2 H), 7.36–7.48 (m, 3 H), 7.60 (m, 2 H); IR (KBr) 3165, 2099, 1509, 1213 cm^{-1}. Anal. ($C_{23}H_{20}FN$) C, H, F, N.

(*E*)-4-(4-Fluorophenyl)-3-(2-iodoethenyl)-5-methyl-2-(1-methylethyl)-6-phenylpyridine (23o). A mixture of acetylene 17o (1.355 g, 4.1 mmol) and AIBN (20 mg) in tri-*n*-butyltin hydride (2.0 mL) was rapidly heated to 120 °C. After 4 min of heating, the mixture was treated with additional Bu_3SnH (0.6 mL) and the temperature of the reaction was raised to 140 °C. Approximately 20 mg of AIBN was added to the reaction mixture 1 and 2 h after heating was initiated. After 3 h, the mixture was cooled to room temperature, diluted with Et_2O (50 mL), and treated with solid I_2 (3.50 g, 13.8 mmol). The dark reaction mixture was stirred for 45 min and then poured into a 50% saturated $NaHCO_3$ solution containing 6.7 g of $Na_2S_2O_3$. The layers were shaken and separated. The ethereal layer was washed successively with H_2O, 1.7 M NH_4OH, and brine, dried (Na_2SO_4), filtered, and stripped to yield a wet solid. The solid was taken up in Et_2O, filtered through Celite, and stripped. The residue was recrystallized from hexane to give compound 23o (1.335 g) as white crystals. The mother liquor was flash chromatographed (5% EtOAc in hexanes as eluant), and the desired fractions were pooled, stripped, recrystallized, and pooled with the above solid to give a total of 1.637 g (87%) of *trans*-vinyl iodide 23o: mp 148.5–150.0 °C; TLC R_f 0.13 (2% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.29 (d, J = 7.0 Hz, 6 H), 2.00 (s, 3 H), 3.31 (m, 1 H), 6.03 (d, J = 15.2 Hz, 1 H), 7.05–7.22 (m, 5 H), 7.34–7.49 (m, 3 H), 7.59 (m, 2 H); IR (KBr) 2961, 1508, 1221, 841 cm^{-1}. Anal. ($C_{22}H_{21}FIN$) C, H, F, I, N.

(*E*),(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (24o). A solution of *trans*-vinyl iodide 23o (1.400 g, 3.06 mmol) in THF (6 mL) was added over a 5-min period to a −100 °C solution of fresh *tert*-butyllithium (1.7 M in pentane, 3.70 mL, 6.3 mmol) in THF (8 mL). The resulting deep red solution was stirred at −100 °C for 25 min and then added via canula over an 8-min period to a −100 °C solution of phosphonochloridate 22 (prepared as in the example for compound 27v from 3.288 g 21) in THF (15 mL). The resulting yellow mixture was stirred at −100 °C for 5 min and at −78 °C for 25 min and then quenched with 50% saturated NH_4Cl. The solution was warmed to room temperature, diluted with H_2O, and poured into saturated $NaHCO_3$. The aqueous phase was extracted twice with Et_2O. The combined Et_2O layers were washed with brine, dried (Na_2SO_4), filtered, and stripped. The resulting yellow oil was flash chromatographed (50% EtOAc in hexanes as eluant) to afford adduct 24o, a 1:1 mixture of diastereomers, as an off-white foam (1.541 g, 66%): TLC R_f 0.22 (40% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.01 and 1.03 (2 s, 9 H), 1.20–1.31 (m, 7 H), 1.78 (m, 1 H), 1.98 and 2.00 (2 s, 3 H), 2.56 (m, 1 H), 2.81 (m, 1 H), 3.19 (pseudo t, $J_{H,P}$ = 11.5 Hz, 3 H), 3.21 (m, 1 H), 3.59 and 3.61 (2 s, 3 H), 4.38 and 4.52 (2 m, 1 H), 5.01 (dd, J = 17.9, 24.8 Hz, 0.5 H), 5.26 (dd, J = 17.9, 24.3 Hz, 0.5 H), 6.89–7.72 (m, 20 H); IR ($CHCl_3$) 2959, 1740, 1605, 1508, 1223, 1036 cm^{-1}.

(*E*),(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (25o). A solution of compound 24o (1.519 g, 1.98 mmol) in THF (15 mL) was treated with HOAc (640 μL, 671 mg, 11.2 mmol) followed by tetra-*n*-butylammonium fluoride (1.0 M in THF, 10.0 mL, 10.0 mmol). After being stirred at room temperature for 19 h, the solution was poured into saturated $NaHCO_3$ and extracted with EtOAc. The EtOAc extract was washed with brine, dried (Na_2SO_4), filtered, and stripped to give an oil that was subsequently flash chromatographed (40–60% acetone in hexanes as eluant). Compound 25o (978 mg, 94%) was obtained as a white foam: TLC R_f 0.34 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.30 (d, J = 7.0 Hz, 6 H), 1.68–1.93 (m, 2 H), 2.00 (s, 3 H), 2.57 (m, 2 H), 3.30 (m, 1 H), 3.43 and 3.47 (2 d, $J_{H,P}$ = 4.7 and 4.1 Hz, 3 H), 3.66 and 3.79 (2 d, J = 2.4 Hz each, 1 H, O*H*), 3.72 (s, 3 H), 4.19 and 4.31 (2 m, 1 H), 5.51 (dd, J = 17.6, 24.6 Hz, 0.5 H), 5.52 (dd, J = 17.6, 24.3 Hz, 0.5 H), 7.10–7.65 (m, 10 H); IR ($CHCl_3$) 2961, 1736, 1605, 1510, 1221, 1034 cm^{-1}.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethyl]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (26o). A mixture of compound 25o (494 mg, 0.94 mmol) and 10% Pd on carbon (110 mg) in MeOH (20 mL) was shaken under 50 psi of H_2 for 3 days. The solution was filtered through Celite, stripped, and flash chromatographed (50% acetone in hexanes) to give compound 26o (419 mg, 85%) as a colorless oil: TLC R_f 0.36 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.33 (d, J = 6.6 Hz, 6 H), 1.57–1.91 (m, 4 H), 1.92 (s, 3 H), 2.42–2.59 (m, 2 H), 2.60–2.74 (m, 2 H), 3.25 (m, 1 H), 3.55 and 3.57 (2 d, $J_{H,P}$ = 10.8 Hz each, 3 H), 3.72 (s, 3 H), 3.78 and 3.87 (2 d, J = 3.0 Hz each, 1 H, O*H*), 4.25 and 4.40 (2 m, 1 H), 7.11–7.25 (m, 4 H), 7.33–7.47 (m, 3 H), 7.56 (m, 2 H); IR ($CHCl_3$) 1734, 1509, 1221, 1179, 1040 cm^{-1}.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethenyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (4o). A solution of compound 25o (461 mg, 0.88 mmol) in dioxane (5 mL) was treated with 1 N NaOH (3.2 mL, 3.2 mmol), and the mixture was stirred at 60 °C for 1.5 h. The solvent was evaporated, and the residue was dissolved in H_2O and chromatographed on CHP-20P (25 mm

× 80 mm), eluting in succession with H_2O (150 mL) and 50% MeOH in H_2O (200 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 4o (430 mg, 87%) as a white solid: TLC R_f 0.10 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 1.27 (d, J = 7.0 Hz, 6 H), 1.54 (dd, J = 7.2, 14.5 Hz, 2 H), 1.93 (s, 3 H), 2.33 (m, 2 H), 3.57 (m, 1 H), 4.10 (m, 1 H), 5.85 (dd, J = 18.0, 19.8 Hz, 1 H), 7.07 (pseudo t, J = 18.0 Hz, 1 H), 7.19 (d, J = 7.0 Hz, 4 H), 7.37–7.54 (m, 5 H); MS (FAB) [M – 2 Na + 3 H]$^+$ 498. Anal. ($C_{27}H_{27}FNNa_2O_5P\cdot1.2H_2O$) C, H, F, N, P.

(*S*)-4-[[2-[4-(4-Fluorophenyl)-5-methyl-2-(1-methylethyl)-6-phenyl-3-pyridinyl]ethyl]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (5o). Saponification of ethyl linked phosphinate 26o was similar to that of *trans*-vinyl-linked phosphinate 25o to give 5o in 77% yield: TLC R_f 0.10 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 1.41 (d, J = 7.0 Hz, 6 H), 1.49 (dd, J = 6.0, 12.6 Hz, 2 H), 1.71 (m, 2 H), 1.93 (s, 3 H), 2.35 (m, 2 H), 2.78 (m, 2 H), 3.58 (m, 1 H), 4.25 (m, 1 H), 7.20–7.60 (m, 9 H); IR (KBr) 2961, 1579, 1509, 1405, 1157 cm^{-1}. Anal. ($C_{27}H_{29}FNNa_2O_5P\cdot3.69H_2O$) C, H, F, N, P.

General Procedure for the Synthesis of Phosphonic Monoesters 6. (*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]methoxyphosphinyl]-3-[[(1,1-dimethylethyl)diphenylsilyl]oxy]butanoic Acid, Methyl Ester (29p). A 0 °C solution of phosphonochloridate 22 (from 2.89 g, 4.57 mmol DCHA salt 21) in pyridine (20 mL) was treated with a solution of alcohol 14p (888 mg, 2.54 mmol) in dry pyridine (7.0 mL). The resulting mixture was stirred at 0 °C for 16 h, diluted with EtOAc, and washed with 50% saturated NH_4Cl. The organic layer was then washed with H_2O followed by brine, dried (Na_2SO_4), filtered, and stripped. The amber residue was subject to flash chromatography (30% EtOAc in hexane) to give adduct 29p (1.104 gm, 56%) as a yellow oil: TLC R_f 0.53 (45% EtOAc in hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.70 (m, 3 H), 1.00 (s, 9 H), 1.22–1.38 (m, 8 H), 1.90 and 2.12 (2 m, 1 H), 2.37 (m, 2 H), 2.55 and 2.81 (2 m, 1 H), 3.29–3.39 (m, 4 H), 3.58 (s, 3 H), 4.43 (m, 1 H), 4.59 and 4.71 (2 m, 2 H), 7.02–7.70 (m, 9 H); IR (CH_2Cl_2) 2954, 1740, 1511, 1223, 1015 cm^{-1}.

(*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]methoxyphosphinyl]-3-hydroxybutanoic Acid, Methyl Ester (30p). The silyl protecting group on 29p was removed via the same procedure as that described for compound 24o to give 30p in 90% yield: TLC R_f 0.59 (1:1 acetone–hexanes); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.70 (t, J = 6.8 Hz, 3 H), 1.34 (d, J = 7.0 Hz, 6 H), 1.92 (m, 2 H), 2.39 (q, J = 6.8 Hz, 2 H), 2.57 (d, J = 7.2 Hz, 2 H), 3.43 (m, 1 H), 3.63 (d, $J_{H,P}$ = 10.8 Hz, 3 H), 3.72 (s, 3 H), 4.31 (m, 1 H), 4.85 (m, 2 H), 7.12–7.28 (m, 5 H), 7.39–7.56 (m, 4 H); IR (CH_2Cl_2) 1734, 1636, 1510, 1221 cm^{-1}.

(*S*)-4-[[[5-Ethyl-4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenyl-3-pyridinyl]methoxy]hydroxyphosphinyl]-3-hydroxybutanoic Acid, Disodium Salt (6p). A solution of compound 30p (650 mg, 1.20 mmol) in dioxane (10 mL) was treated with 1 N NaOH (3.7 mL, 3.7 mmol), and the mixture was stirred at 55 °C for 3 h. The solvent was evaporated to give a white solid. The residue was slurried in warm H_2O and chromatographed on CHP-20P (25 mm × 100 mm) eluting in succession with H_2O (200 mL) and 50% MeOH in H_2O (400 mL). The desired fractions were pooled and evaporated, and the residue was taken up in H_2O and lyophilized to give 6p (435 mg, 65%) as a white solid: TLC R_f 0.31 (8:1:1 CH_2Cl_2–HOAc–MeOH); ^1H NMR (CD_3OD, 270 MHz) δ 0.65 (t, J = 6.8 Hz, 3 H), 1.30 (d, J = 7.0 Hz, 6 H), 1.48 (dd, J = 7.6, 16.0 Hz, 2 H), 2.28 (q, J = 6.8 Hz, 2 H), 2.37 (m, 2 H), 3.66 (m, 1 H), 4.19 (m, 1 H), 4.64 (m, 2 H), 7.18–7.50 (m, 9 H); IR (KBr) 2935, 1581, 1510, 1404, 1222, 1020 cm^{-1}. Anal. ($C_{27}H_{29}FNNa_2O_6P\cdot H_2O$) C, H, F, N, P.

Biological Assays. Rat Hepatic HMG-CoA Reductase Inhibition. Rat hepatic HMG-CoA reductase activity is measured using a modification of the method described by Edwards.[21] Rat hepatic microsomes are used as a source of enzyme, and the enzyme activity is determined by measuring the conversion of the ^{14}C-HMG-CoA substrate to [^{14}C]mevalonic acid. Livers are removed from 2–4 cholestyramine-fed, decapitated, Sprague–Dawley rats, and homogenized in phosphate buffer A (potassium phosphate, 0.04 M, pH 7.2; KCl, 0.05 M; sucrose, 0.1 M; EDTA, 0.03 M, aprotinin, 500 KI units/mL). The homogenate is spun at 16000*g* for 15 min at 4 °C. The supernatant is removed and recentrifuged under the same conditions a second time. The second 16000*g* supernatant is spun at 100000*g* for 70 min at 4 °C. Pelleted microsomes are resuspended in a minimum volume of buffer A (3–5 mL per liver) and homogenized in a glass homogenizer. Dithiothreitol is added (10 mM), and the preparation is aliquoted, quick frozen in acetone/dry ice, and stored at −80 °C. The specific activity of a typical microsomal preparation is 0.68 nmol of mevalonic acid/mg of protein per minute. The reductase is assayed in 0.25 mL, which contains the following components at the indicated final concentrations: 0.04 M potassium phosphate, pH 7.2; 0.05 M KCl; 0.10 M sucrose; 0.03 M EDTA; 0.01 M dithiothreitol; 3.5 mM NaCl; 1% dimethyl sulfoxide; 50–200 μg of microsomal protein; 100 μM of ^{14}C-[D,L]-HMG-CoA (0.05 μCi, 30–60 mCi/mmol); 2.7 mM NADPH. Reaction mixtures are incubated at 37 °C. Under conditions described, enzyme activity increases linearly up to 300 μg of microsomal protein per reaction mixture and is linear with respect to incubation time up to 30 min. The standard incubation time chosen for drug studies is 20 min, which results in 12–15% conversion of HMG-CoA substrate to the mevalonic acid product. [D,L]HMG-CoA substrate is used as 100 μM, twice the concentration needed to saturate the enzyme under the conditions described. NADPH is used in excess at a level 2.7 times the concentration required to achieve maximum enzyme velocity. Standardized assays for the evaluation of inhibitors are conducted according to the following procedure. Microsomal enzyme is incubated in the presence of NADPH at 37 °C for 15 min. DMSO vehicle with or without test compound is added, and the mixture further incubated for 15 min at 37 °C. The enzyme assay is initiated by adding ^{14}C-HMG-CoA substrate. After 20 min of incubation at 37 °C, the reaction is stopped by the addition of 25 μL of 33% KOH. [^3H]Mevalonic acid (0.05 μCi) is added, and the reaction mixture allowed to stand at room temperature for 30 min. Fifty microliters of 5 N HCl is added to lactonize the mevalonic acid. Bromophenol blue is added as a pH indicator to monitor an adequate drop in pH. Lactonization is allowed to proceed for 30 minutes at room temperature. Reaction mixtures are layered onto 2 g of AG 1-X8 anion exchange resin (Biorad, formate form), poured in 0.7 cm (i.d.) glass columns, and eluted with 2.5 mL of H_2O. The first 0.5 mL is discarded, and the next 2.0 mL is collected and counted for both tritium and carbon-14 in 10.0 mL of Opti-fluor (Packard) scintillation fluid. Results are calculated as nanomoles mevalonic acid produced per 20 min and are corrected to 100% recovery of tritium. Drug effects are expressed as I_{50} values (concentration of drug producing 50% inhibition of enzyme activity) derived from composite dose response data from 2–5 experiments.

Inhibition of Cholesterol Synthesis in Freshly Isolated Rat Hepatocytes. Inhibitors of HMG-CoA reductase are evaluated for their ability to inhibit [^{14}C]acetata incorporation into cholesterol in freshly isolated rat hepatocyte suspensions using a modification of the methods originally described by Capuzzi.[22] Sprague–Dawley rats (180–220 g) are anesthetized with Nembutal (50 mg/kg). The abdomen is opened, and the first branch of the portal vein is tied closed. Two closing sutures are placed on the distal section of the portal vein, and the portal vein is canulated between the sutures and the first branching vein. The liver is perfused at a rate of 20 mL/min with prewarmed (37 °C) oxygenated buffer A ((HBSS, Hanks' Balanced Salt Solution) without calcium or magnesium containing 0.05% EDTA) after severing the vena cava to allow drainage of the effluent. The liver is additionally perfused with 200 mL of prewarmed oxygenated buffer B (HBSS containing 0.05% bacterial collagenase). Following perfusion with buffer B, the liver is excised and decapsulated in 50 mL of Waymouth's medium, allowing free cells to

(21) Edwards, P. A.; Lemongello, D.; Fogelmann, A. M. Improved Methods for the Solubilization and Assay of Hepatic 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase. *J. Lipid Res.* 1979, *20*, 40–46.

(22) Capuzzi, D. M.; Margolis, S. Metabolic Studies in Isolated Rat Liver Cells: 1. Lipid Synthesis. *Lipids* 1971, *6*, 601–607.

disperse into the medium. Hepatocytes are isolated either by low-speed centrifugation for 3 min at 50g at room temperature or by unit gravity sedimentation at 4 °C for 30–45 min. Pelleted hepatocytes are washed once in Waymouth's medium, counted, and assayed for viability by trypan blue exclusion. These hepatocyte enriched cell suspensions routinely show 70–90% viability. Hepatocytes are resuspended at 5×10^6 cells per 2.0 mL in incubation medium (IM) (0.02 M Tris-HCl (pH 7.4), 0.1 M KCl, 0.33 mM $MgCl_2$, 0.01 mM $MnCl_2$, 0.001 mM sodium succinate, 0.003 mM Coenzyme A, 0.33 mM sodium citrate, 0.67 mM nicotinamide, 0.23 mM NADP, 1.7 mM glucose-6-phosphate). Test compounds are routinely dissolved in H_2O, DMSO, or DMSO–H_2O (1:3) and added to the IM. Final DMSO concentration in the IM is ≤1.0% and has no significant effect on cholesterol synthesis. Incubation is initiated by adding [^{14}C]acetate (58 mCi/mmol, 2 μCi/mL) and placing the cell suspensions (2.0 mL) in 35-mm tissue culture dishes at 37 °C for 2.0 h. Following incubation, cell suspensions are transferred to glass centrifuge tubes and spun at 50g for 3 min at room temperature. Cell pellets are resuspended and lysed in 1.0 mL of H_2O. Lipids are extracted essentially as described by Bligh and Dyer.[22] Following extraction, the lower organic phase is removed and dried under a stream of nitrogen and the residue resuspended in 100 μL $CHCl_3$–MeOH (2:1). The total sample is spotted on silica gel (LK6D) thin-layer plates and developed in CH_2Cl_2–acetone (60:1). Plates are scanned and counted using a BioScan automated scanning system. Radiolabel in the cholesterol peak (R_f 0.28) is determined and expressed as total counts per peak and as a percent of the label in the total lipid extract. Cholesterol peaks in control cultures routinely contain 5000–20000 dpm, and are approximately 30% of the label present in the total lipid extract. Drug effects (percent inhibition of cholesterol synthesis) are determined by comparing the percent of label in the cholesterol peak for control and drug treated cultures. Dose response curves are constructed from composite data from two or more studies and results are expressed as I_{50} values (concentration of drug which inhibits cholesterol synthesis 50%).

Inhibition of Cholesterol Synthesis in Human Skin Fibroblasts. Human skin fibroblasts (passage 7-27) are grown in minimal essential medium (MEM, Gibco) containing 10% fetal calf serum. For each experiment, stock cultures are trypsinized to disperse the cell monolayer, counted, and plated in 35-mm tissue culture wells (5×10^5 cells/2.0 mL). Cultures are incubated for 18 h at 37 °C in 5% CO_2/95% humidified room air. Cholesterol biosynthetic enzymes are induced by removing the serum containing medium, washing the cell monolayers with MEM, adding 1.0 mL of MEM containing 1.0% fatty acid free bovine serum albumin, and incubating the cultures an additional 24 h. Test compounds are dissolved in H_2O, DMSO, or DMSO–EM (1:3) (final DMSO concentration in cell cultures ≤1.0%) and added to the cultures, and the cultures are preincubated for 30 min at 37 °C in 5% CO_2/95% humidified room air. Following preincubation with drugs, sodium [1-^{14}C]acetate (2.0 μCi/mL, 58 mCi/mmol) is added, and the cultures are reincubated for 4 h. After incubation, the culture medium is removed and the cell monolayer is scraped into 1.0 mL of H_2O. Lipids in the lysed cell suspension are extracted as described for hepatocyte suspensions. The organic phase is dried under nitrogen, and the residue is resuspended and analyzed as described for hepatocytes. Cholesterol peaks in control cultures routinely contain 8000–12000 dpm and are approximately 15% of the label present in the total lipid extract.

Inhibition of cholesterol synthesis is determined as described for hepatocytes. Results are expressed as I_{50} values and are derived from composite dose response curves from two or more experiments.

In Vivo Cholesterol Biosynthesis Inhibition in Rats. The methods used for intravenous (iv) and oral (po) drug testing were adapted from a procedure originally described by Sandoz.[24] Male Sprague–Dawley rats (200–300 g) were adapted to a reverse light cycle for 7–10 days and fed Purina rat chow (no. 5001) ad libitum. In order to measure cholesterol synthesis, sodium [1-^{14}C]acetate (1–3 mCi/mmol) (25 μCi/100 g of body weight) was injected intraperitoneally (ip) 2 h before the mid-dark point in the diurnal cycle. Two hours after the mid-dark point animals were anesthetized ip with ketamine/xylazine and bled into EDTA-treated centrifuge tubes from the abdominal aorta. Plasma was obtained by centrifugation at 1100g for 10 min. One-milliliter plasma samples were aliquoted and either processed directly or frozen at −20 °C. For iv testing, the salt forms of test compounds were routinely dissolved in saline and injected iv into the tail vein 5 min before [^{14}C]acetate injection. For po testing, drugs were dissolved in saline and given by gavage 30 min before [^{14}C]acetate injection. Cholesterol synthesis was measured by determining the level of ^{14}C-labeled nonsaponifiable lipid present in 1 mL of plasma; the method used is a modification of the method described by Dugan.[25] One milliliter physiological saline was added to 1 mL of plasma, followed by the addition of 5.0 mL of 10% KOH in absolute ethanol. Samples were mixed and saponified at 75 °C for 1 h. After cooling, approximately 0.02 μCi (44,000 dpm) [1,2-^{3}H]cholesterol (40–60 Ci/mmol) was added to each sample. Samples were extracted once with 5 mL of petroleum ether, and the organic phase was backwashed with 5 mL of saline. This extraction procedure resulted in 50–90% recovery of the added [^{3}H]cholesterol internal standard. The extracts were dried in glass vials, and the residue resuspended in 0.5 mL of $CHCl_3$–MeOH (2:1). Samples were counted for both ^{3}H and ^{14}C in 10 mL of Optifluor scintillation fluid. The [^{3}H]cholesterol internal standard recovery value from each sample was used to correct each sample to 100% recovery of [^{14}C]cholesterol. In early experiments, sample extract residues were redissolved in 100 mL of $CHCl_3$–MeOH (2:1) and chromatographed on silica gel (Whatman LK6D) thin-layer plates using either hexanes–Et_2O–HOAc (75:25:1) or CH_2Cl_2–acetone (60:1). Using either chromatographic system, greater than 90% of the ^{14}C-label cochromatographed with authentic cholesterol. Thus, to simplify the method, the TLC step was omitted in subsequent experiments and results were calculated as ^{14}C-labeled nonsaponifiable plasma lipid values, of which, greater than 90% of the ^{14}C-label is authentic cholesterol. The percent inhibition of cholesterol synthesis was derived by comparing ^{14}C-labeled nonsaponifiable plasma lipid values per milliliter of plasma from control and drug-treated animal groups (4–5 rats/group). Percent inhibition is plotted relative to the log drug dose and a linear best fit regression line is determined for each experiment. Mean ED_{50} values (level of drug required to suppress cholesterol synthesis in vivo by 50%) were calculated from two or more experiments.

(23) Bligh, E. G.; Dyer, W. J. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* 1959 *37*, 911–917.

(24) Wareing, J., U.S. Patent 4,613,610 and PCT Int. Appl., WO 86/00367.

(25) Dugan, R. E.; Slakey, L. L.; Briedis, A. V.; Porter, J. W. Factors affecting the Diurnal Variation in the Level of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase and Cholesterol Synthesizing Activity in Rat Liver. *Arch. Biochem. Biophys.* 1972, *152*, 21–27.

HMG-CoA Reductase Inhibitors: An Exciting Development in the Treatment of Hyperlipoproteinemia

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I. INTRODUCTION

Coronary heart disease (CHD) continues to be one of the major health problems in all the developed countries of the world. A considerable body of clinical and epidemiological data has emerged over the years linking elevated blood levels of total cholesterol, Low Density Lipoprotein Cholesterol (LDL-C), and Very Low Density Lipoprotein Cholesterol (VLDL-C) as important risk factors for the development of coronary heart disease.[1]

For the treatment of elevated LDL-C and VLDL-C, a judicious diet, low in cholesterol and fat with saturated fatty acids replaced by polyunsaturated fatty acids, is the recommended choice. However, for patients nonresponsive

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to dietary intervention, the development of effective and safe therapeutic agents for the treatment of hyperlipoproteinemia remains an important need. This need has gained considerable support as a result of two important events: (1) the results of the Lipid Research Clinic's Coronary Primary Prevention Trial (LRC-CPPT), a multicenter, randomized, double-blind study involving 3806 asymptomatic middle-aged men in the United States with type II hyperlipoproteinemia, that demonstrated that a statistically significant reduction of 19% in the rate of fatal plus nonfatal coronary heart disease was associated with a 9% decrease in blood cholesterol levels,[2] and (2) the recommendation to treat individuals with blood cholesterol above the 75th percentile, which emerged from the consensus panel of the December, 1984 NIH Consensus Development Conference on the lowering of blood cholesterol to prevent coronary heart disease.[3]

In recent years, to achieve this goal of finding effective and safe therapeutic agents to lower LDL-cholesterol, great interest has focused on potent inhibitors of the enzyme β-Hydroxy-β-Methyl-Glutaryl-CoA reductase (HMG-CoA reductase, EC 1.1.1.34), which controls a key step in the endogenous synthesis of cholesterol. Several studies, both in animals and humans, have been reported with HMG-CoA reductase inhibitors: compactin (Mevastatin), CS-514 (Pravastatin, Mevalotin®, Pravachol®), mevinolin (Lovastatin, Mevacor®) and Synvinolin (Simvastatin, Zocor®),[4] which are structurally very closely related to one another. In order to assess fully the potential of HMG-CoA reductase inhibitors as an effective therapeutic intervention for the treatment of hyperlipoproteinemia, it is thus desirable to study in humans a variety of these inhibitors derived from different structural prototypes which can be distinguished in their overall biological profile from one another. This conceptual framework formed the basis for initiating efforts at the Sandoz Research Institute to develop and study a variety of HMG-CoA reductase inhibitors with chemical structures different in several respects from compactin, pravastatin (a hydroxy analog of compactin), lovastatin (a methyl analog of compactin), and simvastatin (a dimethyl analog of compactin), and has led to fluvastatin (XU 62-320), the first totally synthetic HMG-CoA reductase inhibitor currently in Phase III human clinical trials (Fig. 1).

II. DESIGN ASPECT FOR HMG-CoA REDUCTASE INHIBITORS AT SANDOZ RESEARCH INSTITUTE LEADING TO FLUVASTATIN (XU 62-320)

Investigations by Akira Endo with compactin[4] have to be largely credited for the resurgence of the research on cholesterol biosynthesis and the renewed interest in HMG-CoA reductase inhibitors, a field now almost three decades

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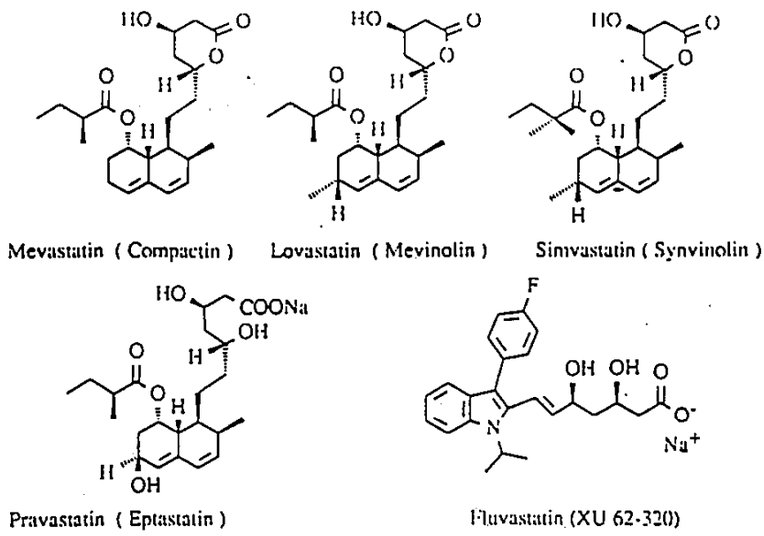


Figure 1

old. While all intensive studies hitherto conducted have been with closely related metabolites, such as compactin, mevinolin, and CS-514 (pravastatin), derived from fungal broths, efforts at the Sandoz Research Institute towards the development of new HMG-CoA reductase inhibitors have been based on synthesis, guided by the following assumptions:

(a) There are two regions at the active site of the enzyme: one with high specific recognition of a 5-carbon unit (C-1 to C-5 as shown below) of the β-OH-β-Methyl-Glutaryl portion, and the other of CoA moiety present in HMG-CoA (Fig. 2).

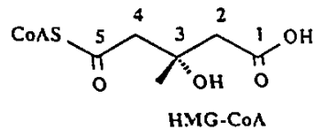


Figure 2

(b) Compactin (R = H, Fig. 3), a known inhibitor of the enzyme, may be regarded as a transition state analog, when in the open dihydroxy acid form.

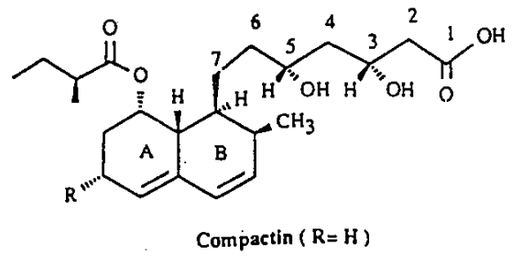


Figure 3

The 5-carbon unit of the side chain present in compactin (Fig. 3) probably occupies the same region as the 5-carbon unit in HMG-CoA (Fig. 2); the bicyclic A-B-ring system, with its substituents in compactin (Fig. 3), possibly sits in the same region or very close to the same region the CoA portion of the substrate HMG-CoA occupies at the active site of the enzyme. However, it is difficult to see any similarity in structure between the bicyclic-ring system of compactin and CoA, when one examines the structure of CoA shown in Fig. 4.

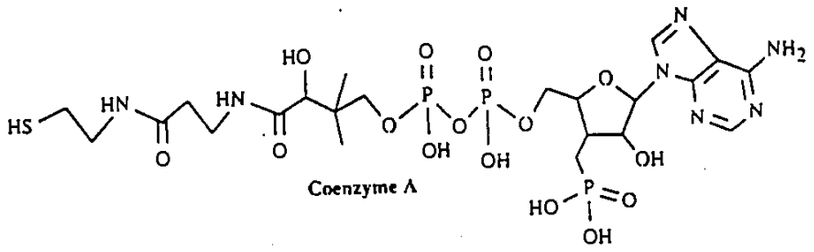


Figure 4

In light of (a) and (b) above, one hoped that it might possible to prepare interesting synthetic inhibitors of HMG-CoA reductase with a very general structure as shown in Fig. 5, with the 5-carbon unit (C-1 to C-5) preferably possessing the absolute configurations of C-3-OH and C-5-OH as present in compactin.

Choice of R and R_1 in Fig. 5 has depended on:

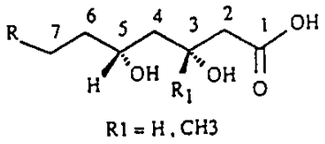


Figure 5

(a) Consideration of the elements of structure of CoA.

(b) Considerations of the overall shape and assumptions of the importance of substituents on Ring A-B of compactin (Fig. 3), first with molecular models and later with computer modelling.

(c) Exploiting the knowledge gained in structure activity relationships with our own Sandoz Research Institute compounds or being reported in literature by outside investigators.

Efforts with the above considerations in mind have led to the development of a variety of novel HMG-CoA reductase inhibitors. Synthesis and Structure Activity Relationships (SAR) of some of these novel inhibitors are discussed below with emphasis on the Phase III candidate, fluvastatin (XU 62-320): [R*,S*-(E)]-(±)-Sodium-3,5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methyl-ethyl-1H-indol-2-yl]-hept-6-enoate (Fig. 1), a mevalonic acid analog more potent than compactin and lovastatin.

III. GENERAL CHEMISTRY APPROACH

Guided by the conviction that the C-3, C-5 dihydroxy acid fragment was the key pharmacophore necessary for the inhibition of HMG-CoA reductase,

our synthetic approach towards the synthesis of compounds of generic structure (Fig. 5) involved:

(a) A convergent synthesis coupling chiral Synthon 1 or racemic or chiral (3R, 5S) C-3, C-5-dihydroxy ester Synthon 2 with a variety of aryl or alkyl fragments 3 (Fig. 6), or

(b) A linear synthesis of the C-3, C-5 dihydroxy acid derivatives wherein the aldehyde 4 is reacted with acetoacetate 5 (Fig. 7) to provide a hydroxyketo ester intermediate, which, with subsequent steps, gives the desired final products of Fig. 5.

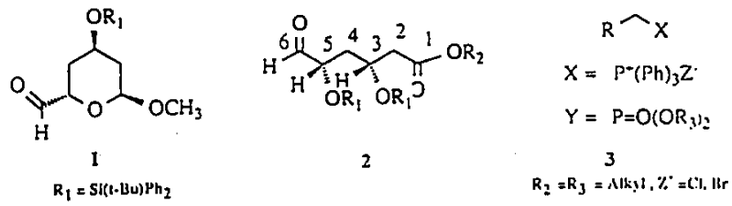


Figure 6

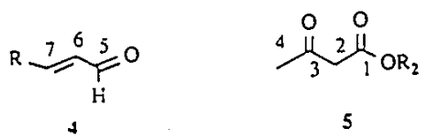
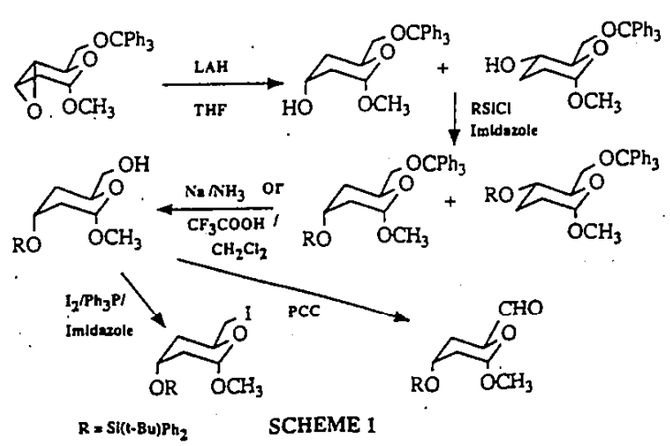


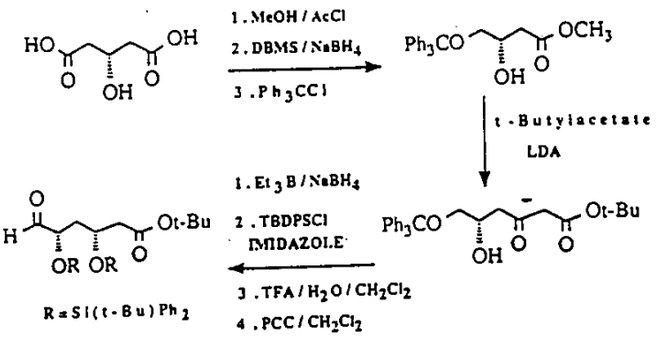
Figure 7

A. Synthesis of Synthon 1 and 2, Fig. 6 (Scheme 1 and Scheme 2)

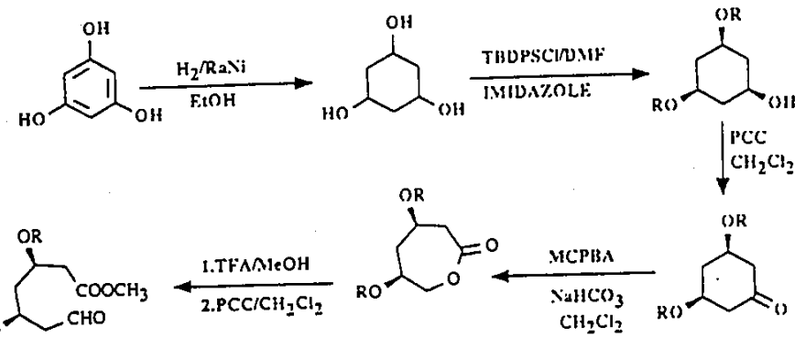
Synthon 1 has been synthesized starting from D-glucose via the key lithium aluminum hydride reductive opening of the epoxide as depicted in Scheme 1.[5] The desired axial alcohol could be separated from the equatorial isomer by preparation of the silyl derivatives. The protected axial alcohol on PCC oxidation gave the desired lactol aldehyde.



SCHEME 1



SCHEME 2



SCHEME 3

Synthesis of chiral Synthon 2 has been accomplished starting from S-malic acid in excellent yields via an eight-step reaction as illustrated in Scheme 2.[6]

On the other hand, an efficient route was developed for the preparation of racemic Synthon 2 starting from 1,3,5-trihydydroxy benzene through a five-step reaction sequence shown in Scheme 3.[7]

B. Choice of R and Synthesis of Intermediates 3, Fig. 6, and 4, Fig. 7

Our initial efforts at the synthesis, and the biological results of C-3, C-5-dihydroxy acid derivatives (Fig. 5) wherein choice of R was based on elements of substructures of coenzyme A (Fig. 4) or the decalin ring structure of compactin (Fig. 3) were not promising.[8] This led us to question the importance and the necessity of the complex stereochemistry and the substituents present in the decalin ring of compactin and turn our attention towards the preparation of C-3, C-5-dihydroxy acid derivatives (Fig. 5) wherein R was a naphthalene ring. During these ongoing efforts, we were being encouraged and helped by two important publications[9] describing mevalonolactone derivatives of the general structure 6 and 7 as inhibitors of HMG-CoA reductase (Fig. 8).

Further exploration of R in Fig. 5 led to the first interesting indolyl derivative (Fig. 9) comparable to compactin in its inhibitory activity against HMG-CoA reductase.[10(a)]

An extensive and rapid analog program allowed the choice of XU 62-320

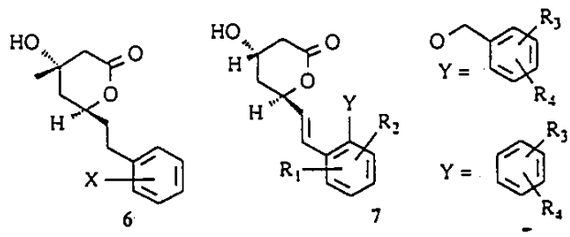


Figure 8

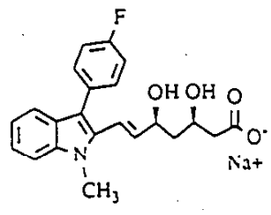


Figure 9

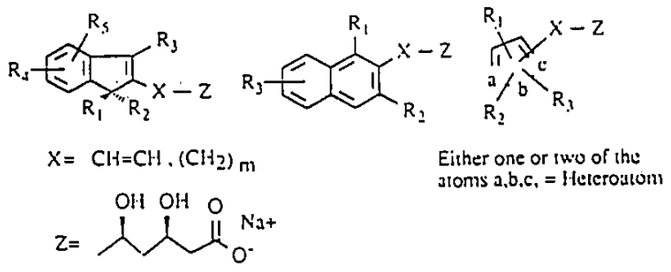
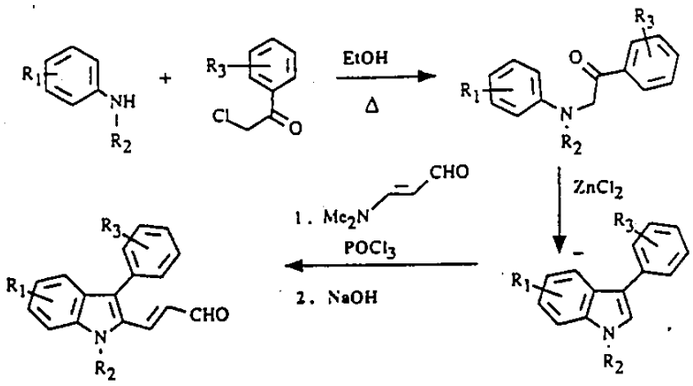


Figure 10

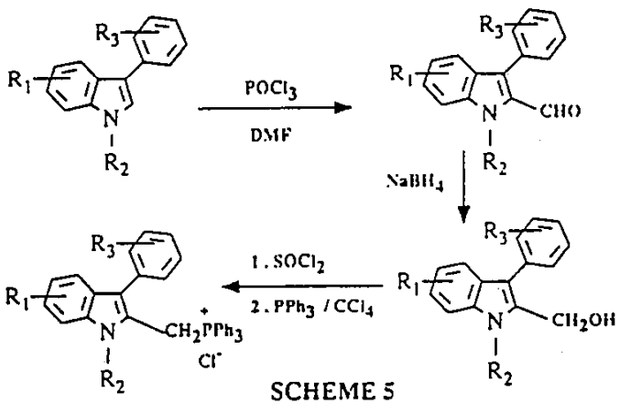
(Fig. 1) as a candidate for extensive biological testing. Currently, fluvastatin (XU 62-320) is in clinical Phase III trials.

With the discovery of XU 62-320, the stage was set for a large number of variations of R in Fig. 5. Extensive work at the Sandoz Research Institute has led to many novel HMG-CoA reductase inhibitors, some of which are discussed in this paper as shown in Fig. 10,[10] and Figs. 12–14.[21–23]

Synthesis of the many interesting fragments 3 (Fig. 6) and 4 (Fig. 7) needed for synthesis of final HMG-CoA-R inhibitors are described in Schemes 4–12 below.[10] Since the appearance of Merck & Co., Inc. and Sandoz patents and publications,[5,9,10(a)] extensive efforts have followed in many laboratories worldwide with semi-synthetic and totally synthetic HMG-CoA reductase inhibitors. A brief overview of these reported activities is presented in Section VIII. It is no wonder that in such a feverish pursuit of finding patentable HMG-CoA reductase inhibitors, review of patent and published literature presents overlapping activities in the laboratories of competing pharmaceutical research companies.



SCHEME 4



SCHEME 5

C. Synthesis of Indole Intermediates

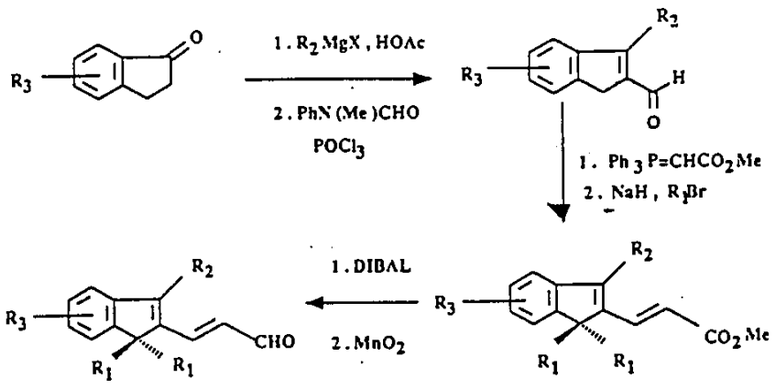
Scheme 4 describes the preparation of α,β-unsaturated aldehydes readily obtained from a variety of 3-phenyl substituted indoles using dimethylamino-acrolein and phosphorous oxychloride, while the triphenyl phosphonium salts of indolyl derivatives are prepared via the 2-formyl and 2-hydroxymethyl indoles using standard procedures (Scheme 5).[10(a)]

D. Synthesis of Indene Intermediates

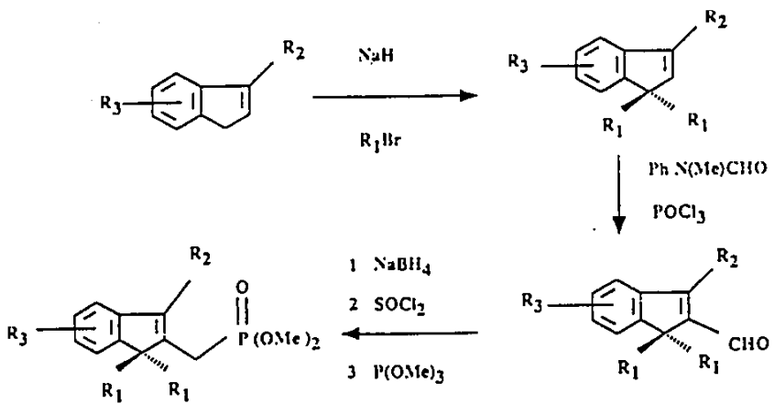
A variety of indenyl-α, β-unsaturated aldehydes and phosphonates have been synthesized via a six-step reaction sequence as depicted in Schemes 6 and 7. The synthesis of these derivatives involves the preparation of the desired indenes from the respective indanones followed by either formylation at C-2 and subsequent alkylations at C-1 or vice versa, and then processing the formyl group through standard reaction sequences to the desired intermediates.[10(b)]

E. Synthesis of Naphthalene Intermediates

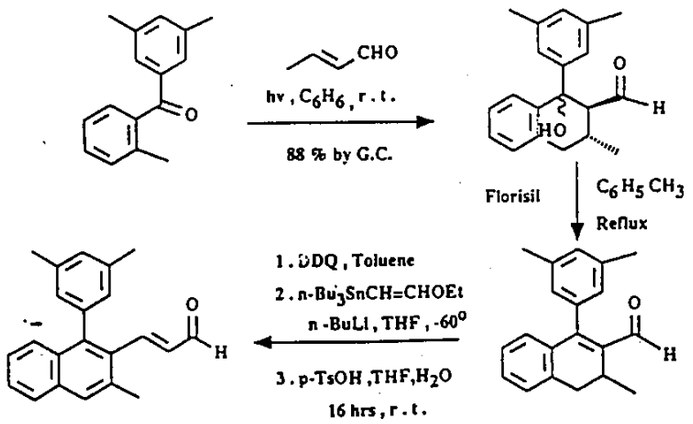
For the preparation of naphthalene derivatives, a novel photochemical route[11] was exploited to give the key hydroxy aldehyde, which on dehydration provides the ene aldehyde. Dehydrogenation of the ene aldehyde and chain



SCHEME 6



SCHEME 7

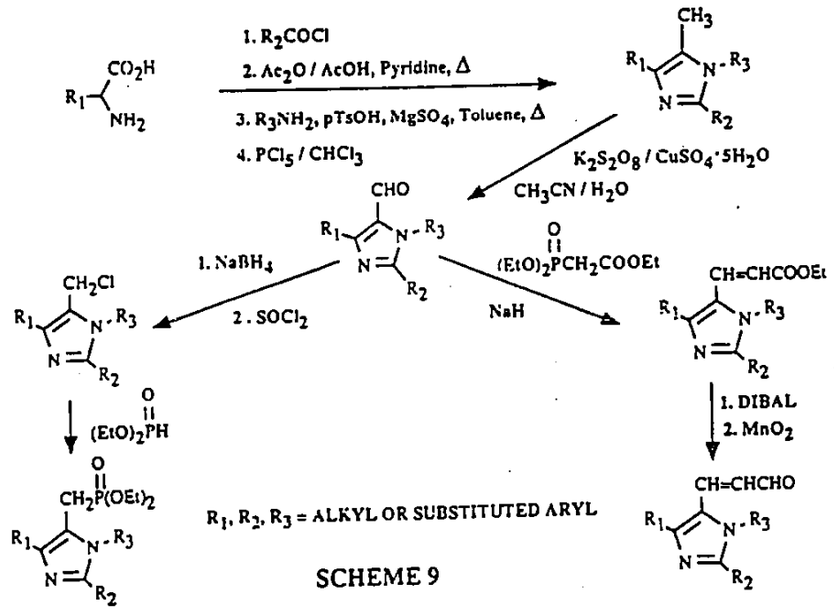


SCHEME 8

extension of the formyl group then leads to the desired α,β-unsaturated aldehydes[10(c)] (Scheme 8).

F. Synthesis of Imidazole Intermediates

Highly substituted imidazole derivatives with the desired functional group at the desired C- or hetero- atom are not well described in the literature. Synthesis of the required imidazole intermediates was best accomplished starting from the respective glycine derivatives as shown in Scheme 9. The key step in the synthetic pathway involves oxidation of the methyl group with potassium persulfate to give the 5-formyl imidazole derivatives, which through standard reaction sequences give the needed α,β-unsaturated aldehydes or the phosphonates.[10(d)]



SCHEME 9

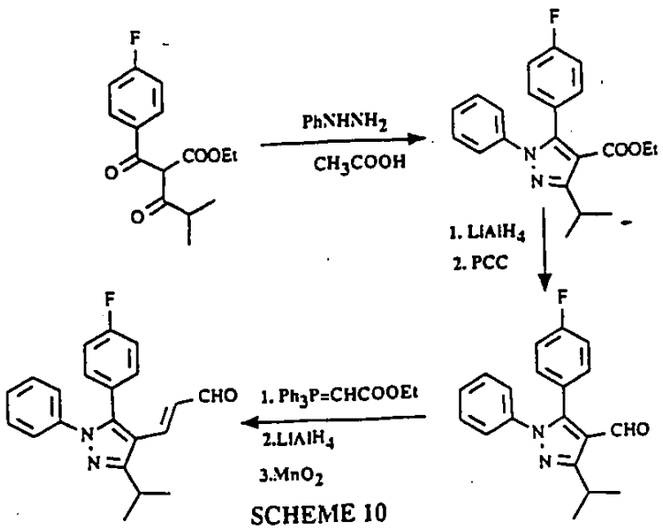
G. Synthesis of Pyrazole Derivatives

A number of pyrazole intermediates have been prepared via procedures dependent on whether one needs the 1,5 (Scheme 10), the 1,3 (Scheme 11), or the 3,4 (Scheme 12) disubstituted pyrazole intermediates. 2,3-disubstituted pyrazole derivatives are obtained through the reaction of the appropriate diketoesters with aryl-hydrazines, requiring separation from the concomitant formation of the corresponding 1,3 isomer (Scheme 10).[10(e)]

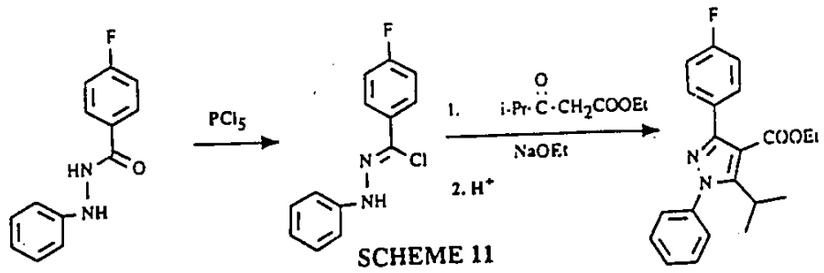
1,3-disubstituted pyrazoles can be best synthesized from the imide chloride on reaction with the acetoacetate derivatives (Scheme 11), while the ring closure of arylhydrazones give the desired 3,4 diaryl pyrazole intermediates (Scheme 12).

H. Synthesis of HMG-CoA-R Inhibitors

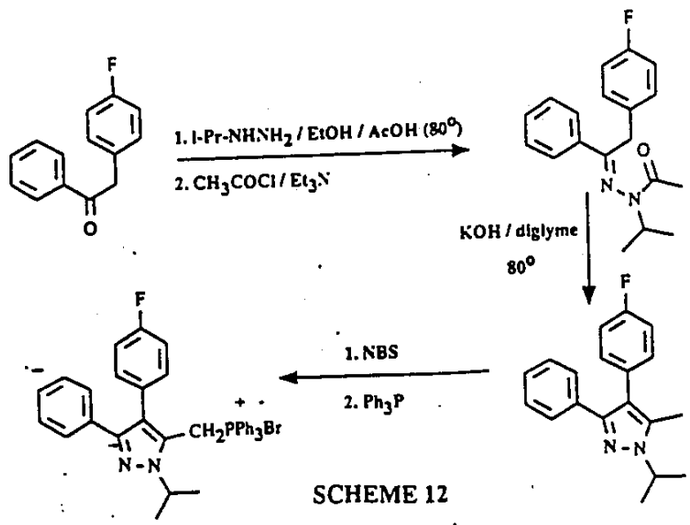
All of the intermediates of the many different prototypes described above in Schemes 4–12 could be converted to the final HMG-CoA reductase inhib-



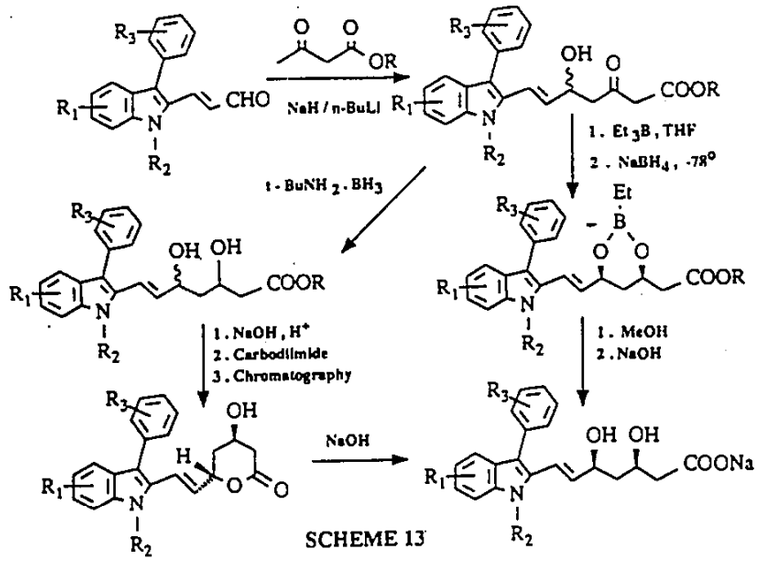
SCHEME 10



SCHEME 11



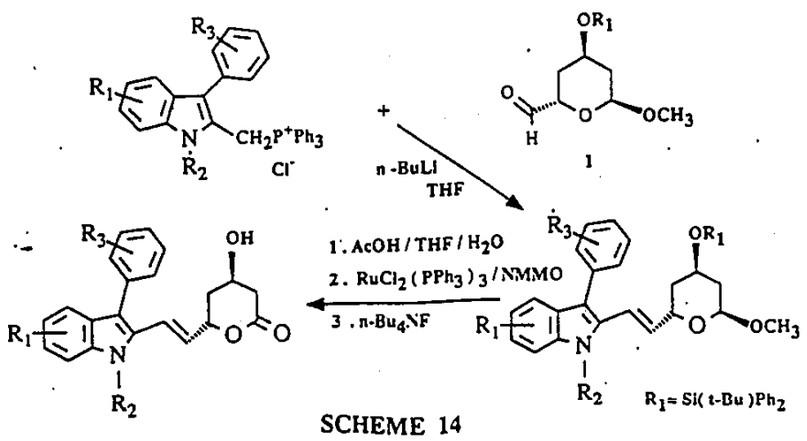
SCHEME 12



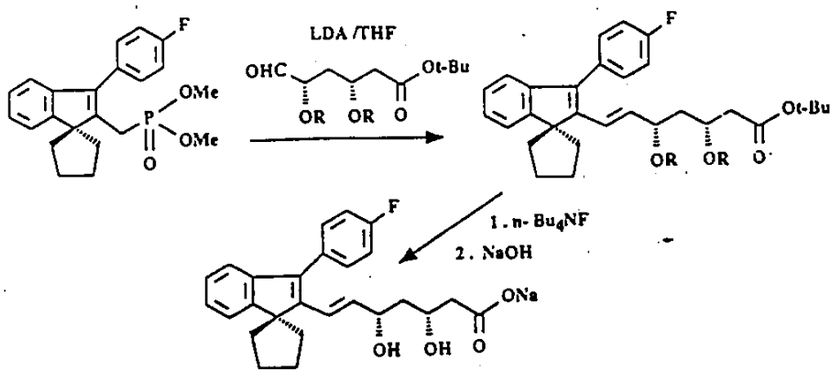
SCHEME 13

itors either using the linear route involving the "dianion chemistry," or the coupling of the respective phosphonates or phosphonium salts with the chiral Synthons 1 and 2 (Fig. 6) or with the racemic Synthon 3 (Fig. 6).

1. *Linear Route.* Synthesis using the linear route is illustrated in Scheme 13 for the preparation of the indolyl HMG-CoA reductase inhibitors. The key step involves the reduction of the hydroxyketoester using trialkylborane/THF/MeOH with sodium borohydride at −78° (Ref. 12) to give the mixture of desired erythro and threo isomers in the ratio of 95–98:5–2%, respectively. In some cases, the boronic esters can be crystallized, which on methanolysis and subsequent hydrolysis with sodium hydroxide provide the desired sodium salts. Nonstereoselective reduction of hydroxyketoester with borane.t-butylamine complex has been used to prepare a mixture of *cis* and *trans* lactones separable on flash chromatography.[10(a)]



SCHEME 14



R = Si (t-Bu) Ph_2

SCHEME 15

2. *Convergent Route.* For illustrative purposes, a convergent route for the preparation of chiral indolyl HMG-CoA reductase inhibitors using the silyl protected Synthon 1 is depicted in Scheme 14. The crucial step in this reaction pathway is the oxidation of lactol with $RuCl_2(PPh_3)_3$/NMMO.[10(f)]

Scheme 15 shows the use of silyl-protected aldehyde Synthon 2 (derived from malic acid) for the synthesis of indenyl HMG-CoA reductase inhibitors.[13]

IV. BIOLOGICAL RESULTS AND DISCUSSION

A. Results in *in vitro* HMG-CoA Reductase Microsomal Assay and in *in vivo* Cholesterol Biosynthesis Assay

All initial studies to assess the inhibitory potency of various compounds against HMG-CoA reductase were conducted with rat liver microsomal suspensions, freshly prepared from male Sprague-Dawley rats, using an assay for HMG-CoA reductase activity as described in Ref. 14. The potency of each compound is expressed as IC_{50} (in μmoles, the concentration which inhibits to the extent of 50% conversion of the substrate HMG-CoA to mevalonate) and for structure activity relationship compared either to compactin = 1 or to XU 62-320 = 1. Tables I–XII summarize the most salient features of structure activity relationships for a few of the varied structural prototypes as HMG-CoA reductase inhibitors being currently studied at the Sandoz Research Institute. In Tables X–XIII, the Relative Potency column is derived from the IC_{50} values of each compound vs. compactin in the *in vitro* rat microsomal HMG-CoA reductase assay.

B. SAR of Fluvastatin (XU 62-320) Analogs

Table I compares the *in vitro* inhibitory activity against HMG-CoA reductase of XU 62-320 with compactin and lovastatin and as their corresponding sodium salts. XU 62-320 is 146- and 52-fold more active than compactin and Lovastatin, respectively. As compared to the respective sodium salts of compactin and Lovastatin, XU 62-320 is 22- and 10-fold more potent in inhibiting HMG-CoA reductase. It is important to note that current clinical studies are being conducted with XU 62-320, which is a dihydroxy acid sodium salt. In contrast,

Table I
Comparison of Microsomal HMG-CoA Reductase Inhibitory Activity

Compound	IC_{50} (μM)	Relative Potency*
XU 62-320	0.0069	146.1
Compactin	1.011	1.0
Lovastatin	0.352	2.8
Na Salt Compactin	0.154	6.5
Na Salt Lovastatin	0.068	14.8

*As compared to Compactin = 1

compactin used in clinical studies and Lovastatin (Mevacor®), now marketed, both exist as the lactone forms (Fig. 1).

Features of the side chain are very important for maximal inhibitory activity as shown in Table II. Erythro configuration, as well as the double-bond configuration, are very important [anti-isomer 17-fold less active and dramatic loss of activity for one (Z) diene isomer]. The dihydro derivative, as well as the ester and the lactone forms, are considerably less active. Maximal inhibitory activity resides in the 3R, 5S antipode.

The importance of the features of the side chain described in Table II for the indole series holds true as well for all the prototypes to be described later and hence, during the discussion of SAR of these prototypes, these aspects will not be reemphasized. HMG-CoA, the substrate for the HMG-CoA reductase, has at C-3 a methyl group. It was important to determine if an analog of XU 62-320 carrying a methyl group at C-3 would be more potent. Surprisingly, introduction of methyl group at C-3 in either of syn- or anti-configuration was considerably less active (Table III).

Studies of the effects of the substituents in the 3-phenyl ring of the indole moiety are given in Table IV. Either electron-withdrawing or electron-donating substituents in the 3-phenyl ring tend to decrease the potency, which is unaffected by the presence of alkyl groups.

Electron-donating or electron-withdrawing substituents (not shown in Table IV) or bulky alkyl groups at C-5 of the indole moiety led to decrease of potency. However, alkyl or alkoxy groups at C-4 and C-6 tend to maintain or enhance the potency slightly (Table V).

Table II
SAR of Variations in the Side Chain

Compound	IC_{50} (μM)	Relative Potency*
XU 62-320	0.0069	1.0
3R, 5S	0.0024	2.8
3S, 5R	0.08	0.086
Na Salt, ANTI	0.12	0.057
Methyl Ester, SYN	0.052	0.13
Trans Lactone	0.029	0.23
CIS(Z) Double Bond	0.62	0.011
Dihydro (Reduced Double Bond)	0.114	0.06

*As compared to XU 62-320 = 1.

Table III
Comparative Activity of XU with the 3-Methyl Analogs

Compound	IC_{50} (μM)	Relative Potency*
XU 62-320	0.0069	1.0
R = CH_3, SYN	0.14	0.049
R = CH_3, ANTI	0.51	0.013

*As compared to XU 62-320 = 1

Table IV
SAR for the Substituents of the 3-Phenyl Ring

R	IC_{50} (μM)	Relative Potency*
4-F	0.0069	1
2-Me	0.14	0.049
2-Me, 4-F	0.004	1.7
3-Me, 4-F	0.009	0.76
3,5-diMe, 4-F	0.02	0.345
3,5-diMe	0.005	1.38
H	0.017	0.40
4-CF_3	0.09	0.076
4-SCH_3	1.152	0.006
4-COONa	>10.0	

*As compared to XU 62-320 = 1

Table V
SAR for the Substituents of the Benzenoid Indole Ring

R	IC_{50} (μM)	Relative Potency*
H (62-320)	0.0069	1.0
4,6-diMe	0.011	0.62
4,6-dii-Pr	0.005	1.38
5-C_6H_{11}	24.0	0.0022
6-OCH_2Ph	0.0026	2.65

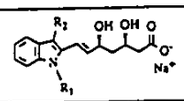
*As compared to XU 62-320 = 1

Table VI
SAR for the Substituents of Indolyl-Nitrogen

R	IC_{50} (μM)	Relative Potency*
i-Pr (62-320)	*0.0069*	*1.0*
CH_3	0.62	0.011
C_2H_5	0.096	0.071
C_6H_{11}	50	0.0001
CH_2CH_2Ph	49.4	0.0001
$CH_2CH(CH_3)_2$	0.245	0.028

*As compared to XU 62-320 = 1

Table VII
SAR for Reversing Substituents at 1 and 3 Positions

	R_1	R_2	IC_{50} (μM)	Relative Potency*
	i-Pr (62-320)	4-FC_6H_4, syn	0.0069	1.0
	4-FC_6H_4	i-Pr, syn	0.0016	4.3
	i-Pr	4-FC_6H_4, anti	0.12	0.057

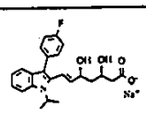
*As compared to XU 62-320 = 1

Most sensitive to the activity is the substituent on the nitrogen of the indole moiety (Table VI). Optimal activity is provided by the isopropyl group, while marked loss in potency results with either bulky alkyl or phenethyl groups.

Reversing the substituents on N-1 and C-3 of the indole moiety to give (Table VII) 3-isopropyl-N-p-fluorophenyl analog of XU 62-320 gives a 4-fold increase in potency.

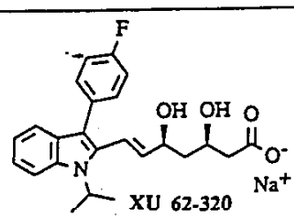
Most of the substances with a reasonable level of activity against HMG-CoA reductase in *in vitro* microsomal assay were studied *in vivo* for their effects on inhibition of sterol biosynthesis. Results are expressed as ED_{50} (mg/kg), effective concentration which inhibits to the extent of 50% incorporation of C^{14} acetate into sterols in rats when administered as appropriate doses of drug substances as compared to controls receiving vehicle alone. Table VIII shows that *in vivo* XU 62-320 is about 40- and 4.5-fold more potent than compactin and Lovastatin, respectively, in inhibiting endogenous cholesterol synthesis in rats. For most substances, although not for all, the relative

Table VIII
Relative Potency for Inhibition of Cholesterol Biosynthesis

	Compound	ED_{50} (mg/kg)	Relative Potency*
	XU 62-320	0.093	37.6
	Compactin	3.5	1.0
	Lovastatin (Monacolin)	0.414	8.4

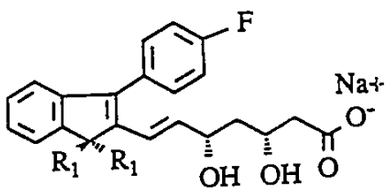
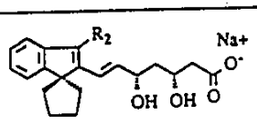
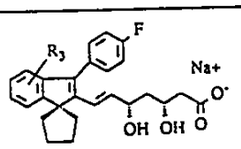
*As compared to Compactin = 1

Table IX
SAR for Cholesterol Biosynthesis Inhibition

	Compound	ED_{50} (mg/kg)	Relative Potency*	
	XU 62-320	0.093	1.0	1.0
	3R, 5S	0.056	1.66	2.9
	3S, 5R	>0.5		0.026
	Na Salt, Anti	1.37	0.067	0.057
	Methyl ester, Syn	0.40	0.23	0.13
	Trans Lactone	0.33	0.28	0.23
	Dihydro (Reduced Double Bond)	1.23	0.075	0.06

*As compared to XU 62-320 = 1

Table X
SAR of Indene Derivatives

	R_1	Relative Potency*
	$(CH_2)_4$	202
	(Racemic)	
	$(CH_2)_4$	337
	(3R, 5S)	
	$(CH_2)_2$	38
	$(CH_2)_5$	1.5†
	CH_2CH_3	<.2
	CH_3	2
	H,iPr	8
	R_2	
	Phenyl	88†
	3,5-Dimethylphenyl	146
	iPr	<0.5
	Cyclohexyl	16.5
	R_3	
	4-Me	114
	6-Me	181
	7-Me	24
	6-OMe	130
	4,6-$(OMe)_2$	60

*As compared to Compactin = 1
†As its Ethyl Ester

potency determined in *in vitro* microsomal assay against HMG-CoA reductase parallels the *in vivo* activity in rats for the inhibition of ^{14}C-acetate into sterols.

As an example, comparison of Tables II and IX reveals the relative potency of several analogs of XU 62-320 when compared in *in vitro* and in *in vivo*. Thus, as compared to XU 62-320, the anti-isomer is ~ 17- (Table II) and ~ 15-fold (Table IX) less active than XU 62-320 in *in vitro* and in *in vivo* assays, respectively. Similarly, close parallelism prevails for the ester (less active ~ 7.5-fold, *in vitro* vs. 4.3-fold, *in vivo*), *trans*-lactone (less active 4.2-fold, *in vitro* vs. 3.5, *in vivo*) and the dihydro derivative (less active 16.5-fold, *in vitro* vs. 13-fold *in vivo*).

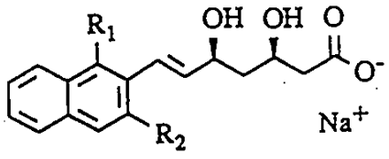
C. SAR of Indene Derivatives

The structure activity relationships for the indene derivatives can be best summarized as follows: Maximal activity is obtained with a spiro cyclopentyl group at C-1, again emphasizing the importance of the bulky group in the vicinity of the dihydroxy acid side chain. At C-3 the best substituent is 4-F-phenyl, while the optimal substituent for the benzenoid portion of the indene moiety is hydrogen (see Table X).

D. SAR of Naphthalene Derivatives

The most interesting part of the structure activity relationships for this group of compounds is the difference observed in the potency of 1-(4-F-

Table XI
SAR of Napthalene Derivatives



R_1	R_2	Relative Potency*
4-F-Ph	H	0.10
4-F-Ph	CH_3	8
4-F-Ph	Et	19
4-F-Ph	i-Pr	22
3,5-diMe-Ph	CH_3	56
Ph	CH_3	2
i-Pr	4-F-Ph	337
i-Pr	Ph	144

*As compared to Compactin = 1

phenyl)-3-isopropyl derivative vs. 1-isopropyl-3-(4-F-phenyl) compound (22 times more potent vs. 337 as compared to compactin) (see Table XI).

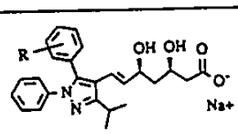
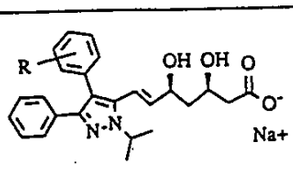
E. SAR of Pyrazole Derivatives

Table XII illustrates the structure activity relationships for a few of the many pyrazole derivatives prepared. Here, too, the optimal substituents are the 4-F-phenyl and isopropyl group adjacent to the dihydroxy acid side chain. The dihydro and the 5-keto derivatives are substantially less potent. 1,3-diaryl-substituted pyrazole derivatives show decreased inhibitory activity (not shown in the table) in contrast to the 1,5 and 3,4-diaryl-substituted compounds, which tend to have comparable potency.

F. SAR of Imidazole Derivatives

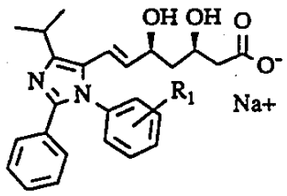
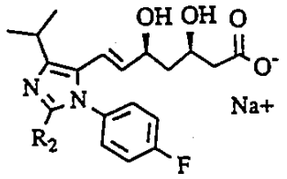
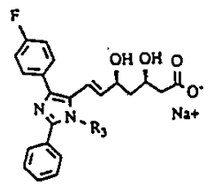
To emphasize the most salient features of the structure activity relationships for the imidazole derivatives, only a few of the derivatives prepared are tabulated in Table XIII. Optimal activity is obtained with 1,2-diaryl derivatives

Table XII
SAR of Pyrazole Derivatives

	R	Relative Potency*
	4-F	60
	4-F (6,7 Dihydro)	5.9
	4-F (5 Keto)	3.5
	H	5.6
	3,5 Dimethyl	4.1
	4-F	30

*As compared to Compactin = 1

Table XIII
SAR of Imidazole Derivatives

		Relative Potency*
	R_1	
	4-F (Racemic)	337
	4-F (3R, 5S)	532
	p-Cl	84
	p-Br	20
	3,5-Di-Me	7
	3,5-Di-Cl	10
	R_2	
	i-Pr	4.4
	t-Butyl	4.4
	cyclohexyl	4.8
	2-Thienyl	202
	1,4-Biphenylyl	35
	p-Dimethylamino-phenyl	56
	p-Nitro-phenyl	72
	R_3	
	i-Pr	1.1
	4-F-Phenyl	<0.1

*As compared to Compactin = 1

with the 4-F substituent preferred in the phenyl ring on nitrogen and H atom being the preferred substituent for the phenyl ring at C-2. Alkyl substituents at C-2 tend to lead to considerable loss of activity. The 1,3-diaryl-substituted imidazole derivatives suffer a dramatic loss of activity when compared to the very potent 1,2-diaryl compounds.

V. EFFECTS OF FLUVASTATIN (XU 62-320) ON PLASMA LIPOPROTEIN LEVELS

Fluvastatin (XU 62-320) has been studied in several species for its effects on serum lipoprotein levels.

Significant and sustained reductions of *rat* serum VLDL + LDL-cholesterol have been observed after treatment of rats with XU 62-320. However, these lipoprotein changes are not observed after chronic dosing of normolipemic rats either with compactin or lovastatin.

In the beagle dog, after three weeks of administration, fluvastatin lowers serum LDL + VLDL-cholesterol to the extent of ~ 47% either at 2 mg/kg/day given once a day or 1 mg/kg/day given twice a day. A comparable effect on VLDL + LDL-cholesterol is observed with compactin at a dose of 20 mg/kg/day

given once a day. In the Rhesus monkey, a reduction of 30% in serum VLDL + LDL-cholesterol is achieved with fluvastatin at a dose of 30 mg/kg/day at the end of three weeks of daily administration.[15]

VI. TOXICOLOGICAL, DRUG METABOLISM, AND PHARMACOKINETIC STUDIES OF FLUVASTATIN (XU 62-320)

The safety, drug metabolism, and pharmacokinetic evaluation of fluvastatin (XU 62-320) has been extensively carried out in acute, subchronic, and chronic rat, dog, monkey, and mouse studies at Sandoz Research Institute. These studies have allowed extensive clinical trials with the first totally synthetic HMG-CoA reductase inhibitor.[16]

VII. HUMAN STUDIES WITH FLUVASTATIN (XU 62-320)

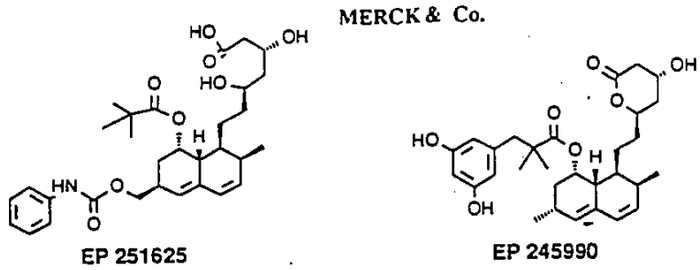
Through completion of Phase II multi-center dose-response and dose-frequency trials, in all 658 subjects have been randomized to treatment with fluvastatin (XU 62-320) in double-blind safety and efficacy trials with another 269 placebo subjects serving as controls. Fluvastatin (XU 62-320) was well tolerated at all doses studied and was free from serious or unexpected adverse effects. Dose-dependent mean reductions of 11% to 21% in total plasma cholesterol and 15% to 28% in LDL-cholesterol were achieved on 5 to 40 mg QPM of fluvastatin. Dose-dependent mean reductions of triglycerides and a drug-related increase in HDL-cholesterol were also observed. Equivalent reductions of LDL-C (22% vs. 23%) were produced by 20 mg per day of fluvastatin when given as a single dose or divided into a BID regimen. A dose of 20 mg once a day at bedtime gave LDL-cholesterol reductions similar in magnitude to that of the marketed agent lovastatin (Mevacor®).

VIII. OVERVIEW OF PUBLISHED LITERATURE ON HMG-CoA REDUCTASE INHIBITORS

A very large number of reviews have described the importance of HMG-CoA reductase inhibitors for the treatment of elevated serum total cholesterol and LDL + VLDL-cholesterol.[4,17] Also, extensive information is available on the pharmacology and clinical efficacy of lovastatin (Mevacor®, MSD), marketed in the United States,[4,18] simvastatin (Zocor®, MSD),[19] marketed in several European countries but not yet available in the United States, and pravastatin (Mevalotin®, Pravachol®, Sankyo, Squibb), yet marketed only in Japan.[20] However, in this section, an overview is presented (Figs. 11–19), describing the attempts in many laboratories towards the discovery of new HMG-CoA reductase inhibitors since the discovery of compactin lovastatin, simvastatin, pravastatin, and fluvastatin. In Figures 11–19, only one specific representative structure is depicted to describe the varied structural prototypes reported in the literature as HMG-CoA reductase inhibitors.

- Scientists at Merck & Co. continue the derivatization efforts towards semisynthetic derivatives using lovastatin as starting material (Fig. 11).[21] Very many wide variants in the acyloxy side chain at C-8 of mevinolin have been executed. Elegant "Barton-type" chemistry has allowed the functionalization of 6-Methyl group in ring A of mevinolin leading to a large number of derivatives with many functional groups at C-6.

MERCK & Co.



EP 251625 EP 245990

Figure 11

• At Sandoz Research Institute, besides the fluvastatin, indenyl, naphthyl, imidazolyl, and pyrazolyl analogs discussed in this paper, a variety of other HMG-CoA reductase inhibitors have been synthesized varying the heterocyclic hydrophobic domain. These derivatives are described in Figs. 12–14.[22–24] The overlapping reports from other companies on similar derivatives are shown as well in Figs. 12 and 13.[22,23]

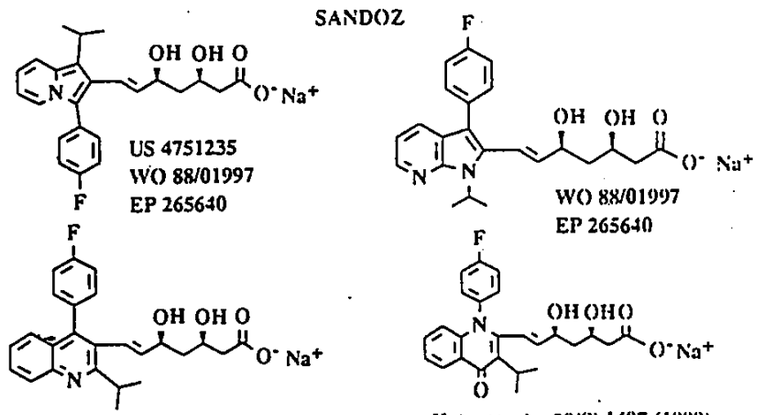
• In addition to the HMG-CoA reductase inhibitors described above, scientists at Hoechst, Baeyer, Warner-Lambert, May & Baker, Rorer, Bristol-Myers, Squibb, and Pfizer have published their efforts and their results in this exciting area (Figs. 15–17).[25–27]

• A set of novel structural prototypes as HMG-CoA reductase inhibitors have been claimed by Pan Medica (Fig. 18).[28] One of the Pan Medica candidates is currently in clinical trials.

• Two groups have focused their efforts towards the development of "regulators of HMG-CoA reductase" rather than towards the development of competitive inhibitors.

• Schroepfer *et al.* have studied extensively Cholest-8(14)-en-15-one as a very interesting hypolipoproteinemic agent. This agent is being studied in

SANDOZ



US 4751235
WO 88/01997
EP 265640

WO 88/01997
EP 265640

Sandoz Patent Pending
W.Lambert US 4761419
Nissan EP 304063A

Heterocycles 29(8) 1497 (1989)

Figure 12

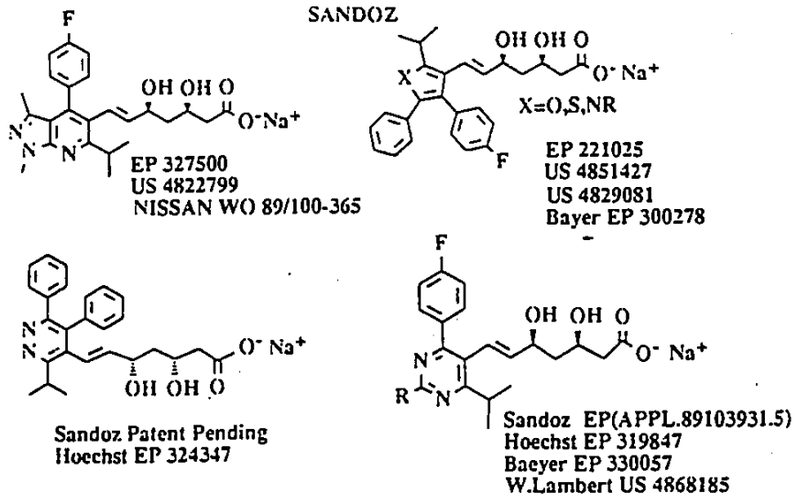


Figure 13

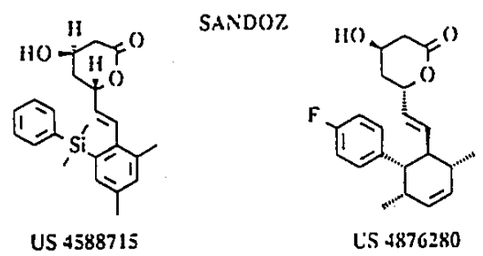


Figure 14

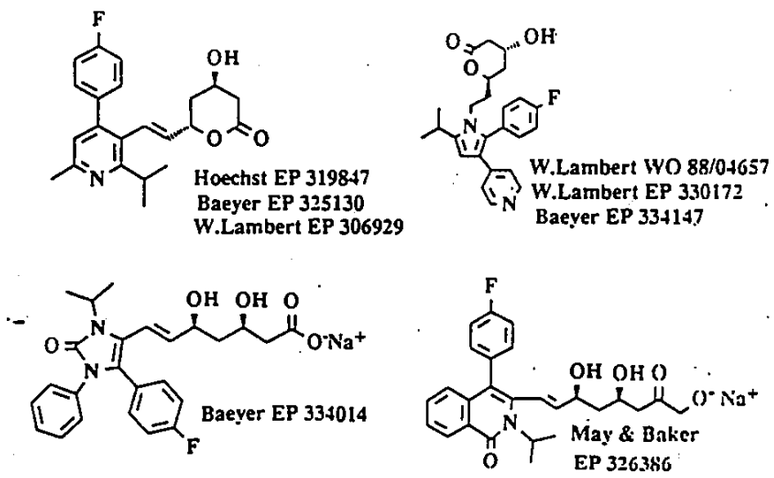


Figure 15

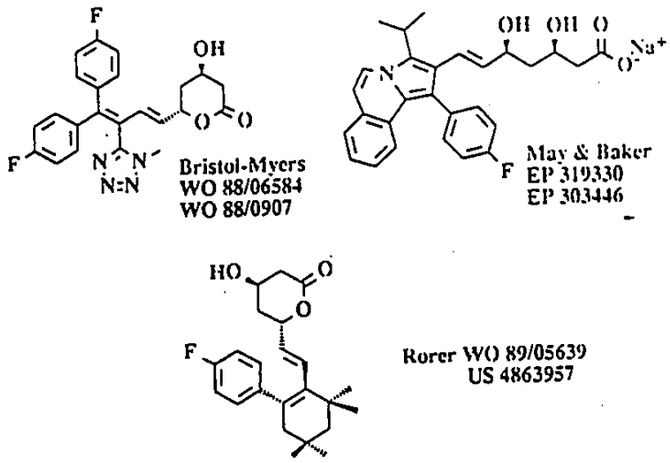


Figure 16

the clinic (Lederle Labs). Taylor *et al.* (DuPont) have attempted to develop inhibitors of HMG-CoA reductase via inhibition of lanosta-8, 24-dien-3 beta-ol-14 alpha-methyldemethylase (Fig. 19).[29]

IX. CONCLUSION

During the discussion on cholesterol biosynthesis inhibitor, Sabine commented, "The development of an effective agent that will lower, and/or prevent a rise in man's level of plasma cholesterol, without accompanying any undesirable side effects, is a pharmacological rainbow at the end of which is an immense pot of gold. Hence, the search for such an agent is conducted with a great deal of vigor, skill, imagination, and money. I myself certainly hope that the attainment of this therapeutic ideal is indeed not a rainbow, but that the possible existence of such an agent is in fact a solid reality and not just a pleasant illusion of light and color."[30]

Since Sabine's remark, HMG-CoA reductase inhibitors have indeed emerged as solid realities and have not remained mere pleasant illusions of light and color. Mevacor®, Zocor®, and Mevalotin® are marketed products showing remarkable efficacy in lowering LDL-cholesterol without serious side effects. Fluvastatin (XU 62-320), being studied intensely in Phase III clinical trials, has shown very good efficacy with no serious adverse effects. Future work will certainly shed more experience not only with Mevacor®, Zocor®, Mevalotin®, and Fluvastatin, but possibly with a host of other HMG-CoA reductase inhibitors reviewed in this paper. Also, in 1989 the worldwide sales of Merck's Mevacor® (launched in September, 1987), being $535 M, speak to the HMG-CoA reductase inhibitor as being the pharmacological rainbow at the end of which is an immense pot of gold.

Excitement has been added to the fascinating story of the development of HMG-CoA reductase inhibitors by the elegant and outstanding work in the laboratories of Nobel laureates Brown and Goldstein, to explain the mechanism of action of these inhibitors. The HMG-CoA reductase inhibitors lower

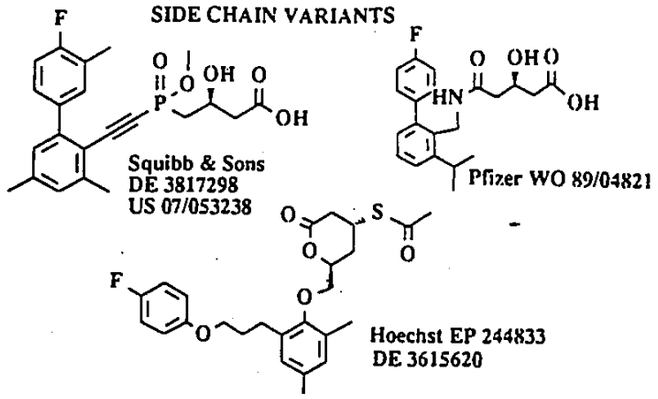


Figure 17

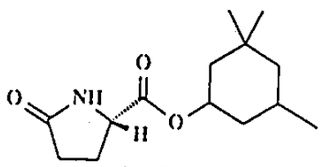


Figure 18

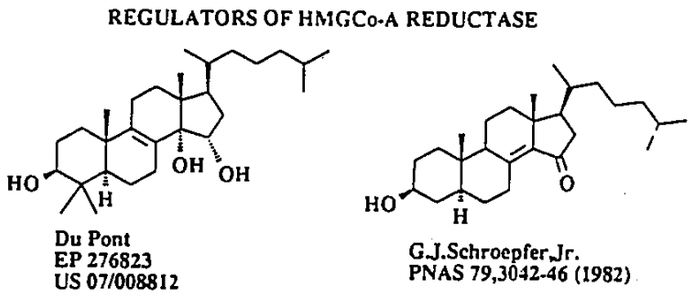


Figure 19

serum lipoprotein levels by up-regulating the lower LDL-receptors. But how do the many different HMG-CoA reductase inhibitors, described in this paper, affect the HMG-CoA reductase activity precisely at the detailed molecular level? The elegant molecular biology efforts in the laboratories of Brown and Goldstein have given us the amino acid sequence of HMG-CoA reductase of several species, but little is known of the detailed tertiary structure of the enzyme. What are the precise conformations of the many HMG-CoA reductase inhibitors, described in this paper, when bound to the active site domain of HMG-CoA reductase? What is the topography of the amino acid residues at the active site of HMG-CoA reductase when one or the other HMG-CoA reductase inhibitors is bound to it? What precise details of molecular recog-

nition are involved and need to be understood to explain the rank-order potency of many of the described analogs of HMG-CoA reductase inhibitors? Fascinating work remains to be done to provide answers to the many interesting unanswered questions in the exciting field of HMG-CoA reductase inhibitors.

ACKNOWLEDGMENTS

I wish to acknowledge the publication of the schemes and tables describing the SAR of the Sandoz compounds by Elsevier in their book *Trends in Medicinal Chemistry '88* (edited by H. van der Goot *et al.*). The extensive work at Sandoz Research Institute on HMG-CoA reductase inhibitors described in part in this paper is truly an outcome of a cohesive team effort of a very large number of dedicated and creative individuals. Most important original contributors to be recognized are: In the Medicinal Chemistry Department, for indole derivatives: H. F. Schuster, R. Stabler, J. Kratunis; for indene derivatives: S. Wattanasin, R. Patel; for naphthalene derivatives: P. L. Anderson, S. W. Meyers, N. A. Paolella; for pyrazole derivatives: J. R. Wareing, M. Martin, C. F. Jewell, Jr., R. Stabler; for imidazole derivatives: J. R. Wareing, J. M. Leginus, J. Linder, G. T. Lee, R. Stabler, M. Martin, L. Widler; for chiral synthon from D-glucose: J. R. Wareing, C. E. Fuller; for synthesis of chiral derivatives using chiral synthon from D-glucose: J. R. Wareing, C. F. Jewell, L. Widler; for coordination of the project: R. E. Damon; in the Process Research and Chemical Development Department, for the chiral synthon from S-malic acid and its use: P. Kapa, K. M. Chen, O. Repic and G. E. Hardtmann; for the racemic synthon and its use: P. Kapa and O. Repic; for large scale preparation and many important improvements of the processes for intermediates and final products: R. E. Walkup, S. Palermo, J. Linder, G. T. Lee, M. Thiede; in the Pharmacology Department, for in *in vivo* testing: R. G. Engstrom, D. B. Weinstein, J. B. Eskesen, M. L. Rucker, R. Miserendino. The success of this work is, in large part, due to our collaboration with Prof. T. Scallen, Department of Biochemistry, University of New Mexico, Albuquerque, New Mexico, who has carried out all the *in vitro* studies. Finally, many thanks are extended for the efforts of J. Birch and P. Schaefer for the preparation of this manuscript.

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II. Personal Information:

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Date of Birth: December 14, 1921

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B.S.	Worcester Polytechnic Institute	1943
M.S.	Worcester Polytechnic Institute	1951
Ph.D.	University of Wisconsin, Madison; Biochemistry	1954

Chester E. Holmlund

III. Academic Activities:

A. Percentage of official time (last three years) devoted to:

Instruction 25% Research 70% Other 5%

B. Positions held:

Higher Education:

Research Assistant, University of Wisconsin, Madison	1951-54
Associate Professor, Dept. of Chemistry, Univ. of Maryland	1967-70
Professor, Dept. of Chemistry, University of Maryland	1970-present

Other than higher education:

Research Chemist, E. I. DuPont deNemours Company	1946-47
Research Chemist, United States Envelope Co.	1948-51
Research Biochemist, Department of Mycology, Lederle Laboratories, American Cyanamid Co.	1954-57
Group Leader, Fermentation Biochemistry & Microbiology Departments, Lederle Laboratories, American Cyanamid Co.	1957-67

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C. E. Holmlund - Publications

IV. Publications:

1. "Bacterial levans of Intermediate Molecular Weight, " J.R. Mattoon, C.E. Holmlund, S.A. Schepartz, J.J. Vavra, and M.J. Johnson, Appl. Microbiol. 3, 321 (1955).

2. "Chemical Hydroxylation of 12α-Deoxytetracycline," C.E. Holmlund, W.W. Andres and A.J. Shay, J. Am. Chem. Soc. 81, 4748 (1959).

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5. "Substrate Specificity in the Microbiological Transformation of Steroids," C.E. Holmlund, L.I. Feldman, R.H. Blank, N. Barbacci, and B. Nielsen, Sci. Repts., 1st Super. Sanita 1, 289 (1961).

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8. "Stereospecific Oxidation of a Methylthiosteroid to the Sulfoxide by Calonectria decora, C.E. Holmlund, K.J. Sax, B.E. Nielsen, R.E. Hartman, R.H. Evans, Jr., and R.H. Blank, J. Org. Chem. 27, 1468 (1962).

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13. "Preparation and Properties of a Steroid Lactonase," C.E. Holmlund and R.H. Blank, Arch. Biochem. Biophys. 109, 29 (1965).

14. "Microbiological Formation of 1α, 2α-Dihydroxysteroids," K.J. Sax, C.E. Holmlund, L.I. Feldman, R.H. Evans, Jr., R.H. Blank, A.J. Shay, J.S. Schultz, and M. Dann, Steroids 5, 345 (1965).

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29. "A Comparison of the Effects of Some Hypocholesteremic Compounds an Squalene Metabolism in Tetrahymena pyriformis and Rat Liver," J.D. Sipe and C.E. Holmlund, Biochim. Biophys. Acta 280, 145 (1972).

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49. R. Pereira, C.E. Holmlund and N. Whittaker, The Effect of AY-9944 on Yeast Sterol and Sterol Ester Metabolism. Lipids, 18, 545-552 (1983).

50. PremKala Prasanna and Chester E. Holmlund, Identification in Tetrahymena pyriformis of 3-Hydroxy-3-Methylglutaryl Coenzyme A Lyase: Its Purification and Properties, International Journal of Biochemistry, 19, (4) 385-389 (1987).

Chester E. Holmlund

Abstracts and other professional papers presented:

"Enhancement of Sterol Synthesis by Saturated and Monounsaturated Phosphatidylcholines in Culture of L-929 Cells," G.N. Thomaidis and C.E. Holmlund, 65th Annual Meeting, Federation of American Societies for Experimental Biology, Atlanta, Georgia, May 12-17, 1981.

"Phospholipid Composition and Sterol Efflux from L-929 Cells," Y. Son and C.E. Holmlund, 73rd Annual AOCS Meeting, Toronto, Canada, May 2-6, 1982.

"Isolation, Characterization, and Chemical Modification of Macrocyclic Trichothenes," B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, C. E. Holmlund, E. P. Mazzola and R. Geohegan, 16th MARM, April 21-23 (1982).

"Effects of SICF-3301 on Growth and Composition of Free and Esterified Sterols in Saccharomyces cerevisiae," C. Jones and C.E. Holmlund, ASBC/AAI Annual Meeting, St. Louis, Mo., June 3-7, 1984.

"Metabolism of β-Hydroxy-β-Methylglutaryl Coenzyme A (HMG-6A) in Tetrahymena pyriformis: Presen e of HMG-CoA Lyase," K. Prasanna and C.E. Holmlund, ASBC/AAI Annual Meeting, St. Louis, MO. June 3-7 (1984).

"Analysis of Free and Esterified Sterols of Crithidia fasciculata at Various Times of Culture," C. Jones and C.E. Holmlund.

C. E. Holmlund - Curriculum Vita

B. Other Creative and Scholarly Activities:

PATENTS:

1. 12α-hydroxy Compounds of the Tetracycline Series. American Cyanamid Co., C.E. Holmlund and W.W. Andres, Ger. 1,092,907. March 5, 1958.

2. 12α-hydroxytetracyclines. American Cyanamid Co., C.E. Holmlund, A. Green and A.J. Shay, Ger. 1,092,906. March 4, 1959.

3. Pregnanes. C.E. Holmlund, L.D. Feldman, H.M. Kissman, and M.J. Weiss, American Cyanamid Co., U.S., 3,047569, July 31, 1962.

4. 12α-hydroxylation of 12αdeoxytetracyclines. C.E. Holmlund and W.W. Andres. American Cyanamide Co. U.S., 3,043,877. July 10, 1962.

5. 16α-hydroxylation of Steroids by Staurophoma Species. C.E. Holmlund, R.J. Blank, and R.H. Evans, American Cyanamid Co. U.S., 3,071,516. January 1, 1963.

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9. Preparation of 1,2-Disubstituted Steroids. L.I. Feldman, C.E. Holmlund, and K.J. Sax. American Cyanamid Co. U.S., 3,297,687. January 19, 1967.

10. Method of Preparing 16-Oxygenated Derivatives of Estr-y-en-3-one. K.J. Sax, R.H. Blank, C.E. Holmlund and R.H. Evans, Jr. American Cyanamid Co. U.S. 3,329,579. July 4, 1967.

11. Fusarium Fermentation, R.H. Evans, Jr., M.P. Kunstmann, C.E. Holmlund, and G.A. Ellestad. American Cyanamid Co. U.S. 3,546,073. December 8, 1970.

12. Preparation of 5,6-dihydro-5-hydroxy-6-propenyl-2-pyrone by Fermentation and Derivatives Thereof. R.H. Evans, J., and C.E. Holmlund, American Cyanamid Co. U.S. 3,701,787. October 31, 1972.

13. Tetracyclic Lactone Antifungal Agents. C.E. Holmlund, R.H. Evans, Jr., and G.E. Ellestad. American Cyanamid Co. U.S. 3,564,019. February 16, 1971.

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Fujikawa et. al.

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Education:

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M.S.	Worcester Polytechnic Institute	1951
Ph.D.	University of Wisconsin, Madison; Biochemistry	1954

Wattanasin : Interference 102,648
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Chester E. Holmlund

III. Academic Activities:

A. Percentage of official time (last three years) devoted to:

Instruction 25% Research 70% Other 5%

B. Positions held:

Higher Education:

Research Assistant, University of Wisconsin, Madison	1951-54
Associate Professor, Dept. of Chemistry, Univ. of Maryland	1967-70
Professor, Dept. of Chemistry, University of Maryland	1970-present

Other than higher education:

Research Chemist, E. I. DuPont deNemours Company	1946-47
Research Chemist, United States Envelope Co.	1948-51
Research Biochemist, Department of Mycology, Lederle Laboratories, American Cyanamid Co.	1954-57
Group Leader, Fermentation Biochemistry & Microbiology Departments, Lederle Laboratories, American Cyanamid Co.	1957-67

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C. E. Holmlund - Publications

IV. Publications:

1. "Bacterial levans of Intermediate Molecular Weight, " J.R. Mattoon, C.E. Holmlund, S.A. Schepartz, J.J. Vavra, and M.J. Johnson, Appl. Microbiol. 3, 321 (1955).

2. "Chemical Hydroxylation of 12α-Deoxytetracycline," C.E. Holmlund, W.W. Andres and A.J. Shay, J. Am. Chem. Soc. 81, 4748 (1959).

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7. "2-Fluoroprednisone," C.E. Holmlund, L.I. Feldman, H.M. Kissman, and M.J. Weiss, J. Org. Chem. 27, 2122 (1962).

8. "Stereospecific Oxidation of a Methylthiosteroid to the Sulfoxide by Calonectria decora, C.E. Holmlund, K.J. Sax, B.E. Nielsen, R.E. Hartman, R.H. Evans, Jr., and R.H. Blank, J. Org. Chem. 27, 1468 (1962).

9. "The Microbiological Preparation of 17-Deoxytriamicinolone," C.E. Holmlund L.I. Feldman, N.E. Rigler, R.H. Evans, Jr., R.H. Blank, and B.E. Nielsen, J. Med. Chem. 6, 611 (1963).

10. "Microbial Formation and Hydrolysis of Testololactone," C.E. Holmlund, R.H. Blank, K.J. Sax, and R.H. Evans, Jr., Arch. Biochem. Biophys. 103, 105 (1963).

11. "Microbiological Transformations of Macrolide Antibiotics," L.I. Feldman, I.K. Dill, C.E. Holmlund, H.A. Whaley, E.L. Patterson, and N. Bohonos, Antimicrobial Agents and Chemotherapy--1963 American Society for Microbiology 54 (1964).

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14. "Microbiological Formation of 1α, 2α-Dihydroxysteroids," K.J. Sax, C.E. Holmlund, L.I. Feldman, R.H. Evans, Jr., R.H. Blank, A.J. Shay, J.S. Schultz, and M. Dann, Steroids 5, 345 (1965).

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40. C. Campagnoni, C.E. Holmlund and N. Whittaker. 1977. Archives Biochem. Biophys., 184, 555.

41. R.B. Field, C.E. Holmlund and N. Whittaker, The Effects of Hyopcholesteremic Compouond 3β-(β-Dimethylaminoethoxy)-androst-5-en-17-one on the Sterol and Steryl Ester Composition of Saccharomyces cerevisiae, Lipids 14(8), 741-747 (1979).

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46. G. Thomaidis and C.E. Holmlund, Effects of Phosphatidylcholines on de novo Synthesis and Excretion of Sterol by L-929 Fibroblasts. Lipids 17, 427-433 (1982).

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Chester E. Holmlund

Abstracts and other professional papers presented:

"Enhancement of Sterol Synthesis by Saturated and Monounsaturated Phosphatidylcholines in Culture of L-929 Cells," G.N. Thomaidis and C.E. Holmlund, 65th Annual Meeting, Federation of American Societies for Experimental Biology, Atlanta, Georgia, May 12-17, 1981.

"Phospholipid Composition and Sterol Efflux from L-929 Cells," Y. Son and C.E. Holmlund, 73rd Annual AOCS Meeting, Toronto, Canada, May 2-6, 1982.

"Isolation, Characterization, and Chemical Modification of Macrocyclic Trichothenes," B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, C. E. Holmlund, E. P. Mazzola and R. Geohegan, 16th MARM, April 21-23 (1982).

"Effects of SICF-3301 on Growth and Composition of Free and Esterified Sterols in Saccharomyces cerevisiae," C. Jones and C.E. Holmlund, ASBC/AAI Annual Meeting, St. Louis, Mo., June 3-7, 1984.

"Metabolism of β-Hydroxy-β-Methylglutaryl Coenzyme A (HMG-6A) in Tetrahymena pyriformis: Presen e of HMG-CoA Lyase," K. Prasanna and C.E. Holmlund, ASBC/AAI Annual Meeting, St. Louis, MO. June 3-7 (1984).

"Analysis of Free and Esterified Sterols of Crithidia fasciculata at Various Times of Culture," C. Jones and C.E. Holmlund.

C. E. Holmlund - Curriculum Vita

B. Other Creative and Scholarly Activities:

PATENTS:

1. 12α-hydroxy Compounds of the Tetracycline Series. American Cyanamid Co., C.E. Holmlund and W.W. Andres, Ger. 1,092,907. March 5, 1958.

2. 12α-hydroxytetracyclines. American Cyanamid Co., C.E. Holmlund, A. Green and A.J. Shay, Ger. 1,092,906. March 4, 1959.

3. Pregnanes. C.E. Holmlund, L.D. Feldman, H.M. Kissman, and M.J. Weiss, American Cyanamid Co., U.S., 3,047569, July 31, 1962.

4. 12α-hydroxylation of 12αdeoxytetracyclines. C.E. Holmlund and W.W. Andres. American Cyanamide Co. U.S., 3,043,877. July 10, 1962.

5. 16α-hydroxylation of Steroids by Staurophoma Species. C.E. Holmlund, R.J. Blank, and R.H. Evans, American Cyanamid Co. U.S., 3,071,516. January 1, 1963.

6. 6β-hydroxy Steroids. C.E. Holmlund, L.I. Feldman, R.H. Evans, S. Berstein, and J.P. Dusza. American Cyanamid Co. U.S., 3,071,516. January 1, 1963.

7. 14α-Hydroxyestrone and Its 3-acetyoxy Ester. C.E. Holmlund, L.I. Feldman, K.J. Sax, and R.H. Evans. American Cyanamid Co. U.S., 3,214,448. Ocotber 26, 1965.

8. Hydroxylation of 19-Norandrostenedione. C.E. Holmlund, L.I. Feldman, K.J. Sax and R.H. Evans, American Cyanamid Co. U.S., 3,243,355. March 29, 1966.

9. Preparation of 1,2-Disubstituted Steroids. L.I. Feldman, C.E. Holmlund, and K.J. Sax. American Cyanamid Co. U.S., 3,297,687. January 19, 1967.

10. Method of Preparing 16-Oxygenated Derivatives of Estr-y-en-3-one. K.J. Sax, R.H. Blank, C.E. Holmlund and R.H. Evans, Jr. American Cyanamid Co. U.S. 3,329,579. July 4, 1967.

11. Fusarium Fermentation, R.H. Evans, Jr., M.P. Kunstmann, C.E. Holmlund, and G.A. Ellestad. American Cyanamid Co. U.S. 3,546,073. December 8, 1970.

12. Preparation of 5,6-dihydro-5-hydroxy-6-propenyl-2-pyrone by Fermentation and Derivatives Thereof. R.H. Evans, J., and C.E. Holmlund, American Cyanamid Co. U.S. 3,701,787. October 31, 1972.

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II. Personal Information:

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Place of Birth: Worcester, Massachusetts

Married, two children

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Phone: 301-454-4412

Education:

B.S.	Worcester Polytechnic Institute	1943
M.S.	Worcester Polytechnic Institute	1951
Ph.D.	University of Wisconsin, Madison; Biochemistry	1954

Wattanasin : Interference 102,648
v. :
Fujikawa et al : EIC M. Sofocleous

Wattanasin : Interference 102,975
v. :
Fujikawa et al : EIC M. Sofocleous

Chester E. Holmlund

III. Academic Activities:

A. Percentage of official time (last three years) devoted to:

Instruction 25% Research 70% Other 5%

B. Positions held:

Higher Education:

Research Assistant, University of Wisconsin, Madison	1951-54
Associate Professor, Dept. of Chemistry, Univ. of Maryland	1967-70
Professor, Dept. of Chemistry, University of Maryland	1970-present

Other than higher education:

Research Chemist, E. I. DuPont deNemours Company	1946-47
Research Chemist, United States Envelope Co.	1948-51
Research Biochemist, Department of Mycology, Lederle Laboratories, American Cyanamid Co.	1954-57
Group Leader, Fermentation Biochemistry & Microbiology Departments, Lederle Laboratories, American Cyanamid Co.	1957-67

Wattanasin : Interference 102,648
v. :
Fujikawa et al : EIC M. Sofocleous

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v. :
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C. E. Holmlund - Publications

IV. Publications:

1. "Bacterial levans of Intermediate Molecular Weight, " J.R. Mattoon, C.E. Holmlund, S.A. Schepartz, J.J. Vavra, and M.J. Johnson, Appl. Microbiol. 3, 321 (1955).

2. "Chemical Hydroxylation of 12α-Deoxytetracycline," C.E. Holmlund, W.W. Andres and A.J. Shay, J. Am. Chem. Soc. 81, 4748 (1959).

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C. E. Holmlund - Curriculum Vita

B. Other Creative and Scholarly Activities:

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3. Pregnanes. C.E. Holmlund, L.D. Feldman, H.M. Kissman, and M.J. Weiss, American Cyanamid Co., U.S., 3,047569, July 31, 1962.

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5. 16α-hydroxylation of Steroids by Staurophoma Species. C.E. Holmlund, R.J. Blank, and R.H. Evans, American Cyanamid Co. U.S., 3,071,516. January 1, 1963.

6. 6β-hydroxy Steroids. C.E. Holmlund, L.I. Feldman, R.H. Evans, S. Berstein, and J.P. Dusza. American Cyanamid Co. U.S., 3,071,516. January 1, 1963.

7. 14α-Hydroxyestrone and Its 3-acetyoxy Ester. C.E. Holmlund, L.I. Feldman, K.J. Sax, and R.H. Evans. American Cyanamid Co. U.S., 3,214,448. Ocotber 26, 1965.

8. Hydroxylation of 19-Norandrostenedione. C.E. Holmlund, L.I. Feldman, K.J. Sax and R.H. Evans, American Cyanamid Co. U.S., 3,243,355. March 29, 1966.

9. Preparation of 1,2-Disubstituted Steroids. L.I. Feldman, C.E. Holmlund, and K.J. Sax. American Cyanamid Co. U.S., 3,297,687. January 19, 1967.

10. Method of Preparing 16-Oxygenated Derivatives of Estr-y-en-3-one. K.J. Sax, R.H. Blank, C.E. Holmlund and R.H. Evans, Jr. American Cyanamid Co. U.S. 3,329,579. July 4, 1967.

11. Fusarium Fermentation, R.H. Evans, Jr., M.P. Kunstmann, C.E. Holmlund, and G.A. Ellestad. American Cyanamid Co. U.S. 3,546,073. December 8, 1970.

12. Preparation of 5,6-dihydro-5-hydroxy-6-propenyl-2-pyrone by Fermentation and Derivatives Thereof. R.H. Evans, J., and C.E. Holmlund, American Cyanamid Co. U.S. 3,701,787. October 31, 1972.

13. Tetracyclic Lactone Antifungal Agents. C.E. Holmlund, R.H. Evans, Jr., and G.E. Ellestad. American Cyanamid Co. U.S. 3,564,019. February 16, 1971.

#38 + #93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN v. FUJIKAWA ET AL.

INTERFERENCE NO. 102,648
INTERFERENCE NO. 102,975

FYI

WATTANASIN CONSOLIDATED RECORD

VOLUME I

[PAGES 1 - 135]

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WATTANASIN CONSOLIDATED RECORD

INTERFERENCE NO. 102,648
INTERFERENCE NO. 102,975

TABLE OF CONTENTS

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(1) WATTANASIN WITNESS INDEX

DECLARATIONS AND CROSS-EXAMINATION DEPOSITIONS

PURSUANT TO 37 CFR §1.672

PAGE

(2) WATTANASIN EXHIBIT INDEX

TITLE	DESCRIPTION	PAGE*
Exhibit A-1:	1984 Proposal of Sompong Wattanasin dated November 28, 1983	23 (138)
Exhibit A-2:	1985 Proposal of Sompong Wattanasin dated November 19, 1984	24 (138)
Exhibit A-3:	Patent Disclosure No. 299/84	24-26 (63, 139, 213, 221-28, 228, 231, 240-41, 257, 266-68, 270-71, 275, 279-81, 283-85, 292-93, 296-98, 301-302, 306, 310, 319-23, 333-335, 341, 380-85, 457)
Exhibit B-1:	Sompong Wattanasin Laboratory Notebook No. 1049, pages 237, 241, 245, 248, 251, and related spectra pages; Sompong Wattanasin Laboratory Notebook No. 1079, pages 22, 24, 27, 30, 33, 34, 39, 105, 106, 110, 111, and related spectra pages	27, 29, 30-44 (58, 142-43, 173, 175-78)
Exhibit B-2:	Sompong Wattanasin Laboratory Notebook No. 1127, pages 5, 9, 11, and related spectra pages	27, 48-52 (58, 104-05, 173, 176-77)

* Page(s) of record where exhibit is introduced into evidence; additional page numbers in parenthesis indicate further locations in record where exhibit is referenced, either by number or subject matter.

TITLE	DESCRIPTION	PAGE
Exhibit Z:	Cover pages of the following representative Sandoz issued patents in the HMG-CoA Reductase Inhibition area:	228-29

Case No. 600-7013:
U.S. Patent No. 4,588,715
(Heptenoic Acid Analogs of HMG-CoA Reductase Inhibitors)
filed June 4, 1984;

Case No. 600-6951/B/CONT:
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U.S. Patent No. 4,613,610
(Pyrazole)
filed June 6, 1985;

Case No. 600-7035/B:
U.S. Patent No. 4,851,427
(Pyrrole)
filed October 15, 1986

Case No. 600-7050:
U.S. Patent No. 4,751,235
(Indolizine)
filed December 23, 1986;

Case No. 600-7028/B/CONT:
U.S. Patent No. 4,755,606
(Imidazolyl)
filed June 29, 1987;

Case No. 600-7064:
U.S. Patent No. 4,822,799
(Pyrazolopyridine)
filed January 27, 1988;

Case No. 600-6955/XN/B/CONT/X:
U.S. Patent No. 4,876,280
(Arylcyclohexane)
filed March 10, 1988;

TITLE	DESCRIPTION	PAGE
	Case No. 600-7022/C: U.S. Patent No. 5,001,255 (Indene) filed March 10, 1988.	

ADDENDUM: WATTANASIN EXHIBITS FOR GIESSER CROSS-EXAMINATION DEPOSITION (REDIRECT)

Title	Description	Page
Exhibit S-1:	Eight loose pages from drafts of Wattanasin application	270 ✓ 372 ✓
Exhibit S-2:	Wattanasin et al., Publication Clearance Request and Abstract dated March 30, 1989; Giesser clearance letter dated April 19, 1989;	
	Publication Clearance Request dated May 17, 1989 and Giesser clearance letter of June 13, 1989	129-30 ✓ 372 ✓
Exhibit S-3:	Case No. 600-7025/CIP: Office action of May 11, 1988; Notice of Abandonment of Jan. 3, 1989; Request for Extension of Time	397 ✓
Exhibit S-4:	Case No. 600-7044/CONT: Notice of Allowance dated January 3, 1989; Notice of Abandonment dated June 19, 1989	402 ✓

(3) CROSS-REFERENCE INDEX OF:
PARTIES' EXHIBITS MARKED FOR IDENTIFICATION
AT CROSS-EXAMINATION DEPOSITIONS
WITH DOCUMENTS OF RECORD

CROSS-EXAMINATION DEPOSITION EXHIBIT	DOCUMENT OF RECORD	PAGE*
... Corresponds to .. [ ] ...		
WATTANASIN EXHIBITS		
W-1	[Wattanasin Exhibits M-1 - M-5]	283 ~~82~~
W-2	[Fujikawa Kitahara Declaration and Supplemental Declaration]	100 ✓
W-3	[Excerpt from Wattanasin Exhibit B-2]	104 ~~283~~
S-1	[Eight loose pages from drafts of Wattanasin application].....	372 ✓
Wattanasin Additional Exhibits		
S-2	Wattanasin et al., Publication Clearance Request and Abstract dated March 30, 1989; Giesser clearance letter dated April 19, 1989; Publication Clearance Request dated May 17, 1989 and Giesser clearance letter of June 13, 1989	372 ✓
S-3	Case No. 600-7025/CIP: Office action of May 11, 1988; Notice of Abandonment of Jan. 3, 1989; Request for Extension of Time	397 ✓
S-4	Case No. 600-7044/CONT: Notice of Allowance dated January 3, 1989; Notice of Abandonment dated June 19, 1989	402 ✓

* Page of record where marked for identification at deposition.

CROSS-EXAMINATION DEPOSITION EXHIBIT	DOCUMENT OF RECORD	PAGE
... Corresponds to .. [	] ...	
FUJIKAWA EXHIBITS		
F-1	[Kassenoff R. 672 Declaration] .	235
F-2	[Wattanasin Exhibit N]	259
F-3	[Wattanasin Exhibit O]	261
F-4	[Wattanasin involved application]	70
F-5	[Wattanasin Request for Interference with Warner-Lambert U.S. Patent No. 4,761,419]	74
F-6	[Wattanasin Rule 672 Supplemental Declaration] ..	79
F-7	[Wattanasin Exhibit P-2]	89
F-8	[Wattanasin Exhibit P-3]	90
F-9	[Rothwell R. 672 Declaration]...	219
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F-22	[Exhibit S]	357

(4) INDEX OF PUBLICATIONS

*Page of record.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v.

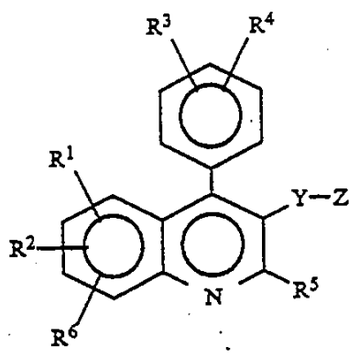
FUJIKAWA et al.

Interference No. 102,975
Examiner-in-Chief: M. Sofocleous

COUNT

[Count 1]

A compound of the formula:



wherein R^1, R^2, R^3, R^4 and R^6 are independently
hydrogen,
C_{1-6} alkyl,
C_{1-6} cycloalkyl,

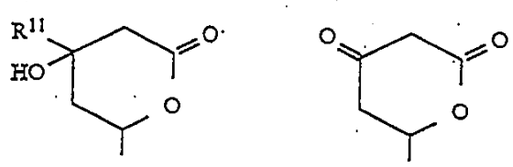
C_{1-3} alkoxy,
n-butoxy,
i-butoxy,
sec-butoxy,
R^7R^8N- (wherein R^7 and R^8 are independently hydrogen or C_{1-3} alkyl),
trifluoromethyl,
trifluoromethoxy,
difluoromethoxy,
fluoro,
chloro,
bromo,
phenyl,
phenoxy,
benzyloxy,
hydroxy,
hydroxymethyl,
$-O(CH_2)_\alpha OR^{19}$ (wherein R^{19} is hydrogen or C_{1-3}alkyl and α is 1, 2 or 3),
or when located at the ortho position to each other, R^3 and R^4 together optionally form -CH=CH-CH=CH-;

R^5 is hydrogen,
C_{1-6} alkyl,
C_{2-3} alkenyl,
C_{3-6} cycloalkyl,
phenyl substituted by R^9 (wherein R^9 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxy, fluoro, chloro, bromo or trifluoromethyl),
phenyl-$(CH_2)_m$- (wherein m is 1, 2 or 3), $-(CH_2)_nCH(CH_3)$-phenyl or phenyl-$(CH_2)_nCH(CH_3)$- (wherein n is 0, 1 or 2).

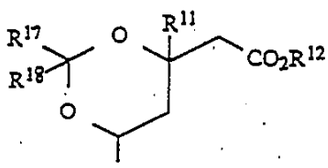
Y is

$-CH_2-$,
$-CH_2CH_2-$,
$-CH=CH-$,
$-CH_2-CH=CH-$, or
$-CH=CH-CH_2-$;

Z is



or



or $-Q-CH_2WCH_2-CO_2R^{12}$ (where R^{12} is hydrogen or R^{14});

Q is $-CH(OH)-$.
$-C(O)-$, or
$-C(OR^{13})_2-$;

W is $-C(R^{11})(OH)-$ (where R^{11} is hydrogen or C_{1-3} alkyl),
$-C(O)-$, or
$-C(OR^{13})_2-$;

the two R^{13} are independently primary or secondary C_{1-6} alkyl; or two R^{13} together form $-(CH_2)_2-$ or $-(CH_2)_3-$;

R^{14} is physiologically hydrolyzable alkyl or M (wherein M is NH_4, sodium, potassium, 1/2 calcium or a hydrate of lower alkylamine, di-lower alkylamine or tri-lower alkylamine); and

R^{17} and R^{18} are independently hydrogen or C_{1-3} alkyl,

The claims of the party Wattanasin which correspond to count 1 are claims 1-7 and 10.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

Interference No. 102,648

v.

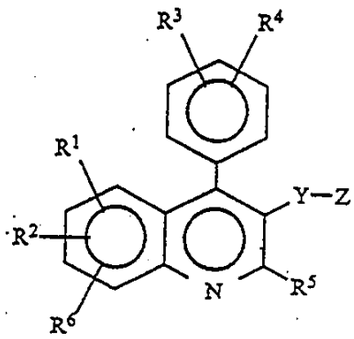
Examiner-in-Chief: M. Sofocleous

FUJIKAWA et al.

COUNT

[Count 3]

A method of inhibiting cholesterol biosynthesis in a patient in need of said treatment comprising administering a cholesterol synthesis inhibiting amount of a compound of the formula:



wherein R^1, R^2, R^3, R^4 and R^6 are independently
hydrogen,
C_{1-6} alkyl,
C_{1-6} cycloalkyl,

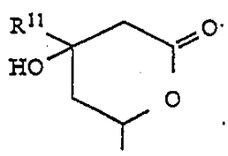
C_{1-3} alkoxy,
n-butoxy,
i-butoxy,
sec-butoxy,
R^7R^8N- (wherein R^7 and R^8 are independently hydrogen or C_{1-3} alkyl),
trifluoromethyl,
trifluoromethoxy,
difluoromethoxy,
fluoro,
chloro,
bromo,
phenyl,
phenoxy,
benzyloxy,
hydroxy,
hydroxymethyl,
$-O(CH_2)_\alpha OR^{19}$ (wherein R^{19} is hydrogen or C_{1-3}alkyl and α is 1, 2 or 3),
or when located at the ortho position to each other, R^3 and R^4 together optionally form $-CH=CH-CH=CH-$;

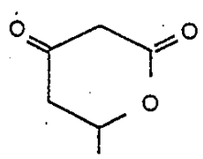
R^5 is hydrogen,
C_{1-6} alkyl,
C_{2-3} alkenyl,
C_{3-6} cycloalkyl,
phenyl substituted by R^9 (wherein R^9 is hydrogen, C_{1-4}alkyl, C_{1-3}alkoxy, fluoro, chloro, bromo or trifluoromethyl),
phenyl-$(CH_2)_m$- (wherein m is 1, 2 or 3),
$-(CH_2)_nCH(CH_3)$-phenyl or phenyl-$(CH_2)_nCH(CH_3)$- (wherein n is 0, 1 or 2).

Y is

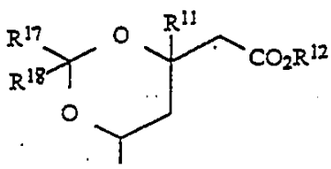
$-CH_2-$,
$-CH_2CH_2-$,
$-CH=CH-$,
$-CH_2-CH=CH-$, or
$-CH=CH-CH_2-$;

Z is





or



or $-Q-CH_2WCH_2-CO_2R^{12}$ (where R^{12} is hydrogen or R^{14});

Q is $-CH(OH)-$.
$-C(O)-$, or
$-C(OR^{13})_2-$;

W is $-C(R^{11})(OH)-$ (where R^{11} is hydrogen or C_{1-3} alkyl),
$-C(O)-$, or
$-C(OR^{13})_2-$;

the two R^{13} are independently primary or secondary C_{1-6} alkyl; or two R^{13} together form $-(CH_2)_2-$ or $-(CH_2)_3-$;

R^{14} is physiologically hydrolyzable alkyl or M (wherein M is NH_4, sodium, potassium, 1/2 calcium or a hydrate of lower alkylamine, di-lower alkylamine or tri-lower alkylamine); and

R^{17} and R^{18} are independently hydrogen or C_{1-3} alkyl;

as defined in combination with pharmaceutically acceptable carrier.

The claims of the party Wattanasin which correspond to count 3 are claims 8 and 9.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF SOMPONG WATTANASIN PURSUANT TO 37 CFR §1.672

I, Sompong Wattanasin, Ph.D., do hereby declare as follows:

(1) That I am the inventor of the subject matter contained in U.S. patent application Serial Number 07/498,301.

(2) That based upon the information provided in this Declaration, I believe that I am entitled to a judgment relative to Fujikawa et al., U.S. patent application Serial No. 07/233,752.

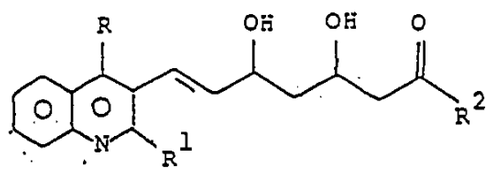
(3) That I am currently a Senior Associate Fellow employed by Sandoz Pharmaceuticals Corporation, 59 Route 10, East Hanover, New Jersey. At the time during which I conceived and reduced the invention of the above-identified patent application to practice, I was a Senior Scientist A. My job responsibilities included the invention and synthesis of compounds which are inhibitors of 3-hydroxy-3-methyl- glutarylcoenzyme A reductase (HMG-CoA Reductase), an enzyme which is involved in cholesterol biosynthesis.

(4) That prior to August 20, 1987, I conceived and reduced to practice my invention in the United States.

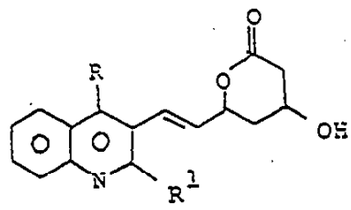
All the activities described in this Declaration took place in the United States.

I. CONCEPTION PRIOR TO AUGUST 20, 1987

(1) On or before November 28, 1983, I conceived of the following compounds:



(I)



(II)

where R = phenyl, 3,5-dimethylphenyl, 4-fluorophenyl, or isopropyl

R^1 = methyl, isopropyl, or 4-fluorophenyl

R^2 = an ester group, a salt, an acid

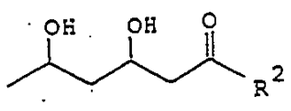
It was preferred that the open chain compounds be in the form of the 3R,5S isomer, or be in the erytho racemic form. For lactone compounds, I preferred the 4R,6S isomer or the trans racemic form.

(2) I made the first drawing or written description of the invention on or before November 28, 1983, when I proposed to Dr. Kathawala to synthesize compounds of the invention from previously synthesized intermediates and commercially available compounds for formulation into compositions for use as HMG-CoA reductase inhibitors.

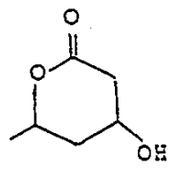
Exhibit A-1 documents my first drawing or written description of my invention, and is a true copy of a research report I authored.

The pages which comprise Exhibit A-1 are written in my handwriting. The first page contains my signature and the date of November 28, 1983 in my handwriting. I sent a copy of this report to Dr. Kathawala on November 28, 1983 and also orally disclosed the substance of the report to Dr. Faizulla Kathawala on or before November 28, 1983.

This exhibit outlines the following year's projects. It explains that the coordinated search for compounds having HMG-CoA reductase activity should be centered in four major areas. On the last page, a compound designated compound 14, which makes up part of this invention is proposed. In this proposal's formulae, "L" indicates either of the side chains:



and



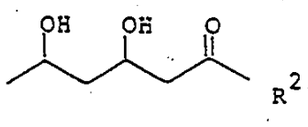
where R^2 = an acid, a salt or an ester.

I also intended that the preferred open chain form be the 3R,5S form or the erythro racemate, and the 4R,6S or trans racemate for lactones.

Exhibit A-2 documents a further written description of my invention, and is a true copy of another research report I authored.

The pages which comprise Exhibit A-2 are written in my handwriting. The first page contains my signature and the date of November 19, 1984 in my handwriting. I sent a copy of this report to Dr. Kathawala on November 19, 1984 and also orally disclosed the substance of the report to Dr. Faizulla Kathawala on or before November 19, 1984.

This report outlines plans for the following year's research. On the first page, the following compounds are proposed. In this proposal's formulae, "L" again indicates these side chains, with R^2 and the stereochemistry the same as above.



and

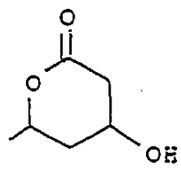
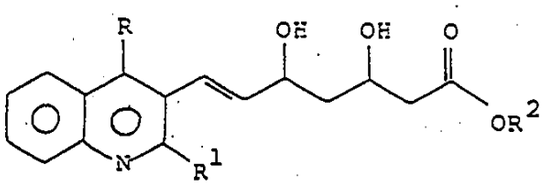
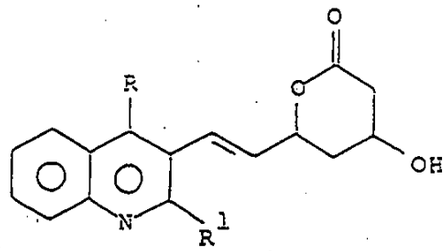


Exhibit A-3 comprises a true copy of an Invention Disclosure Form which I authored. The Form contains my signature and my date of signature of March 16, 1987. I had this document witnessed by Dr. Faizulla Kathawala. I then sent this document to the Sandoz Patent and Trademark Department. Two representative formulae are presented, as follows:



(I)



(II)

where R = 3,5-dimethylphenyl or isopropyl (abbreviated i-Pr)

R^1 = methyl, isopropyl or 4-fluorophenyl

R^2 = ethyl (abbreviated Et).

The second and third pages of the Invention Disclosure were also authored by me and are in my handwriting. I attached these pages to the Invention Disclosure Form before sending it to the Patent and Trademark Department. Two methods of synthesizing compounds are shown on these pages. The method of the second page yields both compounds of Formula I and II, whereas the method on the third page yields only compounds of Formula I.

While the Invention Disclosure Form only indicates the ester compounds, I intended the ester to be a shorthand designation for salts and acids as well. Since the salts and acids are easily made from the esters (i.e. by simple hydrolysis), and since these types of reactions were fully explained in other patent applications on which I am a named inventor, I did not include these in the

Invention Disclosure although I considered them part of my invention. Also at this time, and based on my experience with the chemistry of other HMG-CoA reductase inhibitors, I expected that for a given compound, the lactone would be less active than an open side chain and that the acid form, ester form and salt form would show approximately the same activity. Therefore, I considered the acids, esters and salts to be equivalents and for brevity's sake would only generally draw one of them when referring to all three.

II. ACTUAL REDUCTION TO PRACTICE OF MY INVENTION PRIOR TO AUGUST 20, 1987

(1) Compounds of my invention were actually reduced to practice by me and by chemists working under my supervision in the United States prior to August 20, 1987.

(2) For details of work performed under my supervision, and not by me personally, reference is made to Exhibits F-1 and L-1 hereto, which I have reviewed and which to my knowledge comprises the notebook pages of Rajeshvari Patel.

(3) For details concerning the biological activities of compounds of my invention, reference is made to Exhibit E-1 to E-5 hereto which I have reviewed and which to my knowledge contains the assay work of Dr. Terence Scallen

of the University of New Mexico and Dr. Robert Damon of Sandoz; and to Exhibit K-1 hereto, which I have reviewed and which to my knowledge contains the in vivo data obtained by Mr. Robert Engstrom of Sandoz.

A. FIRST ACTUAL REDUCTION TO PRACTICE PRIOR TO AUGUST 20, 1987

(1) Synthesis of Compound 63-366

On or before May 31, 1984, I began to reduce my invention to practice.

On or before November 15, 1984, I synthesized compound 1079-111-19 (subsequently redesignated compound 63-366, comprising an erythro racemate), a compound within the scope of my invention.

In accordance with standard company procedures, I recorded my laboratory activities relating to the preparation of compounds of the invention in a laboratory notebook. It was my practice to sign and date each notebook page on the same day the work described on the page was performed.

Exhibits B-1 and B-2 hereto comprise true copies of my laboratory notebook pages, which include copies of NMR spectra for the final product synthesized, as well as the intermediates. (On some of the notebook pages, micro-

analysis data were affixed to the laboratory notebook page subsequent to the date the actual synthesis was performed.)

Detailed Description of Laboratory Notebook Pages

i. Designation of Compounds:

Intermediates and final compounds are referred to in the notebooks by a three part number. The first number is the notebook number, the second is the page of the notebook where the compound appears, and the third number is the line of the page. Thus, compound 1049-237-19 is the entity appearing in Laboratory Notebook 1049, page 237, line 19.

ii. Spectra, Microanalyses and TLC:

The spectra and microanalyses were not performed by me, but were performed by an employee of the Physical Organic Chemistry Department of Sandoz Pharmaceuticals Corporation.

Procedures used to obtain these spectra and micro-analyses are detailed in Section V below. Reference is also made to Exhibits C-2, C-3, D-2, G-1, G-2 and H-1 hereto which I have reviewed, and which to my knowledge reflects work performed under the supervision of Dr. S. Barcza of Sandoz.

The copies of the spectra which follow the relevant notebook pages are to my best knowledge, true copies of the results I received from the Physical Organic Chemistry Department. When I received a spectrum from the Physical Organic Chemistry Department, I would file it according to its compound number. For convenience in this Declaration, the spectra have been placed after the relevant notebook pages.

All spectra in Exhibits B-1 and B-2 bear dates prior to August 20, 1987. For microanalyses, the percentages obtained by the Physical Organic Chemistry Department were sent to me and I copied these values into my notebook pages.

All microanalyses in Exhibits B-1 and B-2 were performed prior to August 20, 1987.

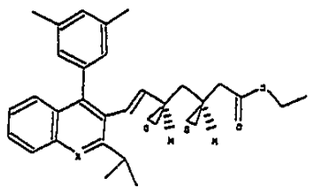
Thin Layer Chromatography (TLC) was performed by me. The entries in the laboratory notebook pages are my drawings of the results I obtained. All the TLCs in Exhibits B-1 and B-2 were performed by me or under my supervision and were recorded in my notebooks prior to August 20, 1987.

Additionally, Section VI. below describes the Sandoz procedure for assigning company numbers to compounds, which I followed; and Section VII. describes the procedures used for determining biological activity of the compounds of the invention.

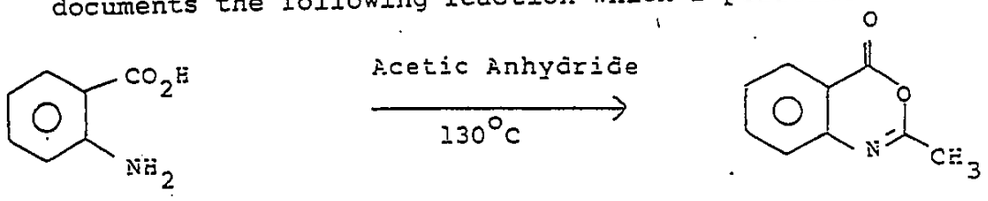
EXHIBIT B-1

Exhibit B-1 comprises true copies of my Laboratory Notebook #1049, pages 237, 241, 248, 251 and Laboratory Notebook #1079, pages 22, 24, 27, 30, 33, 34, 39, 105, 106, 110 and 111 along with copies of spectra, and microanalysis data corresponding to the intermediate and final products.

These pages show the synthesis of the following compound, which was given the designation 63-366:



Notebook #1049, page 237 contains my signature and the date of May 29, 1984 in my handwriting. This page documents the following reaction which I performed.

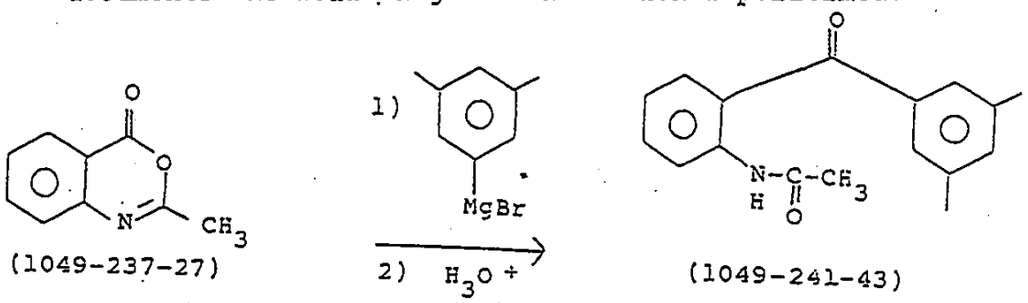


(1049-237-27)

A mixture of 10 g anthranilic acid and 54 ml acetic anhydride was heated at 130°C for 30 minutes at 12:00 P.M.

Then, approximately 30 ml of the acetic anhydride was removed. The residue was cooled to give a yellow solid. Recrystallization from acetic anhydride gave 8.9 g of a pale yellow solid, designated 1049-237-19. 1049-237-19 was dissolved in ether and filtered through a pad of silica gel. Evaporation gave 7.0 g of a colorless solid, melting point 76-78°C, designated 1049-237-27. NMR, IR, and microanalysis were performed on 1049-237-27. The spectra follow page 237, and the results of the microanalysis is reported on page 237. The spectra and microanalysis were judged by me to be consistent with the desired product.

Notebook #1049, page 241 contains my signature and the date of May 31, 1984 in my handwriting. This page documents the following reaction which I performed:



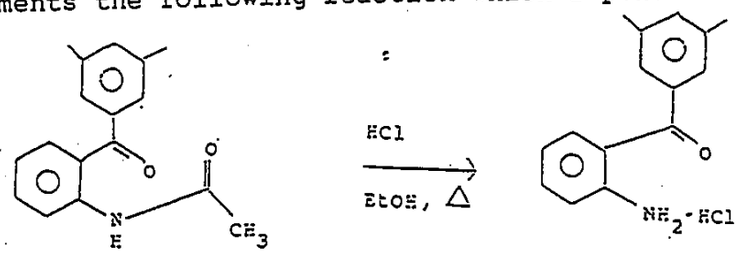
(1049-237-27) (1049-241-43)

To a suspension of 446 mg Mg (0.0186 mol) in 2 ml ether and a few drops of I_2 at room temperature was added a few drops of 1,2-dibromoethane, followed by a solution of 3.44 g (0.0186 mol) 5-bromo-m-xylene in 8 ml ether dropwise (at a rate such that the reaction mixture refluxed gently). This began at 9:05 A.M. and continued until 9:45 A.M. The reaction mixture was then heated at

reflux for 3 hours. Then the Grignard reagent was withdrawn by syringe (approximately 8 ml) and added to a solution of 2 g 1049-237-27 (0.0124 mol) in 10 ml benzene and 2 ml ether dropwise (via a funnel).

The next morning, the reaction mixture was quenched with 3N HCl and extracted with EtOAc and evaporated to give a 3.6 g of a yellow oil, designated 1049-241-31. Preparative TLC of 300 mg of 1049-241-31 gave two products: (a) 128 mg of a colorless oil, designated 1049-241-34; and (b) 24 mg of a white solid designated 1049-241-37. HPLC of the 1049-241-31 gave 1.6 g of a product, designated 1049-241-43. An NMR spectrum was performed on 1049-241-34 and follows Laboratory Notebook #1049, page 241. The spectra was judged by me to be consistent with the desired product.

Notebook #1049, page 248 contains my signature and the date of June 6, 1984 in my handwriting. This page documents the following reaction which I performed:

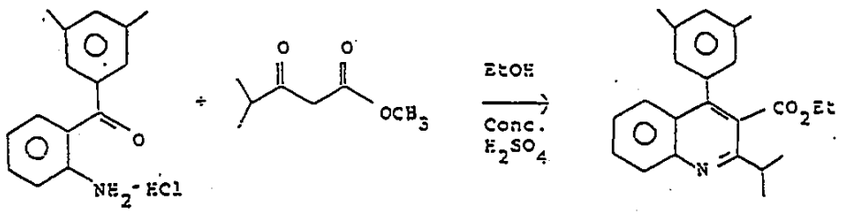


(1049-241-43) (1049-248-24)

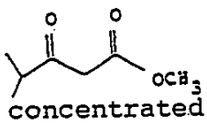
A solution containing 1.6 g of 1049-241-43, 20 ml ethyl alcohol, and 0.5 ml concentrated HCl was heated at 90°C. This began at 8:50 AM and lasted until 4:00 P.M. A TLC showed that there was a very small amount of starting material remaining. The solution was concentrated and the

residue was extracted in ether and filtered to give 1.15 g of a pale yellow solid, which was designated 1049-248-24.

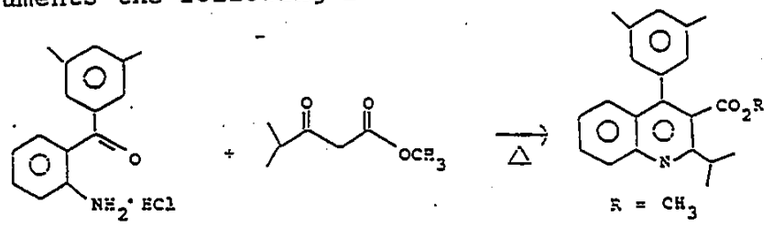
Notebook #1049, page 251 contains my signature and the date of June 8, 1984 in my handwriting. This page documents the following reaction which I performed:



(1049-248-24) (1049-251-29)

500 mg (0.001912 mol) of 1049-248-24, 412 mg of  , 20 ml of ethyl alcohol, and 0.1 ml concentrated HCl were reacted according to the same procedure as set forth in Notebook #1049, page 245 (which is set forth below). The reaction was started at 8:50 A.M. and continued until 12:30 P.M. The product was concentrated, basified with NH_4OH, diluted with H_2O, extracted with ether and evaporated to give 720 mg of an oil. A preparative TLC using 20% ether-petroleum ether showed one main band. The yield was 565 mg which upon standing in the refrigerator solidified into a pale yellow solid with a melting point of 82-83°C, which was designated 1049-251-29. Microanalysis was performed on 1049-251-29 and the results were recorded on page 251. The results of the microanalysis were judged by me to be consistent with the desired product.

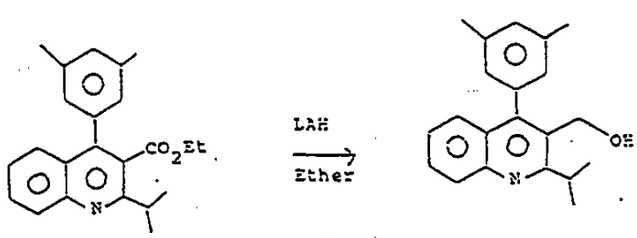
Notebook #1049, page 245, contains my signature and the date of June 5, 1984 in my handwriting. This page documents the following reaction which I performed:



(1049-244-24) (1049-245-23)

The compound on the far left side of the above equation was synthesized and designated 1049-244-24. A solution containing 20 mg of 1049-244-24 (0.0000766 mol), 11 mg (0.000011 mol) of methyl 4-methyl-3-oxopentanoate , 2 ml ethyl alcohol and 1 drop of concentrated H_2SO_4 was heated at reflux. This started at 9:30 A.M. and continued until 5:00 P.M. The product was concentrated, basified with NH_4OH and extracted with ether. The crude oil so obtained was purified by preparative TLC (1:1 ether/petroleum ether) and evaporated to give 17 mg of a colorless oil, designated 1049-245-23. A NMR spectrum was run on 1049-245-23 and follows Laboratory Notebook #1049, page 245. The spectrum was judged by me to be consistent with the desired product.

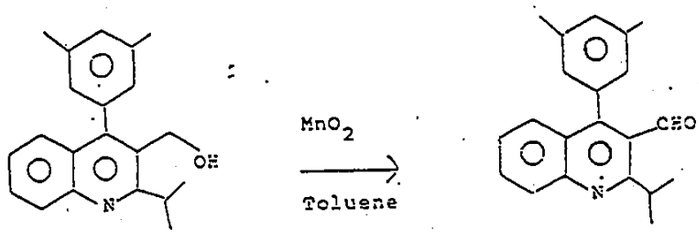
Notebook #1079, page 22 contains my signature and a date of August 10, 1984 in my handwriting. This page documents the following reaction which I performed:



(1049-251-29) (1079-22-28)

To a solution of 535 mg (0.00154 mol) of 1049-251-29 in 8 ml dry ether at room temperature was added 117 mg (0.0091 mol) LAH (lithium aluminum hydride) portion-wise. The mixture was stirred at room temperature and TLC was performed to check the progress of the reaction. The reaction began at 9:15 A.M. and was stopped at 10:15 A.M. when TLC showed that the reaction was complete. The reaction was quenched by pouring into cold water. The product was extracted with ether and evaporated. It solidified upon standing to give 427 mg of a colorless solid with a melting point of 115-118°C, designated 1079-22-28. The IR spectrum of 1079-22-28 was run and the results follow Laboratory Notebook #1074, page 22. The spectrum was judged by me to be consistent with the desired product.

Notebook #1079, page 24 contains my signature and a date of August 10, 1984 in my handwriting. This page documents the following reaction which I performed:

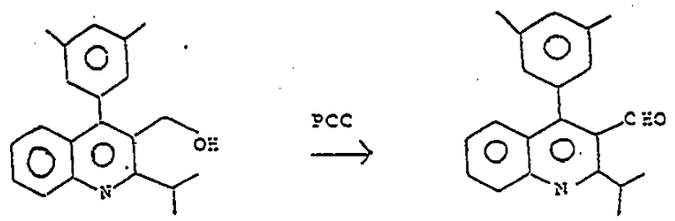


(1079-22-28) (1079-24-24)

A mixture of 420 mg of compound 1079-22-28 and 500 mg of MnO_2 in 6 ml toluene was stirred at room temperature. TLC was performed after 2 days to monitor the progress of

the reaction. The mixture was diluted with ether and filtered through a pad of silica gel. Upon evaporation, a pale yellow solid was obtained, designated 1079-24-24. An NMR spectrum of 1079-24-24 and the results following page 24 indicated that no reaction occurred. A TLC showed that mainly starting material was present, so the crude product, 1079-24-24 was used directly in the reaction set forth below.

Notebook #1079, page 27 contains my signature and the date of August 14, 1984 in my handwriting. This page documents the following reaction which I performed.



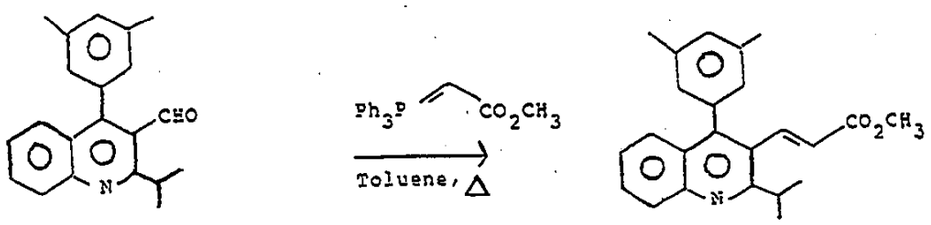
(1079-24-24) (1079-27-25)

382 mg of 1079-24-24, 400 mg DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) and 4 ml toluene are stirred at room temperature overnight. The reaction mixture (a dark red color) was diluted with ether and filtered through a pad of silica gel. Evaporation resulted in a dark red foam gum. TLC indicated no reaction had occurred.

The above gum was dissolved in 10 ml CH_2Cl_2 and 400 mg PCC (pyridinium chlorochromate) and 1 g neutral alumina were added. The mixture was stirred at room temperature

for one hour. TLC showed a complete reaction. The mixture was diluted with ether and filtered through 5 g silica gel. Evaporation of the filtrate gave a pale yellow oil (137 mg) which was designated 1079-27-25. NMR and IR spectra were run on 1079-27-25 and the results follow Laboratory Notebook #1079, page 27. The spectra were judged by me to be consistent with the desired product.

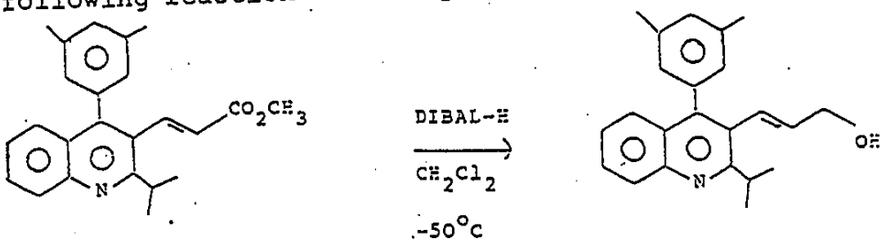
Notebook #1079, page 30 contains my signature and the date of August 17, 1984 in my handwriting. This page documents the following reaction which I performed:



(1079-27-25) (1079-30-23)

140 mg of 1079-27-25, 200 mg of methyl(triphenyl phosphoranylidene)acetate (abbreviated as Ph_3P CO_2CH_3) and 5 ml toluene were heated and refluxed for 3 hours. After cooling, the mixture was diluted with ether and filtered through a pad of silica gel. Concentration gave a semisolid which was further purified by preparative chromatography to give 140 mg of a colorless solid with a melting point of 110-112°C. The product was given the designation 1079-30-23. An NMR spectrum was performed on 1079-30-23 and the results follow Laboratory Notebook #1079, page 30. The spectrum was judged by me to be consistent with the desired product.

Notebook #1079, page 33, contains the date of August 22, 1984 in my handwriting. This page documents the following reaction which I performed:

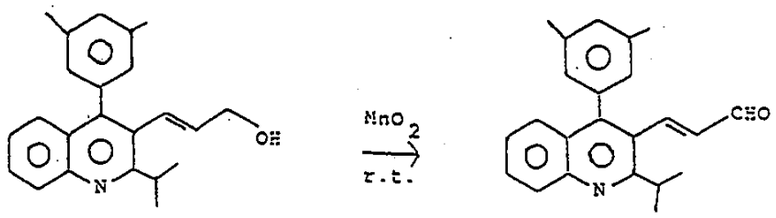


(1079-30-23) (1079-33-19)

To a solution of 130 mg (0.0003768 mol) of 1079-30-23 in 5 ml dry CH_2Cl_2 at -50°C was added 0.5 ml (0.007136 mol) of DIBAL-H. DIBAL-H is the abbreviation I use for diisobutylaluminum hydride. The mixture was stirred at -50°C for 0.5 h. TLC showed that the reaction was complete.

The reaction product was diluted with ether and filtered through a pad of silica gel. Evaporation gave 135 mg of a crude gel which was directly used in the next step. TLC of this crude oil showed only one spot. The reaction product was designated 1079-33-19.

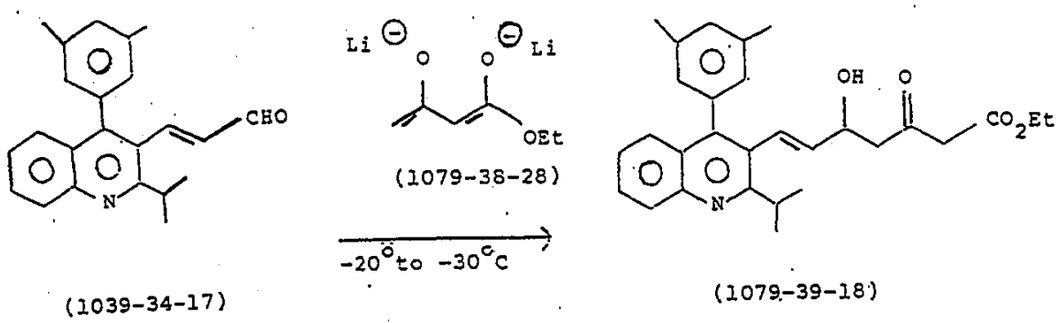
Notebook #1079, page 34 contains my signature and the date of August 23, 1984 in my handwriting. This page documents the following reaction which I performed:



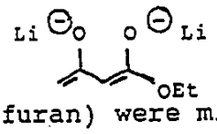
(1079-33-19) (1079-34-17)

A mixture of 135 mg of 1079-33-19 and 300 mg MnO_2 in 5 ml toluene was stirred at room temperature overnight. The result was 107 mg of a pale yellow oil which was designated 1079-34-17. An NMR spectrum was performed on 1079-34-17 and follows Laboratory Notebook #1079, page 34. The spectrum was judged by me to be consistent with the desired product.

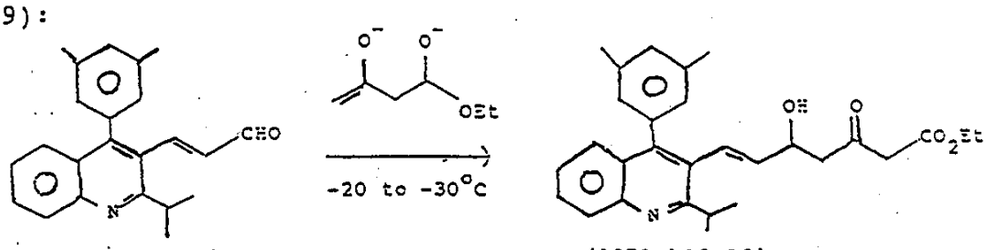
Notebook #1079, page 39 contains my signature and the date of September 5, 1984 in my handwriting. This page documents the following reaction which I performed.



(1039-34-17) (1079-39-18)

At 10:00 A.M., 100 mg of 1079-34-17 (0.0003039 mol), 5 ml of the dianion from 1079-38-28, (≃0.0014 mol) the structure of which is 
and 4 ml THF (tetrahydrofuran) were mixed. By 10:50 A.M. the reaction was complete, as evidenced by one spot on the TLC. The reaction was quenched with saturated NH_4Cl, extracted with ethyl acetate (EtOAc) and evaporated. The result was 177 mg of a yellow oil, which was designated 1079-39-18. The crude 1079-39-18 was reduced in the next step directly without further purification.

Notebook #1079, page 105 contains my signature and the date of November 8, 1984 in my handwriting. This page documents the following reaction which I performed (This is the same reaction described in Notebook #1079, page 39):



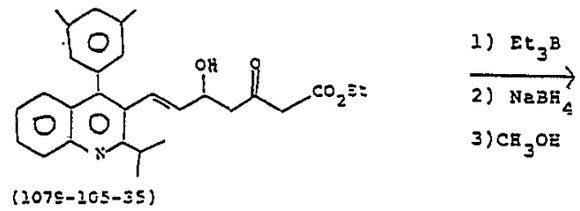
(1079-101-28) (1079-105-35)

The list of reagents on Page 105 includes 0.5 ml (0.00334 mol) diisopropylamine and 1.6 M n-BuLi (2 ml, 0.00334 mol). Upon mixing these reagents, lithium diisopropylamide would be formed, and could be used as set forth below. However, I found that the commercially available lithium diisopropylamide in cyclohexane gave equally satisfactory results compared to the lithium diisopropylamide which I synthesized. Therefore, I used the commercially available reagent.

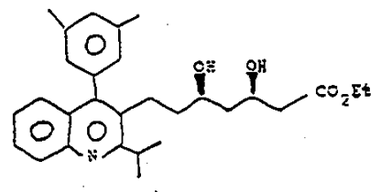
To a solution of lithium diisopropylamide (1.8M in cyclohexane, 1.8 ml) (a commercially available reagent) in THF at -25°C was added 0.22 ml ethyl acetoacetate. The resulting yellow solution was stirred at -20° to -30° for 30 minutes.

4 ml of the above solution was withdrawn by a syringe and added to a solution of 110 mg of the aldehyde designated 1079-101-28 (which was prepared as described for 1079-34-17) in 2 ml THF at -30°C. The solution was stirred at -30° to -10°C for about 20 minutes. The reaction was quenched with 2 ml of saturated NH_4Cl and extracted with EtOAc to give 290 mg of a yellow oil. Prep TLC (1:1 ether-petroleum ether) and evaporation gave 112 mg of a yellow oil, designated 1079-105-35. NMR and IR spectra were performed on this compound and the results follow Laboratory Notebook #1079, Page 105. The results of the spectra were judged by me to be consistent with the desired product.

Notebook #1079, page 106 contains my signature and the date of November 12, 1984 in my handwriting. This page documents the following reaction which I performed:



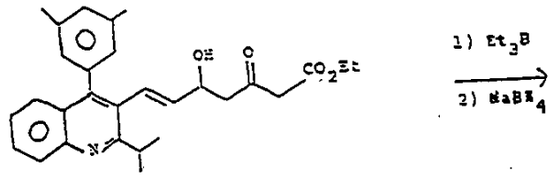
(1079-105-35)



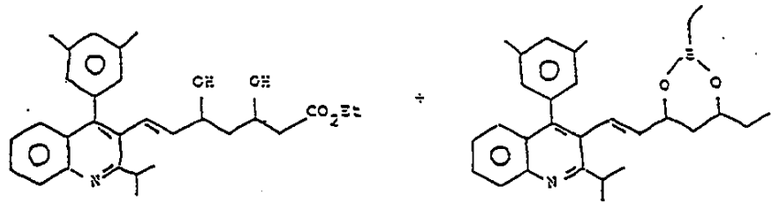
(1079-106-36)

To a solution of 55 mg 1079-105-35 in 2 ml THF at room temperature was added 1M Et_3B (0.0001437 mol) and 1 ml air (by syringe). The solution was stirred at room temperature for 1.5 hr. The solution was then cooled to -78°C and 10 mg $NaBH_4$ was added. The reaction mixture was stirred at -78°C from 10:30 A.M. until 8:30 A.M. the next morning. TLC showed the presence of starting material. 15 mg $NaBH_4$ was added and stirring was continued at -78°C. At approximately 3:20 P.M., the reaction was quenched with dilute HCl and extracted with EtOAc to obtain 50 mg of a crude product designated 1079-106-36, which was used directly in the next step. The crude product probably contained, in addition to the structure shown in the above reaction, some boron-intermediate designated <u>infra</u> as 1079-110-33, and some by-products of the reaction of 1079-106-36 with HCl. 1079-106-36 was believed to be the <u>erythro</u> racemate with the proportion of the 3R,5S isomer to be at least 85% or greater.

Notebook #1079, page 110 contains my signature and the date of <u>November 14, 1984</u> in my handwriting. This page documents the following reaction which I performed, which is similar to that of Laboratory Notebook #1079, page 106:



(1079-105-35)

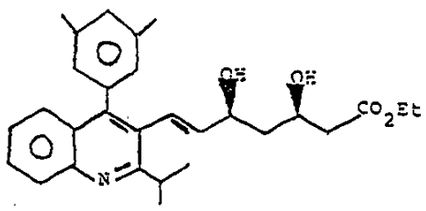


(1079-110-33)

To a solution of 50 mg 1079-105-35 in 2 ml THF at room temperature was added 0.2 ml of 1M Et_3B and 1 ml air (by syringe). The solution was stirred at room temperature from 2:30 to 3:30 P.M., then cooled to -78°C. The following day, the almost colorless reaction mixture was diluted with 4 ml CH_3OH after the cooling bath was removed. After 10 minutes, a slightly fluorescent color occurred, and 1 ml H_2O and a few drops of acetic acid was added. After the evolution of H_2 subsided, the reaction mixture was concentrated. Water was added, the mixture was extracted with ether, and evaporated. The result, 40 mg of an oil which was pale yellow and with some florescence, was designated 1079-110-33 and was used directly in the next step. The oil was believed to contain the two compounds shown, the ester was believed to be the erythro racemate, with at least approximately 85% being the 3R,5S isomer.

Notebook #1079, page 111 contains my signature and the date of November 15, 1984 in my handwriting. This page documents the following reaction which I performed:

1079-110-33 $\xrightarrow{CH_3OH}$



(1079-111-19)

A solution of 40 mg 1079-110-33 in 4 ml CH_3OH was stirred at room temperature for 3 days. TLC (1:1 ether-petroleum ether) showed one main spot of the product. Prep. TLC gave 25.6 mg of a pale yellow oil designated 1079-111-19. IR and NMR spectra were performed on 1079-111-19 and follow Laboratory Notebook, Page 111. The spectra were judged by me to be consistent with the desired product. Compound 1079-11-19 was subsequently redesignated compound 63-366. This redesignation occurred prior to December 13, 1984.

III. TESTING OF COMPOUND 63-366 FOR HMG-COA REDUCTASE INHIBITOR PRIOR TO AUGUST 20, 1987

On or before December 31, 1984, I learned the results of *in vitro* testing of compound 63-366 in an assay for HMG-CoA reductase activity.

Page 111 of my laboratory notebook indicates that on or before November 26, 1984, 14.5 mg of compound 63-366 were sent to Dr. Terence Scallen of the University of New Mexico for testing in his *in vitro* microsomal assay for HMG-CoA reductase inhibition activity.

Compound 63-366 was shown by Drs. Scallen and Damon to inhibit HMG-CoA reductase activity by having an IC_{50} of 1.58.

Having reviewed Exhibit E-5 hereto, my best recollection is that Dr. Damon of Sandoz informed me of this activity either orally or by sending me a copy of the computer printout included in Exhibit E-5 on or before December 31, 1984.

Thus on or before December 31, 1984, I knew that 63-366 had activity in an assay for HMG-CoA reductase activity.

Furthermore, it was my judgment on or before December 31, 1984 that it was likely that said compound 63-366 would have activity in vivo as an HMG-CoA reductase inhibitor, and therefore would have activity when administered to a patient to treat atherosclerosis and other conditions resulting from excessive cholesterol biosynthesis.

By way of background, since prior to December 31, 1984, I had been receiving from Dr. Damon the IC_{50} data he calculated from Dr. Scallen's assays for various other Sandoz compounds being investigated for HMG-CoA reductase inhibitor activity besides the quinoline compounds of my invention.

These other compounds have the same 3,5-dihydroxy heptenoic acid, ester, or salt side chain, or alternatively, lactone form, as the quinoline compounds of my invention. However, these compounds differ by having an organic radical substituent other than a quinoline.

For example, Sandoz compounds containing a substituted napthyl or indole radical, were tested at approximately the same time as compound 63-366, as shown on Exhibit E-5, hereto.

Additionally, on or before December 31, 1984, I knew the IC_{50} values for the compound Mevastatin (Compactin) which was a known HMG-CoA reductase inhibitor for administration to a patient to treat hypercholesterolemia or atherosclerosis. These values were obtained from data generated by Dr. Scallen in the same assays as used to test the quinoline compounds of my invention. (See Exhibit E-5 hereto).

Also, on or before December 31, 1984, I was knew the IC_{50} values for Sandoz compound 62-320/Na (fluvastatin sodium) (see Exhibit E-5), which I knew to be very active in vivo.

[redacted]

Further, on or prior to December 31, 1984, I further knew that there was typically a high correlation between in vitro activity and in vivo activity of Sandoz compounds which had been tested.

Based on my knowledge and experience, it was my judgment since on or prior to December 31, 1984, that Wattanasin compound 63-366 would have activity when administered in vivo to a patient for the treatment of hypercholesteremia or atherosclerosis, and would have activity when administered to a human patient in in a dosage amount recited in my specification at page 35.

It was also my judgment upon receiving the IC_{50} data for each of compounds 63-548, 63-549, 64-933, 64-934/Na, 63-935, and 64-936/Na (0.53), that the quinoline compounds of my invention would have activity as an HMG-CoA reductase inhibitor when administered to a patient, and when administered to a human patient within the dosage amounts taught in my application.

IV. CONTINUING EXPERIMENTAL ACTIVITY UNDER MY SUPERVISION PRIOR TO AND AFTER AUGUST 20, 1987

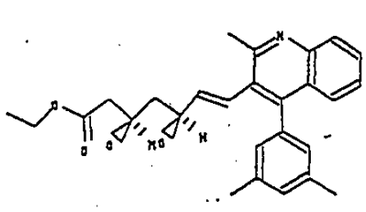
(1) Compounds 53-548 and 53-549

Prior to August 20, 1987, I synthesized compounds 63-548 and 63-549 of the invention.

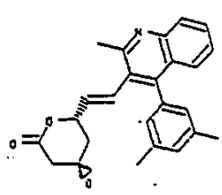
EXHIBIT B-2

Exhibit B-2 comprises true copies of my Laboratory Notebook #1127, pages 5, 9, and 11 along with copies of spectra corresponding to the intermediate and final products. These pages show the synthesis of compounds 63-548 and 63-549. 63-548 is a racemic mixture, with at least 95% being the 3R,5S isomer. Similarly, 63-549 is also a racemic mixture with at least 95% being the 4R,6S isomer. The structures of these compounds are as follows:

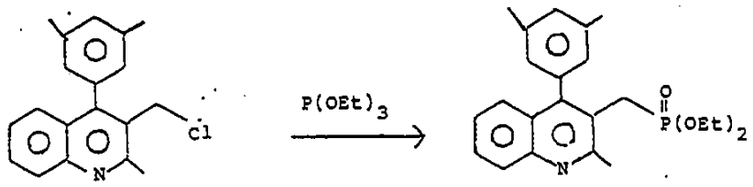
63548



63549



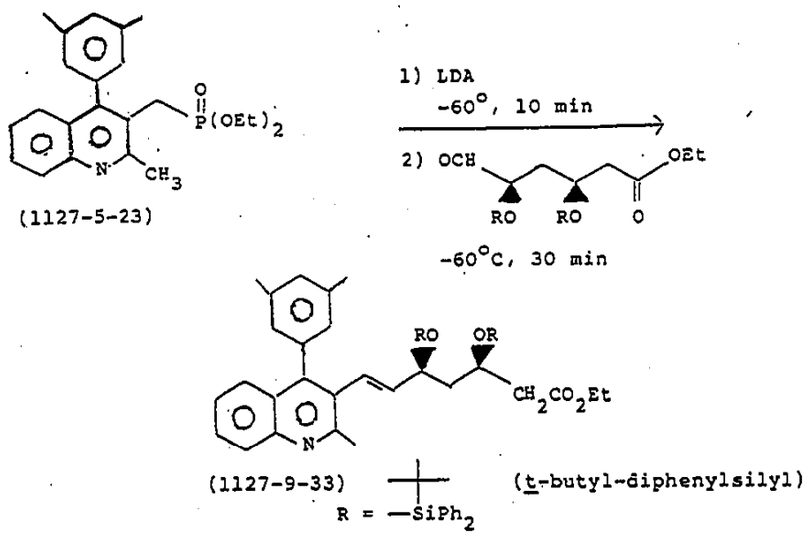
Notebook #1127, page 5 contains my signature and the date of May 2, 1985 in my handwriting. This page documents the following reaction which I performed.



(1079-296-35) (1127-5-23)

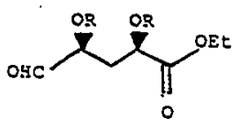
The compound on the left was synthesized by me and given the designation 1079-296-35. A mixture of 1079-296-35 (150 mg) and triethyl phosphite (0.3 ml) in toluene (2 ml) was heated at reflux for approximately 2 hrs. TLC indicated no reaction had occurred. An additional 0.5 ml of triethyl phosphite was added. The reaction was heated at 100°C for 20 hrs. TLC showed a complete reaction. Concentration by distillation at reduced pressure gave 160 mg of an oil which solidified on standing to an almost colorless solid, designated 1127-5-23. Melting point was 105-107° C. NMR and IR spectra were performed on 1127-5-23 and the results follow Laboratory Notebook #1127, page 5. The spectra were judged by me to be consistent with the desired product.

Notebook #1127, page 9 contains my signature and the date of May 6, 1985 in my handwriting. This page documents the following reaction which I performed:



To a solution of 150 mg (0.0003778 mol) of 1127-5-23 in 3 ml THF (tetrahydrofuran) at -55°C was added 0.27 ml (1.2 equivalents) of 1.7 M LDA (lithium diisopropyl amide) in cyclohexanes. The resulting dark orange solution was then stirred at -55°C to -60°C for 10 minutes, from 9:50 A.M. to 10:00 A.M.

The aldehyde having the structure

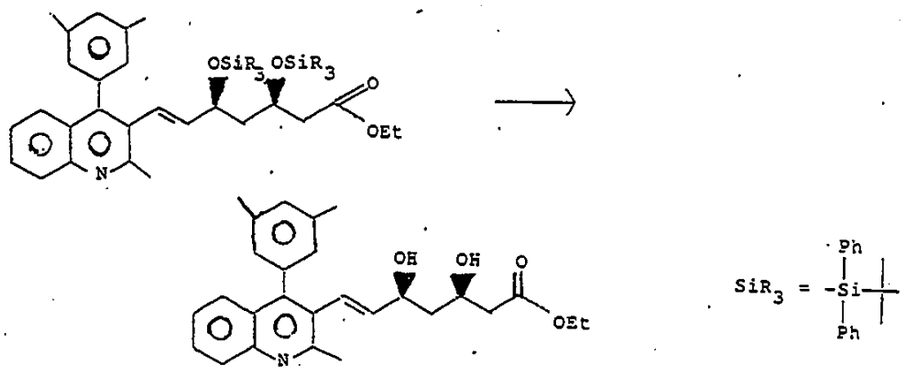


where R= -$SiPh_2$

is termed the "Prasad aldehyde", referring to another chemist at Sandoz, Dr. Prasad Kapa, who made this molecule.

A solution containing 293 mg (0.0004534 mol) of the aldehyde and 2 ml THF is added to the above dark orange solution. A TLC performed after 20 minutes indicated that there was mainly one product formed. After 30 minutes, the reaction was quenched at -60°C with 0.5 ml acetic acid. Then, dilute HCl and H_2O were added, the solution was extracted with EtOAc, and evaporated to yield 500 mg of a yellow oil, designated 1127-9-30. A preparative TLC (1:1 ether/petroleum ether) gave 100 mg of a yellow oil designated 1127-9-33. A NMR was performed on 1127-9-33 and the results follow Laboratory Notebook #127, page 9. The spectrum was judged to be consistent with the desired product.

Notebook #1127, page 11 contains my signature and the date of May 7, 1985 in my handwriting. This page documents the following reaction which I performed:



A mixture of 90 mg (0.0001012 mol) 1127-9-33, 0.61 ml (0.000607 mol) 1M Bu_4NF (tetra-n-butylammonium fluoride) in THF, 0.03 ml (0.0005 mol) HOAc, and 2 ml THF was stirred at room temperature. This began at 9:00 A.M. and a TLC was performed the next morning at 9:00, which indicated that the reaction was not complete. Additional 0.6 ml in Bu_4NF and 0.02 ml of HOAc were added. A second TLC run five days later at 8:30 A.M. indicated that there was a mixture of starting materials and product. The solution was heated at 9:00 A.M. to 50°C to 60°C. A TLC at 11:00 A.M. still showed a mixture of spots. The reaction was stopped at 5:30 P.M. The reaction product was concentrated and the crude oil was purified by a preparative TLC (using ether/HOAc). Two products were obtained; (a) 10 mg of a colorless oil designated 1127-11-34 and (b) 10 mg of an oil designated 1127-11-37. IR and NMR spectra were performed on both of 1127-11-34 and 1127-11-37. The spectra follow Laboratory Notebook #1127, page 11. The spectra were judged by me to be consistent with the desired products.

Compound 1127-11-34 was subsequently renumbered 63-548 and Compound 1127-11-37 was subsequently renumbered 63-549; the renumbering of both compounds occurred on or before March 20, 1985.

[redacted]

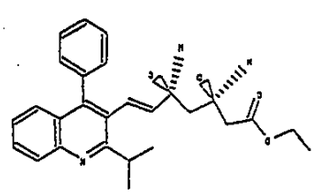
On or prior to May 17, 1985, I sent compounds 63-548 and 63-549 to Dr. Scallen for testing in his in vitro microsomal assay for HMG-CoA reductase inhibition activity. Both compounds were shown by Drs. Scallen and Damon to possess inhibitory activity. Having reviewed Exhibit E-5 hereto, my best recollection is that I learned of the activity of compounds 63-548 and 63-548 from Dr. Damon on or before June 30, 1985.

Based on the in vitro data, it was also my judgment on or before June 30, 1985 that it would be likely that the quinoline compounds of my invention would have activity in vivo as an HMG-CoA reductase inhibitor, and therefore would have activity when administered to a patient to treat atherosclerosis and other conditions resulting from excessive cholesterol biosynthesis.

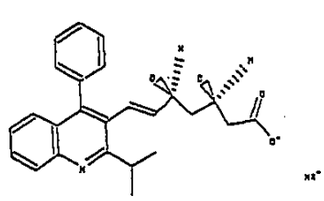
(2) Synthesis of Compounds 64-933, 64-934/Na, 64-935 and 64-936/Na:

Compounds 64-933 and 64-934/Na were synthesized under my direction by Rajeshvari Patel prior to August 20, 1987. These compounds have the following structures:

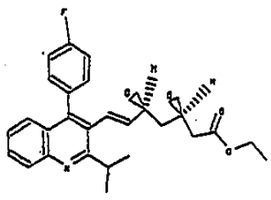
64933



64934



64935



64936

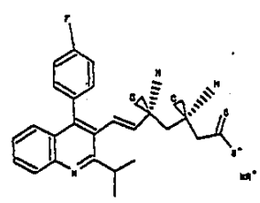


EXHIBIT F-1

Exhibit F-1 comprises pages 130, 137, 145, 153, 158, 166, 172, 175, 176 and 179 of Laboratory Notebook #1206 of Rajeshvari Patel. Each of these pages (except page 179) bear my true signature as a witness.

I witnessed the work performed by Rajeshvari Patel on the pages which I signed. I read and understood the above-numbered laboratory pages which I signed.

Exhibit F-1 indicates that the synthesis of compounds 64-933 and 64-934/Na was commenced on or before June 1, 1987 and was completed on or before August 5, 1987. This is consistent with my general recollection.

I have also reviewed pages 190 and 201 of notebook #1206, which also comprise Exhibit F-1, and Exhibit L-1, hereto. These show the synthesis of compounds 64-935 and 64-936/Na. These pages indicate that the final steps of the synthesis commenced on or prior to August 10, 1987, and it was completed by September 1, 1987. Although I did not perform the work performed on these pages, this time period is consistent with my general recollection as the laboratory supervisor of Rajeshvari Patel.

Testing of Compounds 64-933, 64-934/Na, 64-935 and 64-936/Na

I learned the results of testing of compounds 64-933, 64-934/Na, 64-935 and 64-936/Na in an *in vitro* asssay for HMG-CoA reductase activity.

Compounds 64-933, 64-934/Na, 64-935, and 64-936/Na were sent to Dr. Scallen for testing in his *in vitro* microsomal assay for HMG-CoA reductase inhibition activity. They were shown by Dr. Scallen to possess inhibitory activity.

Having reviewed Exhibit E-5 hereto, my best recollection is that Dr. Damon of Sandoz informed me of this activity either orally or by sending me a copy of the computer printout included in Exhibit E-5 on or before October 31, 1987.

Based on the in vitro data, it was my judgment on or before October 31, 1987 that it was likely that the quinoline compounds of my invention would have activity in vivo as HMG-CoA reductase inhibitors, and therefore would have activity when administered to a patient to treat atherosclerosis and other conditions resulting from excessive cholesterol biosynthesis, and would have activity when administered to a human patient under the dosage conditions recited in my patent application.

I have also reviewed Exhibit K-1 hereto, which contains rat cholesterol biosynthesis data for compounds 64-933, 64-935 and 64-936/Na which were tested by Robert Engstrom. I believe I learned of this data on or before December 9, 1987. This data indicate that the quinoline compounds of my invention would have activity as an HMG-CoA reductase inhibitor when administered to a patient for treatment of hypercholesteremia or atherosclerosis.

V: PROCEDURES FOR OBTAINING SPECTRA AND MICROANALYSES AND MAINTENANCE OF RESULTS

All IR and NMR spectra as well as microanalyses are performed by the Sandoz Physical Organic Chemistry Department. The Department has developed procedures to follow when submitting samples of materials which are to be analyzed. These procedures, described below, were in place prior to and after August 20, 1987, including the time periods referred to herein, and these are the procedures which I followed when I submitted samples of compounds which I made for analysis. For details concerning procedures of the Physical Organic Chemistry Department, reference is made to the work of Dr. Sandor Barcza of Sandoz.

Approximately 1 to 20 mg of the sample was placed in a vial and the vial was labeled with the three-part numerical designation used in the notebooks. A Request Sheet was filled out in duplicate by me or under my supervision, on which it was indicated, among other things, the type of analyses I wished to have performed, and the sample number of the vial.

Exhibit C-1 comprises a copy of a blank Request Sheet.

Upon receipt of the form and sample, the Physical Organic Chemistry Department notes the date of receipt on the form, and assigns its own number to each spectrum run.

This procedure was followed prior to and after August 20, 1987 for each of compounds 64-366, 63-548, 63-549, 64-933, 64-934/Na, 64-935 and 64-936/Na.

Exhibit C-2 comprises true copies of the Sandoz Physical Organic Chemistry Department's Request Sheets for spectra and/or microanalysis before and after the sheets were received and the compounds were assigned a spectrum number. (For the compounds synthesized in Exhibits B-1 and B-2, I was the Requestor. For the compounds synthesized in Exhibits F-1 and L-1, M. Patel is listed as the Requestor.)

Exhibits B-1 and B-2 contain copies of the spectra for the compounds of the invention which I received from the Physical Organic Chemistry Department.

VI. PROCEDURE FOR ASSIGNING COMPANY NUMBERS TO COMPOUNDS

When an end product has been made, an official company number is assigned to the compound and information concerning the compound is entered into the company's computerized database. There is an established procedure in effect for this, both prior to and after August 20, 1987, including the time periods referred to herein, which I followed.

I filled out a "Chemical Information" form for each end product which included such information as the chemical structure, molecular weight, empirical formula, and synthesis procedure as well as the three part number used in the notebook.

Exhibit D-1 comprises a blank "Chemical Information" form.

This form is sent to the Drug Room, which is part of the Sandoz Physical Organic Chemistry Department. Upon receipt of this form, the Drug Room personnel assign the compound a number.

Exhibit D-2 comprises copies of forms submitted by me or under my supervision to the Drug Room for the compounds of the invention. I note that the page for compounds 63-548 and 63-549 bears a date of May 15, 1985 in my handwriting.

Exhibit D-3 comprises a copy of the information which is assembled by the Drug Room personnel and made accessible on the computer database. The "SAH" number is the official compound number. The large type number in the second box in the left column is the internal registry number. The three-part number in the third box in the left column is the notebook, page, and line number. The two-part number in the fourth box in the left column is the number of the patent disclosure which covers the compound.

VI. PROCEDURES FOR DETERMINING BIOLOGICAL ACTIVITY

Sandoz has an established procedure for determining whether end products possess biological activity of interest, e.g. HMG-CoA reductase inhibitor activity which would indicate that they inhibit the biosynthesis of cholesterol and are useful in the treatment of atherosclerosis and other related diseases in a patient.

These procedures were in place prior to and after August 20, 1987, including the time periods referred to herein, and these were the procedures which were followed in determining whether the compounds I invented had such activity.

After the official company number has been assigned to an end product, the compound is tested for biological activity. The Physical Organic Chemistry Department submits the sample of the compounds for biological testing. Some tests are performed in-house; others are performed outside the company. The in vitro testing of my compounds was done by a person who is not employed by Sandoz, Professor Terence Scallen, Department of Biochemistry, School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131.

Dr. Scallen reported his results to Dr. Robert E. Damon at Sandoz. Upon receipt of the reports, Dr. Damon would draw the chemical structure of each test compound on

the report, and calculate the IC_{50} value of each test compound and would write this on the report. Dr. Damon then sent copies of these reports to researchers involved in the project.

Exhibit E-5 comprises true copies of Dr. Scallen's reports which I received, with what I believe to be Dr. Damon's handwritten structures and IC_{50} values, for compounds 63-366, 63-548, 63-549, 64-933, 64-934/Na, 64-935 and 64-936/Na.

. . .

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 13th day of November, 1992.

Sompong Watt——in.
SOMPONG WATTANASIN, Ph.D

Case No. 600-7101/CONT/INT.(1)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

SUPPLEMENTAL DECLARATION OF SOMPONG WATTANASIN, PH.D. PURSUANT TO 37 CFR 1.'672

I, Sompong Wattanasin, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

BACKGROUND

2. Since about 1981, Sandoz Research Institute (SRI) has been engaged in a concerted research effort to develop compounds having utility as HMG-CoA reductase inhibitors for treatment of hypercholesterolemia.

3. Much of this research has focused on compounds which comprise heterocyclic analogs of mevalonolactone and the open chain derivatives thereof.

4. For example, since 1981 SRI has prepared indenyl, indolyl, indolizinyl, imidazolyl, pyrazolopyridinyl, pyrrole, as well as quinolinyl, and other analogs of mevalonolactone and derivatives thereof.

5. The Sandoz research effort culminated in 1992 in the completion of an NDA filing on fluvastatin, i.e. (E)-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2- yl]-3,5-dihydroxy-6-heptenoic acid, sodium salt, which compound is a member of a family of indole analogs of mevalonolactone and the open chain analogs thereof.

6. My laboratory was only one of six laboratories devoted virtually exclusively to the synthesis of HMG-CoA reductase inhibitors. By way of illustration of the large number of HMG-CoA compounds being synthesized at Sandoz, I note that during the period of July 1985 to July 1987, my laboratory alone prepared 60 such compounds. This is evidence of Sandoz' high level of interest in the project and intention since 1981, and including the period of July 1985 to July 1987, to pursue its basic research project in the HMG-CoA reductase area and the inventive concept behind it.

SANDOZ QUINOLINE COMPOUNDS

7. In late March of 1987, I submitted Patent Disclosure 299/84 direct to quinoline analogs of HMG-CoA reductase inhibitors (**Exhibit A-3** hereto) to the Sandoz Patent and Trademark Department.

8. I understand that between April and November of 1987, this disclosure was presented for rating on four occasions at the regular Sandoz patent committee meetings. On each of these occasions, PD 299/84 was rated either "B" or "X", indicating that further information was needed in order to file a patent application thereon (**Exhibits M-1 - M-4** hereto).

9. In the period between July and December 1987, additional compounds of the invention were synthesized under my direction, and they were tested for activity in vitro and in vivo as HMG-CoA reductase inhibitors.

(The synthesis and testing of these compounds are further described in my Declaration of November 13, 1992; the Declaration and Supplemental Declaration of Rajeshvari Patel dated November 13 and 16, 1992; the Declaration of Dr. Terence Scallen dated November 13, 1992; and the Declarations of Robert G. Engstrom and Rodney Slaughter dated November 13, 1992.)

10. I learned shortly after the January 1988 Patent Committee Meeting that my Patent Disclosure 299/84 was rated for filing.

11. 2. On or about February 29, 1988, I sent certain information to Melvyn M. Kassenoff of the Patent Department relating to PD 299/84.

Exhibit O hereto comprises a true copy of the following material which I sent to the Patent Department:

(1) a "post-it" stating "sent to M. Kassenoff. 2/29/88" which is in my handwriting'

(2) 4 pages comprising handwritten reaction schemes and notes bearing my name and a date in my handwriting of February 29, 1988 on the first page (see also **Exhibit P-1**);

12. Additional material which I sent to the Patent Department comprises the following:

Exhibit P-2: 7 pages of computer printouts of specific compounds containing my handwritten notations of the Notebook pages on which they were prepared and relevant physical properties; and

Exhibit P-3: 9 laboratory notebook pages numbered 130, 137, 145, 153, 158, 166, 172, 175 and 176.

13. On November 1, 1988, I printed out the Sandoz database containing the structures of the quinoline compounds of PD 299/84. I subsequently consulted with Robert G. Enstrom about the IC_{50} and ED_{50} values for these compounds, which I wrote on the printout. I sent this printout to the Patent Department. Since the cover page is dated January 4, 1989 in my handwriting, I would have mailed it on or about that date.

Exhibit Y-2 comprises a true copy of the printout bearing my handwritten notations.

14. On or before November 8, 1988, I sent to Mrs. Joanne M. Giesser of the Patent Department a handwritten memorandum outlining a synthesis of the quinoline compounds of my invention according to the procedure identified as "Route I" in my patent disclosure.

Exhibit U-2 comprises a true copy of this memorandum. The front page bears my initials and the date of November 7, 1988 in my handwriting.

15. I received a memorandum dated December 14, 1988 from Mrs. Giesser enclosing a first draft of the patent application on PD 299/84.

Exhibit W comprises a true copy of the memorandum I received.

16. I made handwritten corrections on pages of the draft application and returned them to the Patent Department on or about December 22, 1988.

Exhibit X comprises a true copy of these pages bearing my corrections and my handwritten date of December 22, 1988.

17. On or about January 4, 1989, I returned to Mrs. Giesser a handwritten memorandum and other material in connection with the patent application draft for case 600-7101.

Exhibit Y hereto comprises a true copy of this material, i.e.:

Y-1: 6 pages of handwritten notes on the first draft and a handwritten synthesis step;

Y-2: the computer printout I received from Biology, which I dated January 4, 1989.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this ____ day of February, 1993.

S. Wattanasin 2/9/93.

Sompong Wattanasin, Ph.D.

AL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERFERENCE NOS. 102,648
102,975

WATTANASIN,

vs.

FUJIKAWA, et al.

DEPOSITION OF:
SOMPONG WATTANASIN

Monday, March 22, 1993
Florham Park, New Jersey

A P P E A R A N C E S:

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-and-
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425 Eagle Rock Avenue
Roseland, New Jersey 07068
(201) 228-9280

I N D E X

WITNESS	DIRECT	CROSS	REDIR	RECR
SOMPONG WATTANASIN				
By Mr. Kelber		3		60
By Ms. Furman			31,64	

E X H I B I T S

FOR IDENT.	DESCRIPTION	PAGE
F-4	Patent application	3
F-5	Request for interference with patent under 37 CFR 1.607	7
F-6	Supplemental declaration of Sompong Wattanasin	12
F-7	Document dated 11-26-84 and attachments	22
F-8	Pages 409 to 417	23
W-2	Document entitled "Declaration - Patentably Distinct Subject Matter"	37
W-3	Pages 164, 165 and 166	37

(Before Gary M. Talpins, a Certified Shorthand Reporter and Notary Public of the State of New Jersey, held at the offices of Sandoz Corporation, Patent and Trademark Affairs Department, 25 Hanover Road, Florham Park, New Jersey, on Monday, March 22, 1993, commencing at 11:55 a.m.)

- - - - - - - - - - - - - - - - - -

S O M P O N G W A T T A N A S I N, 11 DiVito Trail, Hopatcong, New Jersey, Sworn.

MR. VILA: Dr. Wattanasin, speak up so everyone here can hear you.

CROSS EXAMINATION BY MR. KELBER:

Q. Doctor, I'm going to hand you a multi-paged document which you can feel free to disassemble as necessary.

MR. KELBER: I would ask the reporter first to mark it as Exhibit F-4, I believe.

(Whereupon the document was received and marked F-4 for identification.)

Q. If you would take a moment to review

the document.

Q. Dr. Wattanasin, do you recognize the document that has been identified as Exhibit F-4?

A. That's something that I have to check because I don't think I remember all of the numbers and so on.

Q. Do you recall seeing a document like this?

A. Oh, yes, definitely, yes.

Q. And can you identify it for me?

MS. FURMAN: By subject matter.

Q. Dr. Wattanasin, is this a patent application prepared by Sandoz?

A. Yes.

Q. And to your recollection, does it name you as an inventor?

A. Yes.

Q. Would you turn to page 54 of F-4.

A. Okay.

Q. Do you see the rather lengthy written passage numbered one there? It continues on to the next page of the document.

A. Yes.

Q. And do you see that that passage, which

Wattanasin - cross

begins with the number one, describes a certain genus of compounds?

A. Yes.

Q. Doctor, when did you first learn that another company had filed for United States patent protection on compounds similar to those set forth in the passage numbered one?

A. From my recollection, I think I saw a patent maybe at the end of '88 from I think Warner-Lambert.

Q. Did you receive an initial draft of the document that's been identified as F-4 prior to its completion in the form it's been presented to you?

A. I believe so.

Q. Do you recall if you became aware of the patent, I believe you identified it as Warner-Lambert patent before you received that draft copy of the application?

A. I don't think so.

Q. Do you recall who first brought the Warner-Lambert patent to your attention?

A. I think my supervisor, I believe so, because we have review, you know, it's a routine process in the department that we review the patent

Wattanasin - cross

applications not only from Warner-Lambert, from other companies that work on HMG-CoA reductase inhibitor at that time.

Q. Do you know whose responsibility it was to secure those patents of other companies?

A. As I say, it's routine practice in our department to circulate abstracts.

Q. Did you draw the existence of the Warner-Lambert patent, did you draw the attention of anybody in the Patent Department at Sandoz to the fact that the Warner-Lambert patent had issued?

A. I may or may not have called someone in the Patent Department saying that okay, this is the patent from Warner-Lambert similar to our case. From a scientific point, I really have no interest in the Warner-Lambert patent.

Q. Do you have any recollection as to what attorney in the Patent Department of Sandoz prepared --

A. At that time, maybe Jody Giesser, I believe, Jody Giesser.

Q. Do you recall at all discussing the Warner-Lambert patent with her?

A. I believe probably just mentioned that

Wattanasin - cross

this is the patent from Warner-Lambert, that's all.

Q. Doctor, I'm going to hand you an exhibit that I would like identified as F-5. It's paper number two from the file, the request for declaration of interference.

(Whereupon the document was received and marked F-5 for identification.)

Q. If you would take just a minute to look at that, doctor.

MR. VILA: Pardon me, can we go off the record.

(Whereupon a discussion took place off the record.)

Q. Doctor, I obtained the document that's been identified as F-5 from the records of the United States Patent and Trademark Office in an application 318773, which identifies you as an inventor, and my question to you is do you recall seeing F-5 prior to this day?

A. I don't think so.

Q. You never saw it prior to today, to the best of your recollection?

A. Yes.

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Q. Do you recall, doctor, at any time discussing the need to bring the Warner-Lambert patent to the attention of the United States Patent and Trademark Office in connection with your application?

A. Yes, I did discuss it sometime, yes, at some point.

Q. Do you recall whether that discussion was before or after the application was filed?

A. Which application?

Q. The original application that is embodied in Exhibit F-4.

A. I did not recall.

Q. Could you take a look at page one of F-5, doctor, the very first page. Do you see the date stamp circle at the very top of the left-hand corner of that page?

A. Yes.

Q. What is that? Can you make out the date that's in there, doctor?

A. March 3, 1989?

Q. Doctor, do you have any knowledge as to whether any patent application besides the application involved in this interference naming

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you as an inventor has ever been involved in an interference in the United States Patent and Trademark Office?

A. Yes.

Q. Would that other application and other interference have occurred prior to the interference that you are testifying in today?

A. Excuse me? I didn't quite understand.

MS. FURMAN: Off the record.

(Whereupon a discussion took place off the record.)

Q. Doctor, has any application for patent been filed by Sandoz Corporation naming you as an inventor other than the application involved in today's interference of --

A. Yes.

Q. Any of those other applications filed naming you as an inventor by Sandoz, of those aplications, to the best of your knowledge, has any been involved in an interference before the United States Patent and Trademark Office?

A. No, I don't think so.

Q. Do you have any recollection of discussing with Ms. Giesser the need for an

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interference in connection with the application involved in today's proceeding prior to its actual filing?

A. Maybe. I cannot say for sure. Maybe, yes, because -- yes.

Q. It's the only interference you have ever been involved in. Is that correct?

A. Yes.

Q. Are you familiar with the nature of an interference, what an interference is?

A. I'm not fully familiar with the legal process.

Q. Did you ever discuss with Ms. Giesser the need to establish a date of invention prior to the Warner-Lambert patent filing date?

A. Yes, I think so.

Q. Do you recall whether that discussion was prior to March 3, 1989?

A. That I don't recall.

Q. Would you flip back to page 54 of F-4, doctor. Do you see the third text line, the second line after the initial formula on that page, where it says, "C_{3-7}cycloalkyl or"? Do you see that line?

A. C_3 --

Q. I'm sorry, counting from the Arabic numeral one on page 54, the third line of text.

A. Okay.

Q. Do you see the recitation C_{3-7}?

A. Yes.

Q. Do you recall having an understanding of what you meant by C_{3-7} at the time this application was originally filed?

A. I believe so, yes.

Q. What was that understanding, doctor?

A. What understanding, can be anything, anything that contains cyclics, having carbon 3 to carbon 7 in it.

Q. That would be five compounds, actually, wouldn't it, doctor, independent of substitutions, that would be five?

A. Yes.

Q. Can you name those compounds for me, what five basic compounds are encompassed by that group C_3 to C_7 cycloalkyl?

A. The name?

Q. The name of the compound.

A. It should be cyclopropane, cyclobutane,

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cyclopentane, cyclohexane and cycloheptane.

Q. Thank you, doctor. Do you have any knowledge as to the level of skill that an initial, an entry category researcher would have in the field of HMG-CoA reductase, what kind, in general, of educational level would be required of such a researcher? By that I mean -- go ahead.

A. I would say it depends on -- I would say at least a Bachelor's degree.

Q. In chemistry?

A. In chemistry, yes.

Q. Would such an individual understand that C_3, in your opinion, that C_3-C_7cycloalkyl included those five basic compounds?

A. Yes.

Q. Thank you, doctor. Doctor, I'm going to hand you a declaration -- sorry, a paper that I would like identified as F-6 and ask you to review that. This one is of record in volume four.

(Whereupon the document was received and marked F-6 for identification.)

MR. VILA: What record page number is that?

MS. FURMAN: Which is it?

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MR. KELBER: Here is my copy.

MS. FURMAN: His declaration.

MR. KELBER: I prefer we not identify what the document is until the witness has a chance to identify it.

MS. FURMAN: Fine.

Q. Doctor, do you recognize this document that's been marked F-6?

A. Yes.

Q. Can you recall the first circumstances under which you saw this document?

A. This is the application that had been filed.

Q. In fact, this document was prepared in connection with this interference, wasn't it, doctor?

A. Yes.

Q. I should say interferences. By interference, I mean Interference 102,648 and 102,975.

A. Right.

Q. Doctor, how many applications, if you know, have been filed by Sandoz naming you as an inventor or co-inventor directed to the field of

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HMG-CoA reductase?

A. At least three including the quinoline case.

Q. Let me turn your attention, doctor, to paragraph seven, page two of Exhibit F-6. Why did you submit patent disclosure 299/84 in late March of 1987?

A. Because I believe that at that time, we felt that we should be able to complete most of the key compounds involved in the quinoline cases.

Q. I'm sorry, doctor, I didn't catch your full response. You thought that you could --

A. At that time, we felt that we should be able to finish making most of the key compounds involved in this case.

Q. In general, why do you file a patent disclosure, submit a patent disclosure to the Patent Department? What criteria do you use to determine when to file a patent disclosure?

A. When we feel that we have a class of compound that we can use --

Q. I'm sorry, if you could continue the answer. When you feel you have a class of compounds that can be used?

A. For this particular objective in our department to find inhibitor of HMG-CoA reductase.

Q. Does that represent a determination by you that these compounds are new?

A. Yes.

Q. Does it represent a determination to you that these compounds may be valuable to the corporation?

A. Yes, that's right.

Q. Did any event subsequent to March of 1987 indicate to you that your decision that the compounds identified in 299/84 were not either new or valuable to Sandoz Corporation?

A. I don't think so.

Q. Let me turn your direction to paragraph eight, doctor. Do you know why during the period April through November of 1987, the Sandoz disclosures were rated, let's take the rating "B" first -- not the Sandoz disclosure, your disclosure, PD 299/84, was rated "B" by the Patent Committee?

A. I'm not in the Patent Committee but I understand it bears on the factor that further information on this case would be needed before the

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application can be filed so more work needs to be done, I think that's the bottom line.

Q. Did you receive notification that the disclosure had been rated "B"?

A. This is by oral, by verbally.

Q. But you did receive that notification?

A. Yes.

Q. What type of extra work needed to be done?

A. Basically, we have to complete the whole set of compounds that need to be prepared.

Q. And why was that, doctor, why did you have to complete the whole set?

A. I think the objective of making, working on any class of compound is to insure that we come up with an optimum structure. In this particular case, we just making only partially part of the set, we are not complete the whole set yet.

Q. Did you expect to find in the set, part of the set that had not been completed a difference, qualitative difference in the compounds in terms of their value to Sandoz Corporation? In other words, you had completed some of the compounds but not all of the compounds of the set.

A. True.

Q. Did you have a personal expectation as to the activity you anticipated from the rest of the compounds?

A. Yes.

Q. And what was that expectation, doctor?

A. My expectation is I expect that I may come up with some compounds that show better activity.

Q. Did you expect that some of the compounds in the set to be completed might have worse activity?

A. Yes, that can be the case.

Q. Did, in fact, you come up with compounds subsequent to March of 1987 that had better activity than the compounds identified in the disclosure?

A. Yes. That's normal.

Q. Did you come up with compounds that were worse?

A. Oh, yes, I come up with a compound worse and compound better.

Q. Let's turn now to the "X" rating. When you received notification that your disclosure has

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been rated "X", what does that mean to you, what does "X" indicate?

A. I think it indicates the same thing to me. I mean as I say, I'm not the one who made this thing but it indicates the same thing, more information will be needed to complete, to complete the whole application process of this case.

Q. Was the information needed in response to an "X" rating different, in your opinion, than the information needed for a "B" rating?

A. No, I don't think so.

Q. Do you see the reference in paragraph nine to additional synthesis and testing between July and December of 1987?

A. Yes.

Q. Was that additional synthesis and testing done responsive to the "B" or "X" rating that your disclosure received?

A. No.

Q. You would have done that, anyway?

A. I would have done that, anyway, yes.

Q. Thank you, doctor. If the disclosure had been rated "A", would you have continued that testing that's referred to in paragraph nine?

A. Yes, I believe so.

Q. Thank you, doctor. In the other applications naming you as an inventor completed by or on behalf of Sandoz Corporation, do you have a recollection as to how long it took between the time you learned that the disclosure had been rated "A" and the time you received the first draft of that application? Do you have any idea?

A. No, I cannot give you that honestly.

Q. Can you tell me was it more than six months?

A. I would say about six months, yes.

Q. About six months?

A. About six months.

Q. Do you know does Sandoz have a written policy regarding responding to questions from the Patent Department for additional information?

A. Yes.

Q. It does have a written policy?

A. Yes, policy as to you have to comply with requests from the Patent Department.

Q. There is such a written policy, you think?

A. I think so, yes.

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Q. If there is such a policy, can you send us a copy to the extent it's not privileged?

THE WITNESS: I --

Q. That's okay, they will get a chance to ask you all about it in not too long a period of time.

Do you have an appreciation based on the experiences of other researchers at Sandoz as to the time it takes for the preparation of a draft application from the time a disclosure is rated "A"? Do you have a general idea?

A. No, no idea.

Q. Let me turn your attention to paragraph 11 on page three of F-6, doctor. Why did you send certain information to Melvyn Kassenoff about February 29 of 1988?

A. I believe that I was requested by Mr. Kassenoff for subsequent information.

Q. You already knew that your disclosure had been rated "A". Is that correct?

A. At that time, yes.

Q. Was it your understanding that the material you sent to Mr. Kassenoff was required or requested -- I'm sorry, requested for the purposes

of preparing that application?

A. Yes.

Q. Did you have occasion, do you recall, to speak with anybody in the Patent Department between February 29 of 1988 and the end of May 1988 regarding the patent application to be prepared on your disclosure?

A. Yes, I think so.

Q. Do you recall who you spoke with?

A. Either Mel Kassenoff or Jody Giesser.

Q. Do you recall the substance of those discussions?

A. Mostly related to specific information as far as the compound, you know, included in the patent.

Q. Did you at any time ask when you might expect a patent application to be prepared?

A. I don't think so.

Q. Let me turn your attention to paragraph 12, pages three and four of F-6. Why did you send that information to the Patent Department?

A. Again, I was requested from the Patent Department for some information.

Q. Do you recall when you sent that

[redacted]

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information?

A. I don't recall when I received the actual copy of the thing I sent to the Patent Department. Generally I would know what date I sent it on the copy.

MS. FURMAN: Could you repeat your last sentence, please.

THE WITNESS: In general, I don't exactly remember the date that I sent any material to anyone but in general, before I send something to someone, I would note the page, I would date the page.

MS. FURMAN: You would date the page.

Q. Let's take them one at a time. I'm going to hand the reporter a document I would like identified as F-7. I will ask you to review that document briefly, doctor.

(Whereupon the document was received and marked F-7 for identification.)

Q. Doctor, does your review of P-2 enable you to determine in any way about when you might have sent that material to the Patent Department?

A. I can tell you that this is after February 29, 1988.

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Q. Do you know would it have been before May of 1988?

A. No.

Q. Did you send it in response to a request from the Patent Department?

A. Yes.

Q. Do you recall who the request came from?

A. I think this is from Mel Kassenoff.

Q. Let me hand you a document, P-3, for identification as Exhibit F-8.

(Whereupon the document was received and marked F-8 for identification.)

Q. Does F-8 contain documents that were sent to the Patent Department as described in paragraph 12 of your declaration?

A. Yes, I think so, yes.

Q. Does review of that document enable you in any way to fix the time you sent those documents to the Patent Department?

A. No.

Q. But you know they were before February of 1988 -- I'm sorry, after February of 1988?

A. After, yes, definitely, yes.

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Q. And you know you sent them in response to a request by Mr. Kassenoff?

A. Yes.

Q. After submission of those documents, but prior to November of 1988, do you recall having any further written or oral communications with the attorneys in the Patent Department at Sandoz regarding your disclosure 299/84?

A. Yes, I think so, yes.

Q. Do you have an actual recollection of it?

A. No, I don't have actual recollection.

Q. Do you have an actual recollection of anything that might have been said or written at that time?

A. Mostly anything that related to the draft or something on it, I see something where they have seen some question that needs to be clarified, I think in general.

Q. But you did not see a draft until November of 1988. Isn't that correct?

A. Yes.

Q. In fact, you didn't see the draft until December of 1988. Is that correct, doctor?

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A. Yes.

Q. Isn't it correct, doctor, that you didn't receive the draft declaration until after you had learned of the existence of a Warner-Lambert patent?

MR. VILA: The declaration?

MR. KELBER: I'm sorry.

Q. Isn't it correct, doctor, that you had received the draft memorandum of your patent application after you had learned of the existence of the Warner-Lambert patent?

A. Let me check the date again. That may be the case, yes.

Q. Do you recall exchanging in writing any communications with Ms. Giesser concerning the Warner-Lambert patent?

A. In writing, no, I don't think so.

Q. Anybody else at the Patent Department, did you exchange correspondence concerning the Warner-Lambert patent?

A. No.

Q. Do you recall publishing the subject matter at item one of page 54-55 of your application, the document that's been marked F-4,

prior to March of 1989?

A. I don't think so.

Q. You had completed the initial set of compounds back in March of 1987. Is that correct?

A. Can you repeat that again?

Q. You had completed the initial set of compounds to which PD 299/84 and subsequently, your application document F-4, pertained, you had completed that initial set of compounds by March of 1987. Is that correct?

A. By March, yes.

Q. And you didn't publish information regarding those compounds until after March of 1989. Is that correct?

A. Yes.

Q. Compounds were interesting to you?

A. Compounds were interesting to me, of course, yes.

Q. Do you think the compounds would have been interesting to other researchers in the field?

A. Of course.

Q. Was there any reason for not publishing that information until after March of 1989?

A. There is no particular reason, I don't

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think so.

Q. When did you become aware that Nissan Chemical Corporation had filed for U.S. patent protection on compounds similar to those identified at item one of page 54 of your application?

A. I don't remember the date exactly but I think it happened after we already, you know, talking about a patent application of this case.

Q. So after the application was filed or before?

A. I don't recall the date. I cannot give you the definite time.

Q. Do you recall having discussed the existence of the Nissan Chemical Company's request for patent protection with Ms. Giesser?

A. Yes.

Q. Subsequent to the classification of your disclosure as "A" in January of 1988, did you at any time express any concern to anyone about the progress made in preparing the application corresponding to that disclosure?

A. No, I don't think so.

Q. In your experience at Sandoz Corporation, the period of January of 1988 till

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March of 1989, is it customary to take that 14 months for preparation of the patent application?

A. That is unusual. That is unusual.

Q. During that time period, were any other applications naming you as an inventor or co-inventor filed by Sandoz Corporation, January of '88 through March of 1989? Do you recall were any other applications naming you as an inventor or co-inventor filed?

A. There are a couple -- I would say there are two other patents involving HMG-CoA reductase inhibitor but I don't recall the exact date.

Q. Have those, either of those patent applications been issued as a U.S. patent?

A. Yes.

Q. Do you know the number offhand?

A. We are in one of four.

MR. KASSENOFF: Off the record.

(Whereupon a discussion took place off the record.)

Q. I want to return just to one subject and that's the question of the information needed in response to a "B" or "X" classification by the Patent Committee. We talked about the need to

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provide more information in response to a "B" classification. What specific type of information is necessary? The synthesis of the compounds, is that required?

A. I think at this time, let me say when you set up on any class of compound, you want to make a few of the compound to find optimum structure and I think at that point in time, we know we are not complete the whole set of compound yet and I think until then, I think we still need further information.

Q. So synthesis and testing of the compound would be required?

A. Synthesis and testing of the compound.

Q. Any of the compounds that are identified in the original disclosure, PD 299/84, did any of those compounds show the type of activity that suggested they might have utility as HMG-CoA reductase inhibitors?

A. Certainly.

Q. Did anything occur between March of 1987 and March of 1989 that suggested that that might not be true, they did not have sufficient activity?

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A. No, I don't think so.

MR. KELBER: Doctor, I really appreciate your patience with me in being here this morning. I have no more questions at this time.

THE WITNESS: Thank you.

MR. VILA: Do you want to take lunch break?

MR. KASSENOFF: Let me ask one question on redirect.

MR. KELBER: I have no objection -- I have discomfort with a witness crossing.

MR. VILA: We will take that question up later.

MR. KELBER: Okay.

MR. VILA: It's a matter of clarifying some things.

MR. KELBER: My only concern is keeping the good doctor longer than we need to. If you have got a lot --

MR. VILA: He is invited to lunch.

MR. KELBER: I kind of hoped you would feed him.

(Whereupon the luncheon recess was taken.)

[redacted]

REDIRECT EXAMINATION BY MS. FURMAN:

Q. Dr. Wattanasin, referring to your testimony concerning the C_3 to C_7 cycloalkyl substituents on the quinoline ring, you testified that that would include, among others, cyclopropyl and you testified that a person of skill in the art would recognize it to include cyclopropyl. Do you think that a person of skill in the art would regard cyclopropyl as being obvious as that structure being obvious in view of isopropyl?

MR. KELBER: Objection. I don't know that the witness -- I don't know how you are using the term "obvious" but I don't know that the witness has demonstrated a knowledge under the 103 sense, if you could rephrase it.

Q. Dr. Wattanasin, do you understand what the term "obvious" means under the patent law or can you give me your definition of the term "obvious"?

A. The obvious, in my term, in the medicinal chemistry term, is a kind of, what do you call it, kind of group that you like to make to cover your hypothesis.

Q. Do you think that someone knowing about

an isopropyl substituted compound, based on that information alone, would be led to prepare a cyclopropyl compound?

A. That's what I mean by obvious because in medicinal chemistry, cyclopropyl would be an obvious analogue of cyclopropyl group. If you look at some of the --

Q. Excuse me, I didn't understand you. Cyclopropyl would what?

A. Cyclopropyl, cyclopropane group would be obvious analogue of cyclopropyl group. Do you understand the word analogue?

Q. Yes. If someone in your lab knew about an isopropyl compound, do you think based on that information, they would be led to prepare a cyclopropyl compound?

MR. KELBER: Objection. You are now asking his opinion as to what others in the laboratory would do.

Q. Would you be led to prepare a cyclopropyl compound?

A. Yes, definitely.

Q. Why would you be led to prepare a cyclopropyl compound?

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A. Because of the, this is according to scientific, basically, when you put the group on any structure, you are looking for two things, two things you are looking for, two properties of that group, sterically and electronically and in this case, cyclopropyl, and cyclopropyl are very similar.

Q. I am talking about cyclopropyl versus isopropyl. Is cyclopropyl similar in chemistry to isopropyl sterically?

A. What I'm saying is sterically and electronically, cyclopropyl group would be very similar to isopropyl group and not only that, you can see from the scheme of the chemistry, chemistry scheme, we have the hardware that can make both compounds quite easily.

MS. FURMAN: I would like to put into evidence as W-2 the declarations that were submitted in this interference of Mr. Kitahara.

MR. KELBER: I will wait until your question but I would object to the extent they would go to anything in the nature of direct questioning.

Q. Dr. Wattanasin, do you recognize the

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structure on page two of the Kitahara declaration?

A. Yes, I do.

Q. What is that structure?

A. This is the structure of isoquinoline derivative.

Q. The R-5 substituent, what is the R-5 substituent?

A. In this case, R-5 can be cyclopropyl or isobutyl.

Q. Going to the test on that declaration, which compares the activity of cyclopropyl with the isopropyl compound, what is your opinion of this activity information?

MR. KELBER: Before you answer, doctor, I'm going to object to that on the grounds that this is in the nature of direct testimony and if you had wanted to submit it, it should have been submitted together with the remainder of your direct testimony. As far as I'm aware, this is our cross on direct and you have not requested rebuttal response or the opportunity to cross our own declarant. I can't stop you from asking your questions but I do definitely object to further questions on this issue.

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MR. VILA: Can I conference with you outside?

MS. FURMAN: Yes.

(Whereupon a brief recess was taken.)

MS. FURMAN: I will go on to a different line of questioning.

BY MS. FURMAN:

Q. Dr. Wattanasin, the patent disclosure on your quinoline compound is numbered 299/84. Do you know how this number was assigned to your patent disclosure?

A. I think this number was assigned on an annual basis, I believe. Before the end of the year, one of the secretaries here send you the patent disclosure form for the next year.

Q. A blank patent disclosure --

A. A blank patent disclosure.

Q. With the number appearing at the top?

A. Yes.

Q. And that was sent to you when?

A. Around the end of the year, in December.

Q. In December of --

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A. Of '83.

Q. Of '83. You synthesized at least one compound by the end of 1984. Is that correct?

A. Yes.

MR. KELBER: Just for clarification, we are talking about the compounds of the disclosure or compounds in general or what?

THE WITNESS: The first compound we are making, one of the first compounds we are making in this case.

Q. After that compound was synthesized, what additional work was done in relation to your quinoline patent disclosure? After the synthesis of 63366, what compounds did you synthesize?

A. There are a number of compounds we synthesized during that period. At that time, we were still working on basically all of them. All of them are HMG-CoA reductase inhibitors and two more compounds, two more compounds were synthesized, the number I believe is 64548 and 64549.

MS. FURMAN: I would like to put into evidence as Exhibit W-3 pages --

MR. KELBER: We started to talk about

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W-2 but we never did get around to marking it. Do you want to have W-2 in or do you want to just mark those as W-2?

MS. FURMAN: Yes, let's put W-2 in.

(Whereupon the document was received and marked W-2 for identification.)

MS. FURMAN: I would like to put into the record as W-3 pages 164 through 166 of the Wattanasin testimony.

(Whereupon the document was received and marked W-3 for identification.)

Q. Dr. Wattanasin, do you recognize those pages?

A. Yes, I do.

Q. Can you describe them?

A. This is reaction, this is a notebook, from my notebook, the synthesis of one of the compounds that later on is designated as 64548.

Q. And what is the date at the top of the page?

A. 5/7/85.

Q. What would the date at the top of the page signify?

A. This is the date that I start doing

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this particular reaction that leads to the synthesis of this particular compound 64548.

Q. So you had synthesized 64548 sometime on or after May 7, 1988?

A. Yes.

Q. Is there an additional compound that you synthesized around that time?

A. The next compound we synthesized is the compound 64549.

Q. Was that also synthesized --

A. Around this date.

Q. Around May of 1985?

A. '85, yes.

Q. Your patent disclosure, which is numbered 299/84, when was that submitted to the Patent Committee by you?

A. I think by March, in March '88.

Q. March of --

A. March of 1988.

Q. Submitted to the Patent --

A. I'm sorry, March of 1987.

Q. What made you submit the patent disclosure in March of '87? Why did you not submit the patent disclosure after you made 64548 or 49?

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A. I think the reason for that is because of we are not complete the whole set of this class of compound yet.

Q. Why had you not completed the whole set?

A. The reason is because, I think one of the key reasons is because of a lack of manpower at that time because I'm the only one working at that time on the HMG-CoA reductase in this lab.

Q. Your lab was the only lab synthesizing quinoline compounds?

A. Yes.

Q. When did you realize you lacked manpower to proceed with the whole series?

A. Actually, at that time, actually 1985 because we are dealing with different classes of HMG-CoA reductase inhibitor compound, quinoline is not the only compound we are making. We are making other, different kind of heterocyclics, as well.

MR. KELBER: I don't know that it raises to the level of an objection, Diane, but to what part of the cross does this line of questioning go to?

MS. FURMAN: I believe you did ask him

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about his activities in that time period.

MR. KELBER: I asked him if anything occurred with regard to the period between the submission and the "A" rating, I asked him if anything occurred to change his mind.

MS. FURMAN: You were discussing the initial set of compounds, you asked whether they were completed by March of 1987 and I was trying to develop that testimony.

MR. KELBER: Okay.

Q. When did you realize there was a manpower shortage?

A. I think around this time, I think sometime in 1985.

Q. How long did it take you to find somebody to fill that position or positions?

A. Normally to get someone, you have got to have approval from your boss and then subsequently, you have got to get approval by your department head and then it also depends on whether or not the opening is available at that time and when you got the actual head count, the opening, then you have got to get approval from your boss, from your department head and then from the head

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of -- from the president of SRI. And then you have to recruit the person. It takes a long time, actually.

Q. How long did it take?

A. You have an opening, after you have an opening, then you have to place an ad and looking for someone, I would say at least six months.

MR. KELBER: I'm going to object because I'm not sure but I don't think the answer was responsive to the question. I think the answer was general and you had a very specific question.

Q. Can you answer the question more specifically. How long did it take you in this case to find somebody?

A. In this case, a whole year.

Q. When did you ultimately find somebody?

A. I got someone to join my lab in January 1987.

Q. What was the name of that person?

A. Miss Patel.

Q. Can you spell out the full name?

A. Rajeshvari Patel, R-a-j-e-s-h-v-a-r-i P-a-t-e-l.

Q. Was she assigned to your lab exclusively?

Wattanasin - redirect

A. Yes.

Q. Did you supervise her work?

A. Yes.

MS. FURMAN: Do you want to continue with questioning or do you want to leave it open?

MR. VILA: Are you finished completely or do you want to come back later?

MS. FURMAN: I'm going to come back later.

BY MR. VILA:

Q. Was there any relationship or significance to the timing of the submission of the patent disclosure to the Patent Department relative to this lack of manpower that you mentioned?

A. Yes, because --

Q. What would that be?

A. Because if I did have the manpower before 1987, some key compounds should have been synthesized before that date, before March 3, 1987.

Q. You mentioned this Miss Patel and she was hired in January of --

A. 1987.

Wattanasin - redirect

Q. -- '87. Do you recall what assignments she was given when she was hired?

A. There were a number of assignments given to her, key projects were given to her and this quinoline project is one of them.

Q. From the start, she --

A. From the start, yes.

Q. -- she was assigned this?

A. Yes.

Q. Having not submitted that disclosure previously, why would you have at that particular time submitted the disclosure?

MR. KELBER: I think that has been asked and answered.

THE WITNESS: Yes.

Q. You can answer it. Go ahead.

A. Because at that time, with additional manpower, I felt that we should be able to complete the whole set of this quinoline case, that's why I file the patent disclosure at that time.

Q. You had testified in response to questions on cross examination with regard to publication of the subject matter of this patent disclosure in this patent application. Would you

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have published on this subject matter prior to March of '89 when the patent application was actually filed?

A. No, I wouldn't.

Q. Why would you have not done that?

MR. KELBER: I'm going to object just to the form. Is the question did he or would he have? I don't understand the subjective tense of the question.

MR. VILA: Would he have. I believe he testified before that he could have --

MR. KELBER: If he didn't, he wouldn't. I mean I don't understand the nature of what -- is there a difference between did and would?

MR. VILA: Yes.

MR. KELBER: Are you asking for a hypothetical situation? We know what he would have done, he did it in this situation. Are you asking --

MR. VILA: Let's go off the record a second.

(Whereupon a discussion took place off the record.)

Q. I will simply ask you did you make any publication on the subject matter of that patent application prior to its filing?

A. No.

Q. Can you explain why you did not make a publication on that subject matter?

A. If I understand, you cannot disclose the information related to the patent disclosure until it was approved by the Patent Department, until it be cleared by the Patent Department.

Q. I believe you testified on cross examination that there was a written policy or you thought there was a written policy with regard to communications with the Patent Office and in particular, responding to requests by the Patent Department.

A. Yes.

Q. Have you ever seen such a written policy?

A. What I meant in that time is this is part of what you call the job description, that you are supposed to comply with all of the requests, information related to the patent application of your discovery.

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BY MS. FURMAN:

Q. You testified concerning the activity of the compounds in the quinoline series. In response to questioning, you indicated that after you did the earliest work, you would have expected some compounds would come up with better activity or worse activity. Is that true?

A. I cannot predict that but it can be seen from the IC_{50} of one of the first compounds, I believe 63366, the IC_{50} of 1.5 micromolar. That, in my judgment, that is comparable to IC_{50} of Compactin and established HMG-CoA reductase inhibitor.

Q. And established HMG-CoA reductase inhibitor?

A. Yes.

Q. So based on the first compound you made, what was the likelihood that the later compounds would have activity in vitro as an HMG-CoA reductase inhibitor?

MR. KELBER: Objection. What later compounds?

MS. FURMAN: 64933, 934, 935 and 936.

A. I cannot predict activity of those

compounds before I make them. However, based on the information, we have learned from closely related analogue of this quinoline compound, I would say that we would have very good chance of being active and as you can see from the IC_{50} of those compounds, again, they are comparable again to Compactin and as you know, going back to the in vivo, as you know, Compactin has a good potency, not only in vitro but also in vivo, as well. So when some of those compounds have IC_{50} similar to Compactin, one would predict that to have a good activity in vivo, as well.

Q. Predicted?

A. One would expect that.

Q. Expect it?

A. Yes.

Q. What level of assurance would you have? How high would be your expectation?

A. Actually, I would say it I would be very certain that the compound should have activity in vivo, as well.

BY MR. VILA:

Q. Would that statement that you just made

apply to 63933, which is part of your mention on page 27 of your original declaration, results on page 27 of the record? Do you know the structure of the compound I referred to as 63933?

A. Yes, I do.

Q. Would that statement apply to that compound?

A. I'm not quite sure. That's project 933, 64933. If I recall, IC_{50} of 64933 is somewhat less active than the first compound I made. However, the statement would apply to the later compound, the number is 64935, which we have better IC_{50} and also have very good potency based on ED_{50} based on in vivo testing.

Q. We know the IC_{50}'s now, I think we are going back to the point when you prepared these compounds and before they were tested, you said that you would have a very high degree of confidence that they would exhibit activity. We don't know the level of the activity.

A. Yes.

Q. Would that high degree of confidence apply to 63933?

A. Yes, I think so.

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Q. And the compound -- I'm sorry, is that --

A. 64933.

Q. I'm sorry, I beg your pardon, correct the record, I'm referring to 64933, correct?

A. Yes.

Q. And that's a compound you know the structure of?

A. Yes.

Q. It's in the record. I would ask the same question with regard to compound 64934. Do you know the structure of that compound?

A. Yes.

Q. Would you have or not have that same degree of confidence as to the activity of that compound at the time it was prepared and before you tested it?

A. I would have the same degree of confidence.

Q. And 64935?

A. Yes.

Q. The same?

A. Same degree of confidence.

Q. In the record that I have observed

Wattanasin - redirect

here, the compound 64933 and 64934 allegedly were prepared --

A. In August, I believe.

Q. -- sometime in July or August of '89.

A. No, '87.

Q. '87, I'm sorry. Yet they were not sent for testing at that point.

MR. KELBER: Objection, assuming facts not in the record of today's deposition. The fact that you may have submitted them elsewhere doesn't make them of record here.

MS. FURMAN: Off the record.

(Whereupon a discussion took place off the record.)

BY MR. VILA:

Q. You testified those compounds were prepared sometime in August of '87 from your recollection.

A. Yes.

Q. Do you recall when they were submitted for testing?

A. I think it's in one of these exhibits. It's definitely. I do recall, yes. I believe it

was submitted for testing on October 2nd, 1987.

Q. And by submitted for testing, what does that mean to you, October 2 of '87?

A. What do you mean this means to me?

Q. You say they were submitted for testing and I asked you what do you mean by submitting, what event took place on October 2, 1987?

A. On October 2, 1987, the compound was shipped to Professor Terry, T-e-r-r-y, Scallen, S-c-a-l-l-e-n.

Q. These compounds were prepared in August, as you say, and they were sent in October. Why weren't they submitted earlier? Do you have a recollection on why they were not submitted earlier to Dr. Scallen?

A. There are basically two key reasons. First of all, doing the process, the compound has to be made and the -- doing the process of the compound being synthesized and purification and characterization, I went to a meeting in New Orleans for over a week and when I came back, I was aware that the next shipment would be on October 2nd and so even though these last three compounds were made before that October 2nd, I would like all

of these compounds to ship for testing together so I can have a better comparison of the potency in the same study.

Q. When you say all of these compounds, you are referring to which ones?

A. 933, 64933, 64934 and 64935 and 64936, as well.

Q. Could you tell me whether you had any particular procedures or arrangements for sending compounds to Dr. Scallen?

A. Yes. Normally after you finish the synthesis and the compound has been purified and the compound had been submitted to different measurements in the physical chemistry department to identify the identity of the compound, then we would, we, I mean the chemists in my lab would then submit the compound to the drug room and then there would be one person responsible for registering the compound into the system and then after the compound had been registered into the system, there would be another person who would be responsible for collecting all of this compound and ship it, ship them for testing.

MR. KELBER: I'm going to renew my

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objection to this line of questioning at this time. I know I didn't go into anything regarding in vivo testing and the procedures therefor on direct.

MR. VILA: I believe you have been into the questions of abandonment and diligence in this area and I think --

MR. KELBER: Certainly not diligence, never. With respect to abandonment, suppression, concealment, that's an issue but it's hardly anything that gives rise to a free-for-all in determining what kind of activities. My understanding of the rules provide that you can ask in areas developed on redirect that were initially explored on cross. I just want to make my objection for the record because the rule requires it to be made now rather than later.

MR. VILA: All right. I think that we are probably finished with that line.

BY MR. VILA:

Q. In January of 1988, your disclosure 299/84 was rated "A" by the Patent Committee, I believe you have testified to that. As a result of

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that rating, what would have been your expectancy with regard to the subject matter in that disclosure?

MR. KELBER: The witness can answer if he can but I admit, I'm totally confused by your question. What is his expectation with regard to this subject matter?

Q. What did that rating mean to you?

A. I think I already answered that question this morning, that the rating doesn't mean to me, it's only my intention to complete the synthesis of one of the key compounds in the quinoline case.

Q. I believe it was also testified this morning that the "A" rating would signal the filing of a patent application.

A. Yes, you are right.

Q. And I would ask you whether that created a certain expectancy in your mind with regard to that filing of a patent application?

A. Yes.

Q. And what would that expectancy be?

A. The expectation would be that the compound should be finished as soon as possible.

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Q. I'm referring to the "A" rating of the decision to file a patent application, whether that decision created a certain expectancy in your mind. Would you have expected that a patent application would have been filed as a result of that "A" rating?

A. Yes.

Q. I would ask you, then, from the period January of 1988, when that was rated "A", and March of 1989, when the patent application was actually filed, whether anything occurred that would have changed your expectancy that a patent application would have been filed?

A. Nothing.

Q. Do you want to verbalize the answer.

A. Can you repeat the question? I'm not quite really understanding the point. Can you repeat the question again, please?

MR. VILA: Do you want to read him the question.

(Whereupon the record was read.)

A. Nothing.

MR. VILA: Let's go off the record for a minute.

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(Whereupon a discussion took place off the record.)

Q. You just testified that you expected a patent application to file. Are you aware of any activities on the part of anybody else that may have indicated any kind of a decision not to file a patent application on that disclosure which had been rated "A" in January of --

A. I was not aware of any.

BY MS. FURMAN:

Q. Did either Mel Kassenoff or Jody Giesser ever indicate to you an intention not to file a patent application?

A. No, definitely not.

Q. You testified earlier that you spoke with Jody Giesser about the Warner-Lambert patent and possibly about the Nissan application. Is that correct?

A. Yes.

Q. I want to ask you again whether you can remember exactly when you spoke to her about those publications. Do you remember for certain that you spoke with her before the filing of the patent

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application?

A. I believe so, yes.

Q. Do you remember exactly when that was?

A. I don't remember exactly when.

Q. Did you arrive at any conclusion based on your talk with her about that?

A. Conclusion about what?

Q. The Warner-Lambert patent. Had you been working on the patent application already when you spoke with her about the Warner-Lambert?

A. Yes.

Q. You were working with her on the draft before you spoke with her about the Warner-Lambert?

A. Yes.

Q. You received a draft of the application in, I believe, December of 1988.

A. December or November.

Q. November of 1988.

A. Yes.

Q. Were you in communication with Jody Giesser before that date concerning the patent application?

A. Yes.

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Q. Were you in communication with her between February and November at any time?

A. Of what year?

Q. 1988.

MR. KELBER: Asked and answered. He said before that day.

MS. FURMAN: More specifically, between February and November.

A. Yes.

Q. Were those communications oral or written?

A. Mostly I believe oral, over the phone.

Q. Dr. Wattanasin, is English your first language?

A. No.

Q. What is your first language?

A. Thai.

Q. Thai?

A. Yes.

Q. Did Jody Giesser ever have trouble understanding you?

A. I don't think so.

Q. You don't think so.

MS. FURMAN: That's about it.

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MR. VILA: I just have one final question.

BY MR. VILA:

Q. During the period sometime in 1985, after you had made the three compounds, the first three compounds, those being, according to the record, 63366, 63548, 63549, that synthesis ending sometime in 1985, and early 1987, when the activities resumed on this quinoline series, was it ever your intention that that earlier work would be considered abandoned in your mind in the sense that it would be no longer of interest?

A. No, definitely not.

Q. And how would you describe the interest that you had in those compounds during that period?

A. My interest in those compounds, I would say very high but as I stated before, that the reason that the gap is somewhat apart is because of two reasons. The first one is because of the manpower that I mentioned before. I think the second thing is because of the priority and the priority is sometimes set by me and most of the time set by my supervisors.

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MR. VILA: I don't think I have any more questions.

MR. KELBER: I have just a few, doctor. I'm sorry to belabor you but I do understand you clearly, I don't think there is a problem there.

RECROSS EXAMINATION BY MR. KELBER:

Q. The very last answer you gave had to do with the manpower shortage and the priority being set on things. Did you set the priority with regard to the compounds in question that you just testified to?

A. The priority was set either by myself or my boss.

Q. In this particular case, do you recall who set the priority?

A. In this particular case, I think -- actually both, I will say both. You see, I mentioned before this is not the only compound, only class of compound we are working with. We are working on different classes of compounds during the HMG-CoA reductase and probably as you have seen from the patent, as well, we have two key

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compounds, very important compounds, indole and indene.

Q. Did those projects receive a higher priority than the project in question?

A. Yes, according to my supervisor, yes.

Q. You also mentioned the kind of arduous process that anybody with supervisory authority is involved with hiring somebody new and you couldn't find anybody for over a year. Is that correct?

A. No, what I'm saying is the process, because of, first of all, before you can hire anyone, you have got to get approval from different people first and once you got approval for hiring someone, then it would take at least six months before you actually get someone to join your lab.

Q. Understood. This manpower shortage, if you will, that was a fairly big problem for you in connection with this?

A. Big problem because I'm the only one working in the lab on a number of compounds, on a number of projects.

Q. Did you speak to anybody in the chain of command, your boss or above, regarding expediting the process of bringing in somebody?

A. Yes, I did speak many times with my bosses about this issue, yes.

Q. To the best of your knowledge, did anybody do anything to expedite it?

A. As I say, the decision not only depend on my boss.

Q. But the decision also included those above your boss?

A. Yes.

Q. And do you recall today making a decision to expedite the search for manpower in this particular case? Did they move faster than the regular procedure in the case that was eventually satisfied by Dr. Patel?

A. That I don't have information to tell you.

Q. Did you ever submit a disclosure relevant to the quinoline derivatives that we have been talking about today for clearance by the Patent Department?

A. Beside quinoline cases?

Q. Besides the patent application and patent disclosure itself, I'm sorry, let me go backwards, during redirect, you spoke that a

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disclosure outside of a patent application can't be released until it's cleared by the Patent Department. Do you recall that testimony?

A. Yes.

Q. Did you, yourself, ever submit a publication for clearance by the Patent Department relative to the subject matter of the application involved?

A. Yes, I prepared some, yes.

Q. And that would have been prior to the filing date?

A. After the filing dates.

Q. You did not submit a disclosure prior to the filing date?

A. That I'm not quite sure. I have to check my record before I can answer to you definitely.

MR. KELBER: Can we ask you to check those records and get back to us.

Q. You testified, doctor, that on the basis of your initial work reflected in the patent disclosure, you had a reasonably high expectation as to the issue of whether the compounds later prepared would exhibit activity.

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A. Yes.

Q. And your expectations weren't disappointed, were they? Your expectations were right on the money, weren't they, doctor?

A. I don't say it's right on the money but it's comparable, yes.

Q. Is there a general formula or thought process that you go through when determining when to submit a patent disclosure? I understand that you submitted this patent disclosure in question, 299/84, because at that point in time, you felt you could complete the rest of the compounds with some expectation of activity.

A. That's right.

MR. KELBER: I have nothing further.

REDIRECT EXAMINATION BY MR. VILA:

Q. Dr. Wattanasin, there seems to be a little uncertainty in your mind with regard to the submission of a publication clearance on the subject matter that became the subject of the patent application that was filed and I believe you testified you are not sure whether you may have submitted a request for publication prior to or

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after the filing of the patent application. Is that correct?

A. Yes.

Q. Would you be still uncertain whether that request was submitted before or after the "A" rating of the disclosure which took place in January 1988, would you have --

A. I would say definitely after, yes.

Q. After the "A" rating?

A. After, yes.

MR. VILA: I have no further questions.

MS. FURMAN: I would just like to ask the general question whether at any time between the synthesis of 63548 and 63549 --

THE WITNESS: 64548 and 64549.

MS. FURMAN: Correct, whether between that synthesis and the synthesis of 64933 and later compounds, whether in that period, you ever had the intention to abandon your invention?

THE WITNESS: No, as I said before, definitely not.

MR. KELBER: Thanks again, doctor. We appreciate it.

THE WITNESS: Thank you.

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MR. KELBER: Before we go off the record, different people have different styles. We have been operating under the situation where you identify an exhibit, you object to it. Just in case you operate under a different fashion, we have exhibits F-1 through F-8 and W-1 through 3. We have objected to W-2. Do you have any objections to any of F-1 through 8?

MS. FURMAN: No.

(Time noted is 2:30 p.m.)

Sompong Watt——n.
SOMPONG WATTANASIN 4/20/93.

Subscribed and Sworn to before me
This 20th day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

[redacted]

ERRATA SHEET

Name of case: Wattanasin v. Fujikawa et al.
Deposition of: Sompong Wattanasin
Date taken: March 22, 1993
Page 1/1

PAGE	LINE	CHANGE	REASON
33	16	Change "hardware" to "pathway".	My best recollection is that I actually spoke the term "pathway," and not "hardware". Also, I would not have said "hardware" because it makes no sense in this context.
46, 46	13 15	Change "and" to "an".	This is an obvious typographical error. The proper word is obviously "an"; the word "and" makes no sense in this context.
38,	5	Change "1988" to "1985".	I believe that the question actually referred to "1985", and that the date of "1988" appears to be a typographical error, since it refers to the date of 5/7/85 on page 37, l. 22.

Sompong Wattanasin,
SOMPONG WATTANASIN
4/20/93

SUBSCRIBED AND SWORN TO BEFORE ME
This 20th day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMSARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

[redacted]

C E R T I F I C A T E

I, GARY M. TALPINS, a Notary Public and Certified Shorthand Reporter of the State of New Jersey, do hereby certify that prior to the commencement of the examination, SOMPONG WATTANASIN was duly sworn by me to testify the truth, the whole truth and nothing but the truth.

I DO FURTHER CERTIFY that the foregoing is a true and accurate transcript of the testimony as taken stenographically by and before me at the time, place and on the date hereinbefore set forth, to the best of my ability.

I DO FURTHER CERTIFY that I am neither a relative nor employee nor attorney nor agent of any of the parties to this action, and that I am neither a relative nor employee of such attorney or counsel, and that I am not interested directly or indirectly in the interference either as counsel, attorney, agent or otherwise.

Gary M. Talpins, C.S.R.
License No. XI00561

Case No. 600-7101/CONT/INT.
Patent

#93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

[redacted]

V. Interference No. 102,975

FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

FYI

WATTANASIN NOTICE OF FILING OF THE RECORD
37 CFR §1.653(c)

MAY 19 1993
RECEIVED IN
BOX INTERFERENCE

Appended is the Wattanasin Consolidated Record for Interference Nos. 102,648 and 102,975.

Respectfully submitted,

Diane Furman 5/17/93
Diane E. Furman
Attorney for the Party Wattanasin
Registration No. 31,104
201-503-7332

Enclosures:

Record
Volumes I, II, III, IV, V

Exhibits
A-1, A-2, A-3; B-1, B-2; C-1, C-2, C-3; D-1, D-2, D-3; E-1, E-2, E-3, E-4, E-5; F-1; G-1, G-2; H-1; I-1; J-1; K-1; L-1; M-1, M-2, M-3, M-4, M-5; N; O; P-1, P-2, P-3; Q; R; S; T; U-1, U-2; V-1, V-2; W; X; Y-1, Y-2; Z; S-1, S-2, S-3, S-4.

SANDOZ CORPORATION
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DEF:rmf
May 17, 1993

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on May 17, 1993
(Date of Deposit)
Diane E. Furman
(Name of applicant, assignee, or Registered Representative)
Diane Furman
(Signature)
5/17/93
(Date of Signature)

CERTIFICATE OF SERVICE

It is hereby certified that a true copy of the paper entitled:

WATTANASIN NOTICE OF FILING OF THE RECORD
37 CFR §1.653(c)

and the Record and Exhibits appended thereto were served on counsel for the party Fujikawa et al., this 17th day of May 1993, by postage pre-paid first-class mail addressed to the following:

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Diane Furman
Diane E. Furman

#38 + #93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN v. FUJIKAWA ET AL.

[redacted]
INTERFERENCE NO. 102,374

FYI

WATTANASIN CONSOLIDATED RECORD

VOLUME II

[PAGES 136 - 226]

MAY 19 1993
RECEIVED IN
BOX INTERFERENCE

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May 15, 1993

S/17/93

"RIBBON COPY FOR PARTY Wattanasin"

86

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF FAIZULLA G. KATHAWALA PURSUANT TO 37 CFR §1.672

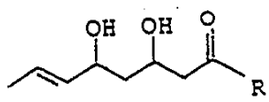
I, Faizulla G. Kathawala, Ph.D., do hereby declare as follows:

(1) That I am employed by Sandoz Pharmaceuticals Corporation as Director of Medicinal Chemistry -- Lipoprotein Metabolism. I am the supervisor of Dr. Sompong Wattanasin. All activities referred to in this Declaration took place in the United States.

(2) For over a decade Sandoz has been involved in an intense effort to discover compounds which have HMG-CoA reductase inhibiting activity. This project began in 1979 when I was named the section head, supervising one other Ph.D. and his technician. Our research team expanded until there are five laboratory units each headed by a Ph.D. and also staffed by 12-15 other scientists. Sompong Wattanasin joined the project in 1982 as a Post-Doctoral level scientist working under my direction, and was later appointed as head of one of the five laboratory units.

(3) That prior to and during the same time as the invention of the quinoline-HMG-CoA reductase inhibitory compounds claimed in the Wattanasin patent application

Serial Number 07/498,301, I and/or other scientists in my department had invented other HMG-CoA reductase inhibitors which were chemically analogous to such quinolines except that the quinoline moiety was replaced by another moiety which included: the pyrazole, pyrimidine, indene, pyrrole, naphthalene and indole systems. By "system" I mean the compound either had the following side chain



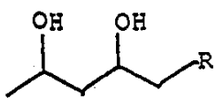
and was a salt form, an acid form, or an ester form, or the compound was in a lactone form. Additionally, it included the 3R,5S forms as well as racemates. Based on the chemistry we learned from these other systems, we expected that if one of these forms showed biological activity, the other forms could be expected to show activity as well. Thus, when a scientist referred to compounds in a generic manner, it was understood by everyone involved to include salts, acids, esters and lactones, even if only one of these was actually drawn.

(4) That when a scientist had an idea for making a new system, he would review the idea with me prior to the start of the synthesis. The proposed synthetic pathways would also be discussed. Dr. Wattanasin reviewed his idea for making a quinoline system with me prior to the synthesis of the first quinoline.

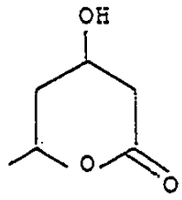
WATTANASIN CONCEPTION PRIOR TO AUGUST 20, 1987

1. On or before November 28, 1983, the subject invention was disclosed to me by Dr. Sompong Wattanasin. On November 28, 1983, I received a report from Dr. Wattansin in which compounds of the Wattanasin patent application were proposed for synthesis.

Exhibit A-1 comprises a true copy of the report I received. I understood the "L" in structure 14 to include the following side chain (where R indicates an acid, salt or ester) and also the lactone form.



and

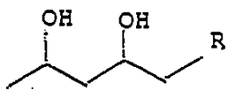


I understood that the open chain compounds were preferably in the 3R,5S form or in an racemic mixture, and that the lactone was preferably in the 4R,6S form or in a trans racemic mixture.

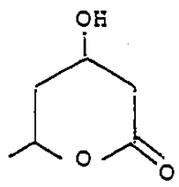
2. On November 19, 1984, I received a report from Dr. Wattanasin, proposing the synthesis of additional compounds which form the subject of this invention.

Exhibit A-2 comprises a true copy of the report I received. For each of the structures drawn on page 1 of Exhibit A-2, I understood "L" to include the following

chain (where R indicates an acid, salt or ester) and also the lactone form.



and



I understood that the open chain compounds were preferably in the 3R,5S form or in an erythro racemic mixture, and that the lactone was preferably in the 4R,6S form or in a trans racemic mixture.

3. On March 16, 1987 I reviewed, understood, and signed and dated as a witness, a disclosure of invention prepared by Dr. Wattanasin for the compounds of this patent application.

Exhibit A-3 is a true copy of the disclosure of invention, bearing my signature as a witness. I understood Compound I of Exhibit A-3 to include the salt and acid forms as well as the ester form shown. I also understood that the preferred stereochemistry for the open chain compound was the 3R,5S or the erythro racemate, and that for the lactone, the 4R,6S form or the trans racemate was preferred.

4. During the time that Drs. Wattanasin and Patel made the compounds described in their laboratory notebooks, I observed their work in my laboratory, and I was in contact with them on a frequent basis concerning their progress and results. Dr. Wattanasin spent a considerable amount of time and effort on this project.

WATTANASIN ACTUAL REDUCTION TO PRACTICE

1. To the best of my knowledge and belief, the Laboratory notebook pages which form Exhibits B-1, B-2, and F-1 are accurate reflections of the work performed in my laboratory.

2. I was aware that certain of the Wattanasin compounds were sent to Dr. Scallen for in vitro biological testing prior to August 20, 1987. I was aware that Dr. Scallen reported the results he obtained to Dr. Robert Damon. Dr. Damon reported these results to me.

Exhibit E-5 contains true copies of reports of activities of compounds which Dr. Damon sent to me and other investigators involved in the HMG-CoA reductase project.

3. That based on the in vitro biological activity, I knew, prior to August 20, 1987, that compounds according to this invention were HMG-CoA reductase inhibitors. I therefore knew that they possessed utility as anti-cholesterol synthesis agents, and therefore as hypolipoproteinemic and anti-atherosclerotic compounds.

[redacted]

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4. I also believed, prior to August 20, 1987, based on the in vitro data for compound 63-366, that compound 63-366 and other compounds of the invention would also have activity as an HMG-CoA reductase inhibitor when administered in vivo, to a patient.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 12 day of Nov., 1992.

Faizulla G. Kathawala
FAIZULLA G. KATHAWALA, Ph.D.

[redacted]

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF NICHOLAS A. PAOLELLA PURSUANT TO 37 C.F.R. §1.672

I, Nicholas A. Paolella, do hereby declare as follows:

(1) That I was a Senior Scientist A employed by Sandoz Pharmaceuticals Corporation from 1960 to 1991. In the course of my employment I synthesized compounds, including HMG-CoA reductase inhibiting compounds and I am familiar with the chemistry employed to make such compounds.

(2) That all activities referred to in this Declaration took place in the United States.

(3) That I have reviewed Sompong Wattanasin's Laboratory Notebook #1049 pages 237, 241, 248, 251, 245, Laboratory Notebook #1079 pages 22, 24, 27, 30, 33, 34, 39, 105, 106, 110 and 111.

(4) That I understood the experiments reported on these pages, and read and understood the aforementioned Laboratory Notebook pages, which I signed as a witness prior to August 20, 1987.

Exhibit B-1 contains true copies of Sompong Wattanasin's Notebook #1049, pages 237, 241, 248, 251, 245 and Notebook #1079, pages 22, 24, 27, 30, 33, 34, 39, 105, 106, 110 and 111 which bear my signature.

It is my recollection that I signed these pages prior to August 20, 1987.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 6th day of November 1992.

Nicholas A. Paolella
NICHOLAS A. PAOLELLA

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

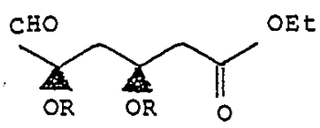
WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF PRASAD KAPA PURSUANT TO 37 CFR §1.672

I, Prasad Kapa, do hereby declare as follows:

(1) That I am a chemist employed by Sandoz Pharmaceuticals Corporation in the Process Research and Development Group. All activities referred to in this Declaration took place in the United States.

(2) That on or prior to July 31, 1983, I synthesized the following racemic compound:



R = t-butyl-diphenyl silyl

(3) That on or prior to May 6, 1985, I supplied this racemate to Dr. Sompong Wattanasin for use in his synthesis of HMG-CoA reductase inhibiting compounds; and that this is the compound referred to as the "Prasad Aldehyde" in Dr. Wattanasin's notebook #1127, page 9, which I have reviewed.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 12th day of November 1992.

PRASAD KAPA, Ph.D.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v. Interference Nos. 102,648, 102,975

FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF RAJESHVARI PATEL PURSUANT TO 37 C.F.R. §1.672

I, Rajeshvari Patel, do hereby declare as follows:

(1) That I am a chemist, who was employed by Sandoz Pharmaceuticals Corporation, 59 Route 10, East Hanover, N.J. during the time when Dr. Sompong Wattanasin was in the process of reducing to practice compounds claimed in U.S. Patent Application Serial Number 07/498,301.

(2) That one of my job responsibilities included the synthesis of certain compounds under the direction and supervision of Dr. Wattanasin.

(3) That all activities referred to in this Declaration took place in the United States of America.

A. SYNTHESIS OF COMPOUNDS 64-933, 64-934/Na, 64-935 AND 64-936/Na

Under the supervision of Sompong Wattanasin, I synthesized compounds 64-933, 64-934/Na, 64-935 and 64-936/Na of the invention. I kept a record of this activity in my Laboratory Notebook #1206.

Exhibit F-1 comprises a true copy of my Laboratory Notebook #1206, Pages 130, 137, 145, 153, 158, 166, 172, 175, 176, 179, 190 and 201.

It was my practice to date the top of each laboratory notebook page on the date I started the experiment reported on the page, and to sign the page and date my signature after the experiment was completed.

General Description of Laboratory Notebook Pages

i. Molecular Weight:

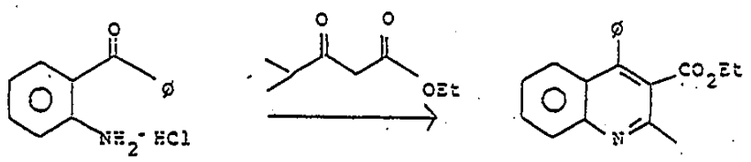
To determine molecular weight of each compound and its intermediates, mass spectrometry was performed. The molecular weight which was determined is the weight of the molecular ion, or $M\text{-}H^+$, where M is the compound of interest. Thus, to calculate the molecular weight of the compound rather than its ion, one must subtract the molecular weight of hydrogen (1) from the molecular weight of the ion. In the notebook pages, I recorded the molecular weight of the ion. Thus, the molecular weight of the compound is 1 less than what I recorded in my notebook.

ii. Spectra and Microanalyses:

The spectra and microanalyses were not performed by me, but were performed by an employee of the Physical Organic Chemistry Department of Sandoz Pharmaceuticals

Corporation. Upon receipt of the spectra from the Physical Organic Chemistry Department, I filed them in their own folder arranged by their compound number. Reference is made to the work of Dr. Sandor Barcza for details concerning analysis procedures.

Notebook #1206, page 130 is dated June 1, 1987 at the top in my handwriting, and contains my dated signature of June 8, 1987, at the bottom of the page. This page documents the following reaction which I performed:



(1206-130-27)

Ø = phenyl group

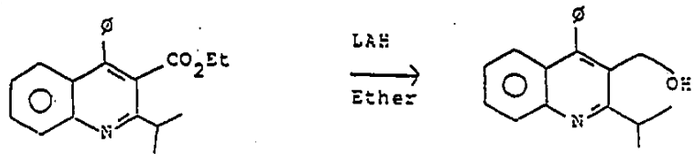
(1206-129-18)

The compound on the left side of the equation was designated 1206-129-18. A mixture of 11.5 g (0.04930 mol) of 1206-129-18, 11.93 ml (0.073958 mol; 1.5 equivalents) of and 105 ml EtOH was heated to reflux for six hours (10:00 A.M. to 4:00 P.M.) and then stirred at room temperature overnight.

The following day, the reaction mixture was evaporated to dryness to give a yellow oil with the rotary evaporator, basified with NH_4OH and extracted with ether, and the ether extract was washed with H_2O and then brine, dried with anhydrous sodium sulfate, and filtered. The filter cake was washed with ether and the washing was

combined with the initial filtrate and evaporated to give 10.21 g of an orange-yellow solid, designated 1206-130-27. IR and NMR spectra were performed and follow Laboratory Notebook #1206, page 130. Yield was calculated to be 64.86%. The spectra were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-130-27).

Notebook #1206, page 137 is dated June 9, 1987 at the top in my handwriting, and contains my dated signature of July 2, 1987 at the bottom of the page. This page documents the following reaction which I performed:



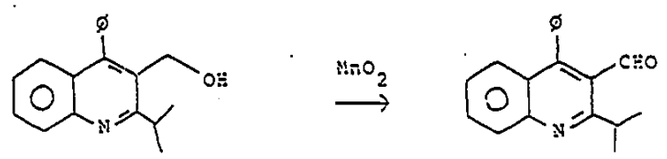
(1206-130-27) (1206-137-31)

To 10.21 g (0.0319621 mol) of 1206-130-27 in 100 ml dry ether with cooling was added 2.43 g (0.063242 mol) LAH (lithium aluminum hydride) portion-wise. The reaction was exothermic and foaming occurred. The mixture was stirred at room temperature for three hours (9:35 A.M. to 12:35 P.M.).

The reaction mixture was poured into ice water (the reaction was strongly exothermic). The result was extracted with ether and the ether extract was washed with water and then brine, dried with anhydrous sodium sulfate and filtered. The filter cake was washed with ether, and

the washing was combined with the initial filtrate. Evaporation gave 8.5 g of a yellow solid, designated 1206-137-31. IR and NMR spectra were performed and the results follow Laboratory Notebook #1206, page 137. The spectra were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-137-31). Yield was calculated at 95.8% of theoretical.

Notebook #1206, page 145 is dated June 17, 1987 at the top in my handwriting, and contains my signature. This page documents the following reaction which I performed:



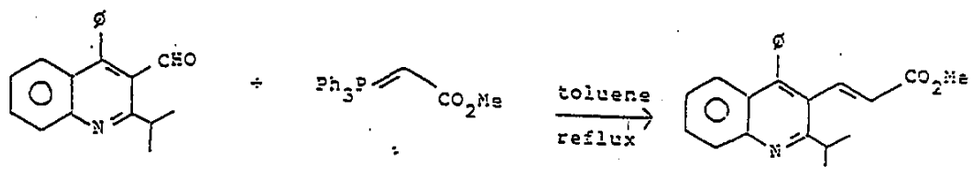
(1206-137-31) (1206-145-25)

To 8.0 g (0.0288392 mol) of 1206-137-31 in 150.0 ml toluene was added 16.0 g activated MnO_2. This was heated to reflux for approximately 3-3/4 hours (11:00 A.M. to 2:45 P.M.). The result was filtered through a pad of silica gel. During filtration, it separated into two bands, which were then filtered separately and evaporated separately. Both were yellow solids: (a) 2.6518 g designated 1206-145-25 with a molecular weight of 276, which was determined to be the desired product; and (b) 4.4663 g, designated 1206-145-26, with a molecular weight of 278, which was determined to be the starting material.

IR and NMR spectra were performed on 1206-145-25 and the results follow Laboratory Notebook #1206, page 145. The spectra were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-145-25).

This process was repeated with 1206-145-26 as recorded in Laboratory Notebook #1206, page 148, and 3.26 g of the same compound as 1206-145-25 was obtained, and designated 1206-148-33. Thus total yield was calculated as 2.6518 g + 3.26 g = 5.91 g. Theoretical yield was 7.91 g, yield was therefore 74.52%.

Notebook #1206, page 153 is dated <u>June 30, 1987</u> at the top in my handwriting, and contains my dated signature of July 6, 1987. This page documents the following reaction which I performed:



(1206-145-25)
(1206-148-33)

(1206-153-40)

Ph = phenyl group
Me = methyl group

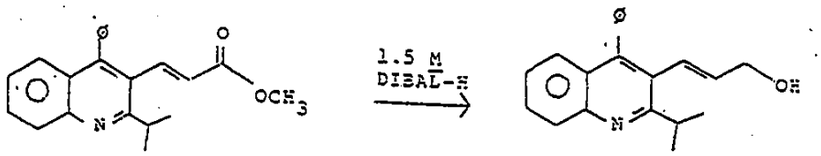
5.91 g of the combination of 1206-145-25 and 1206-148-33 (0.0214909 mol), 8.6135 g of Ph_3P CO_2Me(0.025789 mol) and 85 ml of toluene were heated to

reflux for 1.5 hours. (Before heating this was a yellow heterogeneous mixture). It was then stirred at room temperature overnight.

The following day, the reaction mixture was diluted with 50% ether/petroleum ether and filtered through a pad of silica gel. The filter cake was washed with 50% ether/petroleum ether, the washing was combined with the initial filtrate and evaporated to dryness to give 8.6 g of a yellow crystalline solid. Trituration with methanol gave 5.5198 g of an off-white solid, designated 1206-153-31, molecular weight 331; yield was 77.6%. The mother liquor was evaporated to dryness, leaving a 2.7593 g of a yellow oil, designated 1206-153-34.

Trituration of 1206-153-34 with methanol gave 761.6 mg of a light yellow solid, designated 1206-153-37, with a molecular weight of 331. Evaporation of the mother liquor to dryness resulted in a yellow solid, designated 1206-153-38. 1206-153-31 and 1206-153-37 were combined and designated 1206-153-40. The melting point of 1206-153-40 was found to be 128-130°C. Spectra were run on 1206-153-31 (NMR), 1206-153-37 (NMR) and 1206-153-34 (IR) and the results follow Laboratory Notebook #1206, page 153. The spectra of 1206-153-31 and 1206-153-37 were judged by me and Dr. Wattanasin to be consistent with the desired product.

Notebook #1206, page 158 is dated July 7, 1987 at the top in my handwriting, and contains my dated signature of July 17, 1987. This page documents the following reaction which I performed:

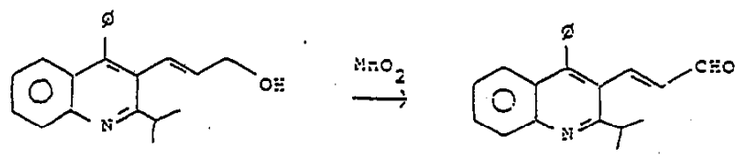


(1206-153-40) (1206-158-41)

To a solution of 6.25 g of 1206-153-40 (0.0188821 mol) in 75 ml CH_2Cl_2 at -78°C was added 25.18 ml of 1.5 M DIBAL-H (diisobutylaluminum hydride) (0.0377642 mol; 2 equivalents) in toluene. This was stirred at -78°C for about three hours (12:15 P.M. to 3:10 P.M.). The reaction was then quenched with 12.5 ml 2 N NaOH, diluted with EtOAc, and stirred at room temperature overnight. A white solid (gel) came out of solution.

The following day, the reaction product was filtered through a pad of silica gel, washed with EtOAc, water, and then brine, dried with anhydrous sodium sulfate and evaporated to dryness. The result was 5.42 g of an off-white solid, designated 1206-158-35. Yield was 73.7% theoretical yield. The solids were dissolved in Et_2O, and the insoluble portion (aluminum oxide) was filtered off. The solution was evaporated to dryness, resulting in 5.22g of white-yellow solids designated 1206-158-37. The solids were dissolved in Et_2O, and the insoluble portion (aluminum oxide) was filtered off. The resulting solution was evaporated to dryness, resulting in 4.2117 g of a yellowish solid, designated 1206-158-41, with a molecular weight of 303 and a melting point of 119-121°C. NMR and IR spectra were run on 1206-158-41 and the results follow Laboratory Notebook #1206, page 158. The spectra were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-158-41).

Notebook #1206, page 166 is dated July 15, 1987 at the top in my handwriting, and contains my dated signature of July 20, 1987. This page documents the following reaction, which I performed:



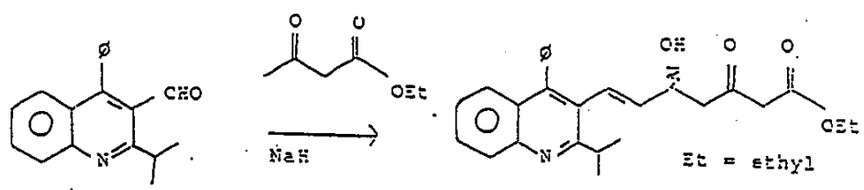
(1206-158-41) (1206-166-30)

To 4.0 g of 1206-158-41 (0.0132013 mol) in 50 ml toluene was added 8.0 g activated MnO_2. This was heated to reflux for one hour (2:00 P.M. to 3:00 P.M.), then stirred at room temperature overnight.

The following day, the reaction product was filtered through a pad of silica gel. Evaporation to dryness gave 3.4946 g of a yellow crystalline material, designated 1206-166-30, with a molecular weight of 301. NMR and IR spectra were run on 1206-166-30 and the results follow Laboratory Notebook #1206, page 166. Yield was 88% theoretical yield. The spectra were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-166-30).

Twelve days later, a microanalysis was performed. Two days later, the melting point was determined to be 98-101°C.

Notebook #1206, page 172 is dated July 20, 1987 at the top in my handwriting, and contains my dated signature of July 21, 1987. Notebook #1206, page 175 is dated July 22, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987. These pages document the following reaction which I performed:



(1206-166-30) (1206-175-4)

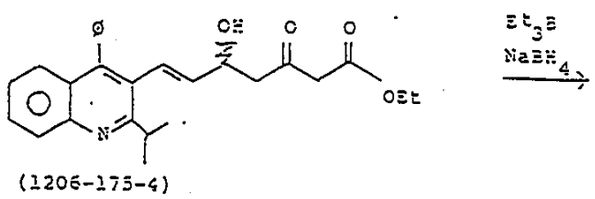
To a solution of 3.5 g (0.0116279 mol) of 1206-166-30 in 40 ml dry THF at -5°C to -10°C was added 38 ml of a previously prepared solution of the dianion of ethyl acetoacetate, the details of the preparation of which are set forth below. The color change from yellow to orange to dark red, suggesting that the reaction had occurred. A TLC (using 50% ether/petroleum ether) run after 15 minutes indicated the reaction was complete. The reaction mixture was stirred for 30 minutes.

The reaction mixture was quenched with NH_4Cl solution, extracted with EtOAc, resulting in two layers. The organic layer was separated and was washed with water then brine, dried with anhydrous sodium sulfate and filtered. Evaporation gave 5.9188 g of a yellow oil, designated 1206-172-41. Yield was 67.87% theoretical.

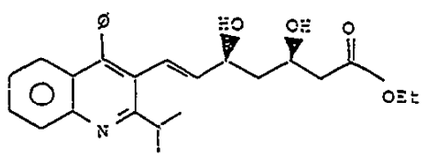
To make the dianion solution used above, the following procedure was used. A solution of 5 ml ethyl acetoacetate in 50 ml dry THF was added 1.9 g of 50% NaH in THF at -5° to 0°C. This was stirred for 15 minutes (the solution was foaming as H_2 was evolved). At -10° to -15°C, 27 ml of 1.6 M n-butyllithium/hexane was added and the mixture was stirred for 20 minutes at -10°C. 92 ml of a yellow homogeneous solution resulted (0.04 mol).

Flash chromatography through silica gel (25% ether/petroleum ether) of 1206-172-41 gave 3.4004 g of a yellow solid, designated 1206-175-4. Melting point was 84-87°C. Yield was 68%. A microanalysis was performed and the results are shown. NMR and IR spectra were run on 1206-175-4 and the results follow Laboratory Notebook #1206, page 172. The spectra were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-175-4).

Notebook #1206, page 176 is dated July 23, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987. This page documents the following reaction which I performed:



(1206-175-4)



(1206-176-41)

To a homogeneous solution of 1.0 g (0.0023201 mol) 1206-175-4 in 10 ml dry THF and 2.5 ml methanol was added 3.5 ml 1 M Et_3B (0.0034801 mol; 1.5 equivalents) in THF. This was stirred at room temperature for one hour (9:45 A.M. to 10:45 A.M.). Then the solution was cooled to -78°C. 0.1315 g of $NaBH_4$ (0.0034810 mol; 1.5 equivalents) was added portion-wise. This was then stirred at -78°C for four hours (11:00 A.M. to 3:00 P.M.).

The reaction was quenched with 5 ml acetic acid at -78°C. Ethyl acetate was then added and the mixture was allowed to warm to room temperature. The organic layer was washed with saturated sodium bicarbonate solution, water, and brine. It was then dried, filtered and evaporated to dryness. The residue was redissolved in methanol and evaporated to dryness. The evaporation process (in methanol) was repeated until TLC showed the desired product was obtained, 1.0914 g of an orange oil, designated 1206-176-39.

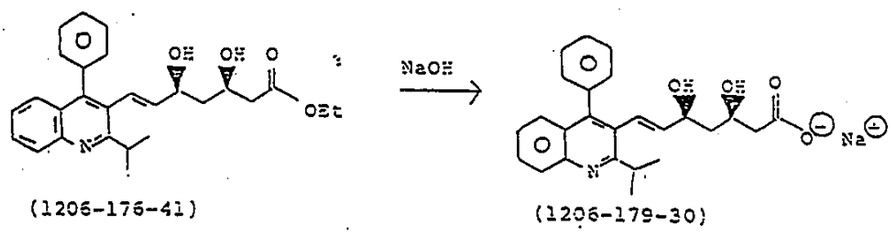
Flash chromatography on silica gel (80% ether/ petroleum ether) gave two products: (a) F_{4-6}, 0.4043 g of a yellow solid, designated 1206-176-41 with a molecular weight of 433 and M.P. 104-106°C, which was shown by HPLC to be 98.3% pure; and (b) F_{7-13}, 0.510 g of a yellow solid designated 1206-176-43, with a molecular weight of 433, which was shown to be 93.2% pure by HPLC. IR and NMR spectra were run on both 1206-176-41 and 1206-176-43 and follow Laboratory Notebook #1206, page 176. Based on these spectra, compound 1206-176-41 was determined to be

Rajeshvari Patel

the desired product. Compound 1206-176-41 was eventually renamed 64-933.

A sample of 64-933 was sent to Dr. Scallen for biological testing in his in vitro microsomal assay for HMG-CoA reductase inhibition activity. It was shown by Dr. Scallen to possess inhibition activity prior to December 7, 1987. I learned of this activity from Dr. Damon. Thus, prior to December 7, 1987, I knew that 64-933 was useful as an anti-cholesterol biosynthesis agent, and would be useful in treating atherosclerosis and other conditions resulting from excessive cholesterol biosynthesis.

Notebook #1206, page 179 is dated July 28, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987. This page documents the following reaction which I performed:

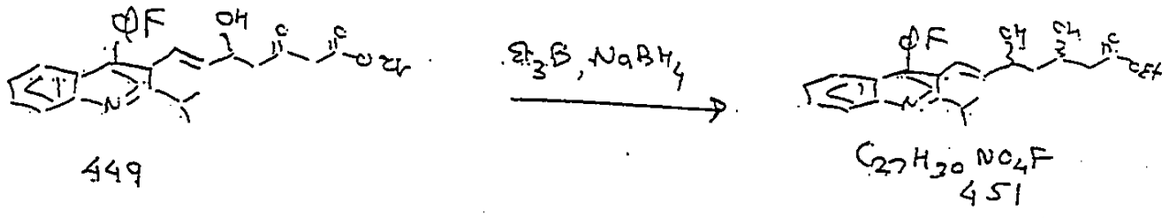


(1206-176-41) (1206-179-30)

To 200.0 mg 1206-176-41 in 5 ml absolute ethanol at 0°C. was added approximately 439 µml of 0.5N NaOH. This was stirred at 0°C. for approximately 1 hour. A yellow oil resulted. The mixture was diluted with ether and evaporated to a yellow oil. This was re-diluted with ether and solids precipitated out of solution. The solids were washed with ether, the ether was decanted, and the solids were dried under vacuum to obtain 178.8 mg of yellow solids designated 1206-179-30. NMR and IR spectra and a microanalysis were performed. The spectra appear after Notebook #1206, page 179, and were judged by me and Dr. Wattanasin to be consistent with the desired product (1206-179-30). The product shrunk at 187°C and the melting point was above 210°C.

1206-179-30 was re-named 64-934. It was submitted to Dr. Scallen for biological testing in his above-mentioned in vitro microsomal assay and was found to be active.

Notebook #1206, page 190 is dated August 10, 1987 at the top in my handwriting, and contains my dated signature of September 1, 1987. This page documents the following reaction which I performed:



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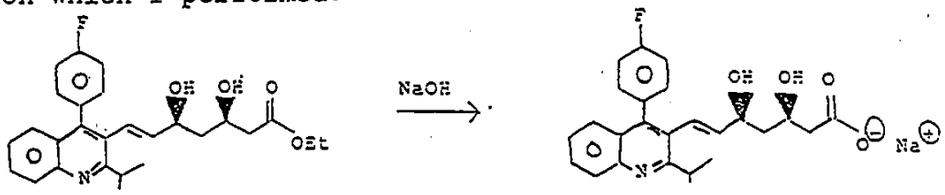
$C_{27}H_{30}NO_4F$
451

To a solution of 400 mg 1206-187-18 in 133.6 ml THF at room temperature was added 1M Et_3B (0.0001437 mol) and 1 ml air (by syringe). The solution was stirred at room temperature for 1 hr. The solution was then cooled to -78°C and 10 mg $NaBH_4$ was added. The reaction mixture was cooled to -78°C, 51 mg $NaBH_4$ was added and stirring was continued at -78°C from 12 noon to 3 p.m. The reaction was quenched and extracted with EtOAc, and washed with saturated $NaHCO_3$, dried, filtered, washed with MeOH five times to give a yellow oil which was chromatographed to give a yellow-orange oil,, 1206-190-38, which was dried over high vacuum to give 206.6 mg of 1206-190-41 which was believed to be the erythro racemate.

1206-190-41 was re-named 64-935. It was submitted to Dr. Scallen for biological testing in his above-mentioned in vitro microsomal assay and was found to be active.

Notebook #1206, page 201 is dated August 25, 1987 at the top in my handwriting, and contains my dated signature of September 1, 1987. This page documents the following reaction which I performed.



(1206-190-41) (1206-201-30)

To 100 mg 1206-190-41 in 5 ml absolute ethanol, at 0°C with stirring was added approximately 217.3 µml 1N NaOH dropwise. The mixture was stirred at 0°C for approximately 3 hours, resulting in a yellow oil.

This was diluted with ether, and evaporated to dryness to produce a yellow oil. Upon the addition of ether, yellow solids precipitated out. These were filtered, washed and dried to give 86.4 mg of a yellow solids designated 1206-201-30.

NMR and a microanalysis were performed on 1206-201-30. The spectrum appears after Notebook #1206, page 201. It was judged by me and Dr. Wattanasin to be consistent with the desired product (1206-201-30). Its melting point was greater than 225°C.

1206-201-30 was renamed 64-936.

Compounds 64-933, 64-934/Na, 64-935 and 64-936/Na were sent to Dr. Scallen for biological testing in his in vitro microsomal assay for HMG-CoA reductase inhibition activity.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 13th day of November, 1992.

Rajeshvari D Patel
RAJESHVARI PATEL

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v. Interference Nos. 102,648, 102,975

FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

SUPPPLEMENTAL DECLARATION OF RAJESHVARI PATEL
PURSUANT TO 37 C.F.R. §1.672

I, Rajeshvari Patel, do hereby declare as follows:

(1) That I am a chemist, who was employed by Sandoz Pharmaceuticals Corporation, 59 Route 10, East Hanover, N.J. during the time when Dr. Sompong Wattanasin was in the process of reducing to practice compounds claimed in U.S. Patent Application Serial Number 07/498,301, and during the time periods referred to in my Declaration pursuant to 37 CFR 1.672 and this Supplemental Declaration pursuant to 37 CFR 1.672.

(2) That one of my job responsibilities included the synthesis of certain compounds under the direction and supervision of Dr. Wattanasin.

(3) That all activities referred to in this Declaration took place in the United States of America.

(4) The contents of my Declaration made Pursuant to Rule 37 CFR 1.672 are hereby incorporated by reference in their entirety.

SYNTHESIS OF COMPOUND 64-935

Under the supervision of Sompong Wattanasin, I synthesized compound 64-935 of the invention. I kept a record of this activity in my Laboratory Notebook #1206.

Exhibit F-1 comprises a true copy of my Laboratory Notebook #1206, Pages 130, 137, 145, 153, 158, 166, 172, 175, 176, 179, 190 and 201.

Exhibit L-1 hereto comprises a true copy of my Laboratory Notebook #1206, Pages 86, 99, 103, 119, 178, 181, 183, 185, 186, and 187.

These pages show the complete synthesis of compound 64-935.

It was my practice to date the top of each laboratory notebook page on the date I started the experiment reported on the page, and to sign the page and date my signature after the experiment was completed.

Notebook #1206, page 86 is dated April 13, 1987 in my handwriting and contains my dated signature of April 14, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-86-387 from benzoxazine according to the method of Suzuki et al., JOC, 1961, 2239, 2241.

Notebook #1206, page 99 is dated April 13, 1987 at the top in my handwriting, and contains my dated signature of April 14, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-99-26 by a process analogous to the one recorded in Notebook #1079, Page 248 in Exhibit B-1.

Notebook #1206, page 103 is dated May 4, 1987 at the top in my handwriting, and contains my dated signature of May 5, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-103-28 by a process analogous to the one recorded my Notebook #1206, Page 130 in Exhibit F-1.

Notebook #1206, page 119 is dated May 20, 1987 at the top in my handwriting, and contains my dated signature of May 27, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-119-30 by a process analogous to the one recorded in my Notebook #1206, Page 137 in Exhibit F-1.

Notebook #1206, page 178 is dated July 27, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-178-31 by a process analaogous to the one recorded in my Notebook #1206, page 145 in Exhibit F-1.

Notebook #1206, page 181 is dated July 29, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-181-26 by a method analogous to the one recorded in my Notebook #1206, page 153 in Exhibit F-1.

Notebook #1206, page 183 is dated August 3, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987 at the bottom of the page. This page documents the synthesis of compound 1206-181-26 by a method analogous to the one recorded in my Notebook #1206, page 158 in Exhibit F-1.

Notebook #1206, page 185 is dated August 4, 1987 at the top in my handwriting. This page documents the synthesis of compound 1206-185-31 by a method analogous to the one recorded in my Notebook #1206, page 166 in Exhibit F-1.

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Notebook #1206, page 186 is dated August 5, 1987 at the top in my handwriting, and contains my dated signature of August 5, 1987 at the bottom of the page. Notebook #1206, page 187 is dated August 5, 1987 at the top in my handwriting and contains my dated signature of September 1, 1987. These pages document the synthesis of compound 1206-187-18 by a method analogous to the one recorded in my Notebook #1206, pages 172 and 175 in Exhibit F-1.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 16th day of November, 1992.

Rajeshvari D Patel 11-16-92
RAJESHVARI PATEL

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF LAWRENCE B. PEREZ PURSUANT TO 37 CFR §1.672

I, Lawrence B. Perez, Ph.D. do hereby declare as follows:

(1) I am an Associate Fellow employed by Sandoz Pharmaceuticals Corporation since July 1987. In the course of my employment I synthesize compounds, including HMG-CoA reductase inhibiting compounds, and I am familiar with the chemistry employed to make such compounds. All activities referred to in this Declaration took place in the United States.

(3) I reviewed and and understood the experiments reported in Rajeshvari Patel's Laboratory Notebook #1206, pages 179, 190 and 201, before signing these pages.

(4) I reviewed and and understood the experiments reported in Rajeshvari Patel's Laboratory Notebook #1206, 86, 99, 103, 119, 124, 167, 173, 177, 178, 180, 181, 183, 185, 186 and 187, before signing these pages.

Exhibit F-1 comprises true copies of Rajeshvari Patel's Notebook #1206, pages 179 and 201, bearing my signature.

Exhibit L-1 comprises true copies of Rajeshvari Patel's Notebook #1206, pages 86, 99, 103, 119, 124, 167, 173, 177, 178, 180, 181, 183, 185, 186 and 187, bearing my signature.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this day of November, 1992.

Lawrence B. Perez, Ph.D.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF LAWRENCE B. PEREZ PURSUANT TO 37 CFR §1.672

I, Lawrence B. Perez, Ph.D. do hereby declare as follows:

(1) I am an Associate Fellow employed by Sandoz Pharmaceuticals Corporation since July 1987. In the course of my employment I synthesize compounds, including HMG-CoA reductase inhibiting compounds, and I am familiar with the chemistry employed to make such compounds. All activities referred to in this Declaration took place in the United States.

(3) I reviewed and and understood the experiments reported in Rajeshvari Patel's Laboratory Notebook #1206, pages 179, 190 and 201, before signing these pages.

(4) I reviewed and and understood the experiments reported in Rajeshvari Patel's Laboratory Notebook #1206, 86, 99, 103, 119, 124, 167, 173, 177, 178, 180, 181, 183, 185, 186 and 187, before signing these pages.

Exhibit F-1 comprises true copies of Rajeshvari Patel's Notebook #1206, pages 179 and 201, bearing my signature.

Exhibit L-1 comprises true copies of Rajeshvari Patel's Notebook #1206, pages 86, 99, 103, 119, 124, 167, 173, 177, 178, 180, 181, 183, 185, 186 and 187, bearing my signature.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19th day of November, 1992.

Lawrence B Perez
Lawrence B. Perez, Ph.D.

[redacted]

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF SANDOR BARCZA PURSUANT TO 37 CFR §1.672

I, Sandor Barcza, Ph.D., do hereby declare as follows:

(1) That I am employed by Sandoz Pharmaceuticals Corporation. My position, both prior to August 20, 1987 and during the time periods thereafter which are referred to herein, was Director of the Department of Physical Organic Chemistry.

(2) That all activities referred to in this Declaration took place in the United States, under my supervision.

(3) That it was the responsibility of personnel working under my supervision to perform various analyses of samples prepared by Sandoz chemists, including the determination or confirmation of chemical structure and purity.

(4) That individuals working under my direction initialed and dated the pages of the IR and NMR spectra which they personally recorded. I have reviewed B-1, B-2 and F-1 hereto, and in my best recollection, the following initials are those of the following named individuals.

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[redacted]

S.D.: Susan DiCataldo; Karl G.: Karl Gunderson; MXK: Michael X. Kolpak; F.M.: Frances McCrink; J.B.: (?); none of whom are now employed by Sandoz.

M.J.S.: Michael J. Shapiro, Fellow, Senior Scientific Staff, and head of the NMR laboratory.

Exhibits B-1, B-2 and F-1 contain true copies of IR and Spectra generated by the Physical Organic Chemistry Department under my supervision.

I. ANALYSIS OF WATTANASIN COMPOUNDS

Sandoz has established procedures which researchers must follow in order for my department to perform various analyses of compounds and mixtures. These procedures are outlined below and were company policy at the time when the samples of Exhibits B-1, B-2, and F-1 were analyzed, i.e., prior to August 20, 1987 and during the other time periods referred to herein.

When a scientist wants to have material analyzed, he completes a Request Sheet.

General Description of Exhibits C-1, C-2, C-3; D-2; G-1, G-2 and H-1

Exhibit C-1 comprises a blank Request Sheet, and is the same as that used during the time that the compounds of this patent application were analyzed, i.e., prior to

August 20, 1987 and during the other time periods referred to herein. Referring to Exhibit C-1, there are areas on the Request Sheet where the type of analysis can be requested, including IR spectrum, NMR spectrum, and microanalysis. Also, there is a space on the Request Sheet where the compound is identified by reference to its notebook number, page number and line number. In addition to filling out the form, the scientist provides a sample of the material in a vial which is also labeled with the notebook number, page number, and line number. The personnel who actually perform the analyses rely on the notebook number-page number-line number designation for identification of the sample, and then assign their own number to the analysis (spectrum).

Exhibit C-2 comprises true copies of the Physical Organic Chemistry Department's copies of Request Sheets for IR spectra.

Upon receipt of a completed Request Sheet and its accompanying sample, the Optical Spectroscopy Laboratory (Infra Red) Laboratory records each request in a laboratory logbook. The Infra Red Laboratory's logbook is kept in a three ring binder. Each sample is treated as a separate entry and is entered sequentially, into two sequential lines.

Exhibit C-3 comprises copies of the Physical Organic Chemistry Department's copies of Request Sheets for NMR spectra.

Exhibit D-2 comprises completed copies of Chemical Information forms for compounds of the invention.

Exhibit G-1 comprises copies from the Infra Red Laboratory's logbook.

Exhibit G-2 contains copies of the Microanalysis Laboratory logbook.

Exhibit H-1 comprises copies of printouts of entries of the computer database. The dates are the dates on which the structures and data were entered.

To my knowledge, the papers which comprise these Exhibits are true copies.

Compounds 63-366, 63-548, 63-549, 64-934/Na, 64-935 and 64-936/Na

The spectra of compounds 63-366, 63-548, 63-549, 64-934/Na, 64-935 and 64-936/Na were recorded under my supervision. The above-listed exhibits show the following:

Exhibit G-1 documents receipt of the Wattanasin compounds by the Infra Red Laboratory on the following dates:

Compounds from Exhibit B-1

Line 1377: receipt of compound 1049-237-27 on 5/31/84
Line 2009: receipt of compound 1079-22-28 on 8/10/84
Line 2029: receipt of compound 1079-27-25 on 8/14/84
Line 2514: receipt of compound 1079-105-35 on 11/ 9/84
Line 2589: receipt of compound 1079-111-19 on 11/21/84

Compounds from Exhibit B-2

Line 1012: receipt of compound 1127-5-23 on 5/ 6/85
Line 1094: receipt of compound 1127-11-34 on 5/17/85
Line 1095: receipt of compound 1127-11-37 on 5/17/85

Compounds from Exhibit F-1

Line 899: receipt of compound 1206-130-27 on 7/ 5/87
Line 922: receipt of compound 1206-137-31 on 7/12/87
Line 1007: receipt of compound 1206-153-34 on 7/16/87
Line 1037: receipt of compound 1206-158-41 on 7/21/87
Line 1052: receipt of compound 1206-175-4 on 7/23/87
Line 1084: receipt of compound 1206-166-30 on 7/30/87
Line 1087: receipt of compound 1206-176-41 on 7/30/87
Line 1093: receipt of compound 1206-179-30 on 7/31/87

The line number of the logbook becomes the assigned spectrum number. The spectrum number is written on the request sheet in the box on the right side marked "do not fill in" by the person who would be running the spectrum, along with that person's initials.

Exhibit C-2 contains the assigned numbers for the compounds:

Compounds from Exhibit B-1:

1049-237-27, assigned spectrum number 1377
1079-22-28, assigned spectrum number 2009
1079-27-25, assigned spectrum number 2029
1079-105-35, assigned spectrum number 2514
1079-111-19, assigned spectrum number 2589

Compounds from Exhibit B-2:

1127-5-23, assigned spectrum number 1012
1127-11-34, assigned spectrum number 1094
1127-11-37, assigned spectrum number 1095

Compounds from Exhibit F-1

1206-130-27, assigned spectrum number 899
1206-137-31, assigned spectrum number 922
1206-153-34, assigned spectrum number 1007
1206-158-41, assigned spectrum number 1037
1206-186-30, assigned spectrum number 1084
1206-175-4, assigned spectrum number 1052
1206-176-41, assigned spectrum number 1087
1206-179-30, assigned spectrum number 1085
1206-179-30, assigned spectrum number 1093

The NMR Laboratory assigns spectra numbers in the following manner: Upon receipt of a Request Sheet and accompanying sample, the request sheet is stamped "Received" with an automatic stamper which dates the request sheet and assigns it a number in sequential order.

Exhibit C-3 contain the assigned spectrum number in the box marked "do not fill in.":

Compounds from Exhibit B-1
1049-237-27, : spectrum number 4716 received on 5/30/84
1049-241-34, : spectrum number 4751 received on 6/ 1/84
1079- 22-28, : spectrum number 6255 received on 8/13/84
1079- 27-25, : spectrum number 6288 received on 8/14/84
1079- 30-23, : spectrum number 6404 received on 8/22/84
1079- 34-17, : spectrum number 6597 received on 9/ 5/84

Compounds from Exhibit B-2
1127- 5-23, : spectrum number 2517 received on 5/ 6/85
1127- 9-33, : spectrum number 2538 received on 5/ 7/85
1127- 11-34, : spectrum number 2683 received on 5/14/85
1127- 11-37, : spectrum number 2686 received on 5/14/85

Compounds from Exhibit F-1

1206-130-27: spectrum number 3256 received on 6/ 5/87
1206-137-31: spectrum number 3326 received on 6/12/87
1206-145-25: spectrum number 3450 received on 6/19/87
1206-145-26: spectrum number 3451 received on 6/19/87
1206-153-31: spectrum number 3596 received on 7/ 2/87
1206-153-37: spectrum number 3615 received on 7/ 7/87
1206-158-41: spectrum number 3677 received on 7/10/87
1206-166-30: spectrum number 3793 received on 7/16/87
1206-175- 4: spectrum number 3874 received on 7/22/87
1206-176-41: spectrum number 3934 received on 7/27/87
1206-176-43: spectrum number 3933 received on 7/27/87

Exhibit G-2 contains copies of the Microanalysis Laboratory's logbook containing the sample numbers listed below. Each sample is entered on one line in a sequential manner and the line number becomes the analysis number:

Compounds from Exhibit F-1:

Line 518: receipt of compound 1206-153-31 on 7/ 9/87
Line 524: receipt of compound 1206-158-41 on 7/15/87
Line 545: receipt of compound 1206-175- 4 on 7/23/87
Line 560: receipt of compound 1206-166-30 on 7/28/87
Line 563: receipt of compound 1206-179-30 on 7/29/87
Line 634: receipt of compound 1206-201-30 on 8/26/87

Compounds from Exhibit B-1:

Line 813: receipt of compound 1049-237-27 on 5/31/84

Upon completion of an IR or NMR spectrum, the chemist is provided with the original spectrum, and no copies are retained by the Physical Chemistry Department. Each spectrum contains information identifying the sample, including the sample's notebook number-page number-line number; the date, the operator, and any other notes which are relevant.

The chemist who requested the sample is primarily responsible for the interpretation of the structure based on the data provided by my department; however, my Department can provide assistance if necessary.

II. PROCEDURES FOR ASSEMBLING THE DATABASE

Another responsibility of the Physical Organic Chemistry Department was the assembly of a computerized database for use only by Sandoz employees which contains information regarding compounds produced by the chemists. The database information regarding the compounds of this patent application were assembled in the following manner. This procedure was the one in use when the compounds of this patent application were submitted.

Upon verification of the structure and purity of a sample, a "Chemical Information" form is completed, and an accompanying sample of the compound is submitted.

Exhibit D-1 is a blank Chemical Information form. The "Date" box at the upper right hand side of the form is filled in by the person registering the compound, and the Compound Number is assigned sequentially by the person registering the compound. (The initials of the person who is registering the compound is recorded on the computer database).

The Chemical Information form also includes a list of "screens" which are standard biological tests which the chemist may request. Abbreviations which appear on the forms (either printed or handwritten in the blank spaces) are as follows: AO= anti-obesity, GHI= growth hormone inhibition, GLUC= glucagon, HG= hypoglycemic, HL= hypolipidemia, PL= platelet, TC= tissue culture cholesterol absorption inhibition test, AM/AV= anti-microbial and/or anti-viral, Tr= Tripanosoma, Agro= agricultural, CSI= cholesterol synthesis inhibition, CSIV= cholesterol synthesis inhibition in vivo.

The Chemical Information Form also includes, at the bottom, a "Chem. No." which is the laboratory notebook-page number-line number of the sample.

Exhibit D-2 contains copies of Chemical Information forms for compounds which are contained in the above-identified patent application. These forms were submitted for compound registration in the database.

Upon receipt of the Chemical Information Form and sample, personnel under my direction enter this information into the Sandoz computer database.

Exhibit H-1 contains copies of printouts of entries of the computer database. The date which is recorded in the database is automatically supplied by the computer; it is not manually entered by the operator, and is not changed once it is generated.

Codes used in the databank are as follows. INT.REG.NO is the unique internal registry number, the number assigned sequentially to this compound in the Sandoz internal database. Information recorded across the top of the printout is as follows. SAH.NO is the "Sandoz Number" or the official Sandoz number for the compound. These numbers are assigned sequentially, and are never deleted. SALTCODE is the code of the type of salt form, if the compound is a salt. CHEM.NO. is the laboratory notebook-page number-line number designation of the sample. SUBMITTED is the date the data were entered into the database. DISCL is the number of the Invention Disclosure form which was submitted to the Patent and Trademark Department.

Each chemist who submits a compound for entry into the database must proofread the entry. The data in the database are considered accurate by the scientists at Sandoz, and the data as recorded in the database are relied upon in the course of further research, testing, and development.

Upon assignment of an official number, the samples are marked with their "SAH" number and are stored in the Drug Room.

III. STORAGE AND INVENTORY PROCEDURES

The Sandoz Drug Room is responsible for the storage of samples of compounds that are catalogued in the database.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 12th day of November, 1992.

Sandor Barcza

SANDOR BARCZA, Ph.D.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF DAVID WEINSTEIN PURSUANT TO 37 CFR §1.672

I, David Weinstein, do hereby declare as follows:

(1) That I am employed by Sandoz Pharmaceuticals Corporation. Presently I am Head of the Department of Lipid and Lipoprotein Metabolism. During the time when Dr. Wattanasin invented the compounds of his invention, I was in charge of the "Drug Room", which is the facility where samples of compounds produced by Sandoz chemists are stored.

(2) That all activities referred to in this Declaration took place in the United States.

TESTING OF WATTANASIN COMPOUNDS

1. That at the time when Dr. Wattanasin supplied the Drug Room with samples of compounds of his invention, both prior to and after August 20, 1987, the following procedure was in place:

A sample of the compound, labeled with its official Sandoz number, was given to the Drug Room personnel, and its receipt was recorded in the computer database.

Exhibit H-1 contains true copies of printouts of the database entries for various compounds of the invention.

Compound 63-366 was entered on November 26, 1984.
Compound 63-548 was entered on May 17, 1985.
Compound 63-549 was entered on May 17, 1985.
Compound 64-933 was entered on September 21, 1987.
Compound 64-934 was entered on September 21, 1987.
Compound 64-935 was entered on September 21, 1987.
Compound 64-936 was entered on September 22, 1987.

At the bottom right hand column of the printout is a box entitled "AMOUNTS,mg". This is the amount of compound which was deposited in the Drug Room. There are also notations in this box if samples were sent to biologists for testing, and whether the Drug Room currently has any of the compound on hand.

Referring to the printout for 63-366, the notation means that a 14.5 mg sample of the compound (the entire amount deposited in the Drug Room) was sent to Dr. Terence Scallen.

For compound 63-548, a 2.0 mg sample (from a total deposit of 4.8 mg) was sent to Dr. Scallen.

For 63-549, the entire 2.0 mg deposit was sent to Dr. Scallen.

For other compounds encompassed by this invention:

64-933: 50.0 mg deposited; 50 mg sent to Dr. Scallen
64-934: 50.0 mg deposited; 50 mg sent to Dr. Scallen
64-935: 20.0 mg deposited; 20 mg sent to Dr. Scallen
64-936: 20.0 mg deposited; 20 mg sent to Dr. Scallen

2. In addition to recording in the computer database, the Drug Room also recorded when a sample was sent to a researcher.

Exhibit I-1 is a true copy of the Drug Room records documenting that Dr. Terence Scallen was sent samples of the compounds as follows:

63-366: December 3, 1984
63-548 and 63-549: June 3, 1985
64-933, 64-934/Na, 64-935 and 64-936/Na: October 2, 1987

3. It has been Drug Room policy, in force since before the dates in question, that when a sample of a compound leaves the Drug Room, it may not be returned to the Drug Room. This policy is meant to eliminate the risk of mis-identifying samples, and prevent contamination of compounds on deposit with the Drug Room. Thus, when a sample is sent from the Drug Room to a researcher, the researcher may rely on the identity of the compound, its purity, and the fact that it has not deteriorated.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 12th day of November, 1992.

David Weinstein

DAVID WEINSTEIN, Ph.D.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF TERENCE J. SCALLEN PURSUANT TO 37 CFR §1.672

I, Terence J. Scallen, M.D., Ph.D., do hereby declare as follows:

(1) That I am a Professor of Biochemistry in the Department of Biochemistry, School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131.

(2) That all activities referred to in this Declaration took place in the United States.

BIOLOGICAL ACTIVITY OF WATTANASIN COMPOUNDS

1. I have done extensive research in the area of cholesterol biosynthesis inhibition and am familiar with compounds which possess cholesterol biosynthesis inhibition activity.

2. I have performed tests of biological activity on compounds supplied to me by Sandoz Pharmaceuticals Corporation both since 1980, and I have reported the results back to Sandoz. The compounds I receive are labeled with only their compound number, and no structural identification of these compounds is given until the testing is completed.

3. The compounds sent to me by Sandoz were tested to determine whether they are competitive inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis, and therefore inhibitors of cholesterol biosynthesis. If a compound possesses this activity, it would be useful for lowering the blood cholesterol level in animals; e.g., mammals and especially larger primates. A compound with this activity would therefore be a hypolipoproteinemic and anti-atherosclerotic agent.

4. There was an established protocol which was used in my laboratory for assaying the samples which I received, which is described on the first page of each of Exhibits E-1 to E-4 (and also for each group of test results in E-5) appended hereto.

In general, the test which I use to determine whether a compound has HMG-CoA reductase inhibition activity is as follows:

> 200 µl aliquots (1.08 - 1.50 mg/ml) of rat liver microsomal suspensions are prepared from male Sprague-Dawley rats (150-225g body weight), in Buffer A with 10 mM dithiothreitol (DTT). "Buffer A" is 0.04M potassium phosphate, pH 7.4, 0.05M KCl, 0.03M EDTA and 0.25M sucrose; (The microsomes were frozen before use.)

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The microsomal suspension is incubated with 10 µl of a solution of the test compound in dimethylacetamide (DMA), as described by Ackerman, et al. 1977 J. Lipid Res. v. 18 p. 408-413. In the assay, the rat microsomes are the source of HMG-CoA reductase enzyme which catalyzes the reduction of HMG-CoA to mevalonate. Rather than using a chloroform extraction procedure as described by Ackerman, et al., supra, a Dowex^{R} 1X8 (200-400 mesh, formate form) ion exchange column is used to separate the product, [^{14}C]mevalonolactone, which is formed by the HMG-CoA reductase reaction from the substrate, [^{14}C]HMG-CoA. [^{3}H]mevalonolactone is added as an internal reference. Inhibition of HMG-CoA reductase is calculated from the decrease in specific activity ([^{14}C/^{3}H]mevalonate ([^{3}H]MVA))) of test groups compared to controls.

5. In vitro assays of biological activity as an HMG-CoA reductase inhibitor, were performed in my laboratory under my supervision on compounds 63-366, 63-548, 63-549, 64-933, 64-934/Na, 64-935 and 63-366/Na; and I reported the results to Dr. Robert Damon of Sandoz.

Compound 63-366

On or before December 13, 1984, an in vitro biological assay of compound 63-366 was performed in my laboratory. I reviewed the results of the assay, and

determined that the compound has HMG-CoA reductase activity. On or before December 20, 1984, I communicated this result to Dr. R. Damon of Sandoz.

Exhibit E-1 comprises true copies of the testing protocol utilized and the Laboratory Notebook pages which recorded the data for compound 63-366.

The first two pages of Exhibit E-1 bear the date of December 13, 1984. It was the practice in my laboratory to date these pages with the date on which the testing of the compound was performed.

The third page of Exhibit E-1 shows the data I obtained for 63-366.

Compounds 63-548 and 63-549

On or before June 13, 1985, *in vitro* biological assays of Compounds 63-548 and 63-549 were performed in my laboratory. I reviewed the results of the assays, and determined that these compounds have HMG-CoA reductase activity. On or before June 30, 1985, I communicated those results to Dr. R. Damon of Sandoz.

Exhibit E-2 contains a true copy of the description of the procedure and the printout showing the data for 63-548 and 63-549. The printout pages bear a date of June 13, 1985. The practice in my laboratory was to date these pages with the date on which the testing of the compound was performed.

The data for compounds 63-548 and 63-549 are on the third page of Exhibit E-2.

Compounds 64-933, 64-934/Na, 64-935 and 64-936/Na

On or before October 8, 1987, in vitro biological assays of compounds 64-933, 64-934/Na, and 64-935 were performed in my laboratory. I reviewed the results of the assays, and determined that these compounds have HMG-CoA reductase activity. On or before October 20, 1987, I communicated these results to Dr. R. Damon of Sandoz.

On or before October 13, 1987, an in vitro biological assay of compound 64-936/Na was performed in my laboratory. I reviewed the results of the assay, and determined that this compound has HMG-CoA reductase activity. On or before October 20, 1987, I communicated these results to Dr. R. Damon of Sandoz.

Exhibit E-3 contains a true copy of the report I sent to Dr. Damon summarizing my results and the printouts for compounds 64-933, 64-934/Na and 64-935. The printout pages bear the date of October 8, 1987. The practice in my laboratory was to date these pages with the date on which the testing of the compound was performed.

Exhibit E-4 contains a true copy of the report I sent to Dr. Damon summarizing my results and the printout for compound 64-936/Na. The printout pages bear the date of October 13, 1987. The practice in my laboratory was to date these pages with the date on which the testing of the compound was performed.

Exhibit E-5 contains true copies (except that structures and IC_{50} values have been added), of the summary of the results of a series of assays which I performed on compounds including 63-366, 63-548, 63-549, 64-933, 64-934/Na, 64-935, and 64-936/Na which I sent to Dr. Damon.

It has been my judgment since prior to August 20, 1987, that the level of in vivo activity of a compound as a cholesterol inhibitor or anti-atherosclerotic when administered to a patient, is typically highly correlatable to its in vitro activity in my HMG-CoA reductase inhibitor assays.

As demonstrated by Exhibit E-5 hereto, since on or prior to December 31, 1984, I was involved in the testing of numerous Sandoz compounds in substantially the same assay as used for the quinoline compounds, to determine in vitro HMG-CoA reductase activity.

These other compounds have the same 3,5-dihydroxy heptenoic acid, ester, or salt side chain, or alternatively have internal ester, i.e. lactone form, as the Wattanasin quinoline compounds at issue. However, these compounds differ by having a different organic radical substituent of the side chain.

For example, I performed in vitro assays of Sandoz compounds having a substituted napthyl or indole substituent, at or about the same time as compound 63-366, as indicated by Exhibit E-5, hereto.

Therefore, I have substantial experience in testing compounds for HMG-CoA reductase activity in vitro; and I

was familiar with the in vivo activity of many of these compounds as a result of my discussions with Dr. Damon and Mr. Engstrom of Sandoz.

On or before December 31, 1984, I also used the assay described herein to determine IC_{50} values for the compound Mevastatin (Compactin) which was a known HMG-CoA reductase inhibitor for administration to a patient to treat hypercholesterolemia or atherosclerosis.

Additionally, on or before December 31, 1984, I determined the IC_{50} values for Sandoz compound 62-320/Na (fluvastatin sodium), which I also knew to be active in vivo on or prior to December 31, 1984.

Therefore, I was able to compare the IC_{50} values for the quinoline compounds to the IC_{50} values for mevastatin and fluvastatin sodium, both of which were known to be active in vivo.

Based on my knowledge and experience, it was my judgment since on or prior to December 31, 1984, that Wattanasin compound 63-366 would be active when administered in vivo to a patient for the treatment of hypercholesteremia or atherosclerosis, in a dosage amount recited by Wattanasin in his patent application. It was

also my judgment after determining the in vitro assay data for each of compounds 63-548, 63-549, 64-934/Na, 63-935 and 64-936/Na, that each of these compounds would also be active in vivo, and would be active when administered to a human patient in the dosage amounts recited in the Wattanasin specification.

. . .

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this day of November, 1992.

Terence J. Scallen 11/13/92
TERENCE J. SCALLEN, M.D., Ph.D.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF ROBERT E. DAMON PURSUANT TO 37 CFR §1.672

I, Robert E. Damon, II, Ph.D., do hereby declare as follows:

(1) That I am a chemist employed by Sandoz Pharmaceuticals Corporation. Among my responsibilities is coordination of the shipping of compounds to Dr. Terence Scallen and receiving data from him concerning the biological activity of new HMG-CoA reductase inhibiting compounds synthesized by Sandoz chemists.

(2) That all activities referred to in this Declaration took place in the United States.

TESTING OF WATTANASIN COMPOUNDS

1. Under my direction, Mrs. Honora Lukas of Sandoz sent samples of compounds stored in the Drug Room to Dr. Scallen for biological activity assaying.

Exhibit I-1 comprises what appear to be true copies of covering sheets accompanying shipments of compounds 63-366, 63-548, 63-549, 64-933, 64-934/Na, 64-935 and 64-936/Na to Dr. Scallen.

2. When Dr. Scallen performed assays on Sandoz compounds, including 63-366, 63-548, 63-549, 64-933, 64-934/Na, 64-935 and 64-936/Na, he sent the data to me.

Exhibit E-5 comprises true copies of reports that I received from Dr. Scallen reporting the results of his assay on the Wattanasin compounds (bearing structures and IC_{50} data handwritten by me after receipt).

As soon as I received the reports, I date-stamped them and initialed the date. The structures and IC_{50} numbers appearing on the reports were also written by me.

The first report I date-stamped on December 20, 1984.
The June 27, 1985 report I date-stamped June 28, 1985.
The October 8, 1987 report I date-stamped October 20, 1987.
The October 20, 1987 report I date stamped October 20, 1987.

3. IC_{50} Data: Based on the data supplied to me in the reports which make up Exhibit E-5, I calculated the IC_{50} value for each compound. IC_{50} is the concentration of the test substance in the assay system calculated to produce a 50% inhibition of HMG-CoA reductase activity. The smaller the IC_{50} value, the more active the compound was in the assay.

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4. I wrote the structural formulae and IC_{50} value for the compounds tested by Dr. Scallen on the reports received from Dr. Scallen.

5. My practice was that, within at most three or four days of receiving a report from Dr. Scallen, I would send the report (containing my handwritten structures and IC_{50} data) to Dr. Wattanasin and other researchers working in the HMG-CoA reductase inhibitor area.

6. I also recorded the data from Dr. Scallen in my laboratory notebooks.

Exhibit J-1 comprises true copies of my Laboratory Notebook #1069, pages 113, 197, 198, and Laboratory Notebook #1238, pages 13, 14, 15, and 16.

It was my practice after receiving a report from Dr. Scallen, to prepare a form containing the structural formula of a compound which was tested by Dr. Scallen. I retrieved the structural formula from the Sandoz computerized database. I affixed the form to a page of my laboratory notebook, and wrote on the form the assay data (including the IC_{50} data) received from Dr. Scallen. Each page bears a date in my handwriting which is the date that Dr. Scallen tested the compound, which I obtained from Dr. Scallen's reports.

Laboratory notebook #1069, page 113, records the biological activity of 63-366. Its IC_{50} (in μM) is 1.58. This page bears a date of December 13, 1984 in my handwriting.

Laboratory notebook #1069, page 197, records the biological activity of 63-549. Its IC_{50} (in μM) is 7.3100. This page bears a date of June 13, 1985 in my handwriting.

Laboratory notebook #1069, page 198, records the biological activity of 63-548. Its IC_{50} (in μM) is 3.7750. This page bears a date of June 13, 1985 in my handwriting.

Laboratory notebook #1238, page 13, records the biological activity of 64-933. Its IC_{50} (in μM) is 2.3700. This page bears a date of October 8, 1987 in my handwriting.

Laboratory notebook #1238, page 14, records the biological activity of 64-934/Na. Its IC_{50} (in μM) is 2.6100. This page bears a date of October 8, 1987 in my handwriting.

Laboratory notebook #1238, page 15, records the biological activity of 64-935. Its IC_{50} (in μM) is 0.4130. This page bears a date of October 8, 1987 in my handwriting.

Laboratory notebook #1238, page 16, records the biological activity of 64-936/Na. Its IC_{50} (in μM) is 0.5300. This page bears a date of October 13, 1987 in my handwriting.

7. On or prior to December 31, 1984, I had already received from Dr. Scallen the in vitro assay data for various other Sandoz compounds being investigated for HMG-CoA reductase inhibitor activity, and had computed the IC_{50} values for such compounds.

These other compounds have the same 3,5-dihydroxy heptenoic acid side chain, or ester, salt or internal lactone form as the Wattanasin quinoline compounds 63-633 et al. However, these compounds differ by having a different organic radical substituent of the side chain. Some of these other compounds were tested approximately the same time as compound 63-366, as indicated by Exhibit E-5, hereto.

I compared the IC_{50} values of the Wattanasin quinoline compounds and other compounds tested by Dr. Scallen to IC_{50} values for the compound Mevastatin (Compactin) which was a known HMG-CoA reductase inhibitor for administration to patients to inhibit cholesterol biosynthesis. Exhibit E-5 also indicates that prior to December 31, 1984, I calculated the IC50 values for Sandoz compound 62-320/Na (fluvastatin sodium), which I also knew to be active in vivo on or prior to December 31, 1984.

Based on my knowledge and experience, it was my judgment on or prior to December 31, 1984, that there was a high probability that Wattanasin compound 63-366 would be active when administered in vivo to a patient to inhibit cholesterol biosynthesis, i.e. for the treatment of hypercholesteremia or atherosclerosis. It was also my judgment based on the in vitro assay data for the other tested quinoline compounds, that there was a high probability that the compounds of Dr. Wattanasin's invention would have activity when administered to a patient to inhibit cholesterol biosynthesis, i.e. for the treatment of hypercholesteremia or atherosclerosis, etc.

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The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 13th day of November, 1992.

Robert E. Damon

ROBERT E. DAMON, II, Ph.D.

[redacted]

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
Fujikawa et al.

Interference No. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF ROBERT G. ENGSTROM PURSUANT TO 37 CFR §1.672

I, Robert G. Engstrom, do hereby declare as follows:

(1) That I have been employed by Sandoz Pharmaceuticals Corporation since 1964 as a Research Scientist. Among my responsibilities has been supervising the testing of new HMG Co-A reductase inhibiting compounds synthesized by Sandoz chemists.

(2) That all activities referred to in this Declaration took place in the United States.

IN VIVO TESTING OF
WATTANASIN COMPOUNDS 64-933, 64-935 and 64-936/Na

1. On or before October 29, 1987, in my laboratory under my supervision, Rodney Slaughter began performing the below-indicated protocol on compounds 64-933, 64-935 and 64-936/Na:

In vivo studies utilized male Wistar Royal Hart rats weighing 150 ± 20 g. which have been kept for 7-10 days on an altered light cycle (6:30 A.M. - 6:30 P.M. dark) housed two per cage and fed powdered Purina Rat Chow and water ad libitum. Three hours before the diurnal maximum of cholesterol systhesis at mid-day the rats were administered the test substances dissolved or as a suspension in 0.5% carboxymethylcellulose in a volume of 1 ml./100 g. body weight. Controls received vehicle alone. One hour after receiving the test substance, the rats were injected intraperitoneally with about 25 μCi/100 g. body weight of sodium [1-^{14}C]acetate 1-3 mCi/mmol. Two hours after mid-dark, blood samples were obtained under sodium hexobarbitol anesthesia, and the serum was separated by centrifugation. The resulting serum samples were saponified and neutralized, and the 3β-hydroxy sterols were precipitated with digitonin basically as described by Sperry et al., J. Biol. Chem. 187,97(1950). The [^{14}C]digitonides were counted by liquid scintillation spectrometry. The assay is based on the conversion of ^{14}C-acetate to ^{14}C-cholesterol in vivo.

2. The counts in DPM of digitonin precipitable sterol (β-hydroxy sterol, mostly cholesterol in the rat) were entered by Rodney Slaughter into my computer program, which converted them to nCi of sterol found per 100 ml. of serum at 4 hours after the injection of the ^{14}C-acetate.

3. I have reviewed **Exhibit K-1** hereto, which comprises true copies of pages 133, 134, and 135, 136, 137 and 138 of R. Slaughter's Laboratory Notebook #917. I witnessed Rodney Slaughter's signature on each of these pages, and each page bears my true signature as a witness.

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4. Notebook pages 133-135 contain true copies of a computer printout for the protocol and results in nCi/dl of Study #H318, which was commenced on October 22, 1987. I initialed the first page of this computer printout on or before October 22, 1987. This computer printout on page 135 indicates that an in vivo assay of compound 64-936 was started on October 22, 1987.

5. Notebook pages 136-138 contain true copies of a computer printout for the protocol and results in nCi/dl of Study #H319, which was commenced on October 29, 1987. I initialed the first page of this computer printout on page 136 on or prior to October 29, 1987. This computer printout on page 137-138 indicates that an in vivo assay of compound 64-933 and 64-935 was started on October 29, 1987.

6. Both studies were completed on or prior to December 9, 1987, the date indicated at the bottom of pages 135 and 138.

7. It was my responsibility to enter the nCi/dl data into a separate computer program which calculates the ED_{50} values of a compound tested in vivo from the reduction in the nCi of sterols formed from test groups compared to controls for each assay, and forms a database of the ED_{50} values. On or before December 9, 1987, I entered the data for compounds 64-933, 64-935 and 64-936/Na.

[redacted]

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8. The 1st page of Exhibit K-1 comprises a true copy of part of the ED_{50} database. This page indicates that the ED_{50} for compounds 64-933, 64-935 and 64-936/Na was in the system as of December 9, 1987. Therefore, the information was available to other Sandoz employees having access to the computer database as of December 9, 1987.

The ED50 for these compounds are:

COMPOUND	ED_{50} (mg/kg)
64-933	0.49
64-935	>1.0
64-936	>1.0

. . .

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 13 day of November 1992.

Robert G. Engstrom

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Case No. 600-7101/CONT/INT.(5)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

SUPPLEMENTAL DECLARATION OF ROBERT G.ENGSTROM PURSUANT TO 37 CFR §1.672

I, Robert G. Engstrom, do hereby declare as follows:

All of the below-indicated activities took place in the United States.

Exhibit Q comprises a true copy of a Biological Activity Data Report dated May 24, 1988 which I sent to the Patent Department concerning the compounds of PD 299/84, together with a computer printout of the Sandoz database dated May 23, 1988. The printout contains IC_{50} and some ED_{50} values for compounds of Patent Disclosure 295/84 and compounds of the subject Patent Disclosure 299/84.

(I note that I became aware of a computer entry error comprising the inadvertent "switching" of the ED_{50} data for compounds 64-933 and 64-935. The corrections on the printout are in my handwriting and would have been made on or about May 23, 1988.)

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful

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false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19 day of February, 1993.

Robert Engstrom

Robert Engstrom

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v. Interference Nos. 102,648, 102,975
FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF RODNEY SLAUGHTER PURSUANT TO 37 CFR §1.672

I, Rodney Slaughter, do hereby declare as follows:

(1) That I have been employed by Sandoz Pharmaceuticals Corporation since 1982, and during the time periods referred to herein, I worked in the Department of Lipid Metabolism.

(2) That it has been my responsibility to carry out an in vivo testing program of various HMG-CoA reductase inhibitor compounds, including Wattanasin compounds 64-933, 64-935 and 64-936.

(3) That all of the below-indicated activities took place in the United States.

IN VIVO TESTING OF
WATTANASIN COMPOUNDS 64-933, 64-935 and 64-936

1. On or before October 29, 1987, I carried out the below-indicated protocol on compounds 64-933, 64-935 and 64-936:

In vivo studies utilized male Wistar Royal Hart rats weighing 150 ± 20 g. which have been kept for 7-10 days on an altered light cycle (6:30 A.M. - 6:30 P.M. dark) housed two per cage and fed powdered Purina Rat Chow and water ad libitum. Three hours before the diurnal maximum of cholesterol systhesis at mid-day the rats were administered the test substances dissolved or as a suspension in 0.5% carboxymethylcellulose in a volume of 1 ml./100 g. body weight. Controls received vehicle alone. One hour after receiving the test substance, the rats were injected intraperitoneally with about 25 μCi/100 g. body weight of sodium [1-^{14}C]acetate 1-3 mCi/mmol. Two hours after mid-dark, blood samples were obtained under sodium hexobarbitol anesthesia, and the serum was separated by centrifugation. The resulting serum samples were saponified and neutralized, and the 3β-hydroxy sterols were precipitated with digitonin basically as described by Sperry et al., J. Biol. Chem. 187,97(1950). The [^{14}C]digitonides were counted by liquid scintillation spectrometry. The assay is based on the conversion of ^{14}C-acetate to ^{14}C-cholesterol in vivo.

2. I entered the counts in DPM of digitonin precipitable sterol (β-hydroxy sterol, mostly cholesterol in the rat) into a computer program, which converted them to nCi of sterol found per 100 ml. of serum at 4 hours after the injection of the ^{14}C-acetate.

3. I have reviewed **Exhibit K-1** hereto, which comprises true copies of pages 133, 134, 135, 136, 137 and 138 of my Laboratory Notebook #917.

4. Notebook pages 133-135 contain true copies of a computer printout for the protocol and results in nCi/dl of Study #H318, which I started on October 22, 1987. These pages contain the date of 10/22/87 at the top in my handwriting.

5. Notebook pages 136-138 contain true copies of a computer printout for the protocol and results in nCi/dl of Study #H319, which I started on October 29, 1987. These pages contain the date of 10/29/87 at the top in my handwriting.

6. Both studies were completed on or prior to December 9, 1987, the date indicated at the bottom of the computer printouts on pages 135 and 138.

7. It was my practice to paste the computer printouts into my notebook and to sign the notebook page when I did this.

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The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 13 day of November 1992.

Rodney Slaughter

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Case No. 600-7101/CONT/INT.(4)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF LINDA ROTHWELL PURSUANT TO 37 CFR §1.672

I, Linda Rothwell, do hereby declare as follows:

All of the below-indicated activities took place in the United States.

1. I have been employed by Sandoz Pharmaceuticals Corporation continuously since 1968 to the present. My position, both currently and during the time periods indicated below, has been Patent Administrator of the Sandoz Patent Department.

2. One of my responsibilities as Patent Administrator has been to type or supervise the typing of the Minutes of each Sandoz Pharmaceutical Corp. Patent Committee Meeting based on notes taken at the meeting by the attending attorney(s). The Minutes serve as the official record for the Sandoz Patent Department of decisions and recommendations made at each Patent Committee Meeting (PCM).

3. Since prior to April 1987, another of my responsibilities as Patent Administrator has been to docket patent disclosures as soon as they are received by the Patent and Trademark Department, for consideration at the following scheduled PCM.

4. Patent Disclosure 299/84 was docketed for initial consideration by the Sandoz Pharmaceuticals Corp. Patent Committee at its April 29, 1987 Meeting.

[redacted]

5. According to Sandoz policy which has been in effect since prior to April 29, 1987, a disclosure which is considered by the Patent Committee and is rated "B", is deferred for reconsideration by the Patent Committee within three months' time. An "X"- rated disclosure is deferred for reconsideration by the Patent Committee within one month's time. A "B" or "X" rating is given when further information is needed before making a decision whether to file a patent application. An "A"- rated disclosure represents a decision to file a patent application on the subject matter of the patent disclosure.

Section 5 of the Minutes is devoted to the rating of newly submitted Patent Disclosures or the re-rating of previously rated Patent Disclosures.

6. **Exhibits M-1 to M-5** appended hereto comprise copies of pages of Patent Committee Minutes prepared in the ordinary course of business by me or under my supervision. Confidential material unrelated to PD 299/84 has been masked. These are true copies with respect to the unmasked material.

The Minutes are maintained under my supervision and control in the files of the Sandoz Patent and Trademark Department in the ordinary course of my employment.

Exhibit M-1 is a masked copy of page 2 of the minutes of the Sandoz Pharmaceuticals Corp. PCM held on Wednesday, April 29, 1987. This page shows that Patent Disclosure 299/84 was rated "B," and was assigned to Frederick H. Weinfeldt ("FHW"), a senior patent attorney in the Sandoz Patent Department.

Exhibit M-2 is a masked copy of page 3 of the minutes of the PCM held on Wednesday, July 29, 1987. This page shows that PD 299/84 was re-rated "B".

Exhibit M-3 is a masked copy of page 3 of the minutes of the PCM held on October 28, 1987. This page shows that PD 299/84 was rated "X".

Exhibit M-4 is a masked copy of page 2 of the minutes of the PCM held on Wednesday, November 25, 1987. This page shows that PD 299/84 was rated "X".

Exhibit M-5 is a masked copy of page 4 of the minutes of the PCM held on Wednesday, January 27, 1988. This page shows that PD 299/84 was rated "A," and was re-assigned to Mrs. Joanne M. Giesser, a patent attorney in the Sandoz Patent Department.

The Patent Department records indicate that no later than about April 1987, Mr. Weinfeldt had taken permanent disability leave (and is now deceased). In August of 1987, Mrs. Giesser joined the Patent Department and assumed a part of Mr. Weinfeldt's docket.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 19th day of February, 1993.

Linda Rothwell

LINDA ROTHWELL

ORIGINAL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERFERENCE NOS. 102,648
102,975

WATTANASIN,

vs.

FUJIKAWA, et al.

DEPOSITION OF:
LINDA ROTHWELL

Monday, March 22, 1993
Florham Park, New Jersey

A P P E A R A N C E S:

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-and-
DIANE E. FURMAN, ESQ.,
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I N D E X

E X H I B I T S

(Before Gary M. Talpins, a Certified Shorthand Reporter and Notary Public of the State of New Jersey, held at the offices of Sandoz Corporation, Patent and Trademark Affairs Department, 25 Hanover Road, Florham Park, New Jersey, on Monday, March 22, 1993, commencing at 2:35 p.m.)

- - - - - - - - - - - - - - - - -

L I N D A R O T H W E L L, 2 Rambling Woods Drive, Morris Township, New Jersey 07960, Sworn.

CROSS EXAMINATION BY MR. KELBER:

Q. Good afternoon, Linda.

A. Hello.

Q. I'm going to have the reporter mark as an Exhibit F-9, a document, and after he marks it and hands it to you, if you would review it briefly.

(Whereupon the document was received and marked F-9 for identification.)

A. Okay.

Q. Is that your signature on page four?

A. Yes, it is.

Rothwell - cross

Q. And did you review this document prior to signing it?

A. Yes.

Q. Miss Rothwell, are you a patent attorney or agent?

A. No, administrator.

Q. If you would turn to page one of that document, F-9, you describe a couple of the responsibilities you have as patent administrator. I would like to focus on the one described in paragraph three, the responsibility to docket patent disclosures. Can you elaborate on that? What is involved in docketing the patent disclosures?

A. Once it's been rated, if it's been rated "A", then it's docketed for three weeks for filing and that's what the docketing procedure is. They get little blue cards.

Q. And after you have docketed it for three weeks, do you have follow-up responsibility?

A. Yes.

Q. Can you describe that?

A. I just go in and check with the attorney.

Rothwell - cross

Q. And if the application has not been prepared, what happens? Let's suppose, I will give you a hypothetical, you docket it for three weeks and do you go in and discuss with the attorney, and the application hasn't been prepared for lack of sufficient information from the inventor, is any further date set for docketing review?

A. No. I would just move it maybe another three weeks or two weeks, if he knows when he is going to get more information.

Q. If he doesn't have any idea when he is going to get more information, is a further date set?

A. No, I would just go back in a couple of weeks.

Q. And do you keep on checking until --

A. Yes.

Q. Do you keep on checking until the application is filed?

A. Yes.

Q. At paragraph four on page one of F-9, you make reference to 299/84. Did you have responsibility for docketing that disclosure for filing after it had been rated "A"?

Rothwell - cross

A. I believe so, yes.

Q. Do you recall checking, as you have just described, with the attorney responsible after the first three weeks in that disclosure?

A. To the best that I can remember, yes.

Q. Do you know who that attorney was?

A. I think at the time, it was Fred Weinfeldt, unless it had already been turned over.

Q. Do you recall checking with any other attorney besides Mr. Weinfeldt with regard to 299/84?

A. It would have to be whoever took over the disclosure.

Q. You don't have a recollection as to who that was?

A. No.

Q. Is there anybody else in the Sandoz Patent Department with responsibility for docketing applications for filing?

A. No.

Q. Just yourself. You mentioned a three week date. Is that generally given all applications?

A. Just if it's rated "A" at the meeting.

Q. I see. So in the course of performing those responsibilities with regard to docketing, have you developed an approximation of on average how long it takes from the time a disclosure is rated "A" to the time an application is filed? Do you have a feeling for that?

A. Not really because some of them are filed quick and others take a little longer for one reason or another.

Q. Would a year be an unusually long time?

A. Yes.

Q. If you are familiar with the procedure, when a disclosure is rated "B" and supplemental information is provided, is it provided to you?

A. No. I would just automatically bring it up at the next meeting.

MR. KELBER: Thank you very much. I appreciate it. I have no further questions. Diane?

MS. FURMAN: I have no questions.

REDIRECT EXAMINATION BY MR. VILA:

Q. You were asked a question with regard to essentially the average time that it would take to file a patent application from the time of an

Rothwell - redirect

"A" rating to disclosure. Would that vary in pattern as you might recognize it among different attorneys in the department?

A. Yes.

Q. With regard to Mr. Kassenoff, would you say that he filed in the average time slower than average, faster than average?

A. Some he would do real quick and others, he would just get held up by some of the inventors.

Q. Were there other reasons for him to be --

A. Not that I would know of.

Q. But in some cases, it would be a longer than average time?

A. Yes.

Q. With regard to Jody Giesser, concerning pharmaceutical patent applications that had been assigned to her, would you have ever had an opportunity to form a judgment there?

A. No.

MR. VILA: Thank you very much.

THE WITNESS: Okay, thank you.

(Time noted is 2:45 p.m.)

Linda Rothwell
LINDA ROTHWELL

Subscribed and Sworn to before me

This 20th day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

C E R T I F I C A T E

I, GARY M. TALPINS, a Notary Public and Certified Shorthand Reporter of the State of New Jersey, do hereby certify that prior to the commencement of the examination, LINDA ROTHWELL was duly sworn by me to testify the truth, the whole truth and nothing but the truth.

I DO FURTHER CERTIFY that the foregoing is a true and accurate transcript of the testimony as taken stenographically by and before me at the time, place and on the date hereinbefore set forth, to the best of my ability.

I DO FURTHER CERTIFY that I am neither a relative nor employee nor attorney nor agent of any of the parties to this action, and that I am neither a relative nor employee of such attorney or counsel, and that I am not interested directly or indirectly in the interference either as counsel, attorney, agent or otherwise.

Gary M. Talpins, C.S.R.
License No. XI00561

#38 + #93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN v. FUJIKAWA ET AL.

INTERFERENCE NO. 102,648
INTERFERENCE NO. 102,975

FYI

WATTANASIN CONSOLIDATED RECORD

MAY 19 1993
RECEIVED IN
BOX INTERFERENCE

VOLUME III

[PAGES 227 - 318]

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May 15, 1993

5/17/93

"RIBBON COPY FOR PARTY Wattanasin"

Case No. 600-7101/CONT/INT.(2)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN
v.
FUJIKAWA et al.

Interference Nos. 102,648, 102,975
Examiner-in-Chief: M. Sofocleous

DECLARATION OF MELVYN M. KASSENOFF PURSUANT TO 37 CFR §1.672

I, Melvyn M. Kassenoff, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

2. I have been employed by Sandoz Corporation in the Patent and Trademark Department since 1972. My current position is Director, Patent and Trademark Affairs. I am an associate counsel of record in these interferences.

3. I have had responsibility for the filing and prosecution of Sandoz patent applications in the HMG-CoA reductase inhibitor area since 1982. However, this area was only a very small portion of my total workload, the bulk of which comprised prosecuting applications in the azo dye area originating from research done by Sandoz AG in Basle, Switzerland.

Since about 1981, Sandoz Research Institute has been engaged in a research effort to develop compounds having utility as HMG-CoA reductase inhibitors for use in the treatment of hypercholesterolemia. This project resulted in numerous patent disclosures being submitted to the Patent Department, including Patent Disclosure 299/84 of Dr. Wattanasin.

Prior to approximately April 1987, when he took permanent leave for health reasons, Mr. Fred Weinfeldt, a senior patent attorney in the Sandoz Patent Department, shared the responsibility of filing of patent applications in the HMG-CoA reductase inhibitor area. In August 1987, Mrs. Joanne M. Giesser joined the Department as a patent attorney and took over a portion of Mr. Weinfeldt's docket of patent disclosures to be filed.

4. Within a week or two following the January 27, 1988 Patent Committee meeting, I was aware that Patent Disclosure 299/84 of Sompong Wattanasin had received an "A" rating. It was my intention that the case would be filed by Mrs. Giesser or myself depending on who was available after existing filing priorities had been completed, inasmuch as following Mr. Weinfeldt's departure, a backlog in unfiled HMG-CoA reductase disclosures had been developing.

5. It is noted that the Sandoz U.S. filings in the HMG-CoA reductase area commenced in about 1982 and continued into 1991. For example, a representative list of Sandoz original (including CIP) U.S. patent application filings in the HMG-CoA reductase inhibitor area comprises the following:

Case 600-6951 filed Nov. 22, 1982 (abandoned)
Case 600-6951/B filed Nov. 4, 1983 (R60 of which) issued as U.S. 4,739,073 (1988)
Case 600-6951/C filed Nov. 22, 1982 (pending)
Case 600-7013 filed June 4, 1984 now U.S. 4,588,715 (1986)
Case 600-7015 filed June 22, 1984 (abandoned)
Case 600-7022 filed Dec. 4, 1984 (abandoned)
Case 600-7025 filed Apr. 12, 1985 (abandoned)
Case 600-7028 filed May 22, 1985 now U.S. 4,668,794 (1988)

Case 600-7015/B filed June 6, 1985 now U.S. 4,613,610 (1986)
Case 600-7035 filed Oct. 25, 1985 (abandoned)
Case 600-7022/B filed Mar. 7, 1986 (abandoned)
Case 600-7041 filed Apr. 30, 1986 (abandoned)
Case 600-7028/B filed May 14, 1986 (R60 of which) issued as U.S. 4,755,606 (1988)
Case 600-7035/B filed Oct. 15, 1986 now U.S. 4,851,427 (1989)
Case 600-7050 filed Dec. 23, 1986 now U.S. 4,751,235 (1988)
Case 600-7025/CIP filed May 5, 1987 (abandoned)
Case 600-7022/C filed Jul. 1, 1988 now U.S. 5,001,255 (1991)
Case 600-7025/CIP/CIP/CIP filed Oct. 6, 1988 (abandoned)
Case 600-7025/CIP/CIP/CIP/CIP filed Jan. 16, 1990 (pending)
Case 600-7041/CIP filed Mar. 6, 1987 (abandoned for R60)
Case 600-7064 filed Jan. 27, 1988 now U.S. 4,822,799 (1989)
Case 600-7041/CIP/CIP filed Mar. 10, 1988 (abandoned)
Case 600-6955/XN//B/CONT/X filed Mar. 10, 1988 now U.S. 4,876,1989 (1989)
Case 600-7087 filed Oct. 13, 1988 (abandoned)
Case 600-7101 filed Mar. 3, 1989 (abandoned for R60 cont.)
Case 600-7087/B filed May 8, 1989 (abandoned)
Case 600-7104 filed May 22, 1989 (abandoned)
Case 600-7041/CIP/CIP/II filed Jul. 13, 1989 now U.S. 4,870,199
Case 600-7104/CIP filed Feb. 20, 1990 (pending)
CAse 600-7087/C filed Sept. 5, 1990 (abandoned)
Case 600-7087/D filed Feb. 26, 1991 (pending)

Appendix Z hereto contains copies of the cover sheets of some of the above-indicated U.S. patents which issued on the above cases.

6. It is my best recollection that in February of 1988, I was in communication with Dr. Wattanasin concerning information

which was needed by the Patent Department in order to prepare an application based PD 299/84. (The application that was subsequently filed was designated as, and is referred to herein as, "Case 600-7101".)

Exhibit N hereto comprises a true copy of a page containing my handwritten notations concerning Case 600-7101 and a handwritten date of February 12, 1988.

These notes comprise a checklist of information items which needed to be developed or confirmed in order to draft Case 600-7101. The fact that these notes were made on the reverse side of the second attachment page to PD 299/84; and furthermore, that paragraph 2 discusses the scope of the disclosure and in sub-paragraph (c), refers to "other substitu [sic] on the quinoline ring," indicates their pertinence to the involved Wattanasin application.

These notes further indicate that I spoke with Sompong Wattanasin ("S.W.") on February 12, 1988 concerning his quinoline compounds and requested that he provide me with certain information.

7. On or about March 1, 1988, I received from Dr. Wattanasin certain reaction schemes which were to be included in case 600-7101.

Exhibit O comprises a copy of material which I received from Dr. Wattanasin for the preparation of Case 600-7101. This shows two different reaction routes to preparing quinoline compounds of the case.

[redacted]

8. It was my practice to request the Sandoz Biology Department to send me IC_{50} and ED_{50} values for compounds I was planning to cover in a patent application, as well as other biological information necessary to properly draft a patent application directed to a pharmaceutical.

Exhibit Q hereto comprises a Biological Data Report and computer printout which I received from the Sandoz Biology Department. The Wattanasin disclosure number, i.e. "299/84" is written in my handwriting on the front page, and the compounds of Patent Disclosure 295/84 as well as PD 299/84 are included in the printout.

The printout bears a date of May 23, 1988.

9. On July 1, 1988 I filed Case 600-7022/C based on PD 295/84, which was indicated for filing ahead of PD 299/84.

Exhibit R hereto comprises a copy of the front page of U.S. Patent No. 5,001,255, which issued on Case 600-7022/C, and indicates a filing date of July 1, 1988.

10. With reference to **Exhibit Y-2**: page 2 of this computer printout bears a date of January 11, 1989 written in my handwriting.

11. At no time subsequent to the "A" rating of Patent Disclosure 299/84 did I or, insofar as I am aware, any other member of the Patent and Trademark Department of Sandoz Corporation, ever have any intention not to file a United States patent application on the quinoline compounds of said patent disclosure in due course.

[redacted]

[redacted]

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19th day of February, 1993.

Melvyn M. Kassenoff

MELVYN M. KASSENOFF

[redacted]

ORIGINAL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERFERENCE NOS. 102,648
102,975

WATTANASIN,

vs.

FUJIKAWA, et al.

DEPOSITION OF:
MELVYN M. KASSENOFF

Monday, March 22, 1993
Florham Park, New Jersey

A P P E A R A N C E S:

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I N D E X

E X H I B I T S

(Before Gary M. Talpins, a Certified Shorthand Reporter and Notary Public of the State of New Jersey, held at the offices of Sandoz Corporation, Patent and Trademark Affairs Department, 25 Hanover Road, Florham Park, New Jersey, on Monday, March 22, 1993, commencing at 10:00 a.m.)

- - - - - - - - - - - - - - - - -

M E L V Y N M. K A S S E N O F F, 3 Shelley Terrace, West Orange, New Jersey 07052, Sworn.

MR. KELBER: Good morning. This is the cross examination of the Sandoz declaration witnesses. The first witness we have today is Mr. Kassenoff.

CROSS EXAMINATION BY MR. KELBER:

Q. Mr. Kassenoff, I'm going to hand you or hand the reporter a document that I would like labeled as F-1 and ask you to take a minute and take a look at that.

(Whereupon the document was received and marked F-1 for identification.)

Kassenoff - cross

Q. Do you recognize that document, Mr. Kassenoff?

A. Yes.

Q. And is that your signature at the end of the document on page six?

A. Yes.

Q. Let me turn your attention first to the very bottom of page one. You see the sentence starting "this project resulted in numerous patent disclosures." Do you have any feel in general numbers for how many disclosures of the type of compounds referred to as having utility as HMG-CoA reductase inhibitors?

A. No, I don't.

Q. You used the word "numerous" in your declaration.

A. Certainly over 10, possibly 20. It wouldn't surprise me; possibly even more than that.

Q. Turning to the top of page two of the declaration that is Exhibit F-1, there is a reference to a Mr. Fred Weinfeldt, who apparently shared the responsibility in that particular technology area. With whom did he share it, sir?

A. With me. In other words, initially he

Kassenoff - cross

was doing the work on it and then obviously, there were too many disclosures so I took over some of them and then eventually, I had primary responsibility.

Q. By primary responsibility, what would primary responsibility entail?

A. Just probably did more of them than anybody else at a particular time.

Q. If there was somebody else doing an application in that field at that particular time, let's pin it down, in the 1987-'88 framework, would you have responsibility for monitoring that other person?

A. Informally but not formally. In other words, I was not reviewing it but if somebody had a question on it, they would obviously come in to me.

Q. Are you familiar with the rating system that was used by the Sandoz Patent --

A. More or less.

Q. I know you know the answers to most of the questions I'm going to ask you but let me finish them for the reporter. I'm pretty clear when I finished the question.

Are you familiar with the rating system

Kassenoff - cross

that was used by the Sandoz Patent Committee during 1987 through '88?

A. Right.

Q. And by "right," you mean --

A. Yes.

Q. What did it mean if a disclosure received a "B" rating?

A. "B" I think is three months, it would come up again in three months.

Q. What criteria would be brought to bear to determine what rating a disclosure would get?

A. Probably ongoing work, it means it wasn't ripe for filing. More detailed than that, I'm not sure.

Q. It wasn't ripe for filing but --

A. It may have been ongoing work, for example.

Q. Would there be any other reasons that a disclosure would receive a "B" rating?

A. Sometimes it was the people there didn't feel qualified but usually that would be -- we would put it off a month if there was nobody there who felt comfortable in making a decision but usually, a "B" rating means it's ongoing work,

Kassenoff - cross

that's the principal reason.

Q. It's ongoing work. In other words, a disclosure would not be rated for more immediate action if the work was ongoing?

A. Well, unless, of course, you had something that was so hot that you had to file on it immediately.

Q. What would --

A. In other words, there are flexible standards involved. It's not an absolute.

Q. By assigning a "B" rating to a disclosure --

A. That means that the thing is of interest but it's not ready for filing yet because of, for example, ongoing work.

Q. Are there any other reasons for assigning it a "B" rating other than ongoing work?

A. I don't recall offhand. I'm not sure. There could be.

Q. You are currently Director of Patent and Trademark Affairs for Sandoz. Is that correct?

A. That's correct.

Q. Is there a Sandoz Patent Committee today?

Kassenoff - cross

A. Yes.

Q. Does it use the same rating system?

A. Yes, it does.

Q. As Director of Patent and Trademark Affairs, I would imagine one of your responsibilities --

A. Yes, I do attend the meetings and have attended on a regular basis for the last year.

Q. In your experience, have disclosures been rated "B" for any other reason than ongoing work?

A. I don't recall of any right now.

Q. You have been with Sandoz since 1972. Is that correct?

A. That's correct.

Q. Let's try and narrow it down. How about in the HMG-CoA reductase inhibitor field, do you recall during your tenure at Sandoz any other disclosure besides the one of interest, 299/84, in that field ever having been rated as "B"?

A. I don't recall. Frankly, I didn't attend the meetings on a regular basis. In fact, probably until the beginning of last year, over the previous 20 years, I probably attended the meeting

Kassenoff - cross

maybe twice and obviously, we have not had any ratings in that field, at least I don't think we have in the last year or so.

Q. If you didn't attend a meeting and a disclosure was rated "B", would you be informed of that fact?

A. Yes. The minutes are published.

Q. Have you reviewed the minutes of the Patent Committee --

A. I look at the minutes. I have got to see if anything is in my area, which I have to file on.

Q. In the period January 1, 1987, to December 31, 1988, did you see any other application --

A. I would not recall.

Q. Please let me finish the question, Mr. Kassenoff.

Do you recall seeing the disclosure 299/84 rated as "B" at any time?

A. I'm sure I saw it.

Q. But you don't recall seeing it now?

A. No.

Q. Are you sure that you might have seen

Kassenoff - cross

any other disclosure in your field rated "B"?

A. I'm sure I would have seen it there but on the other hand, I certainly wouldn't remember it because I would have no reason for remembering it because it didn't require any action.

Q. It didn't require any action. It didn't require any action on your part?

A. On my part.

Q. During the period 1987 through 1988, are you aware did Sandoz employ patent attorneys not employed by Sandoz Corporation directly as full-time employees for the preparation of patent applications?

A. Are you talking about outside?

Q. Outside counsel.

A. Not to write patent applications except possibly once in awhile, we may have an oddball case. Obviously, I wouldn't know about it. In other words, in the pharmaceutical area, I can tell you the answer is no except maybe possibly if there were a very complex interference or something like that but not for normal, we do not hire outside counsel for normal work.

Q. By normal, you would include drafting

Kassenoff - cross

applications?

A. Prosecution and application writing.

Q. Even if there is a crunch in the staff at Sandoz and it is not immediately up to it?

A. No, we don't do that unless -- the only exception being, for example, two years ago, we had -- it was not in the pharmaceutical area but we had a possible sale where we had to rush something, a filing on something which we were about to sell. In the U.S., we didn't have the problem but abroad, we would have had a filing if we didn't get it on filing immediately.

Q. And in that instance, you sent it to outside counsel?

A. Yes. This was not a pharmaceutical case because then you wouldn't have that kind of a problem.

Q. Is there a formal policy that you are aware of that would distinguish between pharmaceutical cases and --

A. There is no formal policy.

Q. How did you find out about the "A" rating that's referred to in paragraph four of the document that's --

Kassenoff - cross

A. The minutes are distributed anywhere from a few days to a week or two after the meeting.

Q. Are the minutes distributed to everybody in the department?

A. Everybody in the department receives the minutes.

Q. Again, I'm going to ask you to let me finish my sentence. I know you are ahead of me on this but you have got to give me a chance to catch up.

How was it determined who was responsible for a particular application that gets an "A" rating?

A. Usually one of the supervisors will decide and it usually will be decided before the meeting and usually it will be people have defined areas, although sometimes, as you can see here, people may share the same area. Obviously, if it's in somebody's area, it will go to that person. If it's in an area that's shared, usually the supervisor will decide who will get it. But things are not done on a formal basis and sometimes things are transferred afterwards.

Q. Was such a decision as to who would be

Kassenoff - cross

responsible for the disclosure that received an "A" rating referred to in paragraph four made?

A. Jody Giesser's initials were on the agenda as well as the minutes for that disclosure.

Q. Does that mean she had responsibility for the preparation of it?

A. That would mean generally she would have responsibility unless, of course, she transferred it to somebody else but at least initially, it was in her bailiwick.

Q. Let me direct you to the last sentence or actually the last phrase in paragraph four, where it indicates a backlog in unfiled HMG-CoA reductase disclosures have been developing. Do you have any idea of how large that backlog was?

A. No, I can't -- I have no idea.

Q. How do you know there was a backlog?

A. Because I can recall that there was some pressure involved in the area and that there were a number of disclosures that were floating around but I do not recall the number.

Q. Aren't there a number of disclosures floating around, weren't there a number of disclosures floating around throughout the 1981

through 1990 time period in that field?

A. At least through the beginning of that time period, probably not at the end of it.

Q. Probably not at the end of it. Let me direct your attention to paragraph five. Do you see the listing of cases that appears in that paragraph?

A. Correct.

Q. Many of those applications have a filing date of 1988 through 1990. Is that correct?

A. A number of them, correct.

Q. In fact, more than half. Isn't that correct?

A. Right, but most of those, if you notice, are CIP's or continuations and the like and would not be the result of new disclosures.

Q. Let's talk about that. The CIP application would not be the result of a new disclosure?

A. That's correct.

Q. How would a CIP application come to be docketed for filing?

A. It's not docketed, it's up to the attorney involved simply to file it without it

being docketed and usually the need for it will become apparent from discussions between the attorney involved and the inventor and/or others in Research and similarly, divisionals would simply come about, those would be decided on by the Patent Committee at the time an issue fee was paid for the earlier case in the series.

Q. Would you help me out. Could you take a look at the list of applications or list of cases, I'm sorry, that are recited there and tell me how many would have come from new disclosures.

A. If it does not have any letter or anything else after the number, that would be a new disclosure.

Q. Could you identify how many of those there are?

A. Starting from which one?

Q. All of the ones in this five, how many came from new disclosures?

A. 6951, 7013, 7015, 7022, 7025, 7028, 7035, 7041, 7050, 7064, 7087, 7101, 7104. There is also I see here 6955 but where is the original on that? There are a number of cases here that probably should be down there for completion but

Kassenoff - cross

are not here. For example, the 6955, what is down here is obviously a later application in the series.

Q. I'm sorry, 6955?

A. 55.

Q. Could you direct me to --

A. March 10th of '88. And there are also a number of other applications in the series which I can see are not down here.

Q. The ones that are down here, you identified 13 that resulted from new disclosures. Is that correct?

A. Correct. You counted them.

Q. That was my count but I'm asking you to confirm that for me.

A. That seems right.

Q. Of those 13 cases, do you have any feel for how many were part of the backlog that is referred to in paragraph four?

A. It was probably 7064 because I wrote that one, 7087, 7101, 7104 and of course, some of the CIP's involved, as well, although those weren't new disclosures but that's part of the backlog of work in this project.

Kassenoff - cross

Q. You indicated after you said 7064 that that would have been part of the backlog because you wrote it.

A. That's why I'm familiar with it.

Q. But you had shared responsibility for that field even before Mr. Weinfeldt's departure. Is that correct?

A. That's correct. I wrote some of the other cases in the series.

Q. Do you know for a fact that 7064 was part of the backlog?

A. Just from the time frame, I do.

Q. That application was filed January 27, 1988. Is that correct?

A. That's what it says here.

Q. But is it correct? You wrote it. Do you know?

A. I don't remember when I filed it. I have to assume that this is correct.

Q. Did you review any documents during the preparation and signing of this declaration?

A. Did I?

Q. Yes.

A. No. I relied on my memory.

Kassenoff - cross

Q. You don't have a memory of the statement that appears here now?

A. Not particularly, not in particular, no.

Q. Did you have a memory at the time you signed it?

A. No. It was to the best of my recollection, it was correct.

Q. And what is that recollection based on, sir?

A. What I remember.

Q. But you don't have a memory of doing it, do you?

A. I have a memory of writing that application and I know it was in that time frame but to say that it was definitely January 27th of '88, I don't know. But that seems right.

Q. Do you know as a matter of personal knowledge that 7064 was part of the backlog referred to in paragraph four?

A. Yes.

Q. 7064 appears to have been filed sometime about January of 1988, according to your recollection.

Kassenoff - cross

A. That's correct.

Q. Do you have any idea when you began preparation of the application at maturity of that filing?

A. No, I do not.

Q. Would it have begun prior to April 1987?

A. Probably not but I really -- without going into the file and looking at whatever notes I have, I can't answer that.

Q. Probably not. Do you have any feel for why you said probably not?

A. Because the time period would have been eight or nine months and I would not have been working on an application that long.

Q. That would be a longer time period than usual for you?

A. For me, yes.

Q. Let's look at 7087. Was that part of the backlog referred to?

A. Yes, it was.

Q. Did you work on that case?

A. Yes, I did.

Q. Do you know how long it had been

Kassenoff - cross

pending before you took over the preparation of that case?

A. No, I do not.

Q. You referred to a backlog in paragraph four and the backlog refers to unfiled disclosures had been accumulating. Is that correct?

A. That's correct.

Q. Were these disclosures that had been rated "A" for filing by the Patent Committee?

A. Yes, otherwise they wouldn't be part of the backlog.

Q. Can you give me an idea of the time delay between the "A" rating received and the delay until action on the disclosure so rated, give me an idea of that time delay involved in the backlog referred to?

A. I really cannot.

Q. What do you mean by "backlog"?

A. It means there were several disclosures which have been pending for more than a month or even probably more than two months.

Q. So your recollection suggests that the backlog was at least two months?

A. More than that, according to my

recollection, but I can't be more specific than that.

Q. I'm a little confused. Mr. Weinfeldt left in approximately April of 1987. Is that correct?

A. That sounds right.

Q. The backlog by January of 1988 had developed to as much as two months or more. Is that correct?

A. It was more than that, probably.

Q. Three months?

A. I'm sure that there were cases that -- in fact, I'm willing to bet that there were cases that were outstanding for longer than that which had not been filed on.

Q. You are willing to bet, is that bet based on your personal knowledge?

A. It's based on my knowledge of how things operate and how things operated in that period as well as currently.

Q. Do you have a specific recollection of a case or cases in that field, the reductase disclosures referred to, that had been pending for more than two months?

Kassenoff - cross

A. I am sure that 7064 was pending for more than two months because of the scope of the application. It was no way that that thing was filed within two months of its being rated "A"; also 7087, which is another case that I wrote, there was no way that that was filed within two months.

Q. We may be talking apples and oranges here. By backlog, I assume you refer to cases that had not been picked up for action. Is that correct?

A. By backlog, I mean cases that had been rated "A" and had not been filed on as yet.

Q. So if an attorney had a particularly difficult case, even though that was the only case the attorney was acting on, under this definition, that would be part of the backlog. Is that correct?

A. That's correct. That is the sense in which I have used the term.

Q. Were there any cases that had been rated "A" but had not received review or attention from an attorney for two months in that backlog?

A. I can't answer that. I can't answer --

Kassenoff - cross

Q. How about for cases assigned to you?

A. Again, I have to assume from the way I operate that within a few weeks of the "A" rating, I would have contacted the inventor and had the inventor or inventors at least start to send me the material required for the application. I would have contacted Biology to get their input and possibly, if relevant, Process Development to get any new processes which I would need for the best mode requirement on it.

Q. In fact, you contacted Dr. Wattanasin, is that the correct pronunciation?

A. Correct.

Q. You contacted Dr. Wattanasin as early as February 1988 regarding this disclosure. Is that correct?

A. That's what the notes in the file show.

Q. Is that customary for what is referred to as the backlog?

A. Yes. That's not saying that in every case, I would do it within a couple of weeks but in that case, I did do it.

Q. You identified earlier four cases that fell into that backlog, cases which had been

designated "A" but not yet filed. Is that correct?

A. That's correct.

Q. Do you recall, did you have personal responsibility for any other cases that might have been in that backlog?

A. No, I did not have any personal responsibility for any other cases.

Q. Besides --

A. In the new filings because filing new applications was only a very small part of my workload.

Q. Besides Ms. Giesser, was there anybody else at Sandoz with responsibility for the preparation of new applications and filing in this field?

A. In the HMG-CoA reductase application?

Q. That's correct.

A. Not to my recollection because I don't think -- Diane picked it up but I think it was after Jody had left.

Q. So for the period 1987 through 1988, after Mr. Weinfeldt's departure --

A. As far as my recollection, as far as I recall, that's correct.

Kassenoff - cross

Q. Do you have any knowledge or understanding of how many backlogged cases, as the term is used here, that Ms. Giesser might have had in this field?

A. No, I do not.

Q. You indicated that you knew from your own personal work that an eight to nine month delay between the receipt of an "A" rating on a disclosure and the filing would have been extraordinary, at least for yourself. Is that correct?

A. Yes. I don't think that I have any cases that were pending that long.

Q. Do you have any feeling for how quickly Miss Giesser would --

A. No, I do not.

Q. But you worked with Miss Giesser in this particular case, 299/84. Isn't that correct?

A. I did some of the spadework initially but that's as far as it goes.

Q. Why did you do the initial spadework if you had your own backlog of cases, sir?

A. Probably because I was ordering, it may have been that I was ordering things from Biology

Kassenoff - cross

for two different cases, it may have been she was so backlogged that I said okay, Jody, I will contact the people involved and start the ball rolling on it for you.

Q. But you don't know if she was backlogged or not?

A. I don't know her workload, if that's your question.

Q. You said she might have been backlogged. Do you have any knowledge that she was?

A. In this particular field?

Q. In this particular situation.

A. No, I really don't, bearing in mind, of course, that each of us has several distinct fields of responsibility.

Q. Let me refer you over for a minute to paragraph six. That spans pages three through four of the declaration that is F-1.

A. Right.

Q. You were in communication with Dr. Wattanasin?

A. That's correct.

Q. Was there communication other than written communication?

Kassenoff - cross

A. As far as this case, I cannot be certain but from the way I operate, that is likely.

Q. But you don't have any knowledge --

A. I don't keep records of my phone calls but I do know that I frequently request for information by telephone.

Q. But you don't have any knowledge of such request, personal knowledge of such a request?

A. No. There is no way I could remember that.

Q. Fair enough. Turning you to maybe two-thirds of the way down on page four, the very last paragraph of paragraph six, these notes indicated that you spoke to Dr. Wattanasin. I have Exhibit N which is referred to and I would like the reporter to mark that as F-2. Once he has done that, if you would take a brief look at that.

(Whereupon the document was received and marked F-2 for identification.)

A. Okay.

Q. Now if you look at the very last line of Exhibit N, is that line in your handwriting?

A. Yes, it is.

Q. And that does indicate "spoke with S.W."?

Kassenoff - cross

A. That's correct.

Q. And is there any other indication in Exhibit N that you spoke with Sompong Wattanasin?

A. No, that's the only indication, I have a date there and it says I spoke with him.

Q. Do you see the very last five words on that line?

A. Yes, I do.

Q. What do those say?

A. "Requested info will be sent."

Q. To what does the information on page b, 392b of Exhibit N, refer to?

A. 392b?

Q. If you look at the very top.

A. That's one of the synthetic routes to the compound, to the quinoline compounds.

Q. Was that the subject matter of your discussion?

A. It might have been but my discussion primarily, at least primarily relates to the material listed at the top of 392a.

Q. Was that the type of information requested, sir?

A. Yes, it was.

Kassenoff - cross

Q. And do you have any recollection of whether that information referred to at the very bottom of the first page of Exhibit N was ever sent?

A. I'm sure it was because I can recall -- let me put it this way: I have seen from the file that some of the material, the lab notebook pages were sent.

MR. KELBER: Let me ask the reporter to identify Exhibit O, this document, as F-3. It bears the legend at the top Exhibit O.

Q. And after the reporter has so identified it, if you would review it for a minute, sir.

(Whereupon the document was received and marked F-3 for identification.)

A. Okay.

Q. Mr. Kassenoff, is the material of Exhibit F-3 responsive to the information that is indicated was requested on Exhibit F-2?

A. Only partially.

Q. In your opinion, Mr. Kassenoff, was the information requested in Exhibit F-2 necessary for the preparation of a full patent application?

Kassenoff - cross

A. Yes, it was.

Q. Am I correct, then, in understanding that additional information that had been requested would have been necessary to prepare the application?

A. That is correct.

Q. And that information would have come from Dr. Wattanasin or somebody working with him. Is that correct?

A. That's correct.

Q. Did you take any further steps to secure that information that was not provided in the --

A. Either Jody did or I did.

Q. Do you have personal recollection of receiving the additional information necessary?

A. No, I do not. The only thing I do know is that in reviewing the file, but of course, this was recently, I did note that there were lab notebook pages in there which were received at a subsequent -- which I think were received at a subsequent time.

Q. This review was made subsequent to the preparation and signing of this declaration?

A. Or concurrently.

Q. You don't recall which?

A. Or both.

Q. Which was it? You executed this declaration on February 19.

A. Right. I did have to look through the file to see these handwritten notes and identify them. Obviously, when I'm looking through the file, I did see other papers there.

Q. But you did not identify those in this declaration? I'm sorry, you did not identify the papers incorporating the additional information that was requested in Exhibit F-2 in this declaration?

A. It doesn't appear there.

Q. Referring you to paragraph eight, Mr. Kassenoff, of Exhibit F-1, as of May 23, 1988, do you have any recollection as to whether you believed you had responsibility for case number 299/84?

A. No, I do not.

Q. Does the fact that you received data from the Sandoz Biology Department with respect to that indicate anything at all to you about who had

Kassenoff - cross

responsibility for that case?

A. No, it does not. The only thing it does reveal is that there was a possibility that I would handle it. However, the case was assigned to Jody Giesser.

Q. Would Biology have known that the case was assigned to Jody Giesser?

A. No, absolutely not.

Q. Were you the only person to receive data from the Biology Department in this field?

A. No, whoever requested it would receive it.

Q. Did you request it?

A. Yes, I did. That's why it was sent to me.

Q. Why did you request it if the case had been assigned to Jody Giesser?

A. Possibly because either Jody was so tied up that I volunteered to do the legwork; possibly because I was asking, also asking for information on other cases; possibly because I might have taken over the case if I had the free time before she did.

Q. Do you have any recollection of which

Kassenoff - cross

of those possibilities it was?

A. No, I do not.

Q. Would a review of the case help refresh your recollection?

A. No. I did go through the case to see if I had any handwritten notes in there, which might help me.

Q. And were there any handwritten notes?

A. No, the only handwritten notes are what you see in front of you.

Q. Is it your custom, sir, to request biological data from the Sandoz Biology Department on cases for which you have no responsibility?

A. Only if there was a possibility that I might pick up the case or if they were tied to cases for which I did have responsibility.

Q. Do you have any feel, any recollection as to when you might have requested the data that is referred to in paragraph eight?

A. No, I do not. The only thing I can say, it was probably at least a week before May 23, 1988. It could have been, however, two months before that.

Q. Would it be customary to have a two

Kassenoff - cross

month delay between a request for biological data and receipt of the same?

A. Usually not but if the person in Biology were tied up, it could be something he forgot about and then I would have to call back and say hey, what about the stuff that I requested.

Q. Do you know who the person in Biology was at this time period?

A. Yes, I would have spoken with Robert Engstrom.

Q. Do you recall what you did with the biological data report for kit PD 299/84?

A. I'm sure I just put it into the notes that I had, the material that I was collecting for this case.

Q. Did you advise Ms. Giesser of the receipt of that data?

A. I have no recollection but it's possible.

Q. But --

A. Because I'm sure that I would have given her everything that I have on the case.

Q. Why would you have done that?

A. Because there would be no -- if she had

responsibility for the case, there certainly would be no sense in each of us having our own file. That would only lead to confusion. So I'm sure that anything I got relating to it, since she had primary responsibility, I would have given to her.

Q. So she had primary responsibility at this period in time?

A. For this particular case because it was assigned to her in the Patent Committee notes.

Q. In your review of the file -- I'm sorry, let me back up. Is it correct, then, that it would have been the practice at the time to have but a single file for the collection of materials for the preparation of the application for PD 299/84?

A. I would hope that would have been the case but I can't guarantee it.

Q. Do you have any familiarity with the procedure of the Sandoz Patent Department in general at that time?

A. There is no general procedure. Each one of us works as our own department.

Q. If each one of you works as your own department, how do you decide who has got primary

Kassenoff - cross

responsibility for the case?

A. That's the Patent Committee's job, to assign the cases, or the supervisor, who will assign it before the Patent Committee meeting.

Q. Do you have any knowledge that Ms. Giesser had a file separate from the file that you reviewed?

A. No, I do not.

Q. In your review of the file that you referred to earlier, did you come across any communications from Ms. Giesser to anyone else at Sandoz regarding the PD 299/84 prior to May 23, 1988?

A. I don't recall, frankly.

MR. KELBER: Is the file available?

MS. FURMAN: Which one?

MR. KELBER: The file that Mr. Kassenoff is referring to.

THE WITNESS: The case file.

MS. FURMAN: Sure.

MR. KELBER: Can we get the case file with reasonable speed and have Mr. Kassenoff review it? I appreciate it. While we are getting that, we can ask some more questions.

Kassenoff - cross

THE WITNESS: Let me just add something on that. Each of us had different methods of operating. What I would do is when I prepared an application, when it got filed, all of my notes relating to that case I would tuck into the file. I have no guarantee that anybody else did that. Some people had supplementary files, some of which were retained, some of which were disposed of; others did not.

Q. Mr. Kassenoff, take a minute to review that file.

A. Sure. Would you please rephrase your question so I know what I'm looking for.

Q. At this point, I'm asking you -- okay. Please review the file specifically with an eye towards determining if there are any written communications reflected there from Ms. Giesser to anybody else in Sandoz prior to May 23, 1988.

A. I don't even see my notes in there. There is nothing in the file here prior to that date, including my notes. I don't know where the originals --

MS. FURMAN: Your notes were not originally in the file.

Kassenoff - cross

THE WITNESS: Where were they?

MS. FURMAN: A bunch of papers were separate from the file that Jody gave me.

THE WITNESS: I think it's clear that there was a supplemental file there of some other notes.

MR. KELBER: Let's go off the record for a minute.

(Whereupon a discussion took place off the record.)

Q. Just to preface the agreement, Mr. Kassenoff, is it correct that your review of the file does not indicate any written communication from Ms. Giesser in the file prior to May 23, 1988?

A. That's correct, nor does it reflect anything from me prior to that date.

MR. KELBER: Diane, we would appreciate it if you would search for any supplemental papers relevant to PD 299/84 and if there is anything in the file prepared by Ms. Giesser for communications to others at Sandoz prior to May 23, 1988, if you would forward us a copy. Is that agreeable to you?

MS. FURMAN: Yes, it is.

Q. To the best of your recollection, Mr.

Kassenoff - cross

Kassenoff, did Ms. Giesser take any action with respect to PD 299/84 prior to May 23, 1988?

A. I have no recollection one way or the other.

Q. Let me direct your attention to paragraph nine of the declaration. You see the phrase "which was indicated for filing ahead of PD 299/84," the very first sentence of paragraph nine, middle of the page?

A. That's correct, yes.

Q. What does it mean to be indicated for filing ahead of?

A. It has no formal meaning, it just simply means that since this was, 7022/C was a CIP application, that I had decided to file it prior to filing, prior to picking up 7101.

Q. So you would work on 7022/C prior to picking up 299/84 if, in fact, you picked up 299 at all?

A. That's correct. I did not do anything as far as writing, that's clear.

Q. I'm trying to get a feeling for what you meant by "picking up" because obviously, you were involved with the file prior to that time.

Kassenoff - cross

A. I was involved with gathering information, that's correct.

Q. You mean picking up for preparation of the application?

A. For preparation, that's correct.

Q. What was the basis of the determination to file 7022 prior to preparing 299 for preparation?

A. I don't recall other than the fact that it was a CIP application so I probably wanted to get that off my desk.

Q. Why?

A. I don't recall. I don't think there was any question of a statutory bar or anything like that. It was probably because I probably had an office action to respond to in the parent application or the parent application was about to issue and I had to get this CIP on file in lieu of a divisional. I don't recall that specifically but I think that's a valid assumption.

Q. Do you recall having prepared any other cases in this field between January 1987 and December 31, 1988?

A. It was clearly 7087; 7041/CIP/CIP was

Kassenoff - cross

not a case I originally handled but I did prepare the most recent CIP in the case; 6955/XN/B/CONT/X was mine but that probably -- I'm not sure how much work I did on it in your time period; the one you just mentioned, 7022/C. Just referring to this list, those are the only ones on the list in that time period which I had prepared myself.

Q. Do you have personal recollection of preparing any other applications in this particular field, the HMG-CoA reductase field, in that time period?

A. In that time period, no.

Q. Let's look at 6955, the suffixes after it. What does the "CONT" designation mean?

A. Continuation.

Q. Would that have been a strict continuation application?

A. Yes.

Q. So no new preparation would have been involved. Is that correct?

A. That's correct.

Q. And 7041 was a CIP of a CIP. Is that correct?

A. That's correct.

Kassenoff - cross

Q. Do you have any recollection of how much additional work was required?

A. That was quite a bit.

Q. By quite a bit, can you give me an idea of how many months it took to prepare the additional information?

A. It probably was about two, three days -- two days work but I don't know over what period of time. It was spread out because there was a significant amount of additional information, totally redrafting of the claims and a significant rewriting of the specification. It was probably more. If I said two days, that's probably incorrect, it probably took me a good three, four days of work on it, now that I'm thinking back on it.

Q. You mentioned 7087.

A. Correct.

Q. That was a new application. Is that correct?

A. That's correct.

Q. Do you have any recollection as to why you would have prepared and filed 7087 prior to 299/84?

Kassenoff - cross

A. I'm not sure of when it was rated but I do know that it related to our potential commercial product, which is now being reviewed by the FDA. It was a process case and it had features that would have related to a commercial process. However, which case was rated "A" first, that I do not recall.

Q. Would the case to be rated first ordinarily receive attention first?

A. Unless there were a reason otherwise.

Q. Do you recall any reasons otherwise with respect to 299/84?

A. The only thing that I do recall is 7087 was a process case and it related to an advanced, at that time advanced research compound and also 7087 was initially assigned to me, whereas 7101 was not assigned to me. So under the totality of the facts, it was clear which one that I was working on first.

Q. In the absence of any reasons for proceeding differently, such as the commercial aspect of 7087, would a case that was rated "A" first get worked on first and then the case that was rated "A" after that get worked on second?

Kassenoff - cross

A. If it were assigned to the same person?

Q. The same person, yes.

A. Probably but I wouldn't say that was always the case.

Q. Is there any standard for proceeding?

A. No.

Q. So --

A. Theoretically, at least, the case that was rated "A" first should be acted on first by the person to whom it's assigned but I would not guarantee that that was followed by everybody at all times.

Q. Was it followed by you?

A. I don't think I can say yes. I think I probably exercised some selection there.

Q. For instance, if a CIP was pending and you were running out of time in response to the parent case --

A. I would pick that up first. That I have no doubt about.

Q. Earlier, we discussed the new applications that had been filed in this time frame, in the 1987-'88 time frame. If the cases

were not prepared and filed by you, after April 1987 through December 31, 1988, is it a necessary conclusion that they would have had to have been prepared and filed by Miss Giesser?

A. After Mr. Weinfeldt left, yes, because I don't recall anybody else working in that area.

Q. I realize you have no personal knowledge but do you have any recollection as to how many more cases in this field, new cases in this field might have been filed than are represented here between April 1987 and December 31, 1988?

A. If there were any, and I'm not sure that there were any, it would have been probably very few.

Q. Did you assist in preparing this list that appears in paragraph five?

A. No, Diane prepared it on her own and I just went through it to make sure that everything there did -- everything listed was a case that was an HMG-CoA reductase case. I did not double-check the dates on them.

Q. Let me direct your attention to paragraph 11 of the declaration, penultimate

paragraph of that form.

A. Okay.

Q. Is it possible for a disclosure never to receive an "A" rating?

A. Of course.

Q. And if that disclosure never receives an "A" rating -- I'm sorry, let me flip it around. Is it a requirement that a disclosure receive an "A" rating before it is prepared as an application for filing within Sandoz?

A. Generally, yes, but there are exceptions. Sometimes an application will be worked on before it actually is formally rated "A".

Q. Did you do any work on PD 299/84, to the best of your recollection, before it was rated "A"?

A. No, I did not. The only work done of which I have any recollection is that reflected by the notes in the file.

Q. Is it a correct statement, Mr. Kassenoff, that if Sandoz intends to file a United States patent application on the basis of a patent disclosure, it first or it simultaneously with that decision rates that disclosure "A"?

Kassenoff - cross

A. In the pharmaceutical area, yes.

Q. Mr. Kassenoff, when did you first become aware, if you recall, when did you first become aware that third parties other than Sandoz had filed for U.S. patent protection on compounds related to those of PD 299/84?

A. I assume it was after Warner-Lambert's patent issue sometime when somebody brought it to my attention or when I noticed it in the OG.

Q. Do you have any recollection as to whether that was before or after November 1987?

A. November --

Q. I'm sorry, November 1988? I apologize.

A. I don't know because if I recall correctly, Warner-Lambert's issue was in June or July or August?

Q. Your recollection is correct, I think it issued in June.

A. If it issued in June, it could have been brought to my attention anywhere from shortly after it issued to several months later. I really do not recall. If it was a question of my noticing it in the OG, I can tell you I did not notice it

Kassenoff - cross

immediately because as Bob will testify, I have a whole stack of OG's sitting around that I have not gone through. If somebody brought it to my attention, it could have been any time.

Q. Just for the record, when you refer to Bob, you are referring to --

A. Bob Honor.

Q. Do you have any recollection of whether you knew, prior to the time that PD 299/84 was filed in March of 1989, whether you knew that a third party had filed for patent protection?

A. Yes, I think I can recall, yes, I did know that.

Q. Have you been involved on behalf of Sandoz in the drafting of an application where you were aware that a third party had filed for patent protection on related subject matter?

A. It depends how you define "related."

Q. Related in the sense that the claims -- I'm sorry, forget claims, the subject matter disclosed in that third party's request for protection were obvious in the sense of 35 U.S.C. 103 with respect to the application you were preparing.

Kassenoff - cross

A. Not that I can recall.

Q. Besides PD 299/84, are you aware of any other situations similar to that within Sandoz?

A. Nothing comes to mind.

Q. As Director of Patent and Trademark Affairs at Sandoz, you attend the Patent Committee meetings as regularly as possible. Is that correct?

A. Yes. That's only since, though, January of last year.

Q. Is it a correct statement to say that you generally have input on how to rate a patent disclosure viewed by the Patent Committee?

A. I open my mouth when warranted.

Q. Fair enough. If you were aware that a third party had filed for U.S. patent protection on subject matter addressed in a disclosure before the Sandoz Patent Committee, would that influence in any way your judgment as to how to rate that disclosure?

A. It probably would.

Q. Give me one second. I'm sorry, one question I meant to ask. What other fields of technology did you have responsibility for besides

Kassenoff - cross

the HMG-CoA reductase field in the period January 1987 through December 1988?

A. 90 percent of my workload at that time consisted of prosecution of dyestuff cases originating in Basle, Switzerland. If you look at my docket, almost all the cases on it would be dyestuff cases.

Q. Prior to Mr. Weinfeldt's retirement or I'm sorry, not retirement but departure from Sandoz, did you have any responsibility in the HMG-CoA reductase field?

A. Yes, I handled some disclosures as early as late 1982.

Q. Prior to Mr. Weinfeldt's departure, did anybody else in the Sandoz Patent Department handle the preparation of applications in the HMG field?

A. Other than me and Fred, no, to my best recollection.

MR. KELBER: Thank you, Mr. Kassenoff. Diane, your witness.

MS. FURMAN: Do you mind if we take a break before cross?

MR. KELBER: Sure.

(Whereupon a recess was taken.)

Kassenoff - cross

MS. FURMAN: I would like to offer into evidence copies of Patent Committee minutes which have been masked as to their proprietary information but left unmasked with respect to information concerning patent disclosure 299/84.

MR. KELBER: Are these the records that are already of record in another declaration?

MS. FURMAN: These comprise exhibits M-1 through M-5 of the testimony already of record.

MR. KELBER: So that would be Rothwell. Go ahead and identify them.

MS. FURMAN: And that would be exhibit, the totality of that would be Exhibit F-4.

MR. KELBER: Can we make a distinction? These are going to be your exhibits to submit and maybe we ought to make it W-1.

MS. FURMAN: Fine.

(Whereupon the document was received and marked W-1 for identification.)

REDIRECT EXAMINATION BY MS. FURMAN:

Q. Mr. Kassenoff, do you recognize the copy of the minutes dated April 29, 1987?

Kassenoff - redirect

A. Yes.

Q. And what information is on the minutes concerning patent disclosure 299/84?

A. That it was rated "B" in April of 1987 and that it was originally assigned to Fred Weinfeldt.

Q. Now I call your attention to the minutes of July 29, 1987. Do you recognize information relating to patent disclosure 299/84?

A. Yes, that it was rated "B" and again, assigned to Fred Weinfeldt.

MR. VILA: Pardon me, is there an exhibit number on that?

MS. FURMAN: Yes, there is.

MR. KELBER: These are all part of W-1.

Q. Look now at the minutes of the October 28, 1987, Patent Committee meeting. What does it say about patent disclosure 299/84?

A. That it was rated "X" and assigned to Fred Weinfeldt.

Q. Again, the Patent Committee minutes of November 25, 1987, what is the rating thereon?

A. It was again rated "X" and is still assigned to Fred Weinfeldt.

Kassenoff - redirect

Q. Finally, I ask you to look at the minutes for the January 27, 1988, PCM. And what is the rating of PD 299/84?

A. It was rated "A" and assigned to Jody Giesser.

Q. To the best of your knowledge, what does a rating of "B" signify?

A. "B" signifies that it will be considered in three months.

Q. What would prompt a rating of "B", would it be not enough information is available to file a patent application or would it be that more information is intended to be developed for the patent?

MR. KELBER: Objection, leading. The question is okay but you can't feed him the answer.

MR. VILA: Cut the question off at the first part.

Q. What is the meaning of the rating of "B"?

A. Generally, it would mean that additional work is being performed on the case.

Q. And what about the rating of "X"?

A. "X" means that it will come up in one

Kassenoff - redirect

month, it can mean one of two things, either one, that the people necessary or the people whose input is required before the disclosure is rated "A" are not at the meeting or that additional work is still ongoing and the results are expected within one month, such that it is anticipated that a decision will be made at the next Patent Committee meeting.

Q. At the time patent disclosure 299/84 was rated, was it within your responsibility to rate patent disclosures?

A. No, it was not.

Q. Once a patent disclosure has been rated by the Patent Committee, can you rerate that disclosure yourself?

A. I can bring it back to the Patent Committee if the need arises.

Q. Is it within your jurisdiction not to file on a patent disclosure that has been rated "A" by the Patent Committee?

A. No, it is not.

Q. Did you at any time intend after the rating of "A" of the patent disclosure not to file a patent application on it either yourself or by someone else in the department?

Kassenoff - redirect

MR. KELBER: Objection. You are asking him for knowledge as to other people's intentions.

Q. By yourself alone?

A. No.

Q. Is there any way to inactivate or retire a patent disclosure once it has been rated "A" by the Patent Committee?

A. The attorney in charge can bring it back to the Patent Committee and request a rerating of it for whatever reasons are deemed relevant.

Q. Absent that, is the patent disclosure considered active until --

A. Yes.

Q. -- the action is taken?

A. It's considered active until the application is filed.

Q. Mr. Kassenoff, I call your attention to your declaration previously made of record as Exhibit F-1 to the list of patent applications filed which is indicated on pages two and three. Do you know or is it within your reasonable belief that all of these applications have now been published in one way or another?

A. As far as I'm aware, every one has been

Kassenoff - redirect

published either in the U.S. or abroad.

Q. Is there a possibility that there may be some applications not on this list in the HMG-CoA area which have not published?

A. There is a possibility but I cannot recall of any specific ones. Actually, there is one that I think I can recall that's not on this list. Whether it was published or not, I don't know. I vaguely recall a case 7044.

Q. Other than that possibility of case 7044, this would constitute the entirety of the HMG-CoA filings?

A. That is not correct. There are a number of other cases that specifically have not been listed here. At least one that comes to mind is 6952. There are cases in the process area like 6957 and its progeny; there are some cases 694 -- I don't recall the last digit on them, some of the early cases. There could be others but I specifically remember those cases.

MS. FURMAN: That concludes my questioning.

MR. KELBER: I have a little bit of --

MR. VILA: Can I ask a couple of

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questions?

MR. KELBER: I have no objection.

BY MR. VILA:

Q. Mr. Kassenoff, I believe you said that you had a substantial involvement in dyestuffs besides the area in question which is the HMG-CoA reductase area. At that time, did you have any other areas of responsibility within the department?

A. Yes, I did.

Q. Would you enumerate those, please.

A. For example, I was keeping track of recent decisions and advising our parent company in Basle on recent decisions in U.S. patent law; I was involved in tracking pending legislation, rule changes and advising our parent company's Patent Department in that regard as well as other members of this department; I had several other projects, for example, in 19 -- it must have been 1987, when the record of understanding between the United States and the Republic of Korea was entered into, I was in charge of preparing all of the declarations. I think that was in early '87

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because if I recall correctly, the five year -- the initial five year period of exclusivity commenced on July 1st of 1987 and that took up a substantial amount of time in that period. There may have been others but those are the ones that come to mind.

Q. When Mr. Weinfeldt left in April of '87, I believe you testified that at that point in time, you and Mr. Weinfeldt had responsibility for the area in question.

A. That's correct.

Q. When Mr. Weinfeldt left, who had responsibility for that area?

A. I had, I would assume, primary responsibility and then Jody Giesser, and I'm not sure exactly when Jody came here, but Jody picked up a good deal of the responsibility sometime in that time period.

Q. Are you saying she picked up a responsibility for existing cases?

A. For existing cases as well as for new disclosures.

Q. And do we know approximately when Jody Giesser was employed?

A. If I recall correctly, it was at the

Kassenoff - redirect

time that Fred was on medical disability.

Q. I believe your declaration mentions that August of '87 is the time that Jody joined the department. Between April, when Mr. Weinfeldt left the department, and August, who else besides yourself would have been handling or responsible for this area?

A. I assume that you had some responsibility but I don't recall if you did any cases on that but other than that, no one.

Q. So as far as you know, you had responsibility for the entire area in that period?

A. As far as I know, that's correct.

Q. When Mrs. Giesser joined the department, did she have any prior experience in this area or in pharmaceutical applications, to your knowledge?

A. She may have had some in pharmaceuticals at the law firm or one of the law firms at which she was previously employed but certainly not in this specific area.

Q. I believe it's on the record that you have substantial long term experience in the pharmaceutical field. How would you describe the

Kassenoff - redirect

degree of effort required in preparing cases in this area in general, would it be routine or easier than routine?

A. The cases were rather lengthy because this is not an area which one could synthesize the compounds in one step reactions. Many of the compounds required five, even ten step syntheses. Consequently -- and often not all of the compounds of a single disclosure could be made by a single route. Consequently, the process description was -- the required process description was extensive and the applications were lengthy.

For example, there were at least a couple of the applications that I wrote were well over a hundred pages and in fact, one may have been close to 150 pages. Of course, some of them were probably on the order of 40 or 50 pages. Those were the shorter ones.

Q. I believe it's been testified that when disclosure 299/84 was rated "A" in January of '88, it had Jody Giesser's initials on the Patent Committee minutes.

A. That's correct.

Q. Indicating the case was her

responsibility. Was there some uncertainty as to who would prepare that case despite that notation?

A. I assume that it was in the back of our minds that there was a possibility that I might do it if I had no other -- if I had the available time because that's the only way I could explain the fact that I did request Dr. Wattanasin to send me some of the Chemical -- the information required from the Chemical side and I did request Biology to send me their input for the application.

Q. Would Mrs. Giesser have had experience in obtaining the type of information which you obtained in 1988 from the Pharmaceutical Research Group?

A. Probably not.

Q. If there were a decision after the "A" rating in January of 1988 not to file a patent application on 299/84, can you tell me what, if anything, would have happened to reflect that decision?

A. It would have been reflected in the subsequent minutes of the Patent Committee.

Q. And how would that procedure have taken place?

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A. The attorney in charge would have requested the Patent Committee to rewrite the disclosure from "A", either into "B", "C", "D" or "X", "D" meaning drop or dead and "X", "C" and "B" being various categories of bringing it up once again.

Q. If there had been a decision not to file the application, what would have been the rating in that case?

A. "D".

Q. Could anybody else other than the patent attorney bring that issue before the Patent Committee?

A. Yes, anybody, any member of the committee could bring it up but generally, it would be done through the attorney, at least directly through the attorney.

Q. Who were the members of the committee?

A. The people of the committee consists of the heads and assistant heads of the Patent Department and members representing Chemistry, Biology, Pharmacy and possibly some other groups in Pharmaceutical Research.

Q. Could members of the committee

Kassenoff - redirect

representing chemistry bring the disclosure back up once it had been rated "A"?

A. Yes, they could.

Q. Members of the Biology group?

A. Yes.

Q. To your knowledge, did anyone, either Patent Department, Chemistry, Biology or anyone else offer this disclosure back up to be given a category other than to be filed upon?

A. Not to my knowledge but then again, I was not participating in the Patent Committee at that time.

Q. If such an action had been taken, would you be aware of it through the Patent Committee minutes?

A. Yes, I would.

Q. Are you aware of any such action?

A. No, I'm not.

MR. VILA: I don't think I have any more questions.

RECROSS EXAMINATION BY MR. KELBER:

Q. Mr. Kassenoff, I believe you testified that between April and August of 1987, you were the

Kassenoff - recross

sole patent attorney or agent at Sandoz responsible for the area of HMG-CoA reductase. Is that correct?

A. Yes, although there is a possibility that Dick Vila here, who is the supervisor of the group, might have filed some responses in some pending cases.

Q. Do you have knowledge of whether he did or not?

A. No, I don't have any knowledge of that.

Q. Between the period April and August of 1987, no patent attorney at Sandoz would have taken up PD 299/84 for any reason, would they have?

A. Between when?

Q. Between the period April and August of 1987.

A. No. It had not been rated "A".

Q. So even if Ms. Giesser had been here in April of 1987, she would have had no reason to pick up that disclosure?

A. That's correct, not until it received an "A" rating or was about to be rated "A".

Q. I believe you testified that at no time

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subsequent to the receipt of the "A" rating on 299/84, you were not aware -- I'm sorry, at no time subsequent to that "A" rating, you personally did not have any intention not to file an application --

A. That's correct.

Q. -- corresponding to PD 299/84. Is that correct?

A. That's correct.

Q. At any time prior to receipt of that rating, did you have any intention to file an application directed to PD 299/84?

A. No. There would be no reason to.

Q. Do you recall whether there was a Patent Committee meeting in December of 1987?

A. Unlikely. At least in the last few years, we have not had December meetings. The Patent Committee invariably meets the last Wednesday of the month and the last Wednesday in December is not a very conducive time to have a meeting.

Q. Are there cases where a disclosure has been rated "A" and additional work is continuing on that particular subject matter?

A. Probably.

Q. Are you aware of any such cases personally?

A. Personally, no, but I'm pretty sure that that's the case.

Q. Isn't it the fact, Mr. Kassenoff, that in the case at issue here, PD 299/84, Dr. Wattanasin continued work in that subject matter subsequent to the "A" rating?

A. That's probably the case.

Q. So the fact that additional work is being performed on a case is not alone reason to rate it only "B" as opposed to "A"?

A. That's correct.

Q. There are other considerations that would go into rating a case as "B" as opposed to "A". Is that correct?

A. Probably.

Q. Can you name some of those other considerations?

A. Yes. For example, if the work done to date shows that the compounds, while being of interest, might not be as interesting as other compounds of the series, although of interest, they

could defer it. For example, if this were the best compound that we had and better than what was available, we would probably not wait for ongoing work. On the other hand, if this compound, that is, the lead compound of this particular series, were good but probably, let's say, maybe not better than anything we already had, we might delay it.

Q. Do you recall whether that was the situation in connection with PD 299/84?

A. I do recall that we had a compound in this series in advanced clinical research at the time and that this compound certainly did not appear to -- the lead compound of the quinoline series certainly did not appear to be better than the compound that was then in clinic. That I do recall.

Q. Is it your --

A. How it compared, it was probably -- I don't know but it was certainly no better.

Q. Is it your testimony, Mr. Kassenoff, that all other things being equal, that lead compound of a disclosure that is not as good, active, without toxicity --

A. Generally --

Kassenoff - recross

Q. Let me finish the sentence, the question.

-- that is not as active as another compound that is already developed and I presume the subject of a patent application, that the application as to the less active compound might be deferred?

A. Put it this way: One, as far as when the disclosures come to the Patent Committee, we generally do not have the tox information available so we are just dealing with the testing of the compound. We have information as to its activity but not as to tox. There are exceptions, of course.

Generally, we will-- I wouldn't say that the applications would be delayed. What I would say is that they wouldn't be expedited.

Q. Now I'm confused. Perhaps I used the wrong word in the term "delay." Would a disclosure be rated "B" for that reason alone?

A. For that reason alone?

Q. The reason you described, that is, less active than another case in testing.

A. No, it would simply mean that since the

Kassenoff - recross

work was ongoing, there was no rush to file it.

Q. If work was ongoing and the lead compound that was the subject of that work was not as active as another compound that you were currently pursuing, would that be sufficient in and of itself to rate a compound -- to rate a disclosure as "B"?

A. I don't know if I could really answer that question. I would say --

Q. What else do you need to know?

A. It was probably a factor but you are saying in and of itself, I really can't answer that.

Q. What other reasons would give rise to rating a disclosure "B"?

A. Other than the ongoing work, probably -- it either would be ongoing work or whether it was of sufficient interest but usually it's ongoing work, it's "B", because if the work had been incompleted, we would be able to make a rating of it.

Q. But PD 299/84 had ongoing work after it was rated "A", wasn't it?

A. That's correct.

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Q. So ongoing work alone is not sufficient --

A. That's correct.

Q. -- to discriminate between "B" and "A". Is that correct?

A. That's correct.

Q. Do you have any idea why PD 299/84 was rated "B" or "X" prior to January of 1988?

A. If I had to make a guess, I would say it's probably because there was some biological testing on what was then the lead compound of the series that had not been completed yet.

Q. But you are guessing?

A. I'm guessing but I would say that's probably the case. It was probably the in vivo testing that had not been completed yet.

Q. Let's go back to the biological data that you requested from Sandoz Biology Department in March of 1988. Is that the type of data that you are talking about?

A. I'm not sure. That certainly is in vitro testing. Whether there was also in vivo testing at the time, I do not recall. If it was, I would have received it. Without looking at the

data, I can't tell you if that's strictly in vitro or whether there is also in vivo testing at that time.

Q. Why would a disclosure be rated "X"?

A. "X" generally means that it will come up in one month and usually either we expect some data to be received during the month or else it means that, in this case it probably would be two months because of the lack of a December meeting, or else it could mean that the people required to make the decision, either the lead person from Chemistry or Biology, without whose input you generally would not want to raise it, was not present at the meeting so it's strictly deferred for a month.

Q. Are there situations where a disclosure can be rated "X" and then not elevated to "A" subsequently?

A. Absolutely.

Q. How long does it take you to prepare the average pharmaceutical application that you spoke to earlier when you are preparing one on behalf of Sandoz?

A. Are you talking about in duration of

Kassenoff - recross

time or actual number of hours?

Q. I'm sorry, duration of time.

A. It really depends on my other workload.

Q. You were able to respond to an issue regarding average applications. Can you give me an average for the period in question?

A. My guess is that it would probably be -- the work would probably require about three months but obviously, I'm doing a lot more in that time period.

Q. Understood.

A. That's a ballpark figure.

MR. VILA: Pardon me.

MR. KELBER: Off the record.

(Whereupon a discussion took place off the record.)

Q. From the time you received notification of an "A" rating on a disclosure to the time you begin preparation of the application, generally how long a time period is that?

A. I don't think I could answer that. It can vary anywhere from days to a month, sometimes even longer.

Q. Why would it be longer?

A. Pressure of other work, particularly a huge docket of applications of office actions to respond to and/or other work. In other words, I'm fitting in my new disclosures on a time available basis between all of my other responsibilities.

Q. And you are careful to take things in turn. Is that correct?

A. As far as new disclosures?

Q. Your work in general.

A. I would give priority to responding to office actions unless there were a statutory bar involved.

Q. You indicated that all of these, almost all of the applications or patents listed in paragraph five of F-1 had, to the best of your recollection, been published by now.

A. Oh, yes.

Q. Do you have any knowledge whether any of them were published before their filing date?

A. Before their filing date?

Q. Before their filing date.

A. Absolutely not.

Q. So it would be the Sandoz policy not to publish material before the application --

Kassenoff - recross

A. Absolutely. That's a clear no-no.

Q. How about PD 299/84, do you have any knowledge specifically in that case as to whether there was any publication prior to its filing date?

A. I have to assume that there would be none because that would not be permitted by our publication clearance procedure. In other words, we will not clear a publication for release until either we filed on it, and generally we will not clear it until it's about to publish out either in the U.S. or abroad or unless the disclosure is rated "D".

Q. So if a disclosure would be rated "B" or "X" --

A. We would not permit a publication, no way.

Q. The synthesis data that you talked about on redirect examination that tends to make pharmaceutical cases lengthy --

A. At least in this particular area. I wouldn't want to generalize it.

Q. In this particular field, does much of that synthesis information come from the individuals responsible for the work on the

Kassenoff - recross

compounds?

A. Yes.

Q. And so that that would not have to be prepared ab initio by the attorney in question?

A. It would have to be prepared by the attorney in that one. Generally, we do not get written up procedures. We get lab notebook, at least I work from lab notebook pages, which means one, I have got to go into the lab notebook pages; two, I have got to, obviously, for the examples, write them up from the lab notebook pages; three, I have got to then check, write up general procedures for it. Sometimes they may come from the inventor, as was the case in this case.

Q. I'm sorry, which case is that, sir?

A. The 7101 case. As you can see, the material, one of the exhibits does have an outline. Then I have got to make sure that for the entire scope agreed upon, that the processes that were provided are operative and if they are not, either we have to modify the scope or we have to provide additional processes such that we have an enabling disclosure for the entire scope.

Q. Do you know offhand whether that was

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necessary in 7101?

A. No, since the case was prepared by Miss Giesser.

Q. You testified, I believe, on redirect with respect to the experience Ms. Giesser had in obtaining data with respect to patent disclosures from other departments within Sandoz. Is that correct?

A. I said -- what I did say is that in all probability, since she was fairly new in the department, she did not have that experience. The basis for that is that most of the work that she did was nonpharmaceutical work. She was handling our seeds work and some agro, as well as some biotech work. She did not, other than the HMG-CoA reductase area, in which she just spent a small amount of her time, she did not spend very much in pharmaceuticals.

Q. But nonetheless, she was charged with responsibility in that field?

A. In these cases.

Q. How difficult is it to request the data in question?

A. Phone call.

Kassenoff - recross

Q. You worked with Ms. Giesser for a period of about two years, three years, is that correct, maybe more?

A. She was here for that period of time, yes.

Q. Did you have an opportunity to judge whether she had the ability to learn how to obtain that data in that period of time?

A. I'm sure to obtain the data didn't require any exercise.

Q. So even though she joined in August of 1987, it wouldn't have taken her too long to learn how to obtain that kind of data?

A. No, but I'm not sure, this could have been -- these probably were the first pharmaceutical cases that she was involved in.

Q. Do you know that one way or the other?

A. I don't know that as a fact but I think it's a valid assumption, since I'm not aware of any other area in which she did any pharmaceutical work.

Q. The applications that were prepared subsequent to April of 1987 in this field that were new cases that were not prepared by you would have

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had to have been prepared by you, wouldn't they?

A. Yes, but if you look at the list, starting with April of 1987, you will see that there aren't very many actually new disclosures.

Q. But there are a few, aren't there?

A. There are a couple.

Q. And you weren't responsible for those entirely, were you?

MR. VILA: Can we go off the record a minute.

(Whereupon a discussion took place off the record.)

Q. You did not prepare all the cases that appear in this list that were filed subsequent to April of 1987. Is that correct?

A. That's correct.

Q. Do you have any knowledge of what type of input was provided to change the rating on PD 299/84 first from "B" to -- I'm sorry -- yes, first from "B" to "X"?

A. I don't know if there was any written input. It probably was oral input at the Patent Committee meeting.

Q. Do you have any knowledge as to what

Kassenoff - recross

that input was?

A. No. I did not attend the meeting at that time.

Q. Do you have any knowledge as to what caused the Patent Committee to change the rating from "X" to "A"?

A. No, I have no specific knowledge of that.

MR. KELBER: I have nothing further at this time.

MR. VILA: Let me clarify the question that was asked. I believe the question was addressed as to Jody Giesser's responsibilities subsequent to April of '87 in this area. Again, when did Miss Giesser join this department?

THE WITNESS: Later in '87, I think. Was it August? Sometime in August. I think it was August of '87.

MR. VILA: So your answer to that question only could have been with reference to the time she actually joined the department, which was later in 1987?

THE WITNESS: That's correct.

MR. KELBER: When Miss Giesser joined

Kassenoff - recross

in August of '87, were the patent disclosures rated "A" waiting to be prepared, assigned to her?

THE WITNESS: I really do not know.

MR. KELBER: Okay.

MS. FURMAN: I have nothing.

MR. KELBER: Thank you, Mr. Kassenoff. I appreciate it. Before we go off the record, we need each of the depositions to be taken today to be prepared in separate transcripts, according to the rules. Don't ask me why. There are lots of rules recited in the CFR about how they have to be prepared and filed.

MS. FURMAN: They are aware of them.

MR. KELBER: Did you take care of it?

MS. FURMAN: Yes.

MR. KELBER: Okay, thank you.

(Time noted is 11:45 a.m.)

Melvyn M. Kassenoff
MELVYN M. KASSENOFF

Subscribed and Sworn to before me
This 21st day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

ERRATA SHEET

Name of case: Wattanasin v. Fujikawa et al.
Deposition of: Melvyn M. Kassenoff
Date taken: March 22, 1993
Page 1

[I have marked the most important changes with an asterisk *.]

PAGE	LINE	CHANGE	REASON
*11	12	Change "filing" to "bar".	This is what I remember saying. Additionally, the sentence at ll. 11-13 makes no sense without this correction.
11	13	Change "filing" to "file".	I am certain I said "file". Also, this change would render the sentence grammatically correct.
4	24	Change the comma (",") to a period (".") and change "it's" to "It's".	This would clarify what I said and render the sentence grammatically correct.
22	4	Change "It" to "There".	This correction reflects what I remember actually saying. Additionally, the sentence at ll. 4-8 is clearly grammatically improper without this change.
23	22	Delete the comma (",") after case and insert a comma (",") after "weeks".	The meaning of my sentence is clarified by this change.
*24	17	Change "application?" to "inhibition field?".	This correction reflects what I remember saying. The word "application" is a clear transcriptional error, since the sentence at l. 17 makes no sense if left uncorrected.
25	13	Change "have" to "had".	This is what I remember saying. Also, without the correction, the sentence at ll. 13-14 does not make grammatical sense.

PAGE	LINE	CHANGE	REASON
31	9	Change "I'm looking" to "I looked".	This is what I remember saying. Also, without the correction, the sentence at ll. 9-10 does not make grammatical sense.
34	13	Delete "kit".	This word is meaningless in the context, and is clearly a transcriptional error. The meaning of the sentence at ll. 12-13 is clear without it.
25	13	Change "have" to "had".	This is what I remember saying. Also, without the correction, the sentence at ll. 13-14 does not make grammatical sense.
31	9	Change "I'm looking" to "I looked".	This is what I remember saying. Also, without the correction, the sentence at ll. 9-10 does not make grammatical sense.
34	13	Delete "kit".	This word is meaningless in the context, and is clearly a transcriptional error. The meaning of the sentence at ll. 12-13 is clear without it.
41	5	After "period;" insert "and".	This change is consistent with my recollection of what I said; the word "and" also provides a basis for my subsequent mention of case 7022/C.
42	4	Change "That" to "There".	This change is consistent with my recollection of what I said. Also, the sentence at l. 4 does not make grammatical sense without this change.

PAGE	LINE	CHANGE	REASON
42	12	Change "totally" to "a total".	This change is consistent with my recollection of what I said. Also, the sentence at ll. 10-13 does not make grammatical sense without this change.
43	16	After "advanced" insert a comma (",").	This insertion would clarify the meaning of my statement.
43	19	Change "working" to "to work".	This change is consistent with my recollection of what I said. Also, the change would clarify my statement.
47	8	After "was" insert "sometime".	This change is consistent with my recollection of what I said. The placement of the word "sometime" in line 9 is a transcriptional error, and it properly belongs in line 8.
47	9	Change "issue" to "issued" and delete "sometime".	This change is consistent with my recollection of what I said. Also, the change would clarify the sentence.
47	17	After "issue" insert "date".	This insertion is consistent with my recollection of what I said. Also, the insertion would clarify the sentence.
60	6	After "area" insert "in".	This insertion is consistent with my recollection of what I said. Also, the insertion would clarify the sentence.
60	12	After "was" (second occurrence) insert a double hyphen ("--").	This would clarify what I said and render the sentence grammatically correct.

60	15	After "wrote" insert "that".	This would clarify what I said and render the sentence grammatically correct.
*62	3	Change "rewrite" to "rerate".	This change is consistent with my recollection of what I said. Also, the sentence does not make sense in the present context without this change.
*69	21	Change "incompleted" to "completed".	This change is consistent with my recollection of what I said.
*71	14	Change "raise" to "rate".	This change is consistent with my recollection of what I said. Also, the word "raise" instead of "rate" would be meaningless in this context.

Melvyn M. Kassenoff
MELVYN M. KASSENOFF

SUBSCRIBED AND SWORN TO BEFORE ME

This 21st day of April, 1993

Antoinette Lombardi
A Notary Public

ANTOINETTE LOMBARDI
Notary Public of New Jersey
My Commission Expires April 3, 1994

C E R T I F I C A T E

I, GARY M. TALPINS, a Notary Public and Certified Shorthand Reporter of the State of New Jersey, do hereby certify that prior to the commencement of the examination, MELVYN M. KASSENOFF was duly sworn by me to testify the truth, the whole truth and nothing but the truth.

I DO FURTHER CERTIFY that the foregoing is a true and accurate transcript of the testimony as taken stenographically by and before me at the time, place and on the date hereinbefore set forth, to the best of my ability.

I DO FURTHER CERTIFY that I am neither a relative nor employee nor attorney nor agent of any of the parties to this action, and that I am neither a relative nor employee of such attorney or counsel, and that I am not interested directly or indirectly in the interference either as counsel, attorney, agent or otherwise.

Gary M. Talpins, C.S.R.
License No. XI00561

#38 + #93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN v. FUJIKAWA ET AL.

INTERFERENCE NO. 102,648
INTERFERENCE NO. 102,975

WATTANASIN CONSOLIDATED RECORD

VOLUME IV

[PAGES 319 - 469]

FYI

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May 15, 1993

5 c Furm 5/17/93

"RIBBON COPY FOR PARTY Wattanasin"

Case No. 600-7101/CONT/INT.(3)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v. Interference Nos. 102,648, 102,975

FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF JOANNE M. GIESSER PURSUANT TO 37 CFR §1.672

I, Joanne M. Giesser, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

2. I was employed by Sandoz Corporation as a patent attorney from August 16, 1987 to November 6, 1992, and during the time periods referred to herein was a member of the Patent and Trademark Department located in East Hanover, New Jersey. (On September 1, 1992, I transferred to the patent department of the Sandoz Crop Protection affiliate of Sandoz Corp. in Palo Alto, California.) I am currently employed as a patent attorney for Amoco Corporation in Naperville, Illinois.

3. I filed the involved Wattanasin continuation application, and I also drafted and filed the parent application thereof, Serial No. 07/318,773 filed on March 3, 1989. As of its filing date, the '773 application received internal docketing number 600-7101, and is hereinafter referred to as "Case 600-7101".

4. Case 600-7101 is based on Patent Disclosure No. 299/84 of Dr. Sompong Wattanasin.

5. At the January 27, 1988 meeting of the Sandoz Corporation Patent Committee, said PD 299/84 was rated "A" for filing. I would have received a copy of the Minutes of the meeting sometime in February 1988.

6. PD 299/84 was assigned to me, although Mr. Kassenoff of the Patent Department and I intended that the case would be filed by either one of us depending on who was available after existing filing priorities had been completed.

7. I received certain materials from Dr. Wattanasin in connection with the filing of Case 600-7101.

Exhibit P comprises a copy of material which the Patent Department received which related to the preparation of Case 600-7101. These materials comprise:

P-1: 4 pages containing handwritten reaction schemes and notes bearing the handwritten name of "S. Wattanasin" and a date of February 29, 1988 on the first page;

P-2: 7 pages of computer printouts of specific compounds containing handwritten notations of the Notebook pages on which they were prepared and relevant physical properties; and

P-3: 9 laboratory notebook pages numbered 130, 137, 145, 153, 158, 166, 172, 175 and 176.

8. When I received the pages which comprise Exhibit P, I made handwritten annotations on some of the pages, which appear on the pages of the Exhibits.

9. It will be noted that in the calendar year 1988, I compiled an airline travel mileage of approximately 75,000 miles. My travel and entertainment expense reports for the period of February 1, 1988 to March 3, 1989, indicate that I was required to be out of the office on business on at least the following dates:

February 21-26.
March 1, 15-16, 20 and 28-31.
April 20-22.
May 2
June 15-16, 24
July 12
August 29-31
September 1, 10-14
October 9-11, 16-17, 27-28
December 6-8
January 8-12
February 21, 28
March 1-2

Exhibit S hereto comprises true copies of travel and entertainment expense reports which I filled out and submitted to the Sandoz Travel Department to obtain reimbursement of my business travel expenses. Each of these reports is in my handwriting and bears my true signature.

10. No later than October 1988, I would have started writing a draft of Case 600-7101.

11. On November 6, 1988, I filed continuation-in-part application, Case 600-7025/CIP/CIP (Serial No. 07/466,083), which was indicated for filing ahead of PD 299/84.

Exhibit T hereto comprises a copy of the filing receipt for Case 600-7025/CIP/CIP/.

12. In early November of 1988, my secretary, Ms. Lorraine M. Chesley, began typing a draft of Case 600-7101.

Exhibit U-1 hereto appears to comprise a copy of the label of the computer disc on which this application is stored, which indicates a starting date of November 3, 1988 and a mailing date of March 3, 1989.

13. Also in about November of 1988, I received a memorandum from Dr. Wattanasin which outlined certain synthesis steps for preparing compounds of Case 600-7101.

Exhibit U-2 comprises a memorandum received from Dr. Wattanasin by the Patent Department, which comprises a cover page and 8 pages containing synthesis steps for preparing compounds covered by PD 299/84.

This memorandum bears a handwritten date of November 7, 1988 and was date stamped November 8, 1988 by the Patent Deparment.

14. On or before November 8, 1988, I requested Mr. Siegfied S. Warhman of Sandoz Information Services to provide correct nomenclature for various compounds of PD 299/84 and starting materials used in their synthesis.

Exhibit V-1 comprises a true copy of my handwritten request, which became the cover page of a responding memorandum from Mr. Henry Mah, also of Sandoz Information Services. The return memorandum is dated November 8, 1988; and the Patent Department date stamp on my request memo indicates that it was received by the Patent Department on November 9, 1988.

Exhibit V-2 is another memorandum which was received by the Patent Department from Mr. Henry Mah which bears a date of November 14, 1988 and is also date stamped November 14, 1988, which provides further nomenclature of the quinoline compounds of the PD 299/84 and their reaction intermediates.

15. On or about December 14, 1988, I sent a first draft of Case 600-7101 to Dr. Wattanasin for his review.

Exhibit W comprises a true copy of the cover letter for the draft application which I sent to Dr. Wattanasin.

15. Further information related to Case 600-7101 which is in the possession of the Patent Department comprises:

Exhibit X: which comprises four pages of reaction diagrams containing notations some of which are written in my handwriting, and the handwritten date of December 22, 1988.

Exhibit Y-1: a handwritten memorandum of changes in a draft of Case 600-7101 bearing a date of January 4, 1989;

Exhibit Y-2: a computer printout of the structures of the compounds of PD 299/84, with handwritten IC50 and/or ED50 values and a handwritten date of January 4, 1989.

16. On March 3, 1989, I filed Case 600-7101, the parent application of the involved Wattanasin application.

[redacted]

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing Declaration this 19 day of February, 1993.

Joanne M. Giesser

JOANNE M. GIESSER

[redacted]

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D-00581-93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
INTERFERENCE NOS. 102,648
102,975

WATTANASIN,	)
	)
vs.	) DEPOSITION OF:
	) JOANNE GIESSER, ESQ.
FUJIKAWA, et al.	)
	)

FRIDAY, APRIL 9, 1993
12:00 P.M. to 4:30 P.M.

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I-N-D-E-X

E-X-H-I-B-I-T-S

Giesser - cross

(Before Paula M. Quetsch, a Certified Shorthand Reporter and Notary Public of the State of Illinois, held at the offices of Amoco Corporation, 55 Shuman Boulevard, Suite 600, Naperville, Illinois, on Friday, April 9, 1993, commencing at 12:00 p.m.)

- -

J O A N N E M. G I E S S E R, 55 Shuman Boulevard, Suite 600, Naperville, Illinois, Sworn.

MR. KELBER: Good morning. This is the cross examination deposition of Joanne Giesser, is that correct --

THE WITNESS: Uh-huh.

MR. KELBER: -- responsive to the declaration filed, I'm here on behalf of Fujikawa, and we have the witness and Diane Furman on behalf of Wattanasin.

CROSS EXAMINATION

By Mr. Kelber

Q. Ms. Giesser, I'm going to hand the reporter a document that I would like identified as F20, and that's just the declaration.

(Deposition Exhibit F20 marked for identification.)

Giesser - cross

Q. If you would, take just a couple of minutes to review that document.

A. Okay.

Q. Is that document familiar to you?

A. Yes, it is.

Q. And on the last page, which is page six of the document and bears the number 373 at the right-hand top corner, is that your signature?

A. Yes, it is.

Q. Did you review any documents before -- any other documents before signing F20?

A. Yes, I did.

Q. Could you describe those documents for me?

A. They were the ones referred to in the declaration.

Q. Were there any other documents that are not identified in the declaration that you reviewed prior to signing this exhibit?

A. Not that I recall.

Q. Let me turn your attention to paragraph three on the first page of that document. Do you see the reference to the involved Wattanasin continuation application and the parent application thereof?

Giesser - cross

A. Yes.

Q. Did you review that application or the parent application prior to signing this declaration?

A. No, I did not.

Q. Do you recall the specifics of that application?

A. I recall the generalities of it.

Q. What was the basis for your conclusion that you filed the involved continuation application if you did not review it?

A. I recall filing it.

Q. How was it -- I'm sorry, if you didn't see it, how was it identified for you?

A. I'm sorry, I don't understand.

Q. Well, you didn't review the actual application itself or the parent application. Did you recall it by serial number, or what was the mechanism for identifying that application?

A. Well, up at the corner of the document it says case 600-7101 continuation.

Q. And that was sufficient to recall it for you?

A. Yes.

Giesser - cross

Q. Ms. Giesser, when did you first become aware that a third party had filed for U.S. patent protection for subject matter similar to that claimed in case number 600-7101?

A. I don't remember the exact date.

Q. Do you remember who identified it for you?

A. Not exactly. I don't recall the specifics of it.

Q. Was the third-party claim brought to your attention by someone in the patent department, do you recall?

A. It would have been likely to have been Mel Kassenoff.

Q. Isn't it correct, Ms. Giesser, that in fact the existence of the third-party patent application was brought to your attention before preparation of the draft of the Wattanasin application?

A. No, that's not how I recall it.

Q. So you recall preparing the draft and then becoming aware of the third-party case?

A. I recall being involved in preparing the draft. It wasn't finished at the time when I learned about the third-party one.

Giesser - cross

Q. Was the initial -- do you have a recollection was the initial draft prepared before learning of it?

A. No, I was in the process of preparing it.

Q. So that would have been before December -- before December 14 --

A. Yes.

Q. -- of 1988?

A. Yes.

Q. At the time you were preparing the draft document for case number 600-7101, had you previously been involved in any interference contests?

A. I had been involved in a minor amount when I was a patent examiner at the patent office. A few cases which I was examining I helped set up the interference, but I didn't do any substantive work on them.

Q. Did anyone at the Sandoz patent and trademark department assist you in requesting the declaration of interference filed in 600-7101?

A. As I recall, I had conversations with Mel Kassenoff and Dick Vila concerning how you would go about setting up such a request and spoke with them

Giesser - cross

while I was compiling necessary documents and such.

Q. Were there any considerations that you took into account in drafting the case 600-7101 by reason of the fact that you would be requesting an interference declaration in connection therewith?

A. I don't recall handling the case any differently from any other case at that point.

Q. Let me turn your attention to page two of the declaration, which is -- and starting at the top at page five -- I'm sorry, paragraph five. Do you have a recollection of actually receiving a copy of the minutes referred to in that paragraph?

A. No, I don't.

Q. Why would you have received a copy?

A. They were routinely distributed to each member of the department after the meetings.

Q. Was there any routine separation between the date of the meeting and the time the minutes were distributed?

A. They were actually distributed within about a week or so, depending on how long it took the secretaries to compile them and type it up.

Q. Were there any occasions where you did not

Giesser - cross

receive minutes of the patent committee during your tenure at Sandoz?

A. No.

Q. How did you know, as stated in paragraph six of F20, that PD 299/84 had been assigned to you?

A. It said so in the minutes.

Q. Said so in the minutes. Was there a specific statement to that effect?

A. The minutes, they would rate the application, and if it was rated A, the attorney who would be responsible for it, their initials would appear.

MS. FURMAN: Not only if it was rated A.

Q. You see the reference to existing filing priorities in paragraph six of page two of the declaration?

A. Yes.

Q. What kind of existing filing priorities were there as of the time you received the assignment of PD 299/84?

A. Mel Kassenoff had a number of cases in the general HMG-CoA Reductase area which had been rated A and which had to be filed, as well.

Giesser - cross

Q. How about yourself?

A. At that time, no.

Q. Did you have any other cases other than case 600-7101 assigned to you for filing at the time that that particular case was assigned to you?

A. I don't recall specifically whether I had or not.

Q. Looking at paragraph six again, what other priorities existed that might preclude you from filing PD 299/84?

A. Well, there were certainly other cases around both from Pharma and for the other companies that I had responsibility for.

Q. Who is Pharma?

A. Sandoz Pharma Company.

Q. Now, by "other cases," other cases to prepare?

A. Yes.

Q. And those cases were assigned a priority in advance of 299/84?

A. I'm not sure whether all those cases were officially rated at that point or not.

Q. Okay. Well, what I'm a little confused

about is that paragraph six indicates that either Mr. Kassenoff or you would take care of it after existing filing priorities had been completed.

Now, you've testified that Mr. Kassenoff had some cases stacked up, if you will, in advance of PD 299/84. Would it be a correct conclusion that if you did not have filing priorities existing as of the time that case was assigned to you that you would have the primary responsibility for filing that case?

A. I did have the primary responsibility for filing this case.

Q. What other tasks or assignments did you have that would take priority on your resources before preparing 299/84 for filing?

A. Well, I was working for -- my responsibilities at Sandoz involved working for a number of different Sandoz companies. Aside from responsibilities in this area of Pharma, I also did a lot of work for the seed companies, which at that time were part of Sandoz' crop protection. I also was getting involved in work with a joint venture that Sandoz was involved called Repligen Sandoz

Research Corporation, or we call it RSRC.

I also had other -- aside from the HMG-CoA Reductase area, I also had other areas which I was responsible for in Pharma.

Q. Now, the other responsibilities that you had identified, and particularly the seed companies and the RSRC, did you have any filing responsibilities for them that would take priority over the filing responsibility for 600-7101?

A. Yes.

Q. Could you describe those responsibilities for me?

A. As it turned out, there were a number of applications which, out of the seed companies, although as of January 1988 had not been decided to be filed upon but later on as the year progressed were coming up against time bars.

Q. So as of January, those cases had not been assigned to you for preparation?

A. Right.

Q. Were they subsequently assigned to you for preparation?

A. Yes.

Giesser - cross

Q. And about when was that?

A. I'm not exactly sure. It was later in the year, though.

Q. Do you have a recollection of approximately how many -- would it have been as early as June?

A. Probably not the seed cases, but probably yes on a number of applications for Sandoz' crop protection.

Q. When you say a number, is that -- help me out. Is that more than five?

A. At least three.

Q. So these cases were designated A after -- and by A, I mean intended for filing -- after 600-7101 but were intended for filing before 600-7101; is that correct?

A. Yes.

Q. And they took priority over 7101 because --

A. Well, certainly, at least as I recall, I think some of the crop protection cases had -- either the scientists had wanted to publish or were scheduled to publish, so there were bars of that sort running on them.

Q. The scientists --

Giesser - cross

A. The inventors.

Q. The inventors had published?

A. No. I believe on the ones at that time they had either submitted, you know, like an abstract to a meeting or something like that -- I don't remember exactly, but I think there were publication concerns involved with some of those.

Q. How many applications did you prepare and file between January -- I'm sorry, between February '88 and March '89?

A. Including March?

Q. Let's take it through the end of February '89.

A. I don't remember exactly. Probably close to 15.

Q. And only one in the HMG-CoA Reductase field; is that correct?

A. No.

Q. What other cases were filed -- did you prepare and file in the HMG-CoA Reductase field?

A. There was a CIP, which I recall was a rather substantial CIP which was filed I believe in October of '88.

Giesser - cross

Q. Let me turn your attention to page three of F20, specifically paragraph 11.

A. Uh-huh.

Q. There is a case referred to there, 7025/CIP/CIP. Is that the case you're referring to?

A. Yes. I believe that date is incorrect. It should be October 6th, 1988, not November.

Q. And that's based on your memory?

A. No, I have since seen copies of a filing receipt for it.

Q. You have since seen copies of the filing receipt. You did not see the filing receipt at the time you signed this declaration?

A. No, I guess I did. It says here -- there's a reference to it here on Exhibit D.

Q. So you think you did see the filing receipt --

A. Uh-huh.

Q. -- at the time that you signed this declaration?

A. Right.

Q. Are there any documents referred to in the declaration that you might not have seen at the time

Giesser - cross

of signing?

A. Again, not that I recall.

MR. KELBER: I'm going to hand the reporter a document that I'd like identified as F21.

(Deposition Exhibit F21 marked for identification.)

Q. Ms. Giesser, is that in fact the filing receipt that you just referred to?

A. Yes, it is.

Q. And that reflects a filing date of when?

A. October 6th, 1988.

Q. My question I guess is, if you reviewed Exhibit T prior to signing this declaration, why does the declaration indicate November 6th?

A. Because it was a mistake.

Q. Are there any other possibilities of date mistakes in this declaration?

A. Not that I've noticed.

Q. If a document was received by the Sandoz patent department on a certain date, how long would it take to circulate to you specifically if you had been designated as a recipient?

A. Generally not very long.

Giesser - cross

Q. A few days?

A. Generally less than that.

Q. Were there instances where it might have been more than that?

A. That would have been very unusual.

Q. Do you have any actual recollection of any such delivery taking more than three days?

A. Not specifically, no.

Q. Let's return to paragraph six of the declaration.

Why was PD 299/84 assigned to you?

A. At that time one of my responsibilities was to help file cases in the HMG-CoA Reductase area.

Q. By "that time," you mean February of 1988?

A. Yes.

Q. Prior to that time, how many cases in the HMG-CoA Reductase field had you filed?

A. Prior to --

Q. Prior to February 1 of 1988.

A. None.

Q. So as of February 1, 1988, what activities had you undertaken in terms of assistance in the field of filing HMG-CoA Reductase cases?

Giesser - cross

A. None.

Q. So this was your first case in that field?

A. Well, it was not the first case that I ended up filing in that field.

Q. Was this the first case -- was this the first instance of assignment of a case to you in that field?

A. It might have been of a new case.

Q. So you had been assigned preexisting cases for re-filing in that field prior to February 1, 1988?

A. I don't recall.

Q. Had you worked on the preparation of any patent applications directed to the field of HMG-CoA Reductase prior to February 1, 1988?

A. By "worked on," you mean --

Q. Had you done work of any type in terms of preparation of a patent application to be filed?

A. Preparation, no.

Q. A patent application in the HMG-CoA Reductase field prior to February 1 of 1988?

A. No.

Q. What work had you undertaken in the HMG-CoA

Giesser - cross

Reductase field prior to February 1, 1988?

A. I don't remember exactly. It's possible I might have done some prosecution of existing -- of cases that had already been filed.

Q. Can you recall any of those cases either by docket number or subject matter or issued patent?

A. Not specifically, no.

Q. When did you first take any action of any type specific to 600-7101 after the assignment of responsibility of that case to you?

A. I don't recall.

Q. Do you recall ever discussing the status of 600-7101 with Linda Rothwell?

A. I don't recall.

Q. Were you acquainted with Linda Rothwell as of February 1, '88?

A. Yes, I was.

Q. And who was Ms. Rothwell?

A. She was our docket clerk.

Q. And do you recall whether or not Ms. Rothwell had responsibility for docketing the filing of new applications that you would be handling?

Giesser - cross

A. Yes, that would be part of her responsibility.

Q. And in fact, wasn't it customary as of February 1, '88, to docket new applications for a three-week date from the date of assignment?

A. I don't know if that was customary, no.

Q. Do you recall whether or not there was a customary date assigned for the filing of new applications? In other words, was there a time space designated from the date a case was assigned to the date it would be first docketed for filing?

A. By "docketed for filing," you mean --

Q. In other words, you indicated that Ms. Rothwell was at least partly responsible for docketing in the patent and trademark department?

A. Uh-huh.

Q. Would she be responsible for tracking the docketing of new applications; would she have been responsible as of February 1, 1988?

A. I'm not sure about the term "docketing of new applications."

Q. Was a date assigned within the patent and trademark department at Sandoz for the anticipation

Giesser - cross

of filing of a new application once that application was designated A and assigned to an attorney?

A. No specific date was given, no.

Q. Would anyone have responsibility for inquiring as to the status of an application to be filed from time to time?

A. I don't know if anyone was particularly responsible. People certainly did inquire, however.

Q. Do you recall anybody inquiring as to the status of 600-7101 between February 1, '88, and March 3, 1989?

A. Not any specific inquiries. Gerald Sharkin, who was the head of the patent department, used to come around periodically, and if he felt that an application has taken awhile to file, he would check on the status of it orally.

Q. Did Mr. Sharkin ever discuss this particular case, 600-7101, with you?

A. Yes.

Q. And was he concerned as to the length of time it was taking to file the case?

A. Yes.

Q. Do you recall when that conversation took

Giesser - cross

place?

A. I recall only one instance.

Q. Do you recall about when that one instance might have taken place?

A. It was at the filing, right when I had filed it.

Q. So he did not inquire prior to your actual filing of the application?

A. I don't recall specifically.

Q. You don't have recollection of anybody else inquiring as to the status of the case prior to March 3, 1989?

A. No specific recollection, no.

Q. Prior to February 1, 1988, had you prepared for filing any application in the field of pharmaceuticals?

A. No.

Q. Would you consider 600-7101 to be directed to pharmaceuticals?

A. Yes.

Q. Was the case that we discussed a moment ago, 600-7025/CIP/CIP, was that directed to pharmaceuticals?

Giesser - cross

A. Yes.

Q. During the period February 1, 1988, to March 3, 1989, did you prepare any other cases directed to pharmaceuticals?

A. Yes.

Q. Can you give me an idea of approximately how many?

A. Maybe three or four.

Q. And those would have been assigned to you after 600-7101; is that correct?

A. Probably.

Q. So they were assigned to you after 600-7101 but filed before 600-7101; is that correct?

A. I'm not sure when all of them were assigned to me, but that may be correct.

Q. Well, I want to double check, because -- and I may have misheard your earlier testimony.

As of February 1, 1988, did you have assigned to you responsibility for preparing and filing any new patent application other than 7101?

A. Not that I can recall.

Q. So any applications that you did prepare and file prior to 7101 -- in other words, prior to

Giesser - cross

March 3, 1989 -- would have been assigned to you after 7101 was assigned to you; is that correct?

A. That might be true. I really don't recall.

Q. But you do positively recall filing cases in the pharmaceutical field before March 3, 1989, and February 1, 1988; is that correct?

A. Yes.

Q. Besides the CIP/CIP case, were any of the other pharmaceutical cases directed to the HMG-CoA Reductase field?

A. Yes.

Q. The cases that you filed in that time period between February 1, 1988, and March 3, 1989, that were in the HMG-CoA Reductase field, why did they receive priority ahead of 600-7101?

A. One of them I believe had a time bar running on it.

Q. By "time bar," could you explain what you mean?

A. From what I recall on this case, the parent application had been allowed, but the research had progressed to where we wanted to add extra information to it, and so we were under a time bar to

Giesser - cross

get the CIP in prior to the paying of the allowance fee.

Q. So that was a CIP case?

A. Yes.

Q. How about the others in the HMG-CoA Reductase field that you prepared?

A. Well, I remember there was one that was specifically -- it was a process case, and I don't recall the circumstances of that one.

Q. Do you recall why it received priority ahead of 600-7101?

A. I believe I was working on the applications at the same time.

Q. But it was filed in advance of 7101?

A. Yes.

Q. Do you recall when that application was assigned to you, the one you were working on at about the same time?

A. No, I don't.

Q. But it was after February '88?

A. Probably.

Q. Was there a time bar involved in that other case, in that case that you were working on

Giesser - cross

simultaneously with 7101?

A. I don't recall the -- I'm sorry, the --

Q. You were working on another case in the HMG-CoA Reductase field at about the same time you were working on 7101; correct?

A. I was actually working on a few of them, yes.

Q. We talked about the one with the time bar involving an allowed parent application.

A. Right.

Q. And then you mentioned a process case.

A. Right. Oh, that one. I don't recall whether that had a time bar or not on there. I believe there might have been a publication that the inventors wanted to get out, but I couldn't -- that's just speculation on my part.

Q. Were there any publications involved with respect to 7101?

A. I don't recall.

Q. If a publication -- if a request for release of a publication had been filed, would that cause the priority assigned to that application to be advanced?

Giesser - cross

A. Generally, yes.

Q. Looking at paragraph seven, when did you receive Exhibit P?

A. Could I see Exhibit P again?

Q. Well, before looking at the exhibits that are described there, do you have any recollection of when you saw them?

A. Originally from Dr. Wattanasin, you mean?

Q. That's correct.

A. No, I don't recall.

Q. Let me hand you part of Exhibit P, which is Exhibit P-1 -- we don't have to make this part of the record -- and ask you if that refreshes your memory as to when you might have first received that document.

A. No, I don't recall.

Q. Do you remember requesting that document?

A. Not specifically, no.

Q. In fact, you didn't request that document at all; did you?

A. I don't think so.

Q. Let me hand you the rest of Exhibit P -- and we don't need to make these a record, either --

Giesser - cross

P-2 and P-3, and ask you if those refresh your recollection as to when you might have received Exhibit P.

A. No, they don't.

Q. Did you request P-2 and/or P-3?

A. I don't recall.

Q. What is your first recollection of actually taking action with respect to case 600-7101?

A. I don't recall the specific time.

Q. Let me direct your attention to paragraph ten, which is on page three of F20.

A. Yes.

Q. How do you know that you started writing the draft no later than October 1988?

A. Well, there was an exhibit that says it's a first draft Wattanasin that was early November. Exhibit U-1 says November 3rd, '88, was when Lorraine started typing it. Due to the other activities I was involved with at the time, it would have taken me at least a month, probably a lot longer -- in fact, I'm sure a lot longer -- to have drafted the application to where I would have had something to give to Lorraine by November 3rd to start typing.

Giesser - cross

Q. She wouldn't have begun typing the draft until you had completed it?

A. At least a large portion of it. It was not a finished draft when I gave it to her.

Q. And it's your recollection that it took you over a month to prepare the draft?

A. Yes.

Q. You indicated that you prepared and filed -- is it correct that you prepared and filed at least five different patent applications between the date February 1, 1988, and March 3, 1989, exclusive of 7101?

A. Yes.

Q. Can you give me an average time of how long it took you to prepare and file those cases from the date assigned to the filing date?

A. No, I couldn't.

Q. Well, you made reference to some other work that you were involved with prior to November 3, 1989. Can you describe for me that other work?

A. At that time I spent a large amount of time working for the seed companies, and it involved a large amount of travel. In fact, the most travel

Giesser - cross

I've done in my career so far basically took place during approximately this year, and so I was out of the office a lot and had to do a lot of preparation for these various trips I was making in relation with the seed companies. So, therefore, it would have taken an extra long time for patent applications to be filed just because of the circumstances of being out of the office so much.

Q. Let me direct your attention to paragraph nine, which lies on page three of F20. Looking at the dates that you were traveling on September and October --

A. Well, actually, the September 1 is a continuation of the August 29th trip.

Q. Okay. As I look at this, you were not out of the office at any time during November; is that correct -- on business travel?

A. Business travel, yes.

Q. During the month of October it seems to me that you were gone seven days on business travel; is that correct?

A. Let's see, it was the 9th through the 11th, 16, 17 and 27th, 28th.

Giesser - cross

Q. So that's about seven days?

A. Uh-huh.

Q. And is that the travel that you referred to a brief moment ago?

A. It's certainly part of it.

Q. And that was all on behalf of the seed companies?

A. Not all, but a lot of it was.

Q. Did the work involving the seed companies, was that assigned priority greater than the preparation and filing of 7101?

A. Yes, some of the deadlines involved were more pressing.

Q. Who assigned those priorities?

A. I'm not sure that things were formally assigned.

Q. Well, who made the determination that those things were more pressing than 7101?

A. Some of it came from management within the various companies, and also a lot of this had interaction with the patent department in Basle and other high-up departments in Basle -- or higher-up figures in Basle.

Giesser - cross

Q. So these management or higher figures in Basle or in the companies of interest would advise you that something had to be done as of a certain date?

A. Correct.

Q. Were these individuals aware that you had been assigned responsibility for 600-7101?

A. I doubt if they specifically knew that.

Q. Do you regard the time from assignment of 600-7101 to the time of filing as average time for you from the date of assignment to the date of preparation and filing of an application at Sandoz?

A. No.

Q. Is it longer than average?

A. Yes.

Q. Did you discuss with anybody at Sandoz at any time the fact that it was taking longer than average to prepare and file 600-7101?

A. No, I don't recall any specific discussions to that effect.

Q. Were you ever concerned with regard to the length of time it was taking to prepare 600-7101?

A. I don't recall specific concerns about it.

Giesser - cross

I knew that after the Warner-Lambert patent had issued that we were certainly under a time restraint to get this application in the office before the Warner-Lambert became 102-B.

Q. Were there any time restraints of any type that you were aware of prior to the Warner-Lambert patent information coming to you in connection with 7101?

A. Not specifically, no.

MR. KELBER: I'm going to hand you a document that I would like marked as F22.

(Deposition Exhibit F22 marked for identification.)

Q. Is in fact Exhibit F22 the document that is referred to in paragraph nine of F20 as Exhibit S?

A. Yes.

Q. Let's take the first page of that document. You see over the right-hand column there's reference to Northrup King, Rogers Brothers and--

A. Zoecon.

Q. -- Zoecon. Thank you. Who or what was Northrup King?

A. Northrup King is a seed company owned by

Giesser - cross

Sandoz.

Q. And Rogers Brothers?

A. Rogers Brothers is also a seed company owned by Sandoz.

Q. And Zoecon?

A. Zoecon is now a part of Sandoz Agro. It's a research facility in California.

Q. And it was a research facility at the time you visited it?

A. Yes.

Q. So you took the -- I'm sure you took the Northwest flight that stops at every city in the Greater Northwest on your way out there?

A. Something like that.

Q. Was it your habit to do business work while flying on behalf of Sandoz -- in other words, the time actually spent in the air?

A. Generally not.

Q. Rest assured, I'm not going to go through each one of these pages, but I do have questions on a few.

The second page which covers the period 3/1/88, what is the NACA patent committee?

Giesser - cross

A. National Agricultural Chemical Association. It's a trade group.

Q. And they have a patent committee?

A. It's a patent law committee.

Q. I see.

A. I was asked to represent Sandoz at one of their patent law committee meetings.

Q. Were you asked by someone within the Sandoz patent and trade department?

A. Yes.

Q. Would that individual have been aware that you had, prior to March 1, been assigned responsibility for 600-7101?

A. Yes.

Q. Let me turn your attention to the fourth page, which refers to a visit with seed committee. What was the seed committee?

A. This was a meeting in Des Plaines. Des Plaines is where the headquarters of what is now Sandoz Agro is. At that time, as I recall, the seed companies were considered part of Sandoz Agro. That's since changed.

During this time frame, the patent

Giesser - cross

office had started issuing patents to various varieties of hybrids which were not genetically engineered, and one of the questions which we were discussing throughout this time period is how this would affect our companies and whether we should look into this as part of the patent policy. This is what involved a lot of the people from very high management.

The seed committee, as it refers to on here, were people who were involved with the seed companies in establishing and recommending patent policies for them.

Q. Would that have included other patent attorneys in addition to yourself?

A. Probably, yes.

Q. So there was an actual meeting of this committee --

A. Yes.

Q. -- during this trip?

A. Uh-huh.

Q. Do you recall participating actively at that meeting?

A. Yes.

Giesser - cross

Q. Let's go actually to the next document -- or the next page in that document. What is the IBA?

A. Industrial Biotechnology Association. It's also a trade group.

Q. What was the nature of the meeting on or about May 2?

A. I don't recall exactly. They have periodic meetings of patent attorneys who are involved with biotechnology companies to discuss various issues of interest.

Q. Was it your habit to participate actively at those meetings?

A. Yes. I only went to a few of them on behalf of Sandoz.

Q. Were you requested by someone at Sandoz to attend those meetings?

A. Yes.

Q. Do you recall who that someone was?

A. Dick Vila.

Q. And he would have been aware of your responsibility for 600-7101; wouldn't he?

A. Yes.

Q. Before we leave the IBA, did anybody else

Giesser - cross

from Sandoz attend those meetings?

A. At that time, no.

Q. Let me turn your attention to the page -- I believe it's the seventh page. It covers the period 8/20 through 9/20/88. And you can identify it because it has in the comments Swiss franc exchange.

A. Yes, okay.

Q. Do you see the reference to Basle patent policy?

A. Yes.

Q. That was a meeting of Sandoz International?

A. It had members from -- the presidents of Northrup King and Rogers Brothers, myself, and members of the Basle patent department.

Q. Nobody else from the U.S. Sandoz patent and trademark department attended that meeting?

A. No.

Q. Let me direct your attention to fourth-from-the-last page; it covers the period 12/1 to 12/31, '88. Do you see the reference to you having delivered a patent lecture to Northrup King?

A. Right.

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Q. Do you recall the nature of that lecture?

A. Yes, it was on general patent law. It took place at the American Seed Trade Association meetings, but it was a closed lecture to Northrup King personnel. A number of the Northrup King breeders who were stationed all over the country usually go to the Chicago meeting.

Q. Can you help me out with the dates over in the left-hand column? How long did this travel last?

A. Let's see, it looks like 12/6 through 12/8.

Q. Did you attend any other functions at the meeting other than delivering the patent lecture?

A. I went to a few of the lectures.

Q. The next-to-last page, which covers the time period February 1 to February 28, there's reference on that in column ten to a lecture to Rogers Brothers.

A. Right.

Q. What was the nature of that lecture?

A. General patent law and how it applied to questions that would arise in the seed industry.

Q. And you were the only person from the Sandoz patent and trademark department for that

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lecture; is that correct?

A. I think -- although I'm not sure, but I think Alan Norris, who is the manager of patents at Palo Alto, I believe he was there, also.

Q. Would he have delivered a lecture, also?

A. If he were there, he would possibly have spoken about international issues and the European system.

Q. Looking at the very last page, it wasn't much of a trip, but you went on up to New York City for the judges' dinner?

A. Yes.

Q. Was that on behalf of Sandoz?

A. Yes.

Q. Somebody at Sandoz suggested or requested that you go?

A. It was basically anyone in the department who wished to go could.

Q. Let me turn your attention to paragraph 14, page four of F20. You asked some information from Mr. Warhman?

A. Yes.

Q. Was that customary for you in the

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preparation of a patent application?

A. When it involved these kind of compounds, yes.

Q. Now, would you have needed that information to prepare the draft application -- the draft of the application in 7101?

A. As far as a completed draft, yes.

Q. Did you provide Mr. Warhman with any written information other than Exhibit V-1?

A. No. What I recall is I just drew the compounds that I wanted to get the correct technical chemical name for and just sent it over to him with a cover sheet.

Q. I'm going to hand you Exhibit V-1 -- I don't think we need to make this a record -- and ask if those are the compounds in question.

A. Yes, they are.

Q. How did you determine those specific compounds for inquiry?

A. As I recall, these were either intermediate or end products that were mentioned in the application.

Q. I'm going to hand you a document which has

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been previously identified in this proceeding as Exhibit F4 that's the application itself and ask you to take a look at that briefly. I'm going to ask you to turn to page 54 of Exhibit F4.

A. Okay.

Q. Do you see in the third line of the text -- I think it's the second line after the initial formula of that page -- the reference to C_{3-7}cycloalkyl?

A. Yes.

Q. Do you have any recollection as to whether that phrase appeared in the initial draft that you prepared?

A. I don't recall.

Q. Do you recall whether you identified that group as a suitable group for a substituent based on your own knowledge alone without reference to other documents?

A. It would not have been from my knowledge.

Q. Is there a name for the moiety or group that corresponds to C_{3-7}cycloalkyl?

A. I'm sorry?

Q. Let me back up and ask some foundation

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questions.

When the document refers to C_{3-7}cycloalkyl, is it correct to understand that that means any cycloalkyl moiety having three through seven carbon atoms?

A. Yes, that's what I intended it to mean.

Q. Do you have an estimate of whether or not those of ordinary skill in the art of making HMG-CoA Reductase field would have interpreted it similarly?

A. I think they would.

Q. Certainly that phrase identifies two possible compounds, one cycloalkyl compound with three carbon atoms and one with seven; is that correct?

A. Yes.

Q. If it had three carbon atoms, would that be cyclopropyl?

A. Yes.

Q. One of skill in this art you feel would similarly interpret it that way?

A. Yes.

Q. Do you recall discussing with anyone whether or not that would be an appropriate

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recitation for the claim in -- that appears -- I guess it begins on page 54?

A. Not specifically, no.

Q. In general would you have discussed the appropriate substituents with anybody in the preparation of an application of this type?

A. Yes.

Q. What persons would that have included?

A. At least the inventor.

Q. I'm going to ask you to turn to page five of F20, which is the declaration. In particular my question pertains to the statements and comments with respect to the exhibit referred to as Y-2. I don't think we need to make that a record, but I will hand it to you for your review.

A. Okay.

Q. Did you obtain that computer printout for the preparation of 7101?

A. Yes.

Q. Why?

A. It was helpful for a number of different reasons. I thought the chemistry involved in this case was very difficult, and sometimes I felt more

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comfortable having very explicit detailed drawings so that I would get the structures right and wouldn't include wrong structures in the case; also, to make sure that I specifically covered all the specific compounds that we thought were important, and also to get the -- some of these had some kind of activity. I guess it's ED_{50s} only -- and IC_{50s}.

Q. Do you know who prepared that document, Y-2?

A. I believe it was -- it says on the document it was from Bob Angstrom. He was a person who did a lot of work in the activity area.

Q. Did you request that document?

A. I don't recall specifically.

Q. Ms. Giesser, can you recall any activity which you undertook between January 4, 1989, and March 3, 1989, with regard to case 600-7101?

A. I have no recollection.

Q. Do you recall receiving any changes, suggestions, additions, information of that type with respect to the draft of 600-7101 after documents that are referred to as Exhibit X, Y-1 and Y-2?

A. I'm sorry, could you repeat that?

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Q. Other than the Exhibits X, Y-1 and Y-2 referred to on page five of F20, do you recall receiving any documents relevant to case 600-7101 subsequent to January 4, 1989, but prior to March 3, 1989?

A. No, I don't recall.

Q. Do you recall reviewing any documents in connection with the preparation and signing of F20, the declaration, that you would have received between January 4 and March 3, 1989?

A. No, I don't.

Q. Would there have been any reason if the information necessary to file 7101 was in your possession as of January 4, 1989, to delay the filing to March 3, 1989, other than final preparation of the application?

A. I believe that at that point I was working on the final draft and getting comments from the inventor and things of that nature.

Q. But you don't recall any specific comments?

A. No.

Q. And you don't recall seeing any written documents to that effect?

A. No.

MR. KELBER: In New Jersey, Diane, we had discussed the possibility of requesting the documents in the file. Any progress with regard to that?

MS. FURMAN: I have searched for the so-called supplemental file, and as I indicated at that deposition, I do have some -- I do have various papers relating to the case. None of such papers bear dates, however, so I do not know whether you would be interested in seeing them. If you are, I will provide them to you.

MR. KELBER: Please do. We had also asked for any requests for publication filed relative to this. Has the search for those documents been done?

MS. FURMAN: Yes, I have isolated two requests for publication, and if you wish, I can enter into evidence now as exhibits the isolated papers that I found on the one hand and the request for publication.

MR. KELBER: Well, I don't know about the need to -- well, let's go ahead and do that.

MS. FURMAN: I have seven loose pages obtained from documents left in the possession of

Giesser - cross

Sandoz by Ms. Giesser which relate to case 600-7101, and I would like them marked as Exhibit S1.

(Deposition Exhibit S1 marked for identification.)

MS. FURMAN: Additionally, I have a second exhibit, S2, comprising, I believe, 22 pages which represent two publication requests related to the subject matter of case 600-7101 in response to Mr. Kelber's request of record.

(Deposition Exhibit S2 marked for identification.)

BY MR. KELBER:

Q. Do you have any feel for how it was determined that the patent and trademark committee of Sandoz, through the 1988 year, how it was determined who would have specific responsibility for a particular application?

A. From what I understood, generally attorneys or agents would be assigned a particular research area, and generally there wasn't too much overlap, the HMG-CoA Reductase area being rather an exception to that rule.

So if an invention disclosure came out

of a particular area, unless there was a reason not to, the attorney or agent who was normally working in that area would be assigned that application.

Q. And you had -- I'm sorry, correct me if I'm wrong. You had not previously been assigned responsibility for filing an application in the HMG-CoA Reductase field as of February 1; is that correct?

A. Right. I had not been at Sandoz for very long at that time.

Q. Did you have occasion to speak with anyone at Sandoz with regard to the volume of responsibility, the volume of work that had been assigned to you in the period February 1, '88, through March '89 -- I'm sorry, specifically with regard to 7101?

A. I don't recall.

Q. With regard to the CIP/CIP case, the 7025 case, did you discuss with anybody the possibility of filing a continuation application to maintain the case pending to allow preparation and filing of the 7101 file first?

A. We really didn't have an option to delay

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the 7025-CIP/CIP case.

Q. Why is that?

A. At that time, as I believe we were filing non-convention, foreign filing non-convention -- and I believe there was an outstanding office action where allowable subject matter had been indicated, but we needed to add some specifics to various other compounds in that case. I don't remember exactly, but I think there was some sort of time bar running vis-a-vis the foreign filing in that case.

Q. Would that have been a time bar in the sense of an outstanding publication imminent?

A. I don't believe there was a publication. I think it might have been -- I forget whether it was allowable subject matter had been indicated, and I know that there was -- I did an extensive amount of work on that case with one of the agents in Basle in preparing the foreign filing text on that one, and I don't remember exactly, but I know there was some sort of time pressure going on with that one.

Q. Were there any cases that you recall that were assigned to you after February 1, 1988, that you prepared and filed prior to March 3, 1989, that did

not involve a time bar?

A. Yes.

Q. Can you describe the field that those applications pertained to?

A. One of them was a plant biotech case that originated in Basle, which they sent the draft over, and I basically had to review the draft and make any changes necessary for filing in the United States. While that had a time bar -- well, no, that was a priority United States filing.

Q. And that case was filed before March 3, '89?

A. Yes, I believe that was filed in December of '88. There were -- as I mentioned before, I believe there were pressures to file the Agro cases, but I don't remember exactly -- it's been awhile.

Q. With regard to the case that came over from Basle in draft form, any particular reason for assigning it a filing priority ahead of 7101?

A. It was a case that did not involve the -- nearly the amount of time or substantive work as 7101, and it was something I could get filed quickly.

Q. You spoke to some kind of pressure involved

in the cases for the seed companies. Can you describe the pressure that was involved there?

A. In March there were 102-B on-use or on-sale bars.

Q. So the bar would have been complete in March of '89?

A. Yes.

Q. You don't recall whether that date would have been before or after March 3, 1989, do you?

A. I believe some were March 3. It was a rather hectic time.

Q. Do you recall during your tenure at Sandoz whether Sandoz ever employed outside patent attorneys, attorneys not regular employees of the patent and trademark department of Sandoz, for assistance in the preparation of patent applications?

A. Very rarely.

Q. Did you have any involvement with such outside attorneys?

A. No.

Q. In the rare cases when it did happen, do you recall why that would be done?

A. Usually it would be a circumstance where we

were licensing a third party's technology and as part of the deal we were prosecuting the patent for them, so in order to avoid any kind of conflict, we'd have a third party do it.

Q. Did you ever encounter a situation where you were assigned a number of specific tasks that had to be completed by a certain date that you simply could not complete by that date -- I'm sorry, while you were at Sandoz?

A. I certainly recall multiple deadlines. Generally they'd all be met somehow.

Q. Did you ever seek help from another individual within Sandoz in that situation where you were facing multiple deadlines?

A. Yes.

Q. Did you ever attempt to seek help with regard to the preparation of 7101?

A. Not insofar as meeting a deadline, but general help involving the chemistry of the case, yes.

Q. Do you recall subsequent to February '88 ever discussing with the patent committee at Sandoz the decision to rate 7101 as A?

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A. I would have not have ever had discussions with the patent committee.

Q. I see. So you -- okay. Do you recall ever suggesting to somebody at the committee or somebody to suggest to somebody at the committee the question of the status of 7101?

A. I did not.

Q. Let me take you back again to the period between when you began at Sandoz and February 1, 1988. Regardless of the field to which it might have pertained, as of February 1, 1988, do you recall whether you had a backlog of cases to prepare and file?

A. I don't think I had a backlog, no.

MR. KELBER: I appreciate your patience with me, and I don't have any further questions at this time.

(WHEREUPON a recess was taken.)

RE-DIRECT EXAMINATION

MS. FURMAN

Q. I would like to ask you a couple of questions first about your experience prior to coming

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to Sandoz, which I believe was raised on cross.

Is it true that you had never written a pharmaceutical patent application prior to coming to Sandoz?

A. Yes.

Q. How would you rate the difficulty of case 600-7101, let's say on a scale of one to ten?

A. With ten being hard?

Q. Correct.

A. Ten.

Q. Why would you say that?

A. It was a multi-step procedure. There were -- it was a long reaction. It's a very complex compound; it has ring substituents as well as side chain substituents, and the stereochemistry is important and is involved.

Q. Were you required to work on other subject matter with which you had no prior familiarity before coming to Sandoz?

A. Yes.

Q. What did that comprise?

A. When I came to Sandoz, basically my first assignment was the prosecution docket from Fred

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Wienfeld, who was not working there at the time; he was on disability. And Fred's docket included a number of different kinds of chemical cases. I don't recall whether there were any HMG-CoA Reductase cases or not, but I do recall cases in areas such as fire retardants, polymers and other different types of chemicals.

Q. How long had you been at Sandoz before receiving case 600-7101 as a patent disclosure for filing?

A. Well, I started in mid August of '87, and the patent committee assigned this the end of January of '88. So middle of September, October, November, December, January -- about five-and-a-half months.

Q. Were you working on any pharmaceutical case as a prosecution matter during those prior five months?

A. I don't recall. It's quite possible, since Fred Wienfeld handled a number of HMG-CoA Reductase cases.

Q. How did you become aware of the A rating of patent disclosure 299/84 at issue?

A. I don't recall. Generally I would become

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aware of them when the minutes of the patent committee meeting would be circulated and I'd receive my copy. I don't recall an exception to that, so I assume that's how I found out.

Q. Was it within your responsibility to rate patent disclosures?

A. No.

Q. Did you have any influence on the patent committee in the rating of disclosures?

A. No.

Q. What rating did the patent committee assign to a disclosure which was not to be filed upon?

A. Ever?

Q. Correct.

A. It would be D.

Q. Did patent disclosure 299/84, to your knowledge, ever receive a D rating?

A. No.

Q. Having received patent disclosure 299/84 for filing, was it within your jurisdiction or ability to -- let me rephrase that -- within your jurisdiction or ability not to file a patent application?

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A. No.

Q. How would you characterize your obligation until the filing?

A. I had no choice but to draft the application.

Q. Is there any way to inactivate or retire a patent disclosure once rated A by the patent committee?

A. Yes.

Q. How is that?

A. You would have to have the disclosure brought up to the patent committee, and they would have to re-rate it.

Q. Did you at any time do that with respect to disclosure 299/84?

A. No.

Q. Did anyone at Sandoz carry out such a process?

A. No.

Q. You indicated that the involved application took perhaps longer to complete than applications you worked on for Sandoz?

A. Yes.

Giesser - re-direct

Q. Can you explain that?

A. Well, it was a combination of factors. One, as I've mentioned before, I considered the chemistry difficult and was very concerned with making sure I had the correct chemistry at the time it actually got filed.

Secondly, I was out of the office a lot traveling on business matters, as went into before, and there were other cases and other issues which at the time seemed to need immediate attention.

And thirdly, this was a rather lengthy patent application. It was 50-some odd pages at least, and just the physical time it would take to write such an application would be long.

Q. Why did Mel Kassenoff collect information on the --

MR. KELBER: Objection, facts not in evidence.

BY MS. FURMAN:

Q. Did Mel Kassenoff collect information --

MR. KELBER: Objection. You're asking her to determine what Mel did. Anything that she says is going to be hearsay.

Giesser - re-direct

BY MS. FURMAN:

Q. How did you receive information relating to case 600-7101?

A. I don't remember exactly. I think that some of it came from Mel, and some of it came from the inventor.

Q. The part that came from Mel, if the case was assigned to you, why did information relating to the case come from Mel?

A. I would expect because the scientists involved in this program were familiar with Mel, since he had been working with them for a number of years in this area.

MR. KELBER: Objection to the degree it's speculation.

Q. Were you familiar with the people to contact and procedures to follow to collect information needed to write case 600-7101 as of February 1988?

A. No.

Q. Did you receive any assistance from anyone in the patent department with respect to the case?

A. Yes.

Giesser - re-direct

Q. Who provided such assistance?

A. Mel Kassenoff.

Q. What was the nature of that assistance?

A. I'm not sure if I can recall everything he did, but he certainly helped me with a lot of chemistry and would provide names of people I had to contact if I needed certain information. For instance, with regard to -- can we go off the record for a second?

MS. FURMAN: Off the record.

(WHEREUPON a discussion was held off the record.)

THE WITNESS: With regard to Exhibit V-1, which is page 448, it's the letter I wrote to Ziggy Warhman asking for the names of the compounds, that's the sort of thing Mel would direct me how to get that information.

BY MS. FURMAN:

Q. Otherwise you would not have known independently how to obtain such information?

A. Correct.

Q. Was it ever your intention not to file a patent application and patent disclosure for 299/84?

Giesser - re-direct

A. No.

Q. What about the Warner-Lambert publication; what was your reaction to this publication, in your best recollection?

A. I don't remember too much about the specifics of finding out about it. I remember being, I guess upset is possibly the best word, when I heard about it.

Q. Why were you upset?

A. Because I knew that what would otherwise be a rather straight forward prosecution of an application suddenly was not.

Q. What is your impression as the involved patent attorney of the interest of the research in the subject matter in view of the Warner-Lambert patent?

MR. KELBER: I'm sorry, could you read that question back?

(The requested testimony was read by the reporter.)

MR. KELBER: Could you specify what you mean; whose interest and involved in what?

BY MS. FURMAN:

Giesser - re-direct

Q. After you became aware of the Warner-Lambert publication, did you request a re-rating of the subject patent disclosure?

A. No.

Q. Why?

A. I don't recall.

Q. Do you recall being directed by anyone in research to drop your work on the involved application?

A. I was never told to drop the work.

Q. You have referred to your activities during the period of February 1988 to March 1989 in the agricultural area for Sandoz. Do you have any special expertise in this area?

A. Yes.

Q. What does that comprise?

A. I hold a master's degree from the Department of Agronomy at Clemson University, and my subspecialty in that area was plant genetics.

Q. Did anyone else at Sandoz have a master's in that specialty?

A. Not that I was aware of; not in the patent department.

Giesser - re-direct

Q. I would like to discuss some of your travel activity during the relevant time period.

You previously testified that you visited Northrup King, Rogers Brothers and Zoecon in February of 1988?

A. Yes.

Q. Approximately how many days -- how many working days were you out of the office in February of '88?

A. It appears from the 21st through the 26th.

Q. That would be how many days?

A. Probably an entire week, five working days.

Q. How many days of preparation for this trip do you estimate was required?

A. I don't recall exactly, but there was more than the usual business trip, since I was not alone on this trip. A member of the Basle patent department, Walter Smolders, who is currently the person in charge of Sandoz Agro and seed patent activities worldwide, accompanied me on this trip.

Q. Was this trip required of you by the Basle patent department?

A. I didn't have any choice in going, if

Giesser - re-direct

that's what you meant.

Q. In March of 1988 you were occupied with patent committee meetings?

MR. KELBER: Objection as to the characterization of the testimony.

BY MS. FURMAN:

Q. What kind of travel activity were you involved in in March of 1988?

A. There was a trip to RSRC in Boston and also a trip to Palo Alto, California.

Q. In March of '88?

A. Yes. Also an one-day trip to Washington, D.C.

Q. Were you required to go on each of these trips in March of 1988?

A. Yes.

Q. Who required you to?

A. Again, it was not an official requirement. Dick Vila had asked me to attend a NACA meeting. I accompanied Dick up to the RSRC visit, and the visit to Palo Alto, I was alone, but it was certainly needed in connection with my activities with Sandoz' crop protection.

Giesser - re-direct

Q. Was there anyone who could substitute for you in the Sandoz patent department at the crop meetings?

A. Certainly Dick could. The point is he asked me to take this over.

Q. Approximately how many days were you out of the office on business in March of 1988?

A. Probably about seven.

Q. How many days of preparation would have been required in total for these trips?

A. The NACA meeting probably wouldn't have required much. I don't recall exactly, but there was certainly some amount of preparation for the RSRC and also the Palo Alto trips.

Q. By the way, you mentioned that Dick could possibly substitute for you. To your knowledge, did Dick Vila have a background in plant genetics?

A. No.

Q. To your knowledge, did he have a degree in agriculture?

A. No.

Q. Did he participate, to your knowledge, in drafting plant policy?

Giesser - re-direct

A. Yes.

Q. In April of 1988 can you summarize how many days you were out of the office on business?

MR. KELBER: Summarize that?

BY MR. FURMAN:

Q. Can you indicate by number?

A. It looks like two.

Q. Two or three?

A. It was in a hotel. I have two nights; so probably three days.

Q. Can you do so similarly for May and June of 1988; can you give me the days out of the office on business?

A. It looks like May was one day; June looks like there was a one-day meeting to Washington and probably a two-day trip to California.

Q. That would be three?

A. Yeah.

Q. On your visit to Palo Alto, did you discuss whether patent disclosures needed to be filed?

MR. KELBER: Which visit is this?

MS. FURMAN: In June of 1988.

A. I don't remember exactly. That was a topic

Giesser - re-direct

that was generally brought up when I was out in California.

Q. If patent disclosures needed to be filed for Palo Alto, who performed such filings?

A. This is a complicated question. There were basically two divisions of research in California, agricultural chemicals and plant biotechnology. The agricultural chemical filings were generally done by the person who was on site there. I think until March of '88 it was Jacqueline Larson. She left the site, and there was no one there for a few months until Alan Norris came over from Basle to take over, which was sometime in the late summer of '88, probably August.

So during that ensuing time, I'm not sure how the chemical cases got filed there, although I was -- I had filed one of the chemical ones. Jackie felt uncomfortable with a lot of the biotech applications, so the idea was that I would be working in that area.

Q. Did a backlog of biotech cases develop?

A. No.

Q. When Palo Alto decided to file a patent

Giesser - re-direct

application and Alan Norris did not do it, who worked on that application?

A. Like I said, generally until Jackie left she handled all the chemical based cases. I was intended to work on the biological based cases from there.

Q. Did Dick Vila work on any of these cases?

A. I know he assisted in some of the biotech cases. I'm not sure of the time frame on those, though.

Q. When did you start writing cases for Palo Alto?

A. I don't remember exactly.

Q. Going to July of '88, how many days were you out of the office on business in that month?

A. It looks like I had a two-day trip to Des Plaines.

MR. KELBER: Can I hear the answer back again?

(The requested testimony was read by the reporter.)

BY MR. FURMAN:

Q. Let's go to August of '88. How many days

Giesser - re-direct

were you out on business?

A. It looks like I had a three-night visit to Palo Alto, so that was probably four days. It was the week that overlapped the last week of August and the first week of September.

Q. In the course of your meetings on seed policy, do you remember when you were first assigned seed cases to work on?

A. Not exactly, no.

Q. Can you give me an estimate?

A. No, I don't recall when they first came up.

Q. Did these cases have statutory bars involved?

A. A number of them did.

Q. So there would have been a time constraint with respect to some of these cases?

A. Yes.

Q. How many such cases do you estimate there were, starting about June of 1988?

MR. KELBER: Objection. You're asking the witness to estimate when you haven't asked her if she knows the exact number.

BY MS. FURMAN:

Giesser - re-direct

Q. Do you remember how many seed cases you worked on with a time constraint?

A. Not the exact number, no.

Q. Do you remember approximately how many?

A. There were quite a number of them. The ones that had bars coming up in March of '89 there were I think about six or so.

Q. These six cases had a required due date -- a filing date in order not to be --

A. They were coming up against the one-year in-public-use or on-sale bar.

Q. I would like to quickly finish up the number of days you were out of the office from September until -- September of 1988 until February of 1989, if you could quickly give me such an estimate for each month.

A. Well, September I had a trip to Basle, and that was four days. October I was out of the office a lot. There was another trip to California that looks like I had two days in a hotel, so probably three days out of the office. Then there was a trip to Wisconsin which looked like another two-day hotel, so probably three days out of the office, and then

Giesser - re-direct

there was a trip do Boulder, Colorado, where there was -- I believe that was two days out of the office.

Q. In what month?

A. That was all October of '88.

Q. How many days in total for October?

A. Probably seven.

Q. November?

A. It looks like I got to stay home in November.

Q. Can you finish up with December through February?

A. Oh, December looks like a one-night -- so possibly two days out of the office in December. January it looks like three hotel nights, so possibly four days out of the office in January. February of '89 it looks like two hotel nights, so probably three days out of the office in February, and then in March of '89 one day -- or two days, one hotel night, so two days.

Q. The judges' dinner in March of '89 occurred after the filing date of the involved application; is that true?

A. Yes.

Giesser - re-direct

Q. Did you have any sick days, to your recollection, out of the office?

A. I don't recall.

Q. Now, what other pressures to file might exist besides a 102-B bar? You testified that you filed CIP applications under certain circumstances.

A. Well, there was the CIP that's 7025-CIP/CIP; I recall there was some sort of foreign filing deadline on that one.

Q. Well, I'm trying to refresh your recollection with Exhibit S3, which comprises a few pages from the prosecution history of --

MR. KELBER: I'm going to object to this exhibit and the questions based thereon as evidence of the type that should have been submitted in direct, but you can ask the witness questions with respect to it.

MS. FURMAN: Since we believe it's necessary for adequate re-direct, we will proceed.

(Deposition Exhibit S3 marked for identification.)

BY MR. FURMAN:

Q. Have you examined Exhibit S3?

Giesser - re-direct

A. Yes.

Q. If you turn to the last page of that exhibit, do you recognize this page?

A. Yes.

Q. Can you describe it?

A. It's the first page of an office action to case number 600-7025/CIP, which is the parent case of 7025/CIP/CIP.

Q. What is the date of mailing?

A. May 11th, 1988.

Q. In order for this case not to go abandoned, under the patent office rules when would a response have had to be filed?

A. It would be six months from that day, or November 11th, '88, assuming the proper fees were paid.

Q. Did you have any interaction with the Basle patent department on this case?

A. Yes, I did.

Q. What did that concern?

A. There was a plan to have a foreign filing of the subject matter of 7025/CIP along with some additional subject matter was planned to be filed

Giesser - re-direct

non-convention.

Q. Did Basle call upon you to assist in the preparation of such a foreign text?

A. Yes.

Q. Was it your responsibility to consult with the inventor in the United States on this case for Basle?

A. Yes.

Q. Did you then have to redraft the application for Basle?

A. No.

Q. How did the foreign text then come about?

A. I would convey any information or comments that the inventor had on the Basle case to the people in Basle. I believe it was Lucian Vallet.

Q. Did it occur to you that it would be necessary to file a continuation-in-part application on this case?

A. Yes.

Q. What would be the purpose of such a filing?

A. To include subject matter that wasn't already present in the parent.

Q. Would you have been required to consult

Giesser - re-direct

with the inventor about that additional subject matter for the foreign text?

A. Yes.

Q. What would be the latest date that you could file a CIP on case 600-7025/CIP in response to the outstanding office action?

A. It appears that the case could have been extended beyond the 11th month if we had chosen to respond to the office action.

Q. Excuse me, the 11th month?

A. I'm sorry, November 11th, '88, would have been the last date we could file if we chose not to respond to the office action.

Q. By file, you mean file a CIP?

A. Right.

Q. Is it fair to say, then, that you were under some degree of time pressure concerning the filing of the CIP on case 600-7025/CIP?

A. Yes.

Q. Was there any standard way in the Sandoz patent department, to your knowledge, of deciding to give priority to an A rated disclosure or a CIP --

A. No.

Giesser - re-direct

Q. -- that had to be filed?

A. I was not aware of any policy on that.

Q. Is it fair to say that you were required to give full time to this case prior to November of 1988 --

MR. KELBER: Which case is that, Diane?

Q. -- 600-7025 for the purpose of assisting Basle in filing the foreign text?

A. Yes.

Q. Is it fair to say that you would have needed the same information to file the CIP?

A. The same --

Q. The same information as needed by Basle?

A. Yes.

Q. Now, you also mentioned a case -- an HMG-CoA case where you were under time pressure because an issue fee was due; is that correct?

A. Yes.

Q. Do you remember the subject matter in particular of that case?

A. Not exactly. I know it was another different heterocycle compound.

MS. FURMAN: I'd like to introduce one

Giesser - re-direct

further exhibit to refresh your recollection for purposes of re-direct, which will be Exhibit S4.

(Deposition Exhibit S4 marked for identification.)

MR. KELBER: I'm going to object to anything and everything with regard to S4. This is a case that's not even referred to in the declaration that constitutes the direct testimony in this case. It's beyond comprehension that it could possibly be necessary for adequate re-direct, since the scope of re-direct is necessarily narrower than the scope of direct.

If you give me a continuing objection, I'll let you ask your questions.

MS. FURMAN: Fine with me.

BY MS. FURMAN:

Q. Do you recognize the second page of this exhibit?

A. Yes.

Q. What does this page comprise?

A. It's the notice of allowance for case 600-7044/CONT.

Q. To your knowledge, was this case issued?

Giesser - re-direct

A. No, this was allowed to go abandoned.

Q. Did you have any involvement in this case or a successor case?

MR. KELBER: Compound question. Why don't you ask them one at a time?

BY MS. FURMAN:

Q. Did you have any involvement in the writing of 600-7044/CONT?

A. I don't recall if I filed a continuation or whether I just received it off of Fred's docket.

MR. KELBER: Objection, that's not responsive to the question.

Q. Then you don't recall whether you were involved in 7044/CONT; is that correct?

A. That is correct.

Q. The second page of this exhibit -- what does this comprise?

A. The second page?

Q. Yes.

A. It's the notice of allowance and issue fee for 600-7044/CONT.

Q. In order to file a continuing application in this case, when would such action have had to be

Giesser - re-direct

taken in order not to abandon the parent?

A. Well, to keep a chain going, it would have to have been by the due date of the issue fee, which would have been April 3rd of '89.

MR. KELBER: Can I hear that question and answer.

(The requested testimony was read by the reporter.)

MR. KELBER: Could you mark that for me?

BY MS. FURMAN:

Q. Did you in fact file a further application on 7044/CONT?

A. I filed a CIP.

Q. Is this the application you were referring to in your prior testimony concerning the need to file a case when an issue fee was due?

A. Yes.

Q. Do you recall when you filed that CIP application?

A. I believe it was in March of '89.

Q. You filed 600-7044/CONT/CIP in March of '89; correct?

A. To my best recollection, yes.

Giesser - re-direct

Q. You filed 600-7025/CIP/CIP about when?

A. October of '88. It was the beginning of October.

Q. You filed how many seed cases under time constraint -- of the seed cases you filed in March '1989, how many were under time constraint?

A. I believe all of them were.

Q. I'm sorry, I don't remember the number you indicated.

A. I think it was five or six.

Q. Would you have had to have been working on the seed cases prior to March of '89 in order to have them on file that month?

A. Yes.

Q. When is your best estimate for beginning work on the earliest of the seed cases?

MR. KELBER: Which seed cases? Why don't you rephrase it?

BY MR. FURMAN:

Q. When do you think you started working on the six seed cases you just indicated were filed in March of 1989?

A. I don't recall.

Giesser - re-direct

Q. Do you have any recollection of working on the seed cases in February of 1989?

A. Yes.

Q. Do you have any recollection in January of 1989?

A. I don't recall.

Q. If I can summarize your most recent testimony on re-direct, you filed case 7025 in October of 1988, and that was under time pressure?

A. Yes.

Q. You filed case 600 7044/CONT/CIP sometime before April of 1989?

A. Yes.

Q. Was that under time pressure?

A. Yes.

Q. The six seed cases that you filed in March of 1989 were under a time pressure, as well?

A. Yes.

Q. Who decided what cases you filed in the seed or Agro area?

A. At the earlier portion of this time period the seed companies would go through the Palo Alto patent committee. The results or the recommendations

Giesser - re-direct

in the Palo Alto patent committee would be noted in the Pharma patent committee or the New Jersey patent committee.

Q. Did the New Jersey patent committee have any influence, to your knowledge, on the patent decisions of Palo Alto?

A. I'm not sure if they could veto something. I know they generally took the recommendations. In the later part of this period Northrup King started having its own -- well, it wasn't a full-blown patent committee, but patent issuings would be discussed at the research management committee meeting, and the results of that would be reported back through the New Jersey patent committee meeting.

Q. Do you recollect having conflicting priorities at times in the period of June of 1988 to February of 1989 between seed and Pharma?

A. There was certainly a lot of items that had to be taken care of within a very short period of time coming from all the different companies, so yes.

Q. An example of which would be patent filings to avoid statutory bars?

A. That would be one part.

Giesser - re-direct

Q. In the period between February of 1988 and March of 1989, what was your general practice in the actual preparation of the patent application? More specifically, were you able to use a computer in drafting these applications?

A. The attorneys at that time didn't have individual work stations. The secretaries had a word processor, so you would have to basically write the application in longhand and give it to the secretary to type.

Q. Is that a practice you followed in connection with case 600-7101?

A. Yes.

Q. You indicated that you provided a substantially complete draft to your secretary to be typed?

A. Yes.

Q. Do you recollect about what date that was, based on the testimony of record?

A. I believe it was around November 3rd or so. According to the affidavit exhibit it was November 3rd of '88.

Q. If you provided to her a substantially

Giesser - re-direct

complete copy on November 3rd, when do you think you might have started writing that draft?

A. I don't have a recollection of when I started that.

Q. Would it have taken more than two weeks, in your estimation?

A. Yes.

Q. A month?

A. I would say longer than that.

Q. Now, we are not talking about total activity exclusive of nothing else; we're talking about the time running from the day you started writing it to the day you handed it to your secretary.

A. Yes.

Q. Would it be more than a month?

A. Yes.

Q. What's your best estimate of the length of time it took you to complete the written draft?

MR. KELBER: Objection as to speculation. The witness has already testified she doesn't know.

BY MS. FURMAN:

Q. Would it have been more than a

Giesser - re-direct

month-and-a-half?

A. Yes.

Q. How about two months?

A. I would say more than that.

Q. Well, that would bring us into December; correct?

MR. KELBER: Bring us where?

THE WITNESS: I think we're talking about different things.

MS. FURMAN: The length of time for you to hand write the draft that was given to your secretary to type on the 3rd.

THE WITNESS: I was counting backwards.

MS. FURMAN: That was my purpose.

MR. KELBER: Why don't you ask the questions?

BY MS. FURMAN:

Q. Counting backwards from November 3rd of 1988, how much time did it take to prepare the written draft?

A. I don't know exactly.

Q. What was the two-month figure that you just referred to?

Giesser - re-direct

A. I know I was working on it for at least two months.

Q. Two months prior to November 3rd?

A. Right, which would have been September 3rd. I was working on this prior to that.

Q. Is that as far back before November 3rd that you can recall working on it?

A. As I said, I don't specifically recall. The date which I'm basing this on is I remember that when it came to light that Warner-Lambert had a patent application issued to the same subject matter -- or when their patent issued, I was in the process of writing this at that time.

Q. While you were writing the application, you had other obligations to the other Sandoz divisions; is that correct?

A. Yes.

Q. Do you recognize the pages that comprise Exhibit S1?

MR. KELBER: I'm going to object to reliance by the junior partner on Exhibit S1; you're beyond the scope.

BY MS. FURMAN:

Giesser - re-direct

Q. Do you recognize those pages?

A. Some of them.

Q. What do they concern?

A. They are all pages that were part -- were either part of a draft of the application or in preparation of a draft in the application of 600-7101.

MR. KELBER: To what part of the cross does this questioning pertain?

MS. FURMAN: At some point you indicated whether or not work was being done during a certain time period, which I'm looking for at the moment, and I believe the testimony that -- the relevant period was between January 4th of 1989 and March 3rd of 1989, and my question of Mrs. Giesser is whether she can be certain that she did not generate any of that work during the time period.

MR. KELBER: You're asking her whether she couldn't have generated any of S1 in that time period?

MS. FURMAN: Whether it was -- whether she could be certain that none of it was done during the period of January 4, 1989, to March 3rd of 1989.

Giesser - re-direct

THE WITNESS: I don't know when these papers were generated.

BY MS. FURMAN:

Q. Did you regard it as your continuing obligation to file a patent application on 299/84 as of the A rating of the underlying patent -- of that patent disclosure?

A. Yes.

Q. And that obligation, when was it fulfilled, on what date?

A. The date I filed the application was March 3rd of '89.

MS. FURMAN: That concludes my re-direct.

RE-CROSS EXAMINATION

By Mr. Kelber

Q. Ms. Giesser, you feel that you have at least a few years' experience in the matters of patent prosecution; is that correct?

A. Yes.

Q. When must a continuation application be on file with the United States Patent and Trademark Office in order to claim priority of an earlier U.S. application?

Giesser - re-cross

A. As long as the earlier application is pending.

Q. So that means sometime before it issues; is that correct?

A. Yes.

MR. KELBER: Can you read back the question and answer that was marked earlier?

(The requested testimony was read by the reporter.)

BY MR. KELBER:

Q. Having heard that question and answer, do you still believe that the answer you gave is correct?

A. I think I'm getting confused. If the parent application is about to issue or go abandoned -- was it April 3rd -- then if you're going to file a continuation, it would have to be on file by that date, assuming that you were letting the parent go abandoned.

Q. So your assumption is that the parent was going to go abandoned; is that correct?

A. Yes.

Q. Let me direct your attention to the last

Giesser - re-cross

page of that exhibit that constitutes S3 -- I'm sorry, not S3, S4.

A. That's 7044?

Q. 7044, that's correct.

A. Uh-huh.

Q. As of December 28, 1988, did you intend for the 7044/CONT to go abandoned?

A. I believe by that time that a CIP was going to be filed, so I believe that if not on December 21, '88, certainly after the time we had received the office action and had a chance to reflect upon it, we would have determined that we should abandon the parent.

Q. My question to you is as of December 21, 1988, had you reached the conclusion that the parent was to be abandoned?

A. I don't recall.

Q. Well, in fact, you've read the last page of S4; is that correct?

A. Yes.

Q. The actions taken there are not exactly consistent with a determination to abandon the application; are they?

A. At that point the question is whether we were going to abandon it on December 21st or whether we were going to abandon it eventually.

Q. So you authorized the examiner to undertake measures to place the case into condition for allowance knowing that you were going to abandon the case?

A. I'm not sure when the determination that the case was going to be abandoned was made.

Q. Would you say that the actions in the last page of Exhibit S4 are consistent with a determination to abandon the case?

A. It could be read as such.

Q. Do you see the reference to the cancelation of non-elected claims 18 and 19?

A. Yes.

Q. You could have in fact filed a divisional application directed to those claims consistent with patent office policy and then abandoned this application; couldn't you?

A. That was one option.

Q. That would have preserved the opportunity to file a CIP application tracing its priority back

Giesser - re-cross

to the original application; wouldn't it?

A. You mean file a CIP off of the case containing only 18 and 19?

Q. That's correct.

A. I suppose that could have been an option.

Q. In fact, you could have re-filed 7044/CONT and subsequently filed a CIP off that re-filing and enjoyed claim to priority of the original case; correct?

A. I believe that could have been done.

Q. Let's turn to S3, which I believe is the notice of abandonment and related papers for 7025. Turning to S3, I believe your testimony was that the CIP of 7025/CIP was to be filed abroad as a non-convention case; is that correct?

A. That's the best of my recollection, yes.

Q. What was the nature of the time pressure involved if it was a non-convention case?

A. I think it might have had something to do with publication of the parent, but I couldn't be sure.

Q. Where was the parent being published?

A. Abroad, the parent abroad application.

Giesser - re-cross

Q. Well, wouldn't that publication have an effective date as of May 5, 19 -- I'm sorry, as of May 5, 1987, abroad?

A. I'm sorry?

Q. Let's take the case of Europe. You indicated that the corresponding foreign application to 7025/CIP was about to be published?

A. I think that was what the -- part of what the time pressure was on it.

Q. Now, that would have constituted a bar to filing where?

A. It wouldn't have barred anything.

Q. So what was the nature of the time pressure?

A. It would have made -- part of the problem, I believe, was wanting to get the foreign application on file prior to the publication of the parent case for non-102 type reasons.

Q. You say you have recollection that there was a concern that there might be an objection abroad for a lack of availability of prior art; is that correct?

A. I believe that was one of the

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considerations.

Q. Were there other considerations?

A. I don't recall.

Q. Now, if you look at the third page of document S3, that's a request for extension of time; is that correct?

A. Yes.

Q. Why was the time period for response extended only two months?

A. I assume that was the only extension that was needed at that point.

Q. Needed for what?

A. To keep the parent -- keep the case from going abandoned.

Q. Is it correct, then, that the CIP would have been filed in the U.S. by that date?

A. Yes.

Q. So you actually filed the CIP sooner than you absolutely had to in the United States; is that correct?

A. Yes.

Q. Did you file that non-convention case in Europe before you filed the CIP in the U.S.?

Giesser - re-cross

A. I'm not sure exactly what the time frame was on that.

Q. Well, in fact --

A. It was right around the same time.

Q. Wasn't it your testimony that you did not draft the non-convention filing but rather communicated the information to Basle?

A. Yes.

Q. So you didn't have to actually prepare a rigid specification for that non-convention filing?

A. No, I did not. That was handled by the Basle patent department.

Q. And the filing of the CIP off 7025/CIP would have had no impact on the filing or entitlement -- I'm sorry, on the availability of the publication of the foreign filed parent on the European non-convention CIP; isn't that correct?

A. I'm not sure I understood what you said.

Q. I'm not sure I do, either.

Isn't it correct that the date of filing the CIP in the United States, the CIP of --

A. CIP two.

Q. Isn't it correct that the date of filing

Giesser - re-cross

CIP two would have had no impact on whether or not prior art was available as against the non-convention document, the non-convention application filed abroad?

A. I don't know.

Q. In what way could it have affected the availability of prior art with respect to the non-convention application?

A. I don't know.

Q. Are you familiar with the practices of -- are you familiar with patent practices in Europe?

A. Yes.

Q. Can you imagine any situation where the filing of the CIP, of 7025/CIP, the filing date of that application would have impacted the availability of prior art as against a similar but non-convention application filed in Europe?

A. I'm sorry, I'm losing my focus here.

Q. You've told me that there was a time pressure to file the CIP of 7025/CIP, and you've told Diane the same thing, in part because there was a need to file an application abroad?

A. Yes.

Giesser - re-cross

Q. And that application was a non-convention application?

A. Yes.

Q. My question to you is, how did the need to file that non-convention application impact the need to file the CIP application in the United States?

A. Well, certainly didn't want to file any information in Europe that hadn't been filed in the United States previously.

Q. Why?

A. We would not necessarily have permission to be under export license.

Q. Couldn't you have applied for export license without filing?

A. I suppose we could have.

Q. So it's your testimony that the 7025/CIP was in fact filed before the corresponding non-convention application?

A. I think it was.

Q. Under the Sandoz procedures that existed as of February 1988, could you have proceeded correctly with the preparation of a patent application on a disclosure and operated --

Giesser - re-cross

A. No that would have been incorrect procedure.

Q. So, in fact, it would not have been proper to proceed with the preparation of an application on 299/84 until sometime after January 27, 1988; is that correct?

A. Yes.

Q. I believe it was your testimony that you weren't familiar with the procedures at Sandoz necessary to obtain the information that was a prerequisite to drafting the application for 7101; is that correct?

A. Yes.

Q. In fact, weren't those procedures just a phone call to the person in question?

A. Well, that was the question, who is the person in question.

Q. And it's fairly easy to identify that by asking Mel; wasn't it?

A. That was part of it, yes.

Q. Of the meetings that you attended that are reflected in your schedule that you testified to about at length, what meetings were there that Dick

Giesser - re-cross

Vila could not have adequately represented the interests of Sandoz alone?

A. I'm sure Dick Vila can represent the interests of Sandoz whenever he chooses to.

Q. And would that representation, in your opinion, if he had so chosen, be adequate for the purposes of Sandoz' patent program?

A. Let me say Dick was at a number of these meetings.

Q. Let me turn your attention to paragraph ten on page three of F20, your declaration. I see that paragraph ten indicates you would have started writing the draft of 7101 no later than October 1988?

A. Yes.

Q. A few moments ago in response to questions from Diane you indicated that you must have started before September of 1988; is that correct?

A. Yes.

Q. What gave you the confidence that in fact it was no later than September rather than October as reflected in the declaration?

A. Well, with all the goings on at the office in October, i.e., the number of trips that I had to

make, particularly the trip to Madison and the trip to Boulder took up a lot of time, I remember being involved in drafting the application on this when the Warner-Lambert patent issued and we found out about it, and also just the general amount of time it would take to physically write this case leads me to believe that it would have been actually earlier than September.

Q. Now, of those three aspects of information you just described, which of them did you come into possession of after February 19th, 1993?

A. After February?

Q. Of this year.

A. None.

Q. So you had all that information in front of you, you were aware of all that information before February 19th of this year; weren't you?

A. Yes.

Q. Did you discuss the date on which you must have started drafting the application with Diane during the interval after your cross-examination by me?

A. No.

Giesser - re-cross

Q. Did you consider anything in responding to Diane's questions on re-direct that you did not consider --

A. No.

Q. -- that you did not consider prior to signing your declaration of February 19, '93?

A. I'm sorry, what was that question again?

Q. Did you consider anything in responding to Diane's questions just a few minutes ago that you did not consider when signing the declaration that is F20 on February 19, 1993?

A. No.

Q. Your recollection is clearer now than it was then?

A. Yes.

Q. When did you receive notice of the Warner-Lambert -- issuance of the Warner-Lambert patent?

A. I don't recall exactly, but it was shortly after the patent issued.

Q. Was it in October?

A. No, it would have been shortly afterwards, possibly within a week or two after publication -- or

Giesser - re-cross

after we received the Gazette.

Q. In response to a question I asked you this morning, I believe you testified that you didn't recall when you received notice of the Warner-Lambert patent or how that information came to you.

A. I don't have an exact recollection, no.

Q. You indicated that the receipt of the official Gazette would have been important in fixing the time on which you learned of the Warner-Lambert patent. Why is that?

A. Well, the general procedure would be that after the Gazette was received in the patent office, it would be circulated among the attorneys and agents for general knowledge.

Q. How many attorneys and agents were there in Sandoz in October of 1988?

A. Let me think. Let's see, October of '88, Mel, Tom Doyle, myself, Dick Vila, Bob Awna (phonetic), Gerry Sharkin, Barry Sullivan, Tom McGovern, Walt Jewel, and Jerry Robian (phonetic).

MS. FURMAN: For the record, Barry Sullivan is a trademark attorney.

Q. How long would each attorney take to review

Giesser - re-cross

the official Gazette?

A. It depended on the attorneys. The circulation was such that people who tended to be quick and pass them on would get them first, and people who were not as quick would get them last.

Q. Where did you fit in the scheme of things?

A. I tended to look at it quickly and pass it along.

Q. How long on average would it take that full rotation to complete?

A. I don't know about the full rotation. I tended to be at the top of the list of getting official Gazettes.

Q. Well, how long did it take for an official Gazette to get to you from its date of publication?

A. Generally within a week or so.

Q. Of its publication?

A. I'm sorry. Of its publication, I don't know. It would be about a week or so after we received it.

Q. How long would it take you to review the entire Gazette?

A. I would try to do it in an hour or so. I

ignored the electrical section.

Q. You don't recall how you first became aware of the Warner-Lambert patent, do you?

A. No. It would either be by seeing it in the Gazette or hearing it from Mel, who had seen it in the Gazette.

Q. So you're certain it was either you or Mel?

A. Yes.

Q. Where did Mel fit in the pattern of obtaining it from the Gazette?

A. Since Mel was responsible for informing Basle of any substantive changes to U.S. patent law that would be proposed, he got the Gazettes either first or very close to it, so he would have been among the first ones to get it.

Q. And you knew this when you signed your declaration?

A. Yes.

Q. Do you ever dictate anything for transcription?

A. Not often, no.

Q. Anybody you know in the Sandoz office ever do that between January '88 and March '89?

Giesser - re-cross

A. Dick tended to dictate things a lot.

MS. FURMAN: For the record, his dictation was to his secretary.

MR. KELBER: You can ask her that. That's fact testimony.

THE WITNESS: Oh, were you talking about --

BY MR. KELBER:

Q. Dictation into a microphone?

A. Yeah, Dick tended to do that.

Q. Were you forbidden to do that?

A. No.

Q. That was just personal choice?

A. Yes.

Q. Do you at the present time feel incompetent to tackle the tasks assigned to you at Sandoz in the period January '88 through March '89 in a timely fashion?

A. No.

Q. Did you ever feel like there was a risk that you weren't going to get it all done?

A. At times, yes.

Q. Did you ever tell anybody about that?

A. Daily.

Giesser - re-cross

Q. Who did you tell?

A. I would talk to Dick a lot.

Q. Did you ever tell Dick that there was a chance that you might not get 7101 done and filed in time?

A. In time, meaning --

Q. Whatever you felt was an appropriate time.

A. I know that it was taking a long time to do it, and he was aware of that.

Q. But did you tell him it might take you too long?

A. I don't recall ever using those words.

Q. Dick was, to the best of your knowledge, satisfied with your progress with regard to that application; wasn't he?

A. He was certainly satisfied with my overall progress of handling things.

Q. Did he ever express any dissatisfaction with your progress with respect to 7101?

A. No.

Q. And he was aware that you were responsible for 7101?

A. Yes.

Q. In fact, you talked to him on more than one occasion with respect to 7101 while you were preparing it; correct?

A. I would speak to him in general terms about it. For technical advice I would go to Mel.

Q. You testified that you had no choice but to file a patent application on the basis of the disclosure that became 7101; is that correct?

A. Yes.

Q. Did you have the option to determine when to file that application?

A. No, it was supposed to be given priority.

Q. Priority over what?

A. That's it. When a case was rated A, it meant it was ready to be filed and you were supposed to put forth all effort to file them.

Q. But in fact, there were other things that you had to put forth effort with respect to first?

A. Yes.

Q. Even as to your refreshed recollection of today between February of 1988 and September of 1988, you did not have the opportunity to begin drafting the application; is that correct?

Giesser - re-cross

A. I don't recall when I began drafting the application.

Q. How much of the application had you drafted when you learned of the Warner-Lambert patent?

A. I don't recall.

Q. Did you focus more attention on the application after you learned of the Warner Lambert patent?

A. Not any more than I had been -- I mean, I didn't treat it any differently after I found out than before I found out.

Q. Well, according to the best of your recollection, the best of your recollection tells you that you began drafting no later than September of 1988; is that correct?

A. No, I believe the best of my recollection is that it would have been earlier than that.

Q. August?

A. I would say yes, because I recall that I was working on it when I heard of the Warner-Lambert patent.

Q. July?

A. I don't know exactly.

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Q. So the Warner-Lambert patent issuance really fixes in your mind the knowledge that you were working on the application?

A. Yes.

Q. That was an important event for you in connection with the application; is that correct?

A. Yes.

Q. Do you have any recollection as to whether you were working a long time on this application in terms of drafting before you learned of the Warner-Lambert application?

A. No, I don't have any recollection of that.

Q. So the best information that you have is sometime before the issuance of the Warner-Lambert application you began working?

A. Yes.

Q. That's quite a bit before October 1988; isn't that correct?

A. Yes.

Q. Would there have been any written records of your work of any type prior to October 1988?

A. I don't think there were any in existence then.

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Q. No, I mean at the time.

A. Certainly my handwritten pages that I was drafting.

Q. Would anybody at Sandoz have known you were working on it at that time?

A. I'm sure Mel would have been generally aware that I was working on it. I don't know if he would recall any specific dates as to what I was doing. He was pretty busy with his own stuff at that point.

Q. How about your secretary?

A. I doubt if Lorraine would be able to distinguish between the various chemical cases I was working on in this area.

Q. So she wouldn't have known if you were working on this case or not?

A. I would say that's probably true.

Q. What happened to the case from January 27, 1988, until the time you began working on the draft?

A. From, I'm sorry, January 27th, 1988?

Q. 1988 until you began working on the draft.

A. Well, at that time Mel had collected some information on it. I don't know exactly when that

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information went to him, but there was certainly some sort of information that I got from Mel that had been from the inventors, some preliminary --

Q. What did you do with that information?

A. I incorporated it into the draft.

Q. Before you began working on the draft, what did you do with that information?

A. I would have kept it until I began working on the draft.

Q. Do you recall reviewing it in detail?

A. Yes.

Q. Before working on the draft?

A. Yes.

Q. Do you know when that would have occurred?

A. No.

Q. You just recall reviewing it?

A. Yes.

Q. What was the nature of your review?

A. I was looking at it and trying very hard to understand it.

Q. Now, you said this was a very hard case to write; is that correct?

A. Yes.

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Q. How much of the information on the synthesis did you obtain from the inventor?

A. I don't recall how much was directly from the inventor or how much was through Mel.

Q. Did you receive a lot of information from Mel, as well?

A. Yes.

Q. How much information with regard to the synthesis did you input yourself?

A. Possibly very little.

Q. How about the stereochemistry, the discussion of stereochemistry that appears in Exhibit S4, which is the application; did that come only from you?

A. I wrote that, but I remember discussing it with the inventor.

Q. Did anybody at Sandoz know that prior to the assignment of 7101 you had never written a pharmaceutical patent application?

A. Yes.

Q. Did Dick Vila know?

A. Yes.

Q. Did you feel at the time that you had

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confidence in your ability to write this particular case?

A. Yes.

Q. Did you ever discuss that particular aspect of your experience with him in connection with 7101?

A. No.

Q. The seed cases that you filed in March of 1989, what were the nature of the time bars?

A. They were one of two, on-sale or in-public-use bars.

Q. And when did Sandoz' patent department learn of a need to file these applications?

A. I'm not sure what the time date was on that.

Q. Could it have been as early as January 1988?

A. No, it would have been later than that.

Q. Would it have been later than August of 1988?

A. I think it would have been, yes.

Q. So after you had begun drafting the application in question, was it necessary to put -- to interrupt that drafting in order to attend to the

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seed cases?

A. I'm not sure whether I was -- I actually stopped working on it or whether I was working on it contemporaneously. The filing of the seed cases were in response to a policy change, and some of the discussions on the policy change were at the meeting in Basle in September, so the decision to file on these would have been after that.

Q. Do you recall how much of the application you had written before October -- draft application you had written before October of'88?

A. I would imagine it would have been relatively close to what I had given Lorraine on November 3rd, because I really wasn't in the office a whole lot or working on -- I was working on other projects for a large part of October.

Q. Well, you knew when you signed this declaration that is F20; correct?

A. Yes.

Q. Did you assign originally the date of October '88 as the date you had firm recollection of writing the first draft of 600-7101 that appears in paragraph ten?

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A. I don't recall how that date came about.

Q. Did you receive a draft of the exhibit that is F20 and offer any corrections or advice with respect thereto?

A. Yes.

Q. In the draft that you received, was the date of October 1988 there?

A. I don't think it was.

Q. Was that date inserted in response to a suggestion from you?

A. I said I don't recall exactly the circumstances, but I remember that was an area that we had -- that there was a change from the original one.

Q. Well, it's not all that long ago compared with 1988. If the draft came to you without that information in it, isn't it reasonable to conclude that that information came from you?

A. I'm sorry.

Q. We're talking about a month-and-a-half from February 1993, and it's your testimony that you remember receiving a draft of this declaration that did not have the information in paragraph ten with

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respect to October 1988; is that correct?

A. Yes. I recall that the original version was worded somewhat differently. I don't recall exactly what that wording was.

Q. Do you recall the effect of that wording?

A. I believe October '88 is an earlier deadline than what was originally in the first draft.

Q. An earlier date of starting?

A. Yes.

Q. Was the date of October 1988 suggested by you, then?

A. I don't recall.

Q. Who else besides you discussed the draft and changes thereto?

A. Well, I discussed them with Diane.

Q. Anybody else?

A. Not that I'm aware of. I don't know what Diane discussed.

Q. Do you recall suggesting to Diane that the date cited for beginning the draft was too late?

A. No.

Q. Somebody changed the date, though; is that correct?

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A. Yes, that was changed.

Q. Did you give Diane documents in connection with changing the draft of this application?

A. I didn't send back a marked up document, no.

Q. I'm sorry, any other documents besides the draft declaration itself?

A. Well, she sent me copies of the other documents mentioned as exhibits.

Q. Did you send her any documents besides the documents --

A. Did I send her anything? No. I didn't take any papers connected with this case when I left Sandoz.

Q. The initial preparation of a declaration led to a date for beginning the draft of no than October 1988. You didn't consult any documents that Diane didn't give you in arriving at the date by February. Is it reasonable to conclude that the February 1988 came out of the discussions you had with Diane?

A. Yes.

Q. Isn't it necessary that the identification

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of that date had to come from you? I mean, it was not so long ago you can't reconstruct that discussion?

A. I don't recall that discussion that clearly.

Q. Do you think if Diane knew you had begun as late as October 1988 -- strike that. It's objectionable even from me.

Did you recall when you were correcting the draft of Exhibit F20 that the Warner-Lambert patent of interest had issued while you were drafting the application?

A. Yes.

Q. But it is today your testimony that your drafting must have begun at least two months prior to the date that's reflected in your declaration?

A. Well, that is no later than October '88.

Q. It's your testimony it had to begin two months earlier than the date reflected in the declaration?

A. Yes.

Q. And you consulted no additional documents to arrive at that recollection?

Giesser - re-cross

A. Correct.

Q. Any of the other dates in here not as specifically fixed as they might be in light of your recollection of today?

A. Well, as we spoke earlier, there was a mistake on the filing date in paragraph 11.

Q. How about anything else?

A. Not that I recall.

Q. Is there any reason to believe that the other dates that are recited in here -- for instance, the date of December 14, 1988, that appears in paragraph 15, do you know for a fact that that date is correct?

A. I believe that was on a cover letter of an exhibit, so I would expect that it was correct.

Q. That was the date of the cover letter; is that correct?

A. Yes.

Q. Do you know that you sent it on the date the cover letter was dated?

A. Well, on or about that date.

Q. Could it have been as much as a week later?

A. I don't recall. I don't think so. I think

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if -- it was my general practice that if a letter was delayed for that length of time, I would change the date on the letter to more accurately reflect when it would be sent.

Q. You spoke on re-direct with regard to the existence of conflicting obligations on your time and services between June '88 and March '89; do you recall that testimony?

A. Yes.

Q. How did you resolve those conflicting obligations when they occurred?

A. I would try and put the biggest fire out first.

Q. By "biggest fire," what do you mean?

A. The action that had the most pressing date or had the most possible adverse consequences.

Q. Missing that date would have the adverse --

A. Yes.

Q. The application that is 7101 was filed reasonably contemporaneously with the five or six seed cases that were filed in response to 102 bars in March; is that correct?

A. Yes.

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Q. So they had the same date consequence?

A. Yes. At that point it was ready to be filed.

Q. It took you about three months to go from beginning to completion of the draft application; is that correct?

A. It appears that way, yes.

Q. Now, that's --

A. At least for completion of the first draft to filing.

Q. That's not my question. From initiating work on the first draft to completion of the first draft?

A. I don't remember exactly.

Q. It wasn't as long as four months, was it?

A. I don't recall.

Q. Forgive me for sounding a bit perturbed, but your recollection seems to come and go.

A. It's quite a while ago.

Q. It was a little bit better just a few minutes ago.

A. I remember I was working on the case when the Warner-Lambert patent issued. I don't recall how

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long I was working on it.

Q. Did the issuance of the Warner-Lambert patent change the size of the fire, in your determination, with respect to 7101?

A. It certainly caused a lot of concern, because we were not expecting to see a Warner-Lambert patent issued to the same subject matter -- we were not expecting any patent to be issued to the same subject matter. As I said, my biggest recollection in finding out was thinking how it was going to complicate prosecution of an otherwise straight forward case.

Q. And you certainly had a time bar then with respect to the filing of the application?

A. Yes.

Q. When you signed the declaration that is F20, it was your recollection that you might have written the draft of the application that is 7101 in about a month's time; is that correct?

A. No.

Q. Well, it says no later than October 1988 you would have started writing the draft; that's correct?

Giesser - re-cross

A. Yes, that's correct.

Q. I apologize. I should have said about two months. Is that correct, it would have been two months from the -- your recollection on February 19, 1993, was that it could have been as little as two months from the beginning of the drafting to the completion of the first draft; is that correct?

A. No.

Q. Well, reading paragraph ten literally, it is consistent with the conclusion that you did not start earlier than October 1988; isn't that correct?

A. It seems consistent with the fact that October '88 would have been the latest possible date I could have started.

Q. And you know that you finished the draft prior to December 14; isn't that correct?

A. Well, I know that I gave a first handwritten version to Lorraine November 3rd.

Q. So that's just about a month; isn't it?

A. From October 1st to November 3rd is a little over a month, but as I've testified before, I was working on the application before.

Q. I understand that, but my question is, when

Giesser - re-cross

you signed this declaration, it was your recollection that it could have been as little as a month; isn't that correct?

A. No, when I signed the declaration, I knew that I had remembered working on the application when the Warner-Lambert patent came in.

Q. Did you know when the Warner-Lambert --

A. I knew it was vaguely in August. I don't know the exact date.

Q. It would have been possible to fix paragraph ten with more specificity if you had that information before you; wouldn't it?

A. It could have been possible to do a lot of things.

Q. That's not my question.

A. I didn't know you asked a question. I thought you were making a statement.

Q. It would have been possible to fix the date on which you would have started writing a draft of case 600-7101 with more specificity given the information you recall as of February 19, 1993; wouldn't it?

A. No, I don't recall when I started to work

Giesser - re-cross

on 600-7101.

Q. But you knew it was in fact earlier than September?

A. Yes.

Q. And you chose to recite October as the latest possible start date?

A. Yes.

Q. Any reason for that?

A. It seemed the most conservative.

Q. Is there still a question in your mind as to the possibility?

A. No.

Q. Was there a question in your mind as to the possibility?

A. No.

Q. What do you mean by "conservative" if you were certain that it started earlier than September?

A. I don't have a fixed date in my mind when I started writing it.

Q. But you didn't put a fixed date. You knew it was before September, you just testified?

A. That's true.

Q. So September 1988 would have been no more

Giesser - re-cross

or less conservative than October 1988; would it?

A. That could be true.

Q. Is it true?

A. Yes.

Q. Do you recall speaking to the inventor in this case with regard to 7101 between January 4 and March 3, 1989?

A. Yes.

Q. And what was the subject of those discussions?

A. I believe we went over the draft I gave him.

Q. Did you go over the changes that you had received?

A. Yes.

Q. Do you recall when you prepared the final draft?

A. Not exactly, no.

Q. When you were hired at Sandoz' patent and trademark department, do you have any reason to know -- I'm sorry, when you were hired at Sandoz' patent department, did you inform them of the nature of your prior experience in the patent field?

Giesser - re-cross

A. Yes.

Q. Is it a correct conclusion that for those meetings or trips that Dick Vila requested you to attend in the period reflected in your declaration that is F20 that he requested you to attend those having a general idea of your other obligations on behalf of Sandoz?

A. Yes.

Q. Is it correct to conclude that Sandoz -- or individuals in the Sandoz patent department made the decision to complete the drafting of the CIP application that bears the docket number 7025-CIP/CIP prior to completion of 7101 rather than filing a continuation?

A. I'm not sure that the weight of the two obligations were necessarily compared. I think it was basically decided that a CIP should be filed and should be filed now. I don't think it was --

Q. You had the responsibility for both; correct?

A. Yes.

Q. Do you remember making the decision with respect to that one way or the other?

Giesser - re-cross

A. At that time I was working on both applications. I was working on both the CIP of 7025 and also the draft of 7101.

Q. It's my understanding that of the two, you had to do the 7025 first.

A. Well, like I said, I was working on them contemporaneously.

Q. You filed 7025-CIP/CIP prior to October 11, 1988; correct?

A. Yes.

Q. If you had elected to file a continuation application or respond to the office action of May 11, '88 on 7025-CIP, thereby extending the time in which to file the CIP application, would you have had more time to work on 7101?

A. I'm not sure that was an election that I could have made at the time.

Q. If somebody at Sandoz had made that election, would you then have had more time?

A. If someone had decided that a continuation should be filed, then I would have had more.

Q. Do you recall ever being told that the 7101 case had to be on file by March of 1989?

A. Not specifically.

Q. That was just when you got to it?

A. That was when it was completed.

Q. And it was completed by you; correct?

A. Yes.

MR. KELBER: I have nothing further.

MS. FURMAN: I have a couple more questions.

RE-RE-DIRECT EXAMINATION

By Ms. Furman

Q. Going to case 600-7025, you earlier testified that you were writing the double CIP and transmitting information to Basle on the foreign text at the same time, roughly; is that correct?

A. Yes.

Q. In your opinion, would it have been a more economical use of your time for you to postpone filing the double CIP until sometime in the future?

A. Well, since I was working on the case with Basle, it probably would not have been.

Q. Did you need any information in filing the double CIP beyond that which you provided to Basle for the foreign text?

A. Are you asking whether I consulted the

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inventors on this case?

Q. No, I'm asking whether the information that you got for foreign purposes was sufficient also for the double CIP. In other words, was drafting the double CIP very similar to preparing the draft of the foreign text?

MR. KELBER: Objection. I don't think she prepared the draft of the foreign.

BY MS. FURMAN:

Q. Was the content of the double CIP similar to the content of the foreign text?

A. As far as I recall, it was similar.

Q. It's my understanding that Basle imposed a deadline of October of '88 to file the foreign text; is that true?

A. As much as I can recommend -- excuse me, as much as I can recollect, yes.

Q. So you had no choice but to gather information, at least for preparing the foreign text, prior to October of 1988?

A. Yes.

Q. Are there structures in the involved patent application?

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A. Yes.

Q. How did you dictate structures?

A. I would convey them by drawing them on the piece of paper that I gave to Lorraine. I didn't dictate this case.

Q. In fact, in a case such as this containing structures, would there be an advantage to writing out the case as opposed to dictating it?

MR. KELBER: Objection. The witness has already testified that she doesn't have much experience dictating cases. So how would she know?

MS. FURMAN: She can speculate.

MR. KELBER: Object strongly to any speculation, and the fact that you're inviting her to speculate I think is truly objectionable.

BY MS FURMAN:

Q. Does the phrase "no later than October 1988" include September of 1988?

A. Yes.

Q. Does it include August 1988?

A. Yes.

Q. Was it your intention between January 27th of 1988 and March 3rd of 1989 to file a patent

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application on 299/84?

MR. KELBER: Asked and answered and far beyond the scope.

MS. FURMAN: If it's asked and answered--

A. Yes.

Q. What was the response?

A. Yes

Re-Re-Cross Examination

By Mr. Kelber

Q. What did you do to get the information for Basle's request?

A. I would have spoken with either or both Mel or the inventors of 7025-CIP/CIP.

Q. Did you actually do those things?

A. Yes.

Q. So you spoke with Mel. Couldn't Mel have sent that information on to Basle himself?

A. Well, it was my responsibility. It was not Mel's case.

Q. So you got some information from Mel, and you got some information from at least Dr. Kathawala; is that correct?

A. Yes.

Giesser - re-re-cross

Q. What did you do with that information?

A. I wrote a draft of 7025-CIP/CIP.

Q. I'm sorry, with respect to communication with Basle, what did you do?

A. I had communications in Basle, both on the phone and while I was over in Basle in September of '88.

Q. When you were in Basle for four days in September of '88?

A. Yes, I was there on -- well, on the weekdays it was Monday, Tuesday, Wednesday, and I came home on a Thursday.

Q. So three days. So the communication was over some part of those three days; is that correct?

A. Yes.

Q. How long did it take you to gather that information?

A. I don't recall exactly.

Q. As much as a day?

A. I'm sorry, to gather all the information needed to provide for the --

Q. To answer Basle's inquiry.

A. I'm sure it would have been longer than

that.

Q. Well, you talked to Mel, you said, and you talked to Mr. Kathawala. What else did you do?

A. That would have been it.

Q. I'm talking total time commitment in number of hours. How many hours did it take to talk to Mel and Dr. Kathawala?

A. As I recall, 7025-CIP/CIP was a rather extensive CIP; it was not a simple CIP, so there would have been a lot of information.

Q. I'm not referring to the CIP/CIP. You told me you needed to transmit some information to Basle with regard to additional information with regard to 7025/CIP; is that correct?

A. That was for them to write what would be the foreign counterpart of 7025-CIP/CIP.

Q. And it was your testimony that you did not write the foreign counterpart?

A. I did not write the foreign counterpart.

Q. So you collected the information and communicated it to Basle?

A. Yes. Usually the way that would work would be I would send a draft of my U.S. application, and

Giesser - re-re-cross

they would modify it for the European formats.

Q. In fact, that's not the way it worked on this case; is it?

A. No, this one, as I recall, was more contemporaneous writing of the applications by Basle and myself.

Q. And your discussions in Basle with regard to the information, that involved revising written documents?

A. I didn't do any revising of written documents over there, no.

Q. Did you write any documents over there?

A. Not that I recall.

Q. Your communication was purely oral there?

A. Yes.

Q. Did you write documents for Basle prior to going over there?

A. I don't recall. I imagine there was.

Q. You imagine there were. What type of documents would those have been?

A. Possibly transmitting technical information.

Q. Would you prepare that technical

Giesser - re-re-cross

information yourself?

A. I would compile it. For instance, diagrams of pathways, chemical pathways or such.

Q. Who would provide those pathway diagrams?

A. The inventor.

Q. So your involvement in written communication was the assembly of information received; is that correct?

A. Yes.

Q. And with regard to the non-convention case, you had some oral communication; is that correct?

A. Yes.

Q. Did you ever have another application at Sandoz that took more than a year from the date it was assigned as an A case to filing?

A. I don't recall. I would expect not.

Q. You would expect not?

A. Yes.

Q. You do recall more than one case where it took less than that amount of time?

A. Yes.

MR. KELBER: That's it.

MS. FURMAN: I have two more questions.

Giesser - re-re-re-direct

Re-Re-Re-Direct Examination

By Ms. Furman

Q. You indicated that 600-7025 CIP/CIP involved extensive work. The word extensive I believe is what you used?

A. Yes.

Q. Why do you use that word to describe it?

A. Well, the amount of material that was added, as I recall, was a lot. It wasn't just one extra example or something which you might put into a CIP. The amount of work involved was the equivalent to writing a new case, in my estimation.

Q. Would that be the same material that you needed to provide to Basle for the foreign text?

A. It would have involved the same material, yes.

Q. Do you recollect whether there was any commercial significance to the compounds covered by case 600-7025?

MR. KELBER: Objection, way beyond the scope of -- what are we, re-re-cross? Never even been raised in direct, let alone --

BY MS. FURMAN:

Giesser - re-re-re-direct

Q. Were there any other reasons for filing 7025-CIP/CIP as quickly as possible?

A. The subject matter was considered important.

MS. FURMAN: That's it. I have no more questions.

MR. KELBER: I couldn't improve on that answer myself. I appreciate your tolerance and your attention.

Are you going to take care of filing the original?

MS. FURMAN: Yes.

MR. KELBER: It continues to be your preference that we identify our objections to the declarations in writing?

MS. FURMAN: To the declarations, or the exhibits?

MR. KELBER: Yes, to both, other than Joanne's or the other ones that were subject to cross? Last time I tried to object to a declaration of yours during the deposition, and you said, quote, "we prefer the objections be made in writing." Is that still your desire?

Giesser - re-re-re-direct

MS. FURMAN: My request stands as indicated.

MR. KELBER: Okay. As far as I'm concerned, that's the end of the record.

(Whereupon the deposition was concluded.)

Giesser - re-re-re-direct

ERRATA SHEET

I have read the foregoing transcript of my deposition taken on April 9, 1992, and _____ It is a true and correct transcript of my deposition given on the day and date aforesaid.

[or]

_____ I wish to make the following changes to my deposition: See attached sheet

Page _____ Change _____

Page _____ Change _____

Page _____ Change _____

Page _____ Change _____

Page _____ Change _____

Page _____ Change _____

Page _____ Change _____

Page _____ Change _____

Subscribed and sworn to before me this 5th day of May, A.D. 1993.

Joanne M. Giesser

Thomas W. Tolpin

Notary Public

My commission expires: 4/9/97

[Seal]

ERRATA SHEET

Name of case: Wattanasin v. Fujikawa et al.
Deposition of: Joanne M. Giesser
Date taken: April 9, 1993
Page 1/1

PAGE	LINE	CHANGE	REASON

The following changes are all of a typographical nature, and primarily are concerned with spelling or punctuation:

7	15	Initial capitalize "patent office".	
7	19-20	Initial capitalize "patent and trademark department".	
10	16	Change "Pharma" to "Pharmaceutical".	
11	22	Initial capitalize "crop protection".	
11	24	After "involved" insert "in".	
13	8-9	Initial capitalize "crop protection".	
20	16	Initial capitalize "patent and trademark department".	
20	23-24	Initial capitalize "patent and trademark department".	
33	5	Change "102-B" to "102(b)".	
35	24	Initial capitalize "patent".	
36	2	Initial capitalize "office".	
38	16-17	Initial capitalize "patent and trademark department".	
39	24	Initial capitalize "patent and trademark department".	
40	12	Initial capitalize "judges' dinner".	
45	12	Change "Angstrom" to "Engstrom".	
52	4	Change "102-B" to "102(b)".	
52	16	Initial capitalize "patent and trademark department".	
56	2	Change "Weinfeld" to "Weinfeldt".	
56	20	Change "Weinfeld" to "Weinfeldt".	
60	23	Initial capitalize "patent department".	
62	12	Change "straight forward" to "straightforward".	
65	23	Delete the apostrophe following "Sandoz".	
65	24	Initial capitalize "crop protection".	
72	2	Change "do" to "to".	
72	21	Initial capitalize "judges' dinner".	
73	6	Change "102-B" to "102(b)".	
87	22	Change "partner" to "party".	
92	15	Change "cancelation" to "cancellation".	
103	19-20	Change "Awna" to "Honor" and delete "(phonetic)".	
103	20	Change "Sullivan" to "Solomon".	
103	21	Change "Jerry Robian (phonetic)" to "Joe Borovian".	
103	22	Change "Sullivan" to "Solomon".	

Joanne M. Giesser
JOANNE M. GIESSER, ESQ.

SUBSCRIBED AND SWORN TO BEFORE ME

This 5th day of May, 1993

My commission expires: 4/9/97

Thomas W Tolgas
A Notary Public

STATE OF ILLINOIS)
) SS.
COUNTY OF K A N E)

I, Paula M. Quetsch, C.S.R. No. 084-003733, a Notary Public in and for the County of Kane, State of Illinois, do hereby certify that JOANNE M. GIESSER, ESQ., was duly sworn by me to testify the truth; that the above deposition, Pages 1 through 140 was recorded stenographically be me and reduced to typewriting under my personal direction; and that the foregoing is a true and correct transcript of the testimony given by the said witness at the time and place previously specified.

I further certify that I am not counsel for nor in any way related to any of the parties to this suit, nor am I in any way interested in the outcome thereof.

IN WITNESS WHEREOF I have hereunto set my hand and affixed by notarial seal this 14th day of April, 1993.

OFFICIAL SEAL
STATE OF ILLINOIS
PAULA QUETSCH
NOTARY PUBLIC

Notary Public

My Commission Expires: September 23, 1996

380

Case No. 600-7101/CONT/INT.(6)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN

v. Interference Nos. 102,648, 102,975

FUJIKAWA et al. Examiner-in-Chief: M. Sofocleous

DECLARATION OF LORRAINE M. CHESLEY PURSUANT TO 37 CFR §1.672

I, Lorraine M. Chesley, do hereby declare as follows:

1. All of the below-indicated activities took place in the United States.

2. I have been employed as a secretary in the Patent and Trademark Department of Sandoz Corporation since August 6, 1984 to the present. My current position is Senior Administrative Secretary.

2. I was Mrs. Joanne Giesser's secretary from 1987 to 1991.

3. **Exhibit U-1** hereto comprises a true copy of a computer disc label which is written in my handwriting, indicating that I started typing Case No. 600-7101 on November 3, 1988 and that the case was mailed to the Patent and Trademark Office on March 3, 1989.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

I hereby subscribe my name to the foregoing DECLARATION this 19th day of February, 1993.

Lorraine M. Chesley

LORRAINE M. CHESLEY

#38 & #93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

WATTANASIN v. FUJIKAWA ET AL.

INTERFERENCE NO. 102,648
INTERFERENCE NO. 102,975

FYI

WATTANASIN CONSOLIDATED RECORD

VOLUME V

[PAGES 470 - 562]

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May 15, 1993

5/17/93

"RIBBON COPY FOR PARTY Wattanasin"

HMG-CoA Reductase Inhibitors: An Exciting Development in the Treatment of Hyperlipoproteinemia

F. G. Kathawala

Preclinical Research Department, Sandoz Research Institute, Route 10, East Hanover, New Jersey 07936

I. INTRODUCTION

Coronary heart disease (CHD) continues to be one of the major health problems in all the developed countries of the world. A considerable body of clinical and epidemiological data has emerged over the years linking elevated blood levels of total cholesterol, Low Density Lipoprotein Cholesterol (LDL-C), and Very Low Density Lipoprotein Cholesterol (VLDL-C) as important risk factors for the development of coronary heart disease.[1]

For the treatment of elevated LDL-C and VLDL-C, a judicious diet, low in cholesterol and fat with saturated fatty acids replaced by polyunsaturated fatty acids, is the recommended choice. However, for patients nonresponsive

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CCC 0198-6325/91/020121-26$04.00

to dietary intervention, the development of effective and safe therapeutic agents for the treatment of hyperlipoproteinemia remains an important need. This need has gained considerable support as a result of two important events: (1) the results of the Lipid Research Clinic's Coronary Primary Prevention Trial (LRC-CPPT), a multicenter, randomized, double-blind study involving 3806 asymptomatic middle-aged men in the United States with type II hyperlipoproteinemia, that demonstrated that a statistically significant reduction of 19% in the rate of fatal plus nonfatal coronary heart disease was associated with a 9% decrease in blood cholesterol levels,[2] and (2) the recommendation to treat individuals with blood cholesterol above the 75th percentile, which emerged from the consensus panel of the December, 1984 NIH Consensus Development Conference on the lowering of blood cholesterol to prevent coronary heart disease.[3]

In recent years, to achieve this goal of finding effective and safe therapeutic agents to lower LDL-cholesterol, great interest has focused on potent inhibitors of the enzyme β-Hydroxy-β-Methyl-Glutaryl-CoA reductase (HMG-CoA reductase, EC 1.1.1.34), which controls a key step in the endogenous synthesis of cholesterol. Several studies, both in animals and humans, have been reported with HMG-CoA reductase inhibitors: compactin (Mevastatin), CS-514 (Pravastatin, Mevalotin®, Pravachol®), mevinolin (Lovastatin, Mevacor®) and Synvinolin (Simvastatin, Zocor®),[4] which are structurally very closely related to one another. In order to assess fully the potential of HMG-CoA reductase inhibitors as an effective therapeutic intervention for the treatment of hyperlipoproteinemia, it is thus desirable to study in humans a variety of these inhibitors derived from different structural prototypes which can be distinguished in their overall biological profile from one another. This conceptual framework formed the basis for initiating efforts at the Sandoz Research Institute to develop and study a variety of HMG-CoA reductase inhibitors with chemical structures different in several respects from compactin, pravastatin (a hydroxy analog of compactin), lovastatin (a methyl analog of compactin), and simvastatin (a dimethyl analog of compactin), and has led to fluvastatin (XU 62-320), the first totally synthetic HMG-CoA reductase inhibitor currently in Phase III human clinical trials (Fig. 1).

II. DESIGN ASPECT FOR HMG-CoA REDUCTASE INHIBITORS AT SANDOZ RESEARCH INSTITUTE LEADING TO FLUVASTATIN (XU 62-320)

Investigations by Akira Endo with compactin[4] have to be largely credited for the resurgence of the research on cholesterol biosynthesis and the renewed interest in HMG-CoA reductase inhibitors, a field now almost three decades

---

F. G. Kathawala *obtained his M.Sc. from the University of Bombay, India, and his Ph.D. in 1961 from Technische Hochschule Braunschweig, West Germany (Prof. H. H. Inhoffen), in Synthetic Organic Chemistry. After a few years of postdoctoral work at Harvard (Prof. R. B. Woodward), Wisconsin (Prof. H. Muxfeldt), and Göttingen (Prof. F. Cramer), he joined Sandoz in East Hanover, New Jersey, as a Senior Scientist, in 1969. Currently, he is the Director of Medicinal Chemistry in the area of Lipoprotein Metabolism/Atherosclerosis. His research interests in Medicinal Chemistry are focused towards the discovery of agents affecting lipoprotein metabolism/atherosclerosis.*

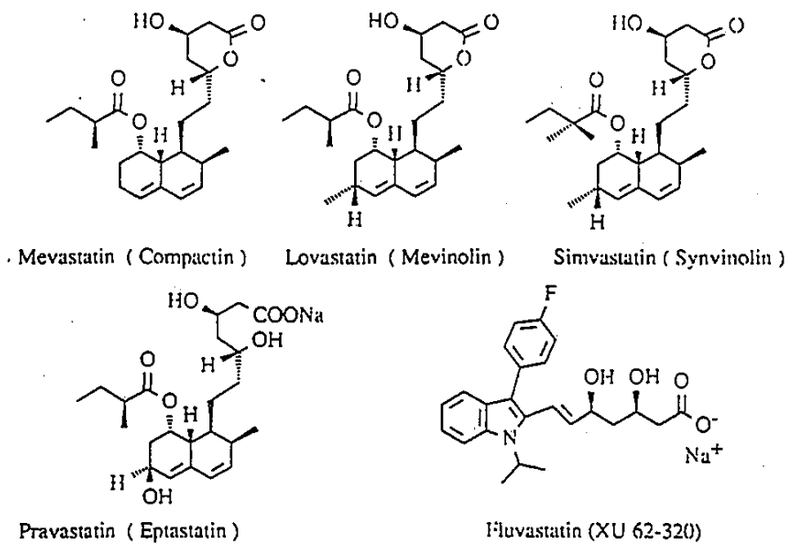


Figure 1

old. While all intensive studies hitherto conducted have been with closely related metabolites, such as compactin, mevinolin, and CS-514 (pravastatin), derived from fungal broths, efforts at the Sandoz Research Institute towards the development of new HMG-CoA reductase inhibitors have been based on synthesis, guided by the following assumptions:

(a) There are two regions at the active site of the enzyme: one with high specific recognition of a 5-carbon unit (C-1 to C-5 as shown below) of the β-OH-β-Methyl-Glutaryl portion, and the other of CoA moiety present in HMG-CoA (Fig. 2).

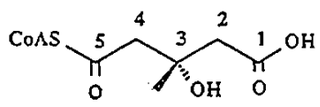


Figure 2

(b) Compactin (R = H, Fig. 3), a known inhibitor of the enzyme, may be regarded as a transition state analog, when in the open dihydroxy acid form.

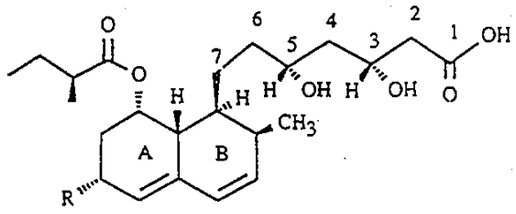
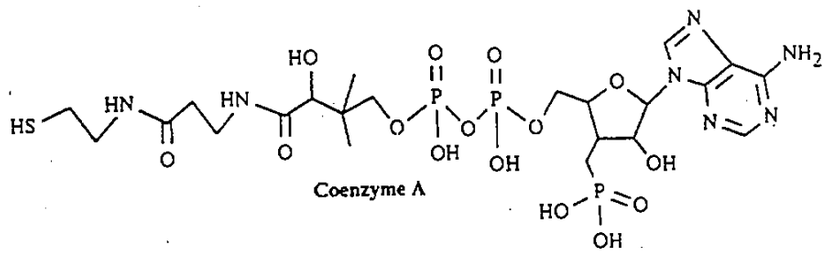


Figure 3

The 5-carbon unit of the side chain present in compactin (Fig. 3) probably occupies the same region as the 5-carbon unit in HMG-CoA (Fig. 2); the bicyclic A-B-ring system, with its substituents in compactin (Fig. 3), possibly sits in the same region or very close to the same region the CoA portion of the substrate HMG-CoA occupies at the active site of the enzyme. However, it is difficult to see any similarity in structure between the bicyclic-ring system of compactin and CoA, when one examines the structure of CoA shown in Fig. 4.

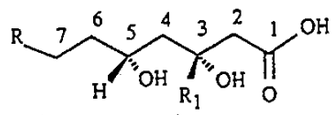


Coenzyme A

Figure 4

In light of (a) and (b) above, one hoped that it might possible to prepare interesting synthetic inhibitors of HMG-CoA reductase with a very general structure as shown in Fig. 5, with the 5-carbon unit (C-1 to C-5) preferably possessing the absolute configurations of C-3-OH and C-5-OH as present in compactin.

Choice of R and R_1 in Fig. 5 has depended on:



R1 = H , CH3

Figure 5

(a) Consideration of the elements of structure of CoA.

(b) Considerations of the overall shape and assumptions of the importance of substituents on Ring A-B of compactin (Fig. 3), first with molecular models and later with computer modelling.

(c) Exploiting the knowledge gained in structure activity relationships with our own Sandoz Research Institute compounds or being reported in literature by outside investigators.

Efforts with the above considerations in mind have led to the development of a variety of novel HMG-CoA reductase inhibitors. Synthesis and Structure Activity Relationships (SAR) of some of these novel inhibitors are discussed below with emphasis on the Phase III candidate, fluvastatin (XU 62-320): [R*,S*-(E)]-(±)-Sodium-3,5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methyl-ethyl-1H-indol-2-yl]-hept-6-enoate (Fig. 1), a mevalonic acid analog more potent than compactin and lovastatin.

III. GENERAL CHEMISTRY APPROACH

Guided by the conviction that the C-3, C-5 dihydroxy acid fragment was the key pharmacophore necessary for the inhibition of HMG-CoA reductase,

our synthetic approach towards the synthesis of compounds of generic structure (Fig. 5) involved:

(a) A convergent synthesis coupling chiral Synthon 1 or racemic or chiral (3R, 5S) C-3, C-5-dihydroxy ester Synthon 2 with a variety of aryl or alkyl fragments 3 (Fig. 6), or

(b) A linear synthesis of the C-3, C-5 dihydroxy acid derivatives wherein the aldehyde 4 is reacted with acetoacetate 5 (Fig. 7) to provide a hydroxyketo ester intermediate, which, with subsequent steps, gives the desired final products of Fig. 5.

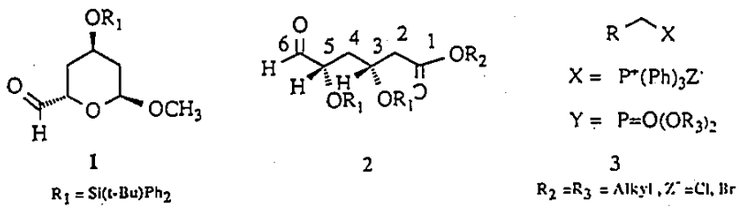


Figure 6

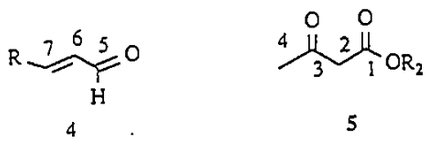
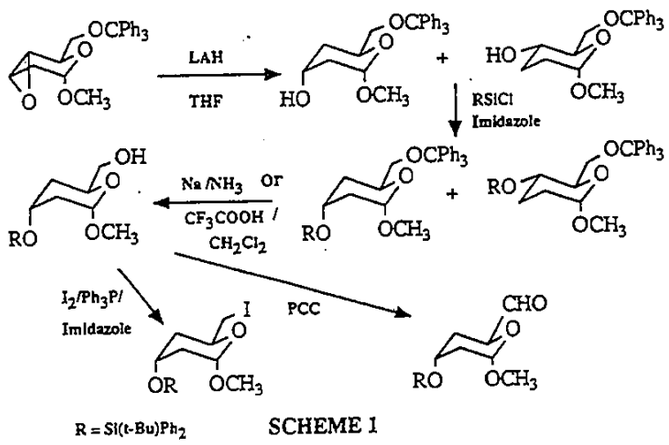


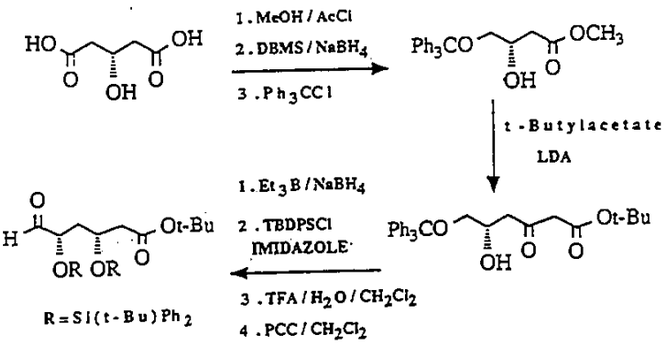
Figure 7

A. Synthesis of Synthon 1 and 2, Fig. 6 (Scheme 1 and Scheme 2)

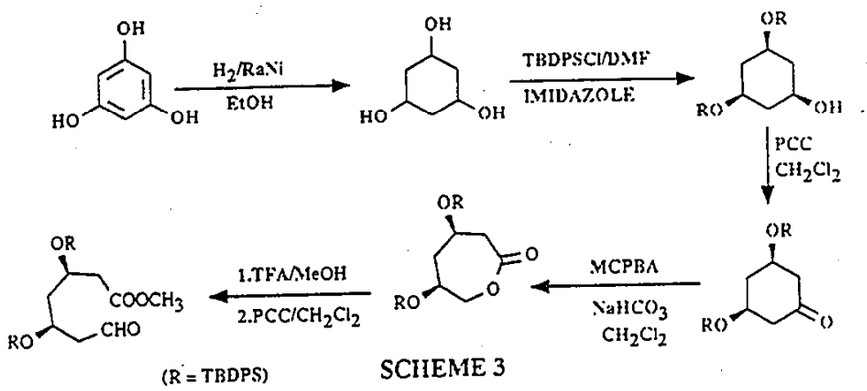
Synthon 1 has been synthesized starting from D-glucose via the key lithium aluminum hydride reductive opening of the epoxide as depicted in Scheme 1.[5] The desired axial alcohol could be separated from the equatorial isomer by preparation of the silyl derivatives. The protected axial alcohol on PCC oxidation gave the desired lactol aldehyde.



SCHEME 1



SCHEME 2



SCHEME 3

Synthesis of chiral Synthon 2 has been accomplished starting from S-malic acid in excellent yields via an eight-step reaction as illustrated in Scheme 2.[6]

On the other hand, an efficient route was developed for the preparation of racemic Synthon 2 starting from 1,3,5-trihydroxy benzene through a five-step reaction sequence shown in Scheme 3.[7]

B. Choice of R and Synthesis of Intermediates 3, Fig. 6, and 4, Fig. 7

Our initial efforts at the synthesis, and the biological results of C-3, C-5-dihydroxy acid derivatives (Fig. 5) wherein choice of R was based on elements of substructures of coenzyme A (Fig. 4) or the decalin ring structure of compactin (Fig. 3) were not promising.[8] This led us to question the importance and the necessity of the complex stereochemistry and the substituents present in the decalin ring of compactin and turn our attention towards the preparation of C-3, C-5-dihydroxy acid derivatives (Fig. 5) wherein R was a naphthalene ring. During these ongoing efforts, we were being encouraged and helped by two important publications[9] describing mevalonolactone derivatives of the general structure 6 and 7 as inhibitors of HMG-CoA reductase (Fig. 8).

Further exploration of R in Fig. 5 led to the first interesting indolyl derivative (Fig. 9) comparable to compactin in its inhibitory activity against HMG-CoA reductase.[10(a)]

An extensive and rapid analog program allowed the choice of XU 62-320

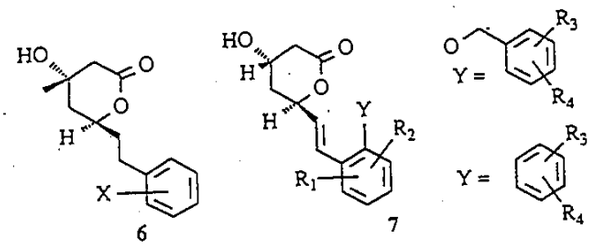


Figure 8

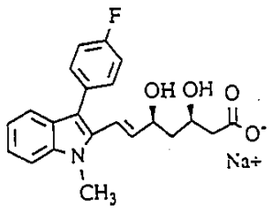


Figure 9

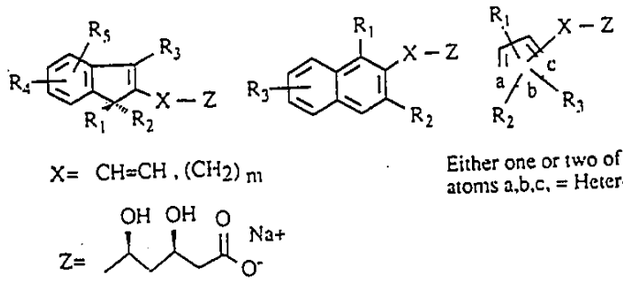
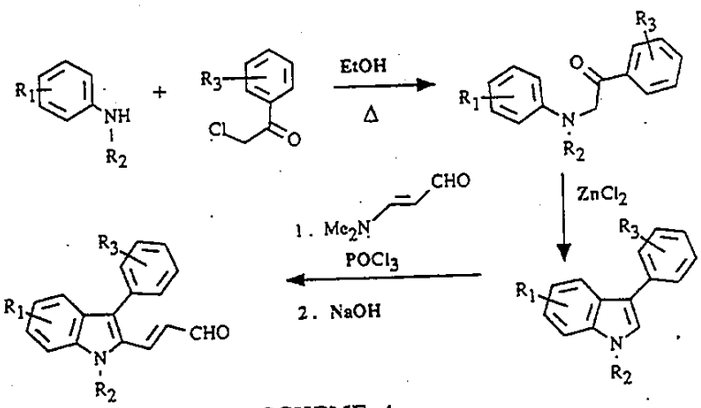


Figure 10

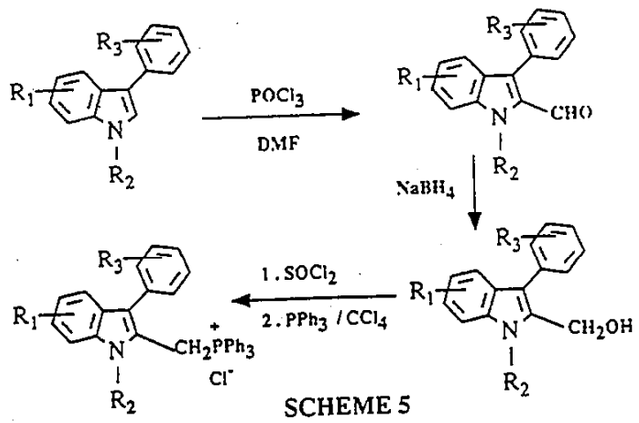
(Fig. 1) as a candidate for extensive biological testing. Currently, fluvastatin (XU 62-320) is in clinical Phase III trials.

With the discovery of XU 62-320, the stage was set for a large number of variations of R in Fig. 5. Extensive work at the Sandoz Research Institute has led to many novel HMG-CoA reductase inhibitors, some of which are discussed in this paper as shown in Fig. 10,[10] and Figs. 12–14.[21–23]

Synthesis of the many interesting fragments 3 (Fig. 6) and 4 (Fig. 7) needed for synthesis of final HMG-CoA-R inhibitors are described in Schemes 4–12 below.[10] Since the appearance of Merck & Co., Inc. and Sandoz patents and publications,[5,9,10(a)] extensive efforts have followed in many laboratories worldwide with semi-synthetic and totally synthetic HMG-CoA reductase inhibitors. A brief overview of these reported activities is presented in Section VIII. It is no wonder that in such a feverish pursuit of finding patentable HMG-CoA reductase inhibitors, review of patent and published literature presents overlapping activities in the laboratories of competing pharmaceutical research companies.



SCHEME 4



SCHEME 5

C. Synthesis of Indole Intermediates

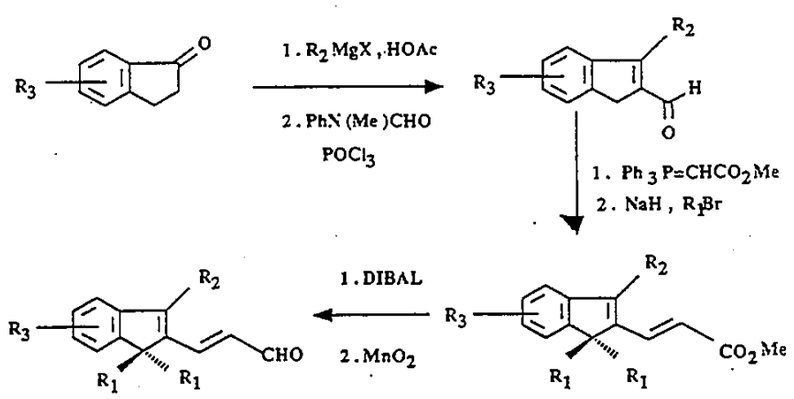
Scheme 4 describes the preparation of α,β-unsaturated aldehydes readily obtained from a variety of 3-phenyl substituted indoles using dimethylamino-acrolein and phosphorous oxychloride, while the triphenyl phosphonium salts of indolyl derivatives are prepared via the 2-formyl and 2-hydroxymethyl indoles using standard procedures (Scheme 5).[10(a)]

D. Synthesis of Indene Intermediates

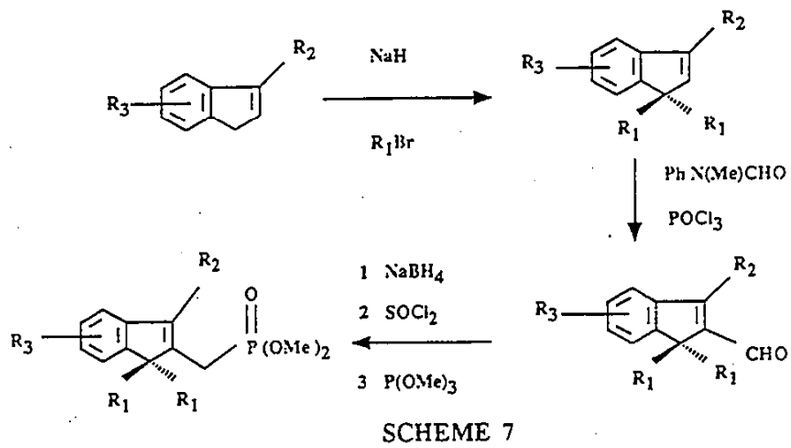
A variety of indenyl-α, β-unsaturated aldehydes and phosphonates have been synthesized via a six-step reaction sequence as depicted in Schemes 6 and 7.-The synthesis of these derivatives involves the preparation of the desired indenes from the respective indanones followed by either formylation at C-2 and subsequent alkylations at C-1 or vice versa, and then processing the formyl group through standard reaction sequences to the desired intermediates.[10(b)]

E. Synthesis of Naphthalene Intermediates

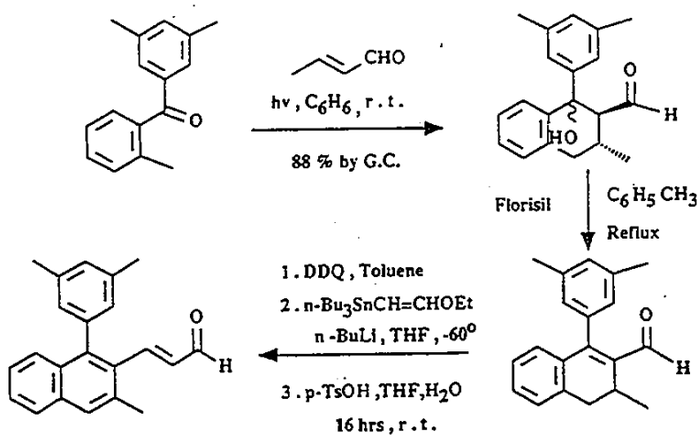
For the preparation of naphthalene derivatives, a novel photochemical route[11] was exploited to give the key hydroxy aldehyde, which on dehydration provides the ene aldehyde. Dehydrogenation of the ene aldehyde and chain



SCHEME 6



SCHEME 7

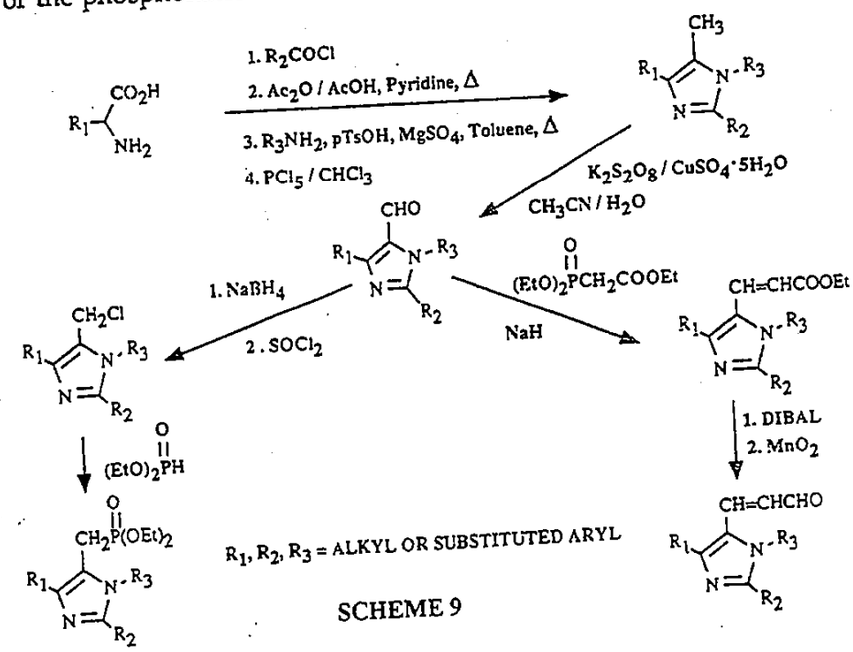


SCHEME 8

extension of the formyl group then leads to the desired α,β-unsaturated aldehydes[10(c)] (Scheme 8).

F. Synthesis of Imidazole Intermediates

Highly substituted imidazole derivatives with the desired functional group at the desired C- or hetero- atom are not well described in the literature. Synthesis of the required imidazole intermediates was best accomplished starting from the respective glycine derivatives as shown in Scheme 9. The key step in the synthetic pathway involves oxidation of the methyl group with potassium persulfate to give the 5-formyl imidazole derivatives, which through standard reaction sequences give the needed α,β-unsaturated aldehydes or the phosphonates.[10(d)]



SCHEME 9

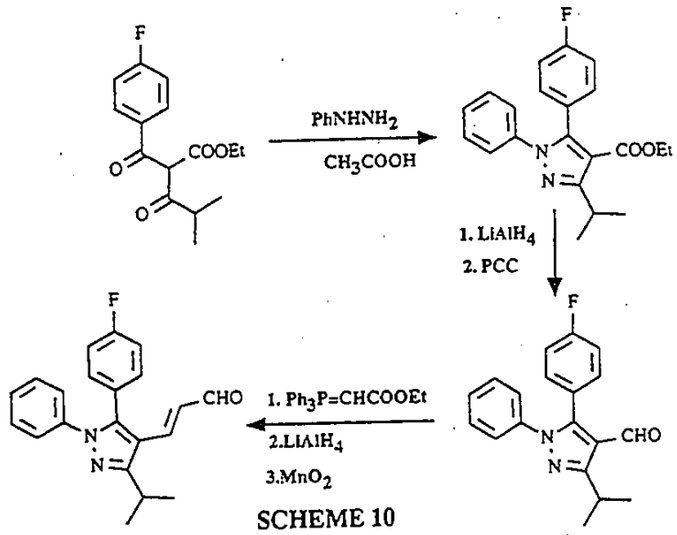
G. Synthesis of Pyrazole Derivatives

A number of pyrazole intermediates have been prepared via procedures dependent on whether one needs the 1,5 (Scheme 10), the 1,3 (Scheme 11), or the 3,4 (Scheme 12) disubstituted pyrazole intermediates. 2,3-disubstituted pyrazole derivatives are obtained through the reaction of the appropriate diketoesters with aryl-hydrazines, requiring separation from the concomitant formation of the corresponding 1,3 isomer (Scheme 10).[10(e)]

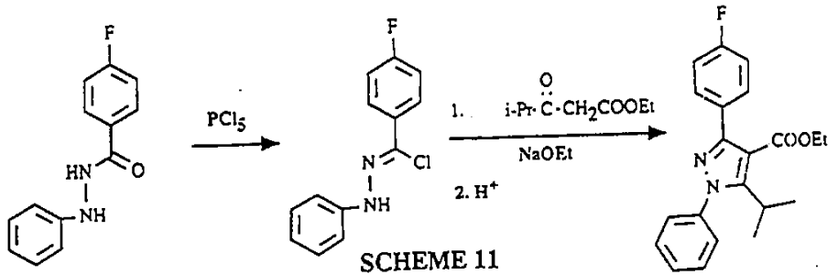
1,3-disubstituted pyrazoles can be best synthesized from the imide chloride on reaction with the acetoacetate derivatives (Scheme 11), while the ring closure of arylhydrazones give the desired 3,4 diaryl pyrazole intermediates (Scheme 12).

H. Synthesis of HMG-CoA-R Inhibitors

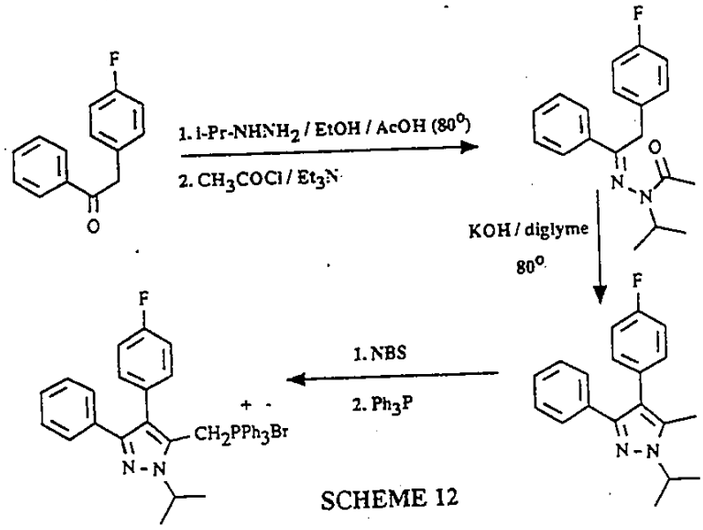
All of the intermediates of the many different prototypes described above in Schemes 4–12 could be converted to the final HMG-CoA reductase inhib-



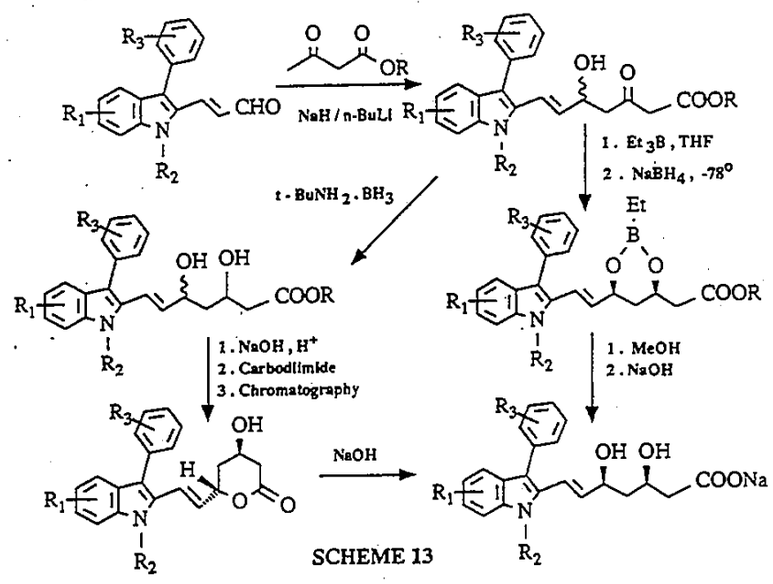
SCHEME 10



SCHEME 11



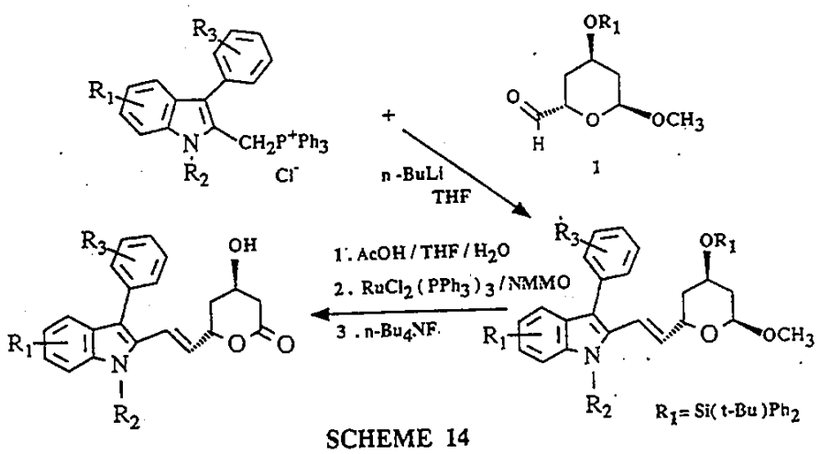
SCHEME 12



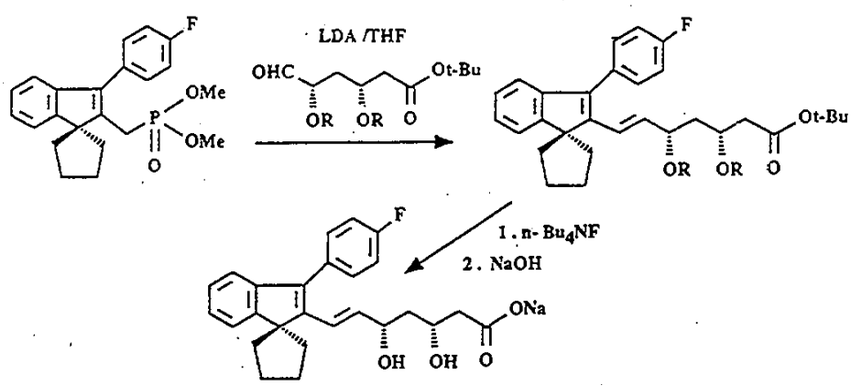
SCHEME 13

itors either using the linear route involving the "dianion chemistry," or the coupling of the respective phosphonates or phosphonium salts with the chiral Synthons 1 and 2 (Fig. 6) or with the racemic Synthon 3 (Fig. 6).

1. Linear Route. Synthesis using the linear route is illustrated in Scheme 13 for the preparation of the indolyl HMG-CoA reductase inhibitors. The key step involves the reduction of the hydroxyketoester using trialkylborane/THF/MeOH with sodium borohydride at −78° (Ref. 12) to give the mixture of desired erythro and threo isomers in the ratio of 95–98:5–2%, respectively. In some cases, the boronic esters can be crystallized, which on methanolysis and subsequent hydrolysis with sodium hydroxide provide the desired sodium salts. Nonstereoselective reduction of hydroxyketoester with borane t-butylamine complex has been used to prepare a mixture of *cis* and *trans* lactones separable on flash chromatography.[10(a)]



SCHEME 14



R = Si (t-Bu) Ph_2 SCHEME 15

2. *Convergent Route.* For illustrative purposes, a convergent route for the preparation of chiral indolyl HMG-CoA reductase inhibitors using the silyl protected Synthon 1 is depicted in Scheme 14. The crucial step in this reaction pathway is the oxidation of lactol with $RuCl_2(PPh_3)_3$/NMMO.[10(f)]

Scheme 15 shows the use of silyl-protected aldehyde Synthon 2 (derived from malic acid) for the synthesis of indenyl HMG-CoA reductase inhibitors.[13]

IV. BIOLOGICAL RESULTS AND DISCUSSION

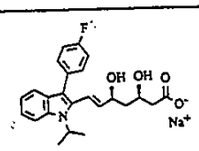
A. Results in *in vitro* HMG-CoA Reductase Microsomal Assay and in *in vivo* Cholesterol Biosynthesis Assay

All initial studies to assess the inhibitory potency of various compounds against HMG-CoA reductase were conducted with rat liver microsomal suspensions, freshly prepared from male Sprague-Dawley rats, using an assay for HMG-CoA reductase activity as described in Ref. 14. The potency of each compound is expressed as IC_{50} (in μmoles, the concentration which inhibits to the extent of 50% conversion of the substrate HMG-CoA to mevalonate) and for structure activity relationship compared either to compactin = 1 or to XU 62-320 = 1. Tables I–XII summarize the most salient features of structure activity relationships for a few of the varied structural prototypes as HMG-CoA reductase inhibitors being currently studied at the Sandoz Research Institute. In Tables X–XIII, the Relative Potency column is derived from the IC_{50} values of each compound vs. compactin in the *in vitro* rat microsomal HMG-CoA reductase assay.

B. SAR of Fluvastatin (XU 62-320) Analogs

Table I compares the *in vitro* inhibitory activity against HMG-CoA reductase of XU 62-320 with compactin and lovastatin and as their corresponding sodium salts. XU 62-320 is 146- and 52-fold more active than compactin and Lovastatin, respectively. As compared to the respective sodium salts of compactin and Lovastatin, XU 62-320 is 22- and 10-fold more potent in inhibiting HMG-CoA reductase. It is important to note that current clinical studies are being conducted with XU 62-320, which is a dihydroxy acid sodium salt. In contrast,

Table I
Comparison of Microsomal HMG-CoA Reductase Inhibitory Activity

	Compound	IC_{50} (μM)	Relative Potency*
	XU 62-320	0.0069	146.1
	Compactin	1.011	1.0
	Lovastatin	0.352	2.8
	Na Salt Compactin	0.154	6.5
	Na Salt Lovastatin	0.068	14.8

*As compared to Compactin = 1

compactin used in clinical studies and Lovastatin (Mevacor®), now marketed, both exist as the lactone forms (Fig. 1).

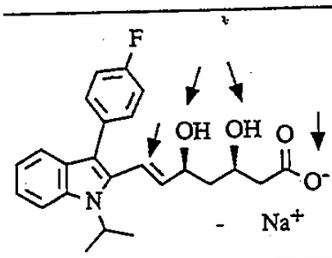
Features of the side chain are very important for maximal inhibitory activity as shown in Table II. Erythro configuration, as well as the double-bond configuration, are very important [anti-isomer 17-fold less active and dramatic loss of activity for one (Z) diene isomer]. The dihydro derivative, as well as the ester and the lactone forms, are considerably less active. Maximal inhibitory activity resides in the 3R, 5S antipode.

The importance of the features of the side chain described in Table II for the indole series holds true as well for all the prototypes to be described later and hence, during the discussion of SAR of these prototypes, these aspects will not be reemphasized. HMG-CoA, the substrate for the HMG-CoA reductase, has at C-3 a methyl group. It was important to determine if an analog of XU 62-320 carrying a methyl group at C-3 would be more potent. Surprisingly, introduction of methyl group at C-3 in either of syn- or anti-configuration was considerably less active (Table III).

Studies of the effects of the substituents in the 3-phenyl ring of the indole moiety are given in Table IV. Either electron-withdrawing or electron-donating substituents in the 3-phenyl ring tend to decrease the potency, which is unaffected by the presence of alkyl groups.

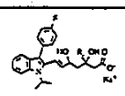
Electron-donating or electron-withdrawing substituents (not shown in Table IV) or bulky alkyl groups at C-5 of the indole moiety led to decrease of potency. However, alkyl or alkoxy groups at C-4 and C-6 tend to maintain or enhance the potency slightly (Table V).

Table II
SAR of Variations in the Side Chain

	Compound	IC_{50} (μM)	Relative Potency*
	XU 62-320	0.0069	1.0
	3R, 5S	0.0024	2.8
	3S, 5R	0.08	0.086
	Na Salt, ANTI	0.12	0.057
	Methyl Ester, SYN	0.052	0.13
	Trans Lactone	0.029	0.23
	CIS(Z) Double Bond	0.62	0.011
	Dihydro (Reduced Double Bond)	0.114	0.06

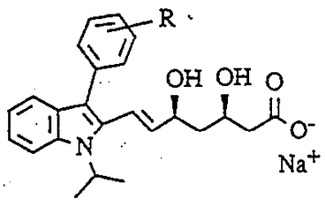
*As compared to XU 62-320 = 1.

Table III
Comparative Activity of XU with the 3-Methyl Analogs

Compound	IC_{50} (μM)	Relative Potency*
		
XU 62-320	0.0069	1.0
R = CH_3, SYN	0.14	0.049
R = CH_3, ANTI	0.51	0.013

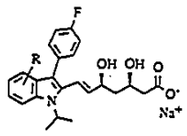
*As compared to XU 62-320 = 1

Table IV
SAR for the Substituents of the 3-Phenyl Ring

R	IC_{50} (μM)	Relative Potency*
		
4-F	0.0069	1
2-Me	0.14	0.049
2-Me, 4-F	0.004	1.7
3-Me, 4-F	0.009	0.76
3,5-diMe, 4-F	0.02	0.345
3,5-diMe	0.005	1.38
H	0.017	0.40
4-CF_3	0.09	0.076
4-SCH_3	1.152	0.006
4-COONa	>10.0	

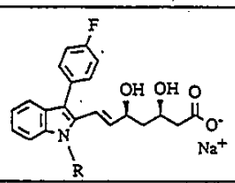
*As compared to XU 62-320 = 1

Table V
SAR for the Substituents of the Benzenoid Indole Ring

R	IC_{50} (μM)	Relative Potency*
		
H (62-320)	0.0069	1.0
4,6-diMe	0.011	0.62
4,6-dii-Pr	0.005	1.38
5-C_6H_{11}	24.0	0.0022
6-OCH_2Ph	0.0026	2.65

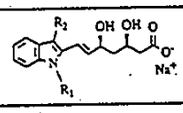
*As compared to XU 62-320 = 1

Table VI
SAR for the Substituents of Indolyl-Nitrogen

R	IC_{50} (μM)	Relative Potency*
		
i-Pr (62-320)	0.0069	1.0
CH_3	0.62	0.011
C_2H_5	0.096	0.071
C_6H_{11}	50	0.0001
CH_2CH_2Ph	49.4	0.0001
$CH_2CH(CH_3)_2$	0.245	0.028

*As compared to XU 62-320 = 1

Table VII
SAR for Reversing Substituents at 1 and 3 Positions

	R_1	R_2	IC_{50} (μM)	Relative Potency*
	i-Pr (62-320)	4-FC_6H_4, syn	0.0069	1.0
	4-FC_6H_4	i-Pr, syn	0.0016	4.3
	i-Pr	4-FC_6H_4, anti	0.12	0.057

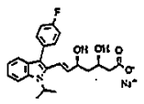
*As compared to XU 62-320 = 1

Most sensitive to the activity is the substituent on the nitrogen of the indole moiety (Table VI). Optimal activity is provided by the isopropyl group, while marked loss in potency results with either bulky alkyl or phenethyl groups.

Reversing the substituents on N-1 and C-3 of the indole moiety to give (Table VII) 3-isopropyl-N-p-fluorophenyl analog of XU 62-320 gives a 4-fold increase in potency.

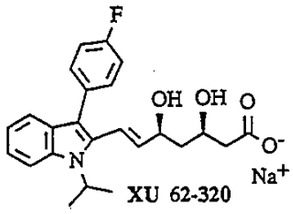
Most of the substances with a reasonable level of activity against HMG-CoA reductase in *in vitro* microsomal assay were studied *in vivo* for their effects on inhibition of sterol biosynthesis. Results are expressed as ED_{50} (mg/kg), effective concentration which inhibits to the extent of 50% incorporation of C^{14} acetate into sterols in rats when administered as appropriate doses of drug substances as compared to controls receiving vehicle alone. Table VIII shows that *in vivo* XU 62-320 is about 40- and 4.5-fold more potent than compactin and Lovastatin, respectively, in inhibiting endogenous cholesterol synthesis in rats. For most substances, although not for all, the relative

Table VIII
Relative Potency for Inhibition of Cholesterol Biosynthesis

	Compound	ED_{50} (mg/kg)	Relative Potency*
	XU 62-320	0.093	37.6
	Compactin	3.5	1.0
	Lovastatin (Monacolin)	0.414	8.4

*As compared to Compactin = 1

Table IX
SAR for Cholesterol Biosynthesis Inhibition

	Compound	ED_{50} (mg/kg)	Relative Potency*
	XU 62-320	0.093	1.0
	3R, 5S	0.056	1.66
	3S, 5R	>0.5	
	Na Salt, Anti	1.37	0.067
	Methyl ester, Syn	0.40	0.23
	Trans Lactone	0.33	0.28
	Dihydro (Reduced Double Bond)	1.23	0.075

XU 62-320

*As compared to XU 62-320 = 1

Table X
SAR of Indene Derivatives

R_1	Relative Potency*
$(CH_2)_4$	202
(Racemic) $(CH_2)_4$	337
(3R, 5S)	38
$(CH_2)_2$	1.5†
$(CH_2)_5$	<.2
CH_2CH_3	2
CH_3	8
H,iPr	

R_2	
Phenyl	88†
3,5-Dimethylphenyl	146
iPr	<0.5
Cyclohexyl	16.5

R_3	
4-Me	114
6-Me	181
7-Me	24
6-OMe	130
4,6-$(OMe)_2$	60

*As compared to Compactin = 1
†As its Ethyl Ester

potency determined in *in vitro* microsomal assay against HMG-CoA reductase parallels the *in vivo* activity in rats for the inhibition of ^{14}C-acetate into sterols.

As an example, comparison of Tables II and IX reveals the relative potency of several analogs of XU 62-320 when compared in *in vitro* and in *in vivo*. Thus, as compared to XU 62-320, the anti-isomer is ~ 17- (Table II) and ~ 15-fold (Table IX) less active than XU 62-320 in *in vitro* and in *in vivo* assays, respectively. Similarly, close parallelism prevails for the ester (less active ~ 7.5-fold, *in vitro* vs. 4.3-fold, *in vivo*), *trans*-lactone (less active 4.2-fold, *in vitro* vs. 3.5, *in vivo*) and the dihydro derivative (less active 16.5-fold, *in vitro* vs. 13-fold *in vivo*).

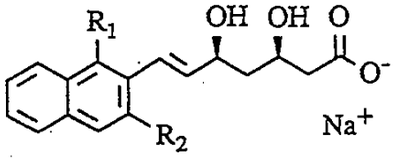
C. SAR of Indene Derivatives

The structure activity relationships for the indene derivatives can be best summarized as follows: Maximal activity is obtained with a spiro cyclopentyl group at C-1, again emphasizing the importance of the bulky group in the vicinity of the dihydroxy acid side chain. At C-3 the best substituent is 4-F-phenyl, while the optimal substituent for the benzenoid portion of the indene moiety is hydrogen (see Table X).

D. SAR of Naphthalene Derivatives

The most interesting part of the structure activity relationships for this group of compounds is the difference observed in the potency of 1-(4-F-

Table XI
SAR of Napthalene Derivatives



R_1	R_2	Relative Potency*
4-F-Ph	H	0.10
4-F-Ph	CH_3	8
4-F-Ph	Et	19
4-F-Ph	i-Pr	22
3,5-diMe-Ph	CH_3	56
Ph	CH_3	2
i-Pr	4-F-Ph	337
i-Pr	Ph	144

*As compared to Compactin = 1

phenyl)-3-isopropyl derivative vs. 1-isopropyl-3-(4-F-phenyl) compound (22 times more potent vs. 337 as compared to compactin) (see Table XI).

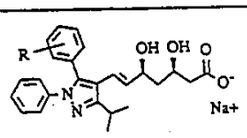
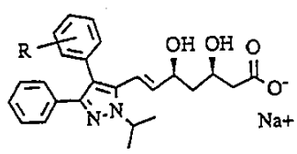
E. SAR of Pyrazole Derivatives

Table XII illustrates the structure activity relationships for a few of the many pyrazole derivatives prepared. Here, too, the optimal substituents are the 4-F-phenyl and isopropyl group adjacent to the dihydroxy acid side chain. The dihydro and the 5-keto derivatives are substantially less potent. 1,3-diaryl-substituted pyrazole derivatives show decreased inhibitory activity (not shown in the table) in contrast to the 1,5 and 3,4-diaryl-substituted compounds, which tend to have comparable potency.

F. SAR of Imidazole Derivatives

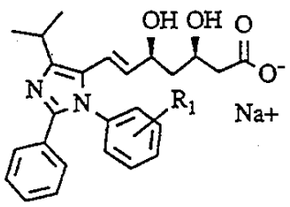
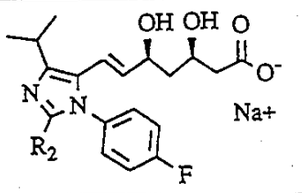
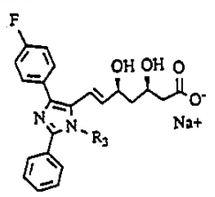
To emphasize the most salient features of the structure activity relationships for the imidazole derivatives, only a few of the derivatives prepared are tabulated in Table XIII. Optimal activity is obtained with 1,2-diaryl derivatives

Table XII
SAR of Pyrazole Derivatives

	R	Relative Potency*
	4-F	60
	4-F (6,7 Dihydro)	5.9
	4-F (5 Keto)	3.5
	H	5.6
	3,5 Dimethyl	4.1
	4-F	30

*As compared to Compactin = 1

Table XIII
SAR of Imidazole Derivatives

		Relative Potency*
	R_1	
	4-F (Racemic)	337
	4-F (3R, 5S)	532
	p-Cl	84
	p-Br	20
	3,5-Di-Me	7
	3,5-Di-Cl	10
	R_2	
	i-Pr	4.4
	t-Butyl	4.4
	cyclohexyl	4.8
	2-Thienyl	202
	1,4-Biphenylyl	35
	p-Dimethylamino-phenyl	56
	p-Nitro-phenyl	72
	R_3	
	i-Pr	1.1
	4-F-Phenyl	<0.1

*As compared to Compactin = 1

with the 4-F substituent preferred in the phenyl ring on nitrogen and H atom being the preferred substituent for the phenyl ring at C-2. Alkyl substituents at C-2 tend to lead to considerable loss of activity. The 1,3-diaryl-substituted imidazole derivatives suffer a dramatic loss of activity when compared to the very potent 1,2-diaryl compounds.

V. EFFECTS OF FLUVASTATIN (XU 62-320) ON PLASMA LIPOPROTEIN LEVELS

Fluvastatin (XU 62-320) has been studied in several species for its effects on serum lipoprotein levels.

Significant and sustained reductions of *rat* serum VLDL + LDL-cholesterol have been observed after treatment of rats with XU 62-320. However, these lipoprotein changes are not observed after chronic dosing of normolipemic rats either with compactin or lovastatin.

In the beagle dog, after three weeks of administration, fluvastatin lowers serum LDL + VLDL-cholesterol to the extent of ~ 47% either at 2 mg/kg/day given once a day or 1 mg/kg/day given twice a day. A comparable effect on VLDL + LDL-cholesterol is observed with compactin at a dose of 20 mg/kg/day

given once a day. In the Rhesus monkey, a reduction of 30% in serum VLDL + LDL-cholesterol is achieved with fluvastatin at a dose of 30 mg/kg/day at the end of three weeks of daily administration.[15]

VI. TOXICOLOGICAL, DRUG METABOLISM, AND PHARMACOKINETIC STUDIES OF FLUVASTATIN (XU 62-320)

The safety, drug metabolism, and pharmacokinetic evaluation of fluvastatin (XU 62-320) has been extensively carried out in acute, subchronic, and chronic rat, dog, monkey, and mouse studies at Sandoz Research Institute. These studies have allowed extensive clinical trials with the first totally synthetic HMG-CoA reductase inhibitor.[16]

VII. HUMAN STUDIES WITH FLUVASTATIN (XU 62-320)

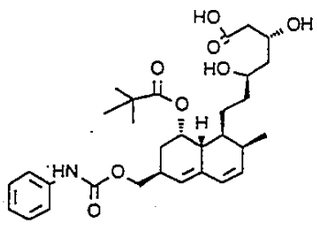
Through completion of Phase II multi-center dose-response and dose-frequency trials, in all 658 subjects have been randomized to treatment with fluvastatin (XU 62-320) in double-blind safety and efficacy trials with another 269 placebo subjects serving as controls. Fluvastatin (XU 62-320) was well tolerated at all doses studied and was free from serious or unexpected adverse effects. Dose-dependent mean reductions of 11% to 21% in total plasma cholesterol and 15% to 28% in LDL-cholesterol were achieved on 5 to 40 mg QPM of fluvastatin. Dose-dependent mean reductions of triglycerides and a drug-related increase in HDL-cholesterol were also observed. Equivalent reductions of LDL-C (22% vs. 23%) were produced by 20 mg per day of fluvastatin when given as a single dose or divided into a BID regimen. A dose of 20 mg once a day at bedtime gave LDL-cholesterol reductions similar in magnitude to that of the marketed agent lovastatin (Mevacor®).

VIII. OVERVIEW OF PUBLISHED LITERATURE ON HMG-CoA REDUCTASE INHIBITORS

A very large number of reviews have described the importance of HMG-CoA reductase inhibitors for the treatment of elevated serum total cholesterol and LDL + VLDL-cholesterol.[4,17] Also, extensive information is available on the pharmacology and clinical efficacy of lovastatin (Mevacor®, MSD), marketed in the United States,[4,18] simvastatin (Zocor®, MSD),[19] marketed in several European countries but not yet available in the United States, and pravastatin (Mevalotin®, Pravachol®, Sankyo, Squibb), yet marketed only in Japan.[20] However, in this section, an overview is presented (Figs. 11–19), describing the attempts in many laboratories towards the discovery of new HMG-CoA reductase inhibitors since the discovery of compactin lovastatin, simvastatin, pravastatin, and fluvastatin. In Figures 11–19, only one specific representative structure is depicted to describe the varied structural prototypes reported in the literature as HMG-CoA reductase inhibitors.

- Scientists at Merck & Co. continue the derivatization efforts towards semisynthetic derivatives using lovastatin as starting material (Fig. 11).[21] Very many wide variants in the acyloxy side chain at C-8 of mevinolin have been executed. Elegant "Barton-type" chemistry has allowed the functionalization of 6-Methyl group in ring A of mevinolin leading to a large number of derivatives with many functional groups at C-6.

MERCK & Co.



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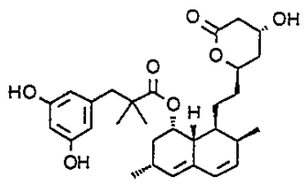

EP 245990

Figure 11

- At Sandoz Research Institute, besides the fluvastatin, indenyl, naphthyl, imidazolyl, and pyrazolyl analogs discussed in this paper, a variety of other HMG-CoA reductase inhibitors have been synthesized varying the heterocyclic hydrophobic domain. These derivatives are described in Figs. 12–14.[22–24] The overlapping reports from other companies on similar derivatives are shown as well in Figs. 12 and 13.[22,23]
- In addition to the HMG-CoA reductase inhibitors described above, scientists at Hoechst, Baeyer, Warner-Lambert, May & Baker, Rorer, Bristol-Myers, Squibb, and Pfizer have published their efforts and their results in this exciting area (Figs. 15–17).[25–27]
- A set of novel structural prototypes as HMG-CoA reductase inhibitors have been claimed by Pan Medica (Fig. 18).[28] One of the Pan Medica candidates is currently in clinical trials.
- Two groups have focused their efforts towards the development of "regulators of HMG-CoA reductase" rather than towards the development of competitive inhibitors.
- Schroepfer *et al.* have studied extensively Cholest-8(14)-en-15-one as a very interesting hypolipoproteinemic agent. This agent is being studied in

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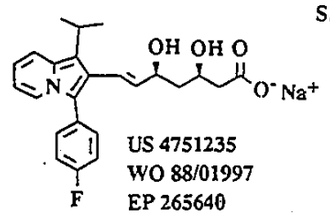

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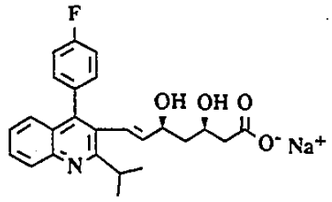
WO 88/01997
EP 265640



Sandoz Patent Pending
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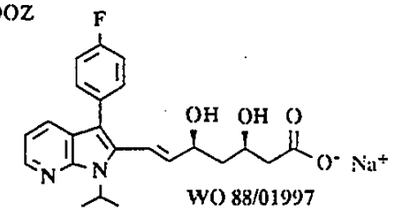
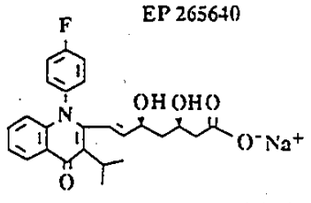
Nissan EP 304063A



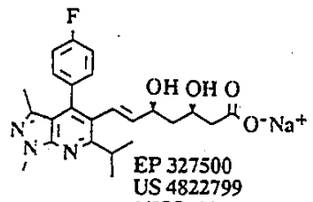
WO 88/01997
EP 265640



Heterocycles 29(8) 1497 (1989)

Figure 12

SANDOZ



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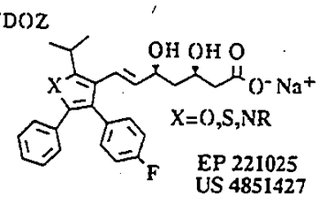
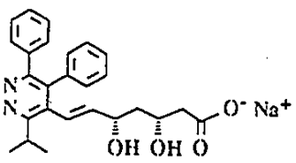
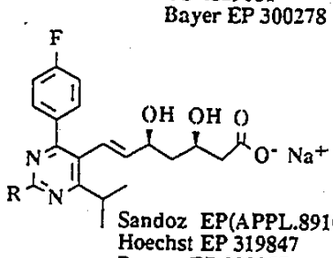


X=O,S,NR

EP 221025
US 4851427
US 4829081
Bayer EP 300278



Sandoz Patent Pending
Hoechst EP 324347



Sandoz EP(APPL.89103931.5)
Hoechst EP 319847
Baeyer EP 330057
W.Lambert US 4868185

Figure 13

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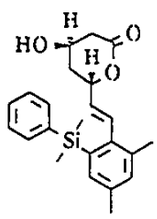
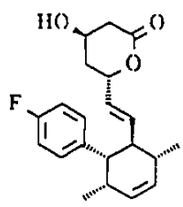
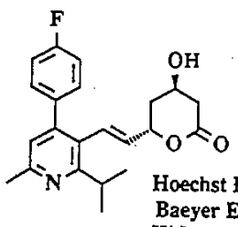


US 4588715

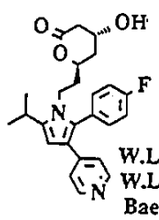


US 4876280

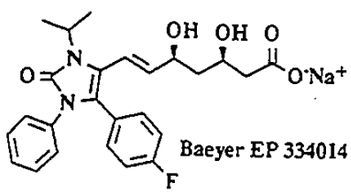
Figure 14



Hoechst EP 319847
Baeyer EP 325130
W.Lambert EP 306929



W.Lambert WO 88/04657
W.Lambert EP 330172
Baeyer EP 334147



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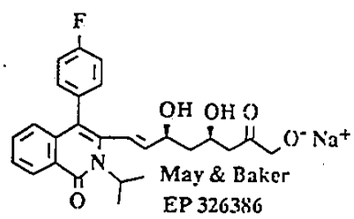

May & Baker
EP 326386

Figure 15

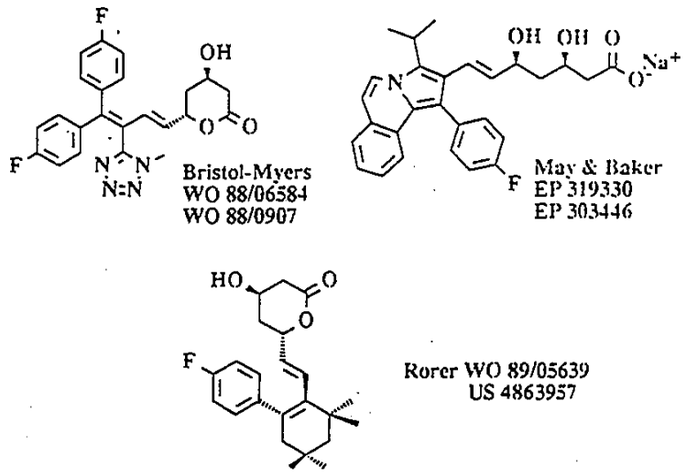

Figure 16

the clinic (Lederle Labs). Taylor *et al.* (DuPont) have attempted to develop inhibitors of HMG-CoA reductase via inhibition of lanosta-8, 24-dien-3 beta-ol-14 alpha-methyldemethylase (Fig. 19).[29]

IX. CONCLUSION

During the discussion on cholesterol biosynthesis inhibitor, Sabine commented, "The development of an effective agent that will lower, and/or prevent a rise in man's level of plasma cholesterol, without accompanying any undesirable side effects, is a pharmacological rainbow at the end of which is an immense pot of gold. Hence, the search for such an agent is conducted with a great deal of vigor, skill, imagination, and money. I myself certainly hope that the attainment of this therapeutic ideal is indeed not a rainbow, but that the possible existence of such an agent is in fact a solid reality and not just a pleasant illusion of light and color."[30]

Since Sabine's remark, HMG-CoA reductase inhibitors have indeed emerged as solid realities and have not remained mere pleasant illusions of light and color. Mevacor®, Zocor®, and Mevalotin® are marketed products showing remarkable efficacy in lowering LDL-cholesterol without serious side effects. Fluvastatin (XU 62-320), being studied intensely in Phase III clinical trials, has shown very good efficacy with no serious adverse effects. Future work will certainly shed more experience not only with Mevacor®, Zocor®, Mevalotin®, and Fluvastatin, but possibly with a host of other HMG-CoA reductase inhibitors reviewed in this paper. Also, in 1989 the worldwide sales of Merck's Mevacor® (launched in September, 1987), being $535 M, speak to the HMG-CoA reductase inhibitor as being the pharmacological rainbow at the end of which is an immense pot of gold.

Excitement has been added to the fascinating story of the development of HMG-CoA reductase inhibitors by the elegant and outstanding work in the laboratories of Nobel laureates Brown and Goldstein, to explain the mechanism of action of these inhibitors. The HMG-CoA reductase inhibitors lower

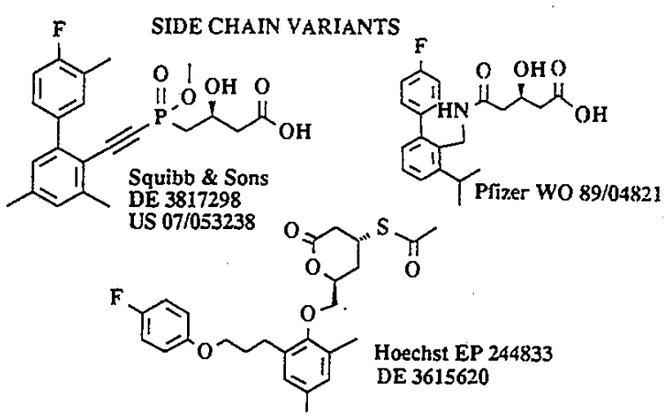

Figure 17

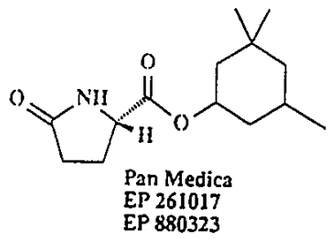

Figure 18

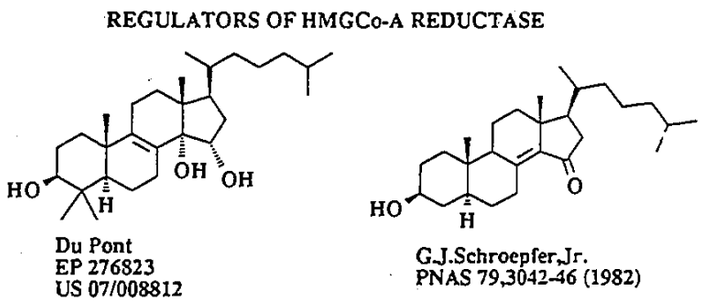

Figure 19

serum lipoprotein levels by up-regulating the lower LDL-receptors. But how do the many different HMG-CoA reductase inhibitors, described in this paper, affect the HMG-CoA reductase activity precisely at the detailed molecular level? The elegant molecular biology efforts in the laboratories of Brown and Goldstein have given us the amino acid sequence of HMG-CoA reductase of several species, but little is known of the detailed tertiary structure of the enzyme. What are the precise conformations of the many HMG-CoA reductase inhibitors, described in this paper, when bound to the active site domain of HMG-CoA reductase? What is the topography of the amino acid residues at the active site of HMG-CoA reductase when one or the other HMG-CoA reductase inhibitors is bound to it? What precise details of molecular recog-

nition are involved and need to be understood to explain the rank-order potency of many of the described analogs of HMG-CoA reductase inhibitors? Fascinating work remains to be done to provide answers to the many interesting unanswered questions in the exciting field of HMG-CoA reductase inhibitors.

ACKNOWLEDGMENTS

I wish to acknowledge the publication of the schemes and tables describing the SAR of the Sandoz compounds by Elsevier in their book *Trends in Medicinal Chemistry '88* (edited by H. van der Goot *et al.*). The extensive work at Sandoz Research Institute on HMG-CoA reductase inhibitors described in part in this paper is truly an outcome of a cohesive team effort of a very large number of dedicated and creative individuals. Most important original contributors to be recognized are: In the Medicinal Chemistry Department, for indole derivatives: H. F. Schuster, R. Stabler, J. Kratunis; for indene derivatives: S. Wattanasin, R. Patel; for naphthalene derivatives: P. L. Anderson, S. W. Meyers, N. A. Paolella; for pyrazole derivatives: J. R. Wareing, M. Martin, C. F. Jewell, Jr., R. Stabler; for imidazole derivatives: J. R. Wareing, J. M. Leginus, J. Linder, G. T. Lee, R. Stabler, M. Martin, L. Widler; for chiral synthon from D-glucose: J. R. Wareing, C. E. Fuller; for synthesis of chiral derivatives using chiral synthon from D-glucose: J. R. Wareing, C. F. Jewell, L. Widler; for coordination of the project: R. E. Damon; in the Process Research and Chemical Development Department, for the chiral synthon from S-malic acid and its use: P. Kapa, K. M. Chen, O. Repic and G. E. Hardtmann; for the racemic synthon and its use: P. Kapa and O. Repic; for large scale preparation and many important improvements of the processes for intermediates and final products: R. E. Walkup, S. Palermo, J. Linder, G. T. Lee, M. Thiede; in the Pharmacology Department, for in *in vivo* testing: R. G. Engstrom, D. B. Weinstein, J. B. Eskesen, M. L. Rucker, R. Miserendino. The success of this work is, in large part, due to our collaboration with Prof. T. Scallen, Department of Biochemistry, University of New Mexico, Albuquerque, New Mexico, who has carried out all the *in vitro* studies. Finally, many thanks are extended for the efforts of J. Birch and P. Schaefer for the preparation of this manuscript.

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(B^+); IR (KBr) 3600–3000 (NH_2, OH), 1750, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 253 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 11.05–10.95 (s, 1 H, 7-OH, D_2O exchangeable), 7.10–6.90 (br, 2 H, NH_2, D_2O exchangeable), 4.95–4.80 (m, 1 H, H-1′), 4.70–4.50 (br, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.32–1.55 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{10}H_{14}N_6O_2 \cdot 1.25H_2O$) C, H, N.

(±)-*cis*-[4-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2-cyclopentenyl]carbinol (11a). Compound 9a (267 mg, 1 mmol) was processed as described for compound 6a with a reaction time of 20 h at 60 °C. The residual mixture was absorbed onto silica gel (2 g); it was packed into a column (2.0 × 10 cm) and eluted by $CHCl_3$–MeOH (15:1) to yield 11a as white crystals, 204 mg (83%). The crude product was recrystallized from ethanol–water (2:1) to yield 11a: mp 240–242 °C dec; MS (30 eV, 240 °C) *m/e* 247 (M^+), 229 ($M^+ - 18$), 217 ($M^+ - 30$), 151 (B^+); IR (KBr) 3600–3100 (NH_2, OH), 1700, 1650, 1600 cm^{-1} (C=O, C=C, C=N); UV λ_{max} 253, 283 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.80–7.20 (br, 2 H, NH_2, D_2O exchangeable), 6.50–6.30 (s, 2 H, NH_2, D_2O exchangeable), 6.15–6.10 and 5.95–5.90 (dd, 2 H, CH=CH vinyl, J = 5.0 Hz), 5.65–5.55 (m, 1 H, H-1′), 4.75–4.65 (t, 1 H, CH_2OH, D_2O exchangeable), 3.55–3.40 (m, 2 H, CH_2OH), 2.95–2.85 (m, 1 H, H-4′), 2.65–2.55 (m, 1 H, CHH′), 1.90–1.80 (m, 1 H, CHH′). Anal. ($C_{10}H_{13}N_7O \cdot H_2O$) C, H, N.

(±)-*cis*-[3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)cyclopentyl]carbinol (11b). Compound 9b (268 mg, 1 mmol) was processed as described for 9a to yield 220 mg of 11b (88%), which was recrystallized from ethanol–water (1:2) to afford pink-white crystals: mp 223–225 °C; MS (30 eV, 250 °C) *m/e* 249 (M^+), 218 ($M^+ - 31$), 151 (B^+); IR (KBr) 3600–3100 (NH_2, OH), 1700, 1600 cm^{-1} (C=C, C=N); UV λ_{max} 253, 283 nm in 0.1 N HCl; NMR (dimethyl-d_6 sulfoxide) δ 7.85–7.25 (br, 2 H, NH_2, D_2O exchangeable), 6.50–6.30 (s, 2 H, NH_2, D_2O exchangeable), 4.95–4.85 (m, 1 H, H-1′), 4.65–4.60 (t, 1 H, CH_2OH, D_2O exchangeable), 3.50–3.40 (d, 2 H, CH_2OH), 2.35–1.60 (m, 7 H, H-4′, CH_2CH_2, CHH′). Anal. ($C_{10}H_{15}N_7O$) C, H, N.

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Registry No. 1a, 61865-50-7; 1b, 65898-98-8; 2a, 122624-72-0; 2b, 78795-20-7; 3a, 122624-73-1; 3b, 122624-74-2; 4a, 122624-75-3; 4b, 122624-76-4; 5a, 122624-77-5; 5b, 122624-78-6; 6a, 118237-87-9; 6b, 118237-86-8; 7a, 118353-05-2; 7b, 112915-00-1; 8a, 118237-88-0; 8b, 120330-36-1; 9a, 122624-79-7; 9b, 122624-80-0; 10a, 122624-81-1; 10b, 122624-82-2; 11a, 122624-83-3; 11b, 122624-71-9; 2-amino-4,6-dichloropyrimidine, 56-05-3; *p*-chloroaniline, 106-47-8.

Inhibitors of Cholesterol Biosynthesis. 1. *trans*-6-(2-Pyrrol-1-ylethyl)-4-hydroxypyran-2-ones, a Novel Series of HMG-CoA Reductase Inhibitors. 1. Effects of Structural Modifications at the 2- and 5-Positions of the Pyrrole Nucleus

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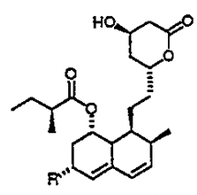
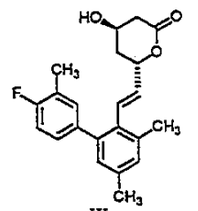
A novel series of *trans*-6-(2-pyrrol-1-ylethyl)-4-hydroxypyran-2-ones and their dihydroxy acid derivatives were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. A systematic study of substitution at the 2- and 5-positions of the pyrrole ring revealed that optimum potency was realized with the 2-(4-fluorophenyl)-5-isopropyl derivative 8x (Table III), which possessed 30% of the in vitro activity of the potent fungal metabolite compactin (I). A molecular modeling analysis led to the description of a pharmacophore model characterized by (A) length limits of 5.9 and 3.3 Å for the 2- and 5-substituents, respectively, as well as an overall width limit of 10.6 Å across the pyrrole ring from the 2- to the 5-substituent and (B) an orientation of the ethyl(ene) bridge to the 4-hydroxypyran-2-one ring nearly perpendicular to the planes of the parent pyrrole, hexahydronaphthalene, and phenyl rings of the structures examined (Figure 3, θ = 80–110°). Attempts to more closely mimic compactin's polar isobutyric ester side chain with the synthesis of 2-phenylpyrroles containing polar phenyl substituents resulted in analogues (Table III, 8m–p) with equal or slightly reduced potencies when compared to the 2-[(unsubstituted or 4-fluoro)phenyl]pyrroles, supporting the hypothesis that inhibitory potency is relatively insensitive to side-chain polarity or charge distribution in this area.

The discovery that the fungal metabolites compactin (I)[1] and mevinolin (II)[2] are not only potent inhibitors of the enzyme HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, but are also effective hypocholesterolemic agents in man[3] has led to a plethora of publications describing synthetic and biological studies of close structural analogues.[4]



I: R = H (compactin)
II: R = CH_3 (mevinolin)



III

The disclosure of a series of very potent 6-(*o*-biphenylyl)-substituted 4-hydroxypyran-2-ones (III) by Willard et al.[5] led us to hypothesize that the key structural

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Scheme I[a]

Method A

$$R_1COCH{=}CH_2 \;(1) + R_2CHO \;(2) \xrightarrow{a} R_1COCH_2CH_2COR_2 \;(3)$$

Method B

$$R_2COCH_2CO_2CH_3 \xrightarrow{b,c} 3$$

[a] (a) 3-Benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, Et_3N, 70 °C. (b) NaH, R_1COCH_2Br. (c) NaOH, CH_3OH.

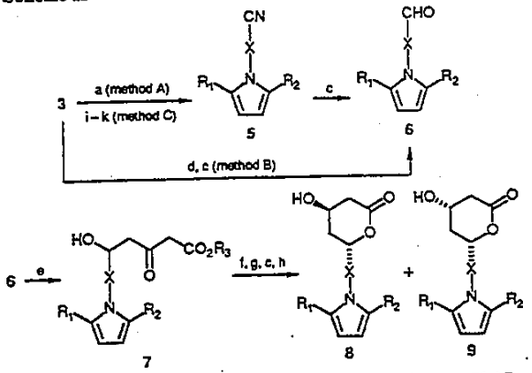
feature possessed by all of these agents was a large lipophilic group held in a particular spatial relationship with respect to the 4-hydroxypyran-2-one moiety. Indeed, examination of CPK models of these inhibitors suggested that the ortho phenyl ring might occupy the same space as the isobutyric ester moiety of compactin and mevinolin. This hypothesis is supported by the 100-fold loss in potency found on hydrolysis of the isobutyric ester group,[6] as well as the suggestion by Nakamura and Abeles that this portion of mevinolin fits into a lipophilic pocket in the active site of HMGR normally occupied by coenzyme A.[7] If this were true, then any connecting group that served to hold the lactone and the lipophilic moiety in the correct spatial relationship might be sufficient for potent inhibition. To investigate this, we selected the pyrrole ring as the anchor for various connecting groups, since there appeared to be sufficient synthetic methodology to allow for the simultaneous introduction of a variety of 2- and 5-substituents. By varying the steric and electronic properties of these substituents, modifying the connecting group, and employing a molecular modeling analysis, we hoped to discern, at least in part, the optimal spatial relationship between the lipophilic group and the 4-hydroxypyran-2-one moiety and use this information in the design of potent HMGR inhibitors.

We herein present our initial investigations into this series of inhibitors that define the structure–activity relationships at the 2- and 5-positions of the pyrrole nucleus and in the connecting group to the lactone ring. Also reported is the molecular modeling study and associated pharmacophore model, which describe conformational requirements of the side chain and steric requirements at the 2- and 5-positions of the pyrrole ring.

Chemistry

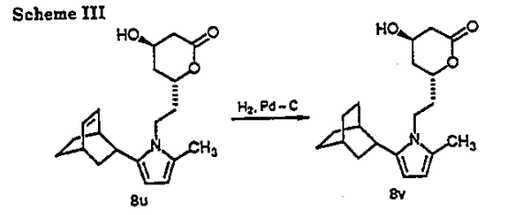
Our general synthetic strategy entailed the preparation of a suitable 1,4-diketone (3, Table I), either by the thiazolium salt chemistry developed by Stetter (Scheme I, method A)[8] or by alkylation of a β-keto ester with an α-halo ketone followed by hydrolysis and decarboxylation (method B). The Stetter reaction proved to be the more versatile and generally higher yielding of the two. Paal–Knorr cyclization with 3-aminopropionitrile or an ω-amino acetal provided the pyrroles in good yield (Scheme II). The one exception was 1-(4-fluorophenyl)-5,5-dimethyl-

Scheme II[a]

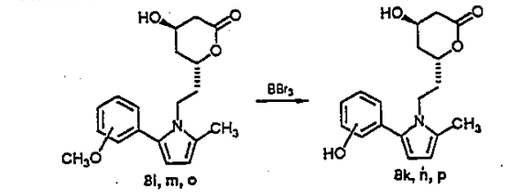


[a] (a) H_2N–X–CN, HOAc, reflux. (b) DIBAL-H, toluene, −78 °C. (c) aqueous HCl. (d) H_2N–X–$CH(OEt)_2$, toluene, cat. p-TSA, reflux. (e) $^-CH_2CO^-CHCH_3CH_3$, THF, −78 °C. (f) n-Bu_3B, $NaBH_4$, −78 °C. (g) H_2O_2, OH^-. (h) Toluene, reflux. (i) H_2N–X–OH, HOAc. (j) CH_3SO_2Cl, pyr. (k) KCN, DMF–H_2O, 100 °C.

Scheme III



Scheme IV



hexane-1,4-dione (3q), which was extremely resistant to cyclization. After considerable experimentation, it was found that treatment with ethanolamine in acetic acid resulted in an exothermic reaction from which the pyrrole was isolated in 84% yield. Mesylation and displacement with potassium cyanide in DMF/H_2O afforded the requisite nitrile. Reduction of the nitriles 5 with DIBAL-H produced the desired aldehydes 6 in good yields (Table II). Condensation of 6 with the dianion of methyl or ethyl acetoacetate under the conditions of Weiler[9] afforded the corresponding alcohols 7. Sih et al.[10] reported the reduction of a related δ-hydroxy-β-keto ester in their synthesis of compactin in which little stereoselectivity (2:1 erythro:threo) was found employing either sodium or zinc borohydride. We, and others,[5b] have found excellent selectivity (>10:1 erythro:threo) employing the procedure of Narasaka and Pai,[11] in which 7 was complexed with a trialkylborane prior to treatment with borohydride at low temperature. The resultant boronate was hydrolyzed with

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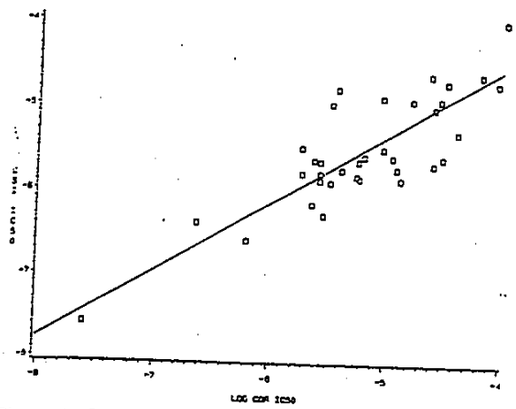


Figure 1. Correlation between CSI and COR IC_{50}'s.

Table I. Substituted 1,4-Diketones

$R_1COCH_2CH_2COR_2$

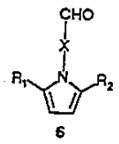
3

no.	R_1	R_2	bp (mmHg), °C	% yield[a] (procedure)
3a[b]	Ph	CH_3	100 (0.1)	80 (A)
3b	4-FC_6H_4	CH_3	46–8	66 (A)
3c	4-PhC_6H_4	CH_3	109–112	73 (A)
3d[c]	4-ClC_6H_4	CH_3	116–8 (1.0)	44 (A)
3e[c]	4-$CH_3OC_6H_4$	CH_3	*b*	57 (A)
3f	3-$F_3CC_6H_4$	CH_3	*b*	38 (A)
3g	3-$CH_3OC_6H_4$	CH_3	143–5 (0.2)	80 (A)
3h	2-$CH_3OC_6H_4$	CH_3	133–5 (1.0)	51 (A)
3i	2-naphthyl	CH_3	87–8	55 (A)
3j	1-naphthyl	CH_3	105 (0.1)	83 (A)
3k		CH_3	114–6 (1.0)	76 (A)
3l		CH_3	*b*	98 (A)
3m[d]	cyclohexyl	CH_3	110 (4)	88 (A)
3n	Ph_2CH	CH_3	*b*	61 (A)
3o	4-FC_6H_4	C_2H_5	*b*	89 (A)–55 (B)
3p	4-FC_6H_4	$CH(CH_3)_2$	133–5 (1.0)	58 (A)
3q	4-FC_6H_4	$C(CH_3)_3$	108–9 (0.2)	56 (A)
3r	4-FC_6H_4	$CH(C_2H_5)_2$	132–3 (0.2)	54 (A)
3s	4-FC_6H_4	cyclopropyl	*b*	75 (A)
3t	4-FC_6H_4	cyclobutyl	132–5 (1.0)	65 (A)
3u	4-FC_6H_4	cyclohexyl	150–5 (0.1)	51 (A)
3v	4-FC_6H_4	CF_3	*b*	25 (B)
3w	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	79–83 (0.2)	53 (A)
3x	3-FC_6H_4	$CH(CH_3)_2$	*b*	90 (B)
3y	2-FC_6H_4	$CH(CH_3)_2$	*b*	95 (A)
3z	2,4-$F_2C_6H_3$	$CH(CH_3)_2$	*b*	77 (A)
3aa	2-$CH_3OC_6H_4$	$CH(CH_3)_2$	138–141 (0.2)	71 (A)
3bb	2,6-$(CH_3O)_2C_6H_3$	$CH(CH_3)_2$	160–2 (2)	68 (B)

[a] All spectral data were consistent with assigned structures. [b] Purified by silica gel chromatography.

aqueous peroxide and base.[12] The dihydroxy acids were then lactonized by refluxing in toluene with azeotropic removal of water. Generally, the lactones were crystalline, such that the small amounts of the cis lactone stereoisomer 9 present were easily removed by recrystallization, providing >95% of the racemic trans stereoisomer (8). The conversion of 8u to 8v was accomplished by hydrogenation over Pd–C at 1 atm (Scheme III). Finally, the phenol analogues 8k, 8h, and 8p were prepared from the corresponding methyl ethers 8i, 8m, and 8o by BBr_3-mediated demethylation (Scheme IV).[13]

Table II. 2,5-Disubstituted Pyrrol-1-yl Carbox- or Benzaldehydes



6

no.	X	R_1	R_2	% yield[a,b] (method)
6a		4-FC_6H_4	CH_3	63 (A)
6b		4-FC_6H_4	CH_3	56 (A)
6c		4-FC_6H_4	CH_3	35 (A)
6d	$-CH_2CH_2CH_2-$	4-FC_6H_4	CH_3	65 (A)
6e	$-CH(CH_3)CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	34 (C)
6f	$-CH_2CH_2-$	4-FC_6H_4	CH_3	45 (A)
6g	$-CH_2CH_2-$	Ph	CH_3	27 (A)
6h	$-CH_2CH_2-$	4-PhC_6H_4	CH_3	60 (A)
6i	$-CH_2CH_2-$	4-$CH_3OC_6H_4$	CH_3	32 (A)
6j	$-CH_2CH_2-$	4-ClC_6H_4	CH_3	56 (A)[c]
6k	$-CH_2CH_2-$	3-$F_3C_6H_4$	CH_3	37 (A)
6l	$-CH_2CH_2-$	3-$CH_3OC_6H_4$	CH_3	68 (A)
6m	$-CH_2CH_2-$	2-$CH_3OC_6H_4$	CH_3	58 (A)
6n	$-CH_2CH_2-$	2-naphthyl	CH_3	50 (A)
6o	$-CH_2CH_2-$	1-naphthyl	CH_3	23 (A)
6p	$-CH_2CH_2-$	cyclohexyl	CH_3	60 (A)
6q	$-CH_2CH_2-$		CH_3	63 (A)
6r	$-CH_2CH_2-$		CH_3	22 (A)
6s	$-CH_2CH_2-$	Ph_2CH	CH_3	32 (A)
6t	$-CH_2CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	92 (A)
6u	$-CH_2CH_2-$	4-FC_6H_4	$C(CH_3)_3$	42 (C)
6v	$-CH_2CH_2-$	4-FC_6H_4	$CH(C_2H_5)_2$	46 (A)
6w	$-CH_2CH_2-$	4-FC_6H_4	cyclopropyl	25 (A)
6x	$-CH_2CH_2-$	4-FC_6H_4	cyclobutyl	34 (A)
6y	$-CH_2CH_2-$	4-FC_6H_4	cyclohexyl	22 (A)[d]
6z	$-CH_2CH_2-$	4-FC_6H_4	CF_3	55 (A)
6aa	$-CH_2CH_2-$	3-FC_6H_4	$CH(CH_3)_2$	29 (A)
6bb	$-CH_2CH_2-$	2-FC_6H_4	$CH(CH_3)_2$	17 (A)
6cc	$-CH_2CH_2-$	2,4-$F_2C_6H_3$	$CH(CH_3)_2$	20 (A)
6dd	$-CH_2CH_2-$	2-$CH_3OC_6H_4$	$CH(CH_3)_2$	42 (A)
6ee	$-CH_2CH_2-$	2,6-$(CH_3O)_2C_6H_3$	$CH(CH_3)_2$	36 (A)[e]
6ff	$-CH_2CH_2-$	2,5-$(CH_3)_2C_6H_3$	$CH(CH_3)_2$	43 (A)
6gg	$-CH_2CH_2-$	2-[$(CH_3)_2CHO$]C_6H_4	$CH(CH_3)_2$	79 (A)
6hh	$-CH_2CH_2-$	2-ClC_6H_4	$CH(CH_3)_2$	46 (A)
6ii	$-CH_2CH_2-$		$CH(CH_3)_2$	41 (C)
6jj	$-CH_2CH_2-$	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	60 (A)

[a] Isolated yields after chromatography on silica gel. [b] All compounds possessed ^1H NMR spectra in accord with assigned structure (aldehydic proton, singlet, δ 8.95–9.65). [c] Mp 70–3 °C. [d] Mp 104–6 °C. Anal. C, H, N. [e] Mp 105–7 °C. Anal. C, H, N.

Biological Results

The target lactones (8, Table III) were saponified and tested for their ability to inhibit HMGR employing two protocols. Method I[14] (cholesterol synthesis inhibition screen, or CSI) measured the rate of conversion of [^{14}C]-

(12) A detailed examination of this reaction has appeared: Kathawala, F.; Prager, B.; Prasad, K.; Repic, O.; Shapiro, M.; Stabler, R.; Widler, L. *Helv. Chim. Acta* 1986, *69*, 803–5.

(13) McOmie, J.; Watts, M.; West, D. *Tetrahedron* 1968, *24*, 2289.

(14) Dugan, R.; Slakey, L.; Briedis, A.; Porter, J. *Arch. Biochim. Biophys.* 1972, *152*, 21–7.

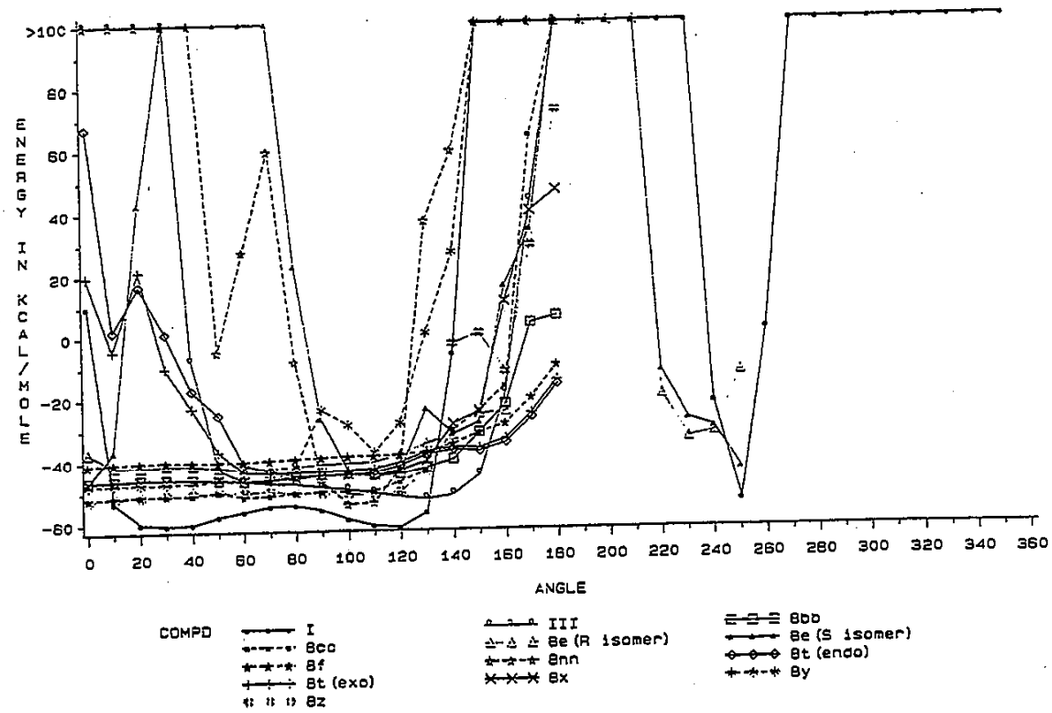


Figure 2. CAMSEQ-II energies calculated for comparable orientations of the lactone side chain. Dashed lines represent less potent analogues (8j, 8z, 8bb, 8cc, and 8nn; CSI $IC_{50} > 5$ μM).

acetate to cholesterol employing a crude liver homogenate derived from rats fed a chow diet containing 5% cholestyramine. Method II[15] (CoA reductase inhibition screen, or COR) was a more specific screen employing a partially purified microsomal enzyme preparation to measure the direct conversion of D,L-[^{14}C]HMG-CoA to mevalonic acid. The biological activities are reported as IC_{50} values and as a ratio to compactin, which was employed as the internal standard in each testing protocol. Compactin consistently displayed an IC_{50} between 0.02 and 0.03 μM. The IC_{50} values from the two assays were moderately correlated (eq 1,[16] Figure 1).

$$\log (IC_{50}, \text{COR}) = 0.81\ (\pm 0.09) \log (IC_{50}, \text{CSI}) - 1.32 \quad (1)$$

$$n = 36,\ r^2 = 0.70,\ F = 81,\ s = 0.39$$

Structure–Activity Relationships

As very little was known about heterocycle-containing inhibitors at the outset of this study, our strategy was to systematically examine each portion of the structure, keeping the 4-hydroxypyran-2-one ring intact. Initially, the optimum chain length between the lactone and the pyrrole ring was determined. A two-carbon bridge (8f) was superior to either a three-carbon (8d) or aryl spacer (8a–c) (Table III). This is consistent with the findings of Stokker et al.[5b]

Holding the bridge constant as ethyl, the structure–activity relationships of the 2 and 5 pyrrole substituents were explored. With 5-methyl substitution (8f–w), high potency was conferred by bulky cycloalkyl 2-substitutents (8s–v). Among 2-(substituted-phenyl)-5-methyl derivatives (8f–r), aside from a length limitation of the 2-substituent (see the molecular modeling section below), no obvious structure–activity relationships could be discerned. Optimum potency resided in the 4-fluorophenyl analogue, 8f. With 2-substitution held constant as the optimal 4-fluorophenyl, potency increased with increasing length of the 5-substituent from methyl (8f) through cyclopentyl (8aa) to a maximum with isopropyl (8x) (length = 2.5 Å; see modeling section below). Potency decreased thereafter to a low of >100 μM with 5-cyclohexyl substitution (8cc).

With 5-substitution held constant as the optimal isopropyl, additional variation of the 2-phenyl substituents, now keeping within the length limit of 5.9 Å suggested by the modeling analysis (8ee–mm), failed to improve the potency over the 2-(4-fluorophenyl)-5-isopropyl derivative, 8x. Indeed, an additional "front-to-back" width limitation (Figure 3) may be apparent with 8ii and 8mm, which project significantly greater bulk in these directions than the other analogs. Finally, of interest is the 2-(4-fluorophenyl)-5-trifluoromethyl analogue 8dd, whose high potency may be due in part to stabilization of the pyrrole ring by the electron-withdrawing trifluoromethyl group, an aspect to be addressed in future communications.

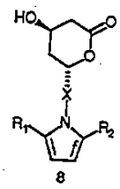
These results, combined with results from the molecular modeling study, confirmed our belief that 8x possessed the optimum substitution pattern, since structural modifications at the 2- and 5-positions, as well as variation of the bridge to the lactone ring, led to decreased potency. A similar conclusion can be inferred from the examination of other 5-membered ring heterocycles reported in the patent literature.[17]

(15) Kita, T.; Brown, M.; Goldstein, J. *J. Clin. Invest.* 1980, *66*, 1094–1100.

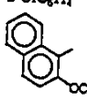
(16) Compounds 8c and 8cc were assigned IC_{50} values of 100 μM so they could be included in the correlation.

(17) Kathawala, F. G. WIPO Patent WO 84/02131, 1984.

Table III. *trans*-6-(2-Pyrrol-1-ylalkyl or -aryl)-4-hydroxypyran-2-ones



8

no.	X	R_1	R_2	mp, °C	% yield	formula[a]	IC_{50},[b,c] μM, CSI	log IC_{50}, CSI	relative potency,[d] CSI	IC_{50},[c,e] μM, COR	log IC_{50}, COR
8a		4-FC_6H_4	CH_3	155–7	32	$C_{22}H_{20}FNO_3$	20	−4.7	0.10	–	–
8b		4-FC_6H_4	CH_3	54–7	29	$C_{22}H_{20}FNO_3$	24	−4.6	0.01	63	−4.2
8c		4-FC_6H_4	CH_3	142–5	21	$C_{22}H_{20}FNO_3$	>100	−4.0	<0.01	>100	−4.0
8d	$-CH_2CH_2CH_2-$	4-FC_6H_4	CH_3	oil	41	$C_{19}H_{22}FNO_3$	53	−4.3	0.02	–	–
8e	$-CH(CH_3)CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	167–9	30	$C_{21}H_{26}FNO_3$	5.0	−5.3	0.50	40	−4.4
8f	$-CH_2CH_2-$	4-FC_6H_4	CH_3	oil	32	$C_{18}H_{20}FNO_3$	0.51	−6.3	0.90	2.8	−5.6
8g	$-CH_2CH_2-$	Ph	CH_3	89–91	29	$C_{18}H_{21}NO_3$	1.4	−5.9	0.40	13	−4.9
8h	$-CH_2CH_2-$	4-PhC_6H_4	CH_3	104–7	35	$C_{24}H_{25}NO_3$	23	−4.6	0.10	23	−4.6
8i	$-CH_2CH_2-$	4-$MeOC_6H_4$	CH_3	95–96	50	$C_{19}H_{23}NO_4$	12	−4.9	0.10	28	−4.6
8j	$-CH_2CH_2-$	4-ClC_6H_4	CH_3	118–121	28	$C_{18}H_{20}ClNO_3$	10	−5.0	0.20	3.2	−5.5
8k	$-CH_2CH_2-$	4-HOC_6H_4	CH_3	161–2	–	$C_{18}H_{21}NO_4$	2.6	−5.6	1.0	6.3	−5.2
8l	$-CH_2CH_2-$	3-$F_3CC_6H_4$	CH_3	oil	65	$C_{19}H_{20}F_3NO_3$	1.5	−5.8	0.30	5.4	−5.3
8m	$-CH_2CH_2-$	3-$MeOC_6H_4$	CH_3	106–9	21	$C_{19}H_{23}NO_4$	2.5	−5.6	0.60	11	−5.0
8n	$-CH_2CH_2-$	3-HOC_6H_4	CH_3	144–5	–	$C_{18}H_{21}NO_4$	1.9	−5.7	1.40	12	−5.0
8o	$-CH_2CH_2-$	2-$MeOC_6H_4$	CH_3	112–3	38	$C_{19}H_{23}NO_4$	2.1	−5.7	0.90	25	−4.6
8p	$-CH_2CH_2-$	2-HOC_6H_4	CH_3	140–2	–	$C_{18}H_{21}NO_4$	2.5	−5.6	1.10	30	−4.5
8q	$-CH_2CH_2-$	2-naphthyl	CH_3	foam	30	$C_{22}H_{23}NO_3$[f]	16	−4.8	0.10	3.6	−5.4
8r	$-CH_2CH_2-$	1-naphthyl	CH_3	137–8	21	$C_{22}H_{23}NO_3$	1.8	−5.8	0.70	4.0	−5.4
8s	$-CH_2CH_2-$	cyclohexyl	CH_3	129–130	25	$C_{18}H_{27}NO_3$	0.69	−6.2	0.50	2.2	−5.6
8t	$-CH_2CH_2-$		CH_3	125–6	20	$C_{19}H_{21}NO_3$	1.4	−5.8	1.10	5.8	−5.2
8u	$-CH_2CH_2-$		CH_3	135–8	13	$C_{20}H_{27}NO_3$[g]	1.3	−5.9	1.60	3.2	−5.5
8v	$-CH_2CH_2-$		CH_3	135–9	68	$C_{20}H_{29}NO_3$	2.3	−5.6	1.10	2.3	−5.6
8w	$-CH_2CH_2-$	Ph_2CH	CH_3	129–132	33	$C_{25}H_{27}NO_3$	13	−4.9	0.10	8.9	−5.4
8x	$-CH_2CH_2-$	4-FC_6H_4	$CH(CH_3)_2$	105–6	34	$C_{20}H_{24}FNO_3$	0.40	−6.4	30.2	0.23	−6.6
8y	$-CH_2CH_2-$	4-FC_6H_4	$C(CH_3)_3$	117–8	24	$C_{21}H_{26}FNO_3$	1.6	−5.8	1.70	1.8	−5.7
8z	$-CH_2CH_2-$	4-FC_6H_4	$CH(C_2H_5)_2$	107–8	36	$C_{22}H_{28}FNO_3$	20	−4.7	0.10	32	−4.5
8aa	$-CH_2CH_2-$	4-FC_6H_4	cyclopropyl	foam	22	$C_{20}H_{22}FNO_3$	2.2	−5.7	1.30	2.6	−5.6
8bb	$-CH_2CH_2-$	4-FC_6H_4	cyclobutyl	88–9	5	$C_{21}H_{24}FNO_3$	17	−4.8	0.20	–	–
8cc	$-CH_2CH_2-$	4-FC_6H_4	cyclohexyl	64–6	30	$C_{23}H_{28}FNO_3$	>100	−4.0	<0.01	>100	−4.0
8dd	$-CH_2CH_2-$	4-FC_6H_4	CF_3	oil	58	$C_{18}H_{17}F_4NO_3$	0.25	−6.6	8.0	0.63	−6.2
8ee	$-CH_2CH_2-$	3-FC_6H_4	$CH(CH_3)_2$	87–9	40	$C_{20}H_{24}FNO_3$	1.3	−5.9	1.8	2.6	−5.6
8ff	$-CH_2CH_2-$	2-FC_6H_4	$CH(CH_3)_2$	oil	9	$C_{20}H_{24}FNO_3$[h]	3.2	−5.5	0.9	1.8	−5.8
8gg	$-CH_2CH_2-$	2,4-$F_2C_6H_3$	$CH(CH_3)_2$	75–7	8	$C_{20}H_{23}F_2NO_3$	1.6	−5.8	1.5	2.6	−5.2
8hh	$-CH_2CH_2-$	2-$MeOC_6H_4$	$CH(CH_3)_2$	oil	16	$C_{21}H_{27}NO_4$	2.2	−5.6	1.0	5.6	5.2
8ii	$-CH_2CH_2-$	2,6-$(MeO)_2C_6H_3$	$CH(CH_3)_2$	foam	36	$C_{22}H_{29}NO_5$	19	−4.7	0.2	87	−4.1
8jj	$-CH_2CH_2-$	2,5-$Me_2C_6H_3$	$CH(CH_3)_2$	oil	25	$C_{22}H_{29}NO_3$[i]	12	−4.9	0.2	16	−4.8
8kk	$-CH_2CH_2-$	2-$iPrOC_6H_4$	$CH(CH_3)_2$	oil	12	$C_{23}H_{31}NO_3$[j]	3.2	−5.5	0.9	–	–
8ll	$-CH_2CH_2-$	2-ClC_6H_4	$CH(CH_3)_2$	foam	25	$C_{20}H_{24}ClNO_3$	3.2	−5.5	0.5	9.1	−5.0
8mm	$-CH_2CH_2-$		$CH(CH_3)_2$	oil	34	$C_{25}H_{29}NO_4$[k]	9.6	−5.0	0.2	25	−4.6
8nn	$-CH_2CH_2-$	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	oil	20	$C_{21}H_{33}NO_3$	>100	−4.0	<0.01	–	–
I	compactin						0.026	−7.6	100	0.025	−7.6

[a] Analytical results are within ±0.4% of theoretical values unless otherwise noted. [b] Cholesterol synthesis inhibition screen; a measure of the rate of conversion of [^{14}C]acetate to cholesterol employing a crude liver homogenate. [c] IC_{50} values were determined with four dose levels of each inhibitor in the assay systems described in ref 14 (CSI) and 15 (COR). [d] Calculated as follows: (IC_{50} of test compound)/(IC_{50} of compactin determined simultaneously) × 100. [e] CoA reductase inhibition screen; a measure of the direct conversion of D,L-[^{14}C]HMG-CoA to mevalonic acid employing a partially purified microsomal enzyme preparation. [f] C: calcd, 75.62; found, 75.12. [g] C: calcd, 72.92; found, 72.50. [h] C: calcd, 69.54; found, 71.37; H: calcd, 7.01; found, 7.54. [i] C: calcd, 74.33; found, 74.78. [j] C: calcd, 71.66; found, 72.09. [k] C: calcd, 73.69; found, 72.09.

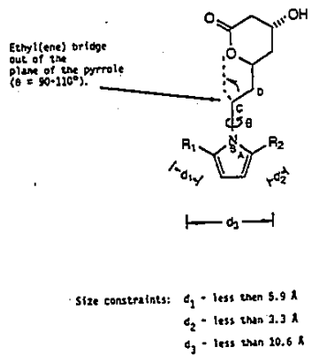


Figure 3. Summary of conclusions from the molecular modeling study.

Molecular Modeling

In order to identify the required spatial relationship between the lipophilic group (represented by the substituted pyrrole, phenyl, and hexahydronaphthalene ring systems) and the 4-hydroxypyran-2-one moiety, quantify steric tolerances across the pyrrole ring, and evaluate the relationship between potency and the polarity (charge distribution) of the side chains, selected analogues from Table III, compactin (I), and the potent biphenyl inhibitor III were modeled by using the CAMSEQ-II program package[18,19] (Table IV; see the Experimental Section). Conformational preferences of the ethyl (or ethylene) bridge to the lactone ring, size of the R_1 and R_2 substituents (Table IV), and charge distribution were compared to potency in the CSI screen (at the outset of this study, affinities in the COR screen were unavailable for the majority of the analogues studied) in order to develop a pharmacophore model for HMGR inhibition.

Lactone Side Chain Conformations. For reference purposes, calculated energies for the 0°, 90°, 180°, and lowest energy conformations of θ are summarized in Table IV. Figure 2 depicts the calculated energies for individual conformations. From Figure 2, all of the modeled compounds, including compactin (I), the biphenyl analogue III, and the less potent analogues **8z**, **8bb**, **8cc**, and **8nn**, can adopt an eneretically favorable conformation where the ethyl(ene) bridge is nearly perpendicular to the parent pyrrole, benzene, or hexahydronaphthalene ring systems. Indeed, for the potent derivatives 8t and III, the calculations show that the out of plane ($\theta \approx 80$–$110°$) orientation is the only one allowed. In addition, the reduced potency of the *tert*-butyl (8y) over the isopropyl (8x) analogue may be explained by the fact that the out of plane conformation ($\theta = 110°$) of 8y is calculated to be energetically disfavored over the in-plane ($\theta = 0$–$70°$) orientations.

Thus, it is concluded that a conformation of the ethyl(ene) bridge to the 4-hydroxypyran-2-one ring out of the plane (90–120°) of the parent ring systems is consistent with increased potency as a HMGR inhibitor. Interestingly, this corresponds to the calculated minimum energy and not the X-ray conformation[1b] of compactin. The X-ray conformation represents a secondary minimum at θ = 24.6°, 1.2 kcal/mol higher in energy, probably due to packing interactions.

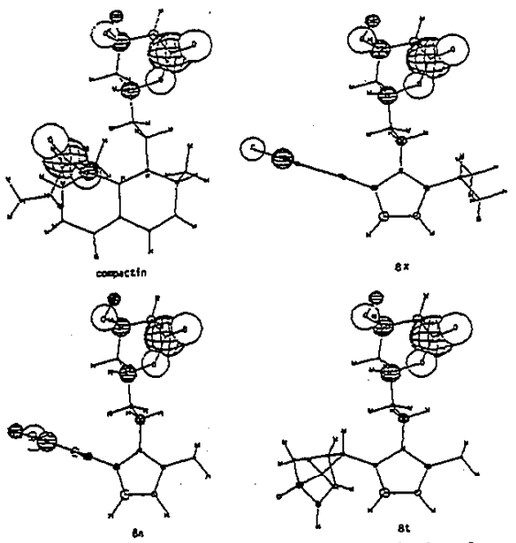


Figure 4. Charge distribution of compactin and selected analogues. Hatched and open spheres represent positive and negative charges, respectively. Sphere size is proportional to the magnitude of the atomic charge.

Steric Tolerances. In determining steric tolerances, the substituents were somewhat arbitrarily assigned. Larger substituents such as substituted phenyl, norbornenyl, and the isobutyric ester on compactin were placed at R_1 (Table IV); small alkyl groups were assigned to R_2. Changing the assignment would affect the conclusions regarding these tolerances. Low-energy, extended conformations of the substituents were used in the distance calculations; other orientations of flexible groups such as $CH(C_2H_5)_2$ could produce different distances.

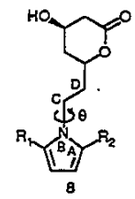
The maximum lengths of R_1 and R_2 and the overall width of the molecule across the parent ring system from R_1 to R_2 are given in Table IV. The calculations show a clear dependence of CSI potency on all three distances summarized in Figure 3. High potency ($IC_{50} < 1.6$ μM) is observed only for those analogues whose (a) maximum length of R_1 (Figure 3, d_1) is <5.9 Å (Table IV: compare 8f and 8j), (b) maximum length of R_2 (Figure 3, d_2) is <3.3 Å (compare 8x and 8z or **8nn**), and (c) overall width (Figure 3, d_3) is <10.6 Å (compare 8y and **8bb**). Other analogues not included in Table IV reinforce the length constraints at R_1: the 2-naphthyl analogue 8q (d_1 = 6.40 Å) is less potent than the 1-naphthyl (d_1 = 4.20 Å), and the para-substituted derivatives 8h and 8i possess reduced potency.

Charge Distribution. Initially, it was hypothesized that the spatial orientation of polar regions with relatively large partial charges within the molecule might be connected to CSI potency. Compactin contains two distinct regions of relatively large partial charges corresponding to the 4-hydroxypyran-2-one ring and the isobutyric ester side chain (Figure 4). The potent inhibitors 8f and 8x also present relatively large partial charges, albeit weaker in strength, in roughly the same region as this side chain. However, attempts to increase potency by more closely mimicking the polar regions associated with the isobutyric ester of compactin with the more polar 2- and 3-(methoxy and hydroxy)phenyl analogues 8m–p resulted in equipo-

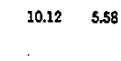
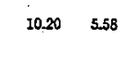
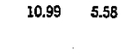
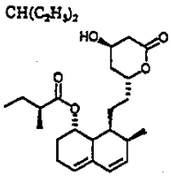
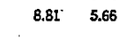
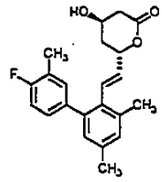
(18) (a) Potenzone, R., Jr.; Cavicchi, E.; Weintraub, H. J. R.; Hopfinger, A. J. *Comput. Chem.* 1977, *1*, 187. (b) Potenzone, R., Jr.; Hopfinger, A. J. *A Demonstration of the CAMSEQ-II Software System* In DHEW Publ. (FDA) (U.S.), Issue FDA 78-1046, Structural Correlations of Carcinogenesis and Mutagenesis, 1978, pp 102–103.

(19) In-house conversion of the program to run on an IBM 3033 under MVS/TSO (J. W. Vinson, unpublished work).

Table IV. Results of Modeling Studies on Compactin and Substituted Pyrroles



8

no.	R_1	R_2	IC_{50},[a] μM	lactone side chain rotations, CAMSEQ energies[b] 0°	90°	180°	min en conf	maximum overall width, Å (R_1 to R_2)	maximum lengths, Å R_1	R_2	other rotations[c]
8e	4-FC_6H_4 (α-Me)[d]	$CH(CH_3)_2$	5.0	−37.10[e]	−41.43[e]	100[e]	60°, −42.92[e]	10.12	5.58	2.48	also bond from α-Me to lactone side chain from 0° to 60° by 20°
8e	4-FC_6H_4 (α-Me)[f]	$CH(CH_3)_2$	5.0	−46.93[e]	−27.09[e,g]	100[e]	0°, −46.93[i]	10.12	5.58	2.48	as above
8f	4-FC_6H_4	CH_3	0.51	−40.92	−39.27	−10.03	0°, −40.92	7.66	5.58	1.50	methyl group (R_2) from 0° to 60° by 10°
8j	4-ClC_6H_4	CH_3	10					9.33	5.89	1.50	as above
8t[h]		CH_3	1.4	67.11	−44.98	−16.40	90°, −44.98	7.22	3.64	1.50	bond from R_1 to pyrrole from 0° to 360° by 20°
8t[i]		CH_3	1.4	19.63	−43.65	−15.01	70°, −44.65	7.87	4.27	1.50	as above
8x	4-FC_6H_4	$CH(CH_3)_2$	0.40	−46.64	−45.06	46.29	0°, −46.64	10.12	5.58	2.48	
8y	4-FC_6H_4	$C(CH_3)_3$	1.6	−47.77	−24.10[j]	100	0°, −47.77	10.20	5.58	2.48	 all bonds from 0° to 60° by 20°
8z	4-FC_6H_4	$CH(C_2H_5)_2$	20	−52.35	−50.97	100	0°, −52.35	10.99	5.58	3.74	 terminal methyls set to a staggered conformation
8bb	4-FC_6H_4	cyclobutyl	17	−46.46	−44.82	6.01	60°, −46.64	10.62	5.58	3.35	bond from R_2 to pyrrole from 0° to 360° by 20°
8cc	4-FC_6H_4	cyclohexyl	100	−51.76	−50.31	100	0°, −51.76	11.92	5.58	4.33	bond from R_2 to pyrrole from 0° to 360° by 20°
8nn	$CH(C_2H_5)_2$	$CH(C_2H_5)_2$	100	100	−47.28	100	100°, −54.31	9.41	3.74	3.74	see compound 8z above
I			0.026	10.17[i]	−56.04[i]	100[i]	120°, −61.74[i]	8.81	5.66	1.50	 terminal alkyl groups set to a staggered conformation
III			0.01	100	−48.89	100	130°, −52.92	8.74	5.52	1.50	bond from R_2 (Me) to phenyl from 0° to 60° by 20°; bond from R1 (4-F,3-MeC_6H_3) to pbenyl from 0° (biphenyl coplanar) to 90° by 15°

[a] CSI screen (see Table III). [b] Counterclockwise rotation of θ from 0 to 180° by 10°, unless otherwise noted, starting from the in-plane conformation shown (atoms A, B, C, D in a cis orientation). Steric and electrostatic (using charges calculated via the CNDO/2 method) terms were used. Energies are in kilocalories/mole. [c] At each conformation of the lactone side chain, rotations were performed on the marked bonds from 0° to 180° by 20°, unless otherwise indicated. Substituted phenyl rings at R_1 were held perpendicular to the pyrrole. [d] *R* stereoisomer. [e] θ was scanned from 0° to 250° by 10°. [f] *S* stereoisomer. [g] θ = 110° conformer, −46.09 kcal/mol. [h] Endo isomer. [i] Exo isomer. [j] θ = 70° conformer, −46.93 kcal/mol. [k] Chair form; equatorial attachment to pyrrole. [l] θ was scanned from 0° to 350° by 10°.

tent, not more potent, analogues. In addition, compounds containing bicyclo moieties at R_1 (8t–v) demonstrated that a polar substituent in this area (or an aryl ring, for that matter) was not required for CSI potency at the 1 μM level. Thus, it is concluded that CSI potency is relatively insensitive to the polarity of the group at R_1.

Conclusions

A series of 6-(2-pyrrol-1-ylethyl)-4-hydroxypyran-2-ones (8) has been identified as inhibiting the enzyme HMG-502

reductase (HMGR). By measuring the inhibition of HMGR in vitro, the 2- and 5-substituents on the pyrrole ring have been optimized, thus obtaining a compound (8x) that possesses 30% of the in vitro potency of the potent fungal metabolite compactin.

From a molecular modeling study, it was determined that so long as the 2- and 5-substituents did not interfere with the ability of the ethyl bridge to the lactone ring to attain an out-of-plane conformation (θ = 90–110°), and the substituents were within the distance contraints given in Figure 3, one could expect to achieve potency at the 1 μm level in the CSI screen. Attempts to enhance potency by mimicking partial charges in the polar isohutyric ester side chain in compactin failed. It is concluded that there are no strong electronic requirements for binding in this area.

In addition, the reduced potency of 8w, 8ii, and 8mm relative to other substituted phenyl derivatives suggests a steric intolerance off of one of the ortho phenyl positions of the R_1 substituent. One other noteworthy observation is that substitution of the 5-isopropyl with trifluoromethyl produced an analogue, 8dd, of essentially equal potency, (Table III: compare 8dd with 8f and 8x). This suggests the desirability of an electron-deficient pyrrole ring and a possible direction for future exploration. Efforts to further optimize the inhibitory potency of this series will be reported in subsequent publications from these laboratories.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. THF was distilled from sodium and benzophenone. All organic extracts were dried over $MgSO_4$ except where otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. NMR spectra were determined on either a Varian EM-390 spectrophotometer or a Varian XL-200 instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses for carbon, hydrogen, and nitrogen were determined on a Perkin-Elmer Model 240C elemental analyzer and are within 0.4% of theory unless noted otherwise. HPLC analyses were performed with a Varian 5500 unit equipped with a Reodyne 7126 loop injector, a Dupont variable wavelength detector, and an octadecylsilane column (Alltech C18 600RP, CH_3CN–H_2O eluant, 60:40, v/v) interfaced to Varian 402 data system for computation of peak areas. All starting materials were commercially available unless indicated otherwise.

Preparation of 1-(4-Fluorophenyl)-5-methyl-1,4-hexanedione (3p). Method A. 1-(4-Fluorophenyl)-2-propen-1-one (43.0 g, 287 mmol) was mixed with 31.2 mL (344 mmol) of isobutyraldehyde, 28 mL (200 mmol) of triethylamine, and 14.5 g (58 mmol) of 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride. The mixture was stirred at 70 °C under nitrogen for 12 h, cooled to room temperature, and partitioned between ether (500 mL) and water (100 mL). The aqueous layer was further extracted with ether (300 mL). The combined ether extracts were washed successively with water (200 mL), 2 M HCl (2 × 100 mL), and brine (100 mL) and dried. Filtration and concentration to dryness in vacuo provided an oil which was distilled (bp 115–120 °C, 0.2 mmHg) to provide 36.7 g (58%) of the title compound which solidified on standing: 90-MHz NMR ($CDCl_3$) δ 1.15 (d, 6 H, J = 7 Hz), 2.7 (septet, 1 H, J = 7 Hz), 2.8 (m, 2 H), 3.05 (m, 2 H), 7.12 (t, 3 H), 7.95 (m, 2 H). An analytical sample could be obtained by recrystallization from hexane, mp 51–3 °C. Anal. ($C_{13}H_{15}FO_2$) C, H, N.

Alternate Synthesis of 3p. A mixture of 2-methyl-4-penten-4-one[8d] (2.0 g, 20 mmol), 4-fluorobenzaldehyde (2.4 g, 20 mmol), 2 mL (14 mmol) of triethylamine, and 1.0 g (4 mmol) of 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride was stirred under nitrogen for 5 h at 70 °C, cooled to room temperature, and partitioned between ether (200 mL) and water (50 mL). The water layer was extracted with ether (200 mL). The ether extracts were combined, washed successively with water (50 mL), 2 M HCl (50 mL), and brine (50 mL), and dried. After concentration to dryness in vacuo, the residue was flash chromatographed on silica gel with hexane–ethyl acetate (20:1 v/v) as eluant, affording 2.6 g of 3p, mp 47–49 °C.

Method B. To a suspension of hexane-washed NaH (6.5 g, 270 mmol) in dry DMF (300 mL) at 0 °C under dry nitrogen was added a solution of methyl 4-methyl-3-oxopentanoate (37.5 g, 260 mmol) in 100 mL of dry DMF. When gas evolution had subsided, a solution of 2-bromo-4′-fluoroacetophenone (260 mmol) in dry DMF (100 mL) was added dropwise over 60 min. The mixture was allowed to warm to 25 °C overnight, poured into ice-cold 2 M HCl (300 mL), and extracted with ether (2 × 200 mL). The organic layer was washed with water (3 × 50 mL) and brine (50 mL) and concentrated to dryness in vacuo. The crude product was dissolved in 800 mL of 3:1 THF–water and treated with NaOH (24 g, 600 mmol), and the mixture was stirred overnight. The solution was made acidic with 6 N HCl and extracted with ether (2 × 300 mL). The ether extracts were washed with water (50 mL), bicarbonate (50 mL), and brine (50 mL) and dried. Distillation provided 40 g (69%) of 3p.

Preparation of 2-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]-1-cyanoethane (5, R_1 = 4-FPh, R_2 = $CH(CH_3)_2$, X = $-CH_2CH_2-$). A mixture of 3p (365 g, 1.65 mol), 3-aminopropionitrile $^1/_2$-fumarate (234 g, 1.83 mol), and 1 g of *p*-TSA in glacial acetic acid (1800 mL) was stirred and heated at reflux for 8 h. After cooling to room temperature, the solution was poured into ice water (3 L). The solid that formed was isolated by suction filtration and recrystallized from isopropyl ether and hexane (212 g, mp 75–78 °C). The filtrate was extracted with ether (2 × 1 L). The combined ether extracts were washed with water (1 L), saturated aqueous sodium bicarbonate (until gas evolution ceased), and brine (500 mL) and dried. Filtration and concentration to dryness in vacuo afforded a solid which was recrystallized from isopropyl ether to provide a further 98 g of the title compound (310 g total, 73%): IR (KBr) 2990, 2249, 1566, 1522, 1484, 1219, 1162, 847, 782 cm^{-1}; 200-MHz NMR ($CDCl_3$) δ 1.30 (d, 6 H, J = 7 Hz), 2.32 (t, 2 H, J = 7 Hz), 2.92 (septet, 1 H, J = 7 Hz), 4.22 (t, 2 H, J = 7 Hz), 6.00 (d, 1 H, J = 3.5 Hz), 6.10 (d, 1 H, J = 3.5 Hz), 7.0–7.4 (m, 4 H). Anal. ($C_{16}H_{17}FN_2$) C, H, N.

Preparation of 3-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]propanal (6t). A stirred solution of the above intermediate (200 g, 780 mmol) in 1500 mL of CH_2Cl_2 at ambient temperature under nitrogen was treated dropwise with 936 mL of a 1.0 M solution of diisobutylaluminum hydride (DIBAL-H) in CH_2Cl_2 over 4 h. The resulting mixture was stirred overnight at room temperature, and then the excess hydride was destroyed by cautious addition of methanol. When gas evolution was complete, the solution was carefully poured into 1500 mL of vigorously stirred ice-cold 2 M HCl (exothermic). The emulsion that resulted was extracted with ether (2 × 1 L), and the combined ether extracts were washed successively with water (500 mL), saturated aqueous sodium bicarbonate (2 × 500 mL), and brine (500 mL) and dried. The solvents were removed in vacuo, and the residue was flasb chromatographed over silica gel, eluting with hexane–ethyl acetate (10:1, v/v) to provide 6t (187 g, 92%) as a colorless oil: IR (film) 2930, 1720, cm^{-1}; 90-MHz NMR ($CDCl_3$) δ 1.25 (d, 6 H, J = 7 Hz), 2.50 (t, 2 H, J = 7 Hz), 2.85 (septet, 1 H, J = 7 Hz), 4.20 (t, 2 H, J = 7 Hz), 5.90 (d, 1 H, J = 2.5 Hz), 6.03 (d, 1 H, J = 2.5 Hz), 6.0–7.3 (m, 4 H), 9.45 (s, 1 H).

Preparation of Methyl 7-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]-5-hydroxy-3-oxoheptanoate (7, R_1 4-FPh, R_2 = $CH(CH_3)_2$, X = $-CH_2CH_2-$). A stirred suspension of hexane-washed NaH (2.17 g, 91 mmol) in anhydrous THF (200 mL) at 0 °C under nitrogen was treated dropwise with a solution of methyl acetoacetate (8.9 mL, 82 mmol) in anhydrous THF (150 mL) over 30 min. When gas evolution was complete, *n*-butyllithium (39 mL of a 2.1 M solution in hexane) was added dropwise. The resulting solution was stirred for 30 min and then treated dropwise over 30 min with a solution of 6t (19.4 g, 74.9 mmol) in anhydrous THF (150 mL). The solution was stirred for an additional 1 h and the reaction was quenched with saturated aqueous NH_4Cl (100 mL), followed by 2 M HCl (100 mL).

The resulting mixture was partitioned between ether (500 mL) and water (100 mL). The water layer was separated and extracted

with ether (300 mL). The ether extracts were combined, washed with brine (50 mL), and dried. The solvents were removed in vacuo, and the residue was flash chromatographed on silica gel, eluting with hexane–ethyl acetate (5:1, v/v) to yield 19.9 g (64%) of the title compound as a colorless oil: 200-MHz NMR ($CDCl_3$) δ 1.28 (d, 6 H, J = 7 Hz), 1.55 (m, 2 H), 2.45 (m, 2 H), 2.6 (br s, 1 H, J = 2.5 Hz), 7.0–7.4 (m, 4 H); IR (film) 3520, 2966, 2873, 1749, 1716, 1518, 1223, 1159, 845, 815, 767 cm^{-1}.

Preparation of *trans*-6-[2-[2-(4-Fluorophenyl)-5-(1-methylethyl)-1*H*-pyrrol-1-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (8x). Air (30 mL) was bubbled by syringe through a stirred solution of *n*-Bu_3B (58 mL of a 1 M THF solution) in dry THF (50 mL) containing 19.9 g (53 mmol) of the above intermediate at room temperature. The solution was stirred for 18 h at room temperature and cooled to −78 °C, and sodium borohydride (2.27 g, 60 mmol) was added in one portion. The mixture was stirred for 60 min at −78 °C and warmed to 0 °C for 90 min. A mixture of water (10 mL) and methanol (10 mL) was carefully added (gas evolution). NaOH (3 M, 60 mL) and 30% H_2O_2 (30 mL) were added simultaneously to the mixture from separate dropping funnels. The vigorously stirred mixture was held at 0 °C for 60 min and then at room temperature for 2 h.

The mixture was partitioned between water (300 mL) and ether (300 mL). The ether layer was extracted with 10% aqueous NaOH (50 mL). The aqueous layers were combined, acidified with concentrated HCl, and extracted with ethyl acetate (2 × 500 mL). The ethyl acetate extracts were combined, washed twice with brine (100 mL), and dried. Removal of the solvents in vacuo yielded 12.5 g of an oil which was dissolved in toluene (500 mL) and heated at reflux with azeotropic removal of water (Dean–Stark trap). The cooled solution was concentrated and the residue flash chromatographed on silica gel, eluting with hexane–ethyl acetate (5:1 v/v) to yield 11 g of a colorless solid. Recrystallization from isopropyl ether yielded 9.5 g (52%) of 8x, mp 104–105 °C, which was a 97:3 mixture of diastereomers by HPLC: 200-MHz NMR ($CDCl_3$) δ 1.30 (d, 6 H, J = 7 Hz), 1.5–1.9 (m, 4 H), 2.60 (m, 2 H), 2.98 (septet, 1 H, J = 7 Hz), 4.0–4.3 (m, 3 H), 4.45 (m, 1 H), 5.98 (d, 1 H, J = 2.5 Hz), 6.08 (d, 1 H, J = 2.5 Hz), 7.10 (m, 2 H), 7.33 (m, 2 H); IR (KBr) 3440, 2966, 2870, 1690, 1518, 1268, 1223, 1075, 837, 773 cm^{-1}. Anal. ($C_{20}H_{24}FNO_3$) C, H, N.

Preparation of 2-[2-(4-Fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-1-cyanoethane (5, R_1 = 4-FPh, R_2 = $C(CH_3)_3$, X = $-CH_2CH_2-$). Glacial acetic acid (125 mL) was added in one portion to a stirred solution of 3q (66 mmol) and ethanolamine (27 mL) at ambient temperature. A vigorous exothermic reaction ensued (the internal temperature rose to 95 °C). When the exotherm had subsided (TLC indicated reaction almost complete), the solution was stirred and heated at reflux for 30 min (TLC indicated all starting material was consumed, but a new high-R_f spot had appeared). The reaction mixture was cooled to room temperature and poured into ice water (200 mL). The aqueous mixture was extracted with ether (2 × 500 mL). The combined ether extracts were washed with water (2 × 200 mL), saturated aqueous bicarbonate (2 × 200 mL), and brine (100 mL), dried, and concentrated to dryness in vacuo. Flash chromatography of the residue on silica gel, eluting the ethyl acetate–hexane (10:1 v/v) provided 10.7 g of 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethanol product (62%) and 5 g of a high-R_f material which appeared to be the corresponding *O*-acetate by NMR (3 H, s, δ 2.05). The high-R_f fraction was stirred with NaOH (2 g) in CH_3OH (50 mL) and water (10 mL) for 2 h. The solution was concentrated, diluted with water (20 mL), and extracted with ethyl acetate (2 × 200 mL). The ethyl acetate extracts were washed with brine (50 mL) and dried. Filtration and concentration to dryness in vacuo provided a further 3.7 g of the above alcohol (14.4 g total, 84%).

Mesyl chloride (1.93 mL, 25 mmol) was added dropwise to a stirred solution of the above alcohol (5 g, 19.1 mmol) in pyridine (15 mL) cooled in an ice bath. The mixture was stirred for 2.5 h at 0 °C, warmed to room temperature, poured into water (300 mL), and extracted with ether (2 × 300 mL). The combined ether extracts were washed with water (50 mL), 2 M HCl (50 mL), bicarbonate (2 × 50 mL), and brine (50 mL), dried, and concentrated to dryness in vacuo. The crude mesylate was used without further purification.

A solution of KCN (1.54 g, 23.6 mmol) and KI (1.16 g, 10 mmol) in water (12 mL) was added dropwise to a stirred, 70 °C solution of the mesylate (4.0 g, 18 mmol) in DMF (36 mL). The resulting solution was heated under reflux for 24 h, cooled, and poured into ice water. The mixture was extracted with ether (2 × 200 mL). The combined ether extracts were washed with water (50 mL), 2 M HCl (25 mL), bicarbonate (2 × 50 mL), and brine (25 mL), dried, and concentrated to dryness in vacuo. Flash chromatography of the residue on silica gel, eluting with hexane–ethyl acetate (20:1, v/v), provided 2.8 g (88%) of the title compound: 90-MHz NMR ($CDCl_3$) δ 1.42 (s, 9 H), 2.20 (t, 2 H), J = 2 Hz), 4.30 (t, 2 H, J = 7 Hz), 5.90 (d, 1 H, J = 4 Hz), 6.00 (d, 2 H, J = 4 Hz), 6.9–7.4 (m, 4 H).

Prepartion of 6-[2-(2-Bicyclo[2.2.2]oct-2-yl-5-methyl-1*H*-pyrrol-1-yl)ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (8v). To a solution of 8u (0.3 g, 0.91 mmol) in ethyl acetate (10 mL) was added 0.03 g of 10% Pd–C. The mixture was evacuated, placed under a balloon of hydrogen (1 atm) at room temperature, and stirred overnight. The suspension was filtered through Celite and concentrated to dryness in vacuo, and the solid residue was recrystallized from isopropyl ether to afford 0.21 g of 8v (68%), mp 135–139 °C. Anal. ($C_{20}H_{29}NO_3$) C, H, N.

General Demethylation Procedure (Preparation of 8n). BBr_3 (11 mmol) was dissolved in 8 mL of CH_2Cl_2 and added dropwise to a solution of 8m (1.2 g, 3.64 mmol) in 100 mL of CH_2Cl_2 at −20 °C under dry nitrogen. The mixture was stirred for 2 h, and then a further 2 mmol of BBr_3 was added. The solution was allowed to warm slowly to 0 °C, poured into saturated aqueous bicarbonate (500 mL), and extracted with ethyl acetate (2 × 200 mL). The combined organic extracts were washed with 10% aqueous bisulfite (50 mL), saturated aqueous bicarbonate (30 mL), and brine (30 mL), dried, and concentrated to dryness in vacuo. Flash chromatography of the residue provided 450 mg of impure phenol. Two recrystallizations from isopropyl ether provided pure 8n, mp 110–111.5 °C. Anal. ($C_{18}H_{21}NO_4$) C, H, N.

HMG-CoA Reductase Inhibition Assay 1: The Cholesterol Synthesis Inhibition Screen (CSI). The procedure is a modification of the protocol developed by Dugan et al.[14] Male rats (type CD from Charles River) weighing 300–400 g were kept in-house for at least 1 week before the day of the experiment. For 3 consecutive days before being used, they were fed a diet of 5% cholestyramine (by weight) in normal ground chow. On the day of the assay, the rats were anesthetized with ether and sacrificed. Their livers were removed, weighed, and placed on Saran Wrap on ice. The entire livers were minced and diluted with 2 volumes of ice-cold pH 7.4 homogenizing buffer (0.1 M KPO_4, 0.004 M $MgCl_2$-$6H_2O$, 0.001 M EDTA, and 0.01 M 2-mercaptoethanol).

Liver homogenates were prepared by use of five to six passes of a Teflon pestle in a 50-mL glass homogenizer. The homogenates were pooled and centrifuged at 5000*g* for 10 min at 4 °C. Initial supernatants were pooled and centrifuged at 20000*g* for 15 min at 4 °C. Final supernatants were carefully drawn off, avoiding the loose pellet and lipid layer, pooled, and kept on ice. One-milliliter aliquots of this crude microsomal preparation were used for the assay.

Compounds were dissolved in 2 mL of toluene and sonicated if not fully soluble. The mixture was treated with 2 mL of 0.1 N NaOH and stirred constantly for 2 h in a water bath at 45–50 °C. Any remaining toluene was blown off under a stream of N_2. Approximately 6 mL of 0.1 N NaOH was added and the saponified drug placed on ice immediately. If the salt had crystallized, it was sonicated to achieve as uniform a suspension as possible. The pH was adjusted to 7.4 with HCl and the volume brought to 10 mL with H_2O. One-milliliter aliquots were frozen in dry ice–acetone and stored at −70 °C.

On the day of the screen, drugs were dissolved in 1 mL of 0.1 N KOH and diluted with 11 mL of homogenizing buffer to make a 2 mM stock solution. If necessary, sonication was used to achieve a solution, or in some cases, a suspension of drug. The 2 mM stock was diluted 1:1 with a mixture of 1 mL of 0.1 N KOH and 11 mL of homogenizing buffer. The resulting 1 mM solution was further diluted with homogenizing buffer alone to produce a series of 10 × stocks from 10^{-6} to 10^{-3} M. The sodium salt of compactin was used as a reference compound in every assay in a concentration range of 10^{-9} to 10^{-6} M.

Assay Conditions. The assay was carried out in duplicate in 16 × 125 mm screw-capped tubes. The reaction mixture contained the following, on ice (initial concentrations): 0.1 mL of 20 mM NAD, 0.1 mL of 20 mM NADP, 0.1 mL of 200 mM glucose 6-phosphate, 0.5 mL of 0.12 mM niacinamide, and 0.2 mL of the 10 × drug stocks. Controls were also run with 0.2 mL of a mixture of 1 mL of 0.1 N KOH, plus 11 mL of homogenizing buffer in place of drug. One milliliter of the crude microsomal preparation was added immediately after the drugs, to give a total volume of 2 mL. Final drug concentrations were 10^{-4} to 10^{-7} M, or in the case of compactin, 10^{-6} to 10^{-9} M. The samples were warmed at 37 °C for 5 min before adding the radioactive precursor. [1-^{14}C]Acetate was used in the amount of 2.88 μCi per sample, plus 98 μmol of sodium acetate as cold carrier. When [^{3}H]-mevalonate was used, the amount of 0.5 μCi per sample with cold carrier was added to make a total of 0.2 μmol per sample. Volume of radiolabel per sample was 100 μL. After receiving radiolabel, samples were incubated at 37 °C for 1 h and treated with 2.5 mL of 10% KOH in ethanol, and the saponification was carried out at 70 °C for 2 h in a water bath. After cooling to room temperature, the nonsaponifiable lipids (cholesterol accounts for approximately 80% of nonsaponifiable lipids; the remainder are methyl sterols) were extracted by shaking the samples with 4.2 mL of hexane for 10 min. After phase separation, 2 mL of the hexane layer was diluted with 8 mL of Handifluor and counted.

Percent inhibition was calculated as follows: 1.0 − (drug cpm/control cpm). Control refers to the samples that received buffer only. From a plot of percent inhibition versus the log of the drug concentration, the IC_{50} was determined. Every assay yielded an IC_{50} for the reference compound, compactin, thus providing a comparison for the other compounds as well as a standard to check for consistency between assays.

HMG CoA Reductase Inhibition Assay 2: Co-A Reductase Inhibition Screen (COR). This procedure is a modification of that reported by Kita et al.[15] Male Charles River (CD) rats weighing 200–300 g were fed a chow diet containing cholestyramine (5%) for 3 days in order to increase levels of liver microsomal HMG–CoA reductase. Between 9 a.m. and 10 a.m., fed animals were anesthetized with ether prior to a midline incision to open the abdomen. Traverse cuts were made to the left and right of abdominal cavity exposing the hepatic portal vein. A syringe with a 22-gauge needle containing 10 mL of exsanguinating buffer (40 mM Tris, 0.25 M sucrose, 0.3 mM EDTA, 5 mM dithiothreitol (DTT), pH 7.2) was injected into the portal vein after cutting the inferior vena cava. Prior to excision, the liver was cleared of blood by perfusion with exsanguinating buffer. Immediately after excision, the liver was added to ice-cold (4 °C) pH 7.4 buffer (0.3 M sucrose, 5 mM DTT, 50 mM leupeptin, 5 mM EGTA, 1 mM PMSF). Approximately 1 g samples were taken from the largest lobe and homogenized with 10 strokes of a tight-fitting Potter-Elvehjem homogenizer. Each homogenate was centrifuged for 15 min at 12000g in a Servall refrigerated-automatic centrifuge (SM-34 rotor). The supernatant was decanted and respun under the same conditions. The resulting supernatant was removed via pipet, with special care being taken not to remove any of the mitochondrial-rich pellet. The supernatants were then pooled and centrifuged with a 50 Ti or 60 Ti rotor in a Beckman L8-80 ultracentrifuge. After ultracentrifugation, the pellet was mixed with ice-cold KH_2PO_4 buffer (0.2 M, pH 7.4), homogenized, and stored in liquid nitrogen at 10 mg/mL microsomal protein. Microsomes maintained in liquid nitrogen retained HMG-CoA reductase activity for up to 1 year. Each pellet was resuspended in a solution of 0.3 M sucrose and 10 mM 2-mercaptoethanol and frozen immediately in liquid nitrogen. The aliquoted samples (500 μL) were then stored at −70 °C for no more than 1 month. For each microsomal isolation, an activity/microgram of microsomal protein curve was determined so that the amount of microsomal protein utilized in each assay was in the linear part of the activity curve.

Assay Conditions. Frozen microsomes (see above) were allowed to slowly thaw on ice. Assay solutions were prepared as follows:

A. Resuspension buffer: 0.2 M KH_2PO_4 buffer, pH 7.4.

B. Incubation buffer: 0.2 M KH_2PO_4 buffer (stock, 3 M $KH_2PO_4 \cdot 3H_2O$, 1 M KH_2PO_4, final 2 M); 0.01 M EDTA, 12 mM dithiothreitol; 40 mM glucose 6-phosphate; 4 mM NADPH; 0.45 μM DL-3-hydroxymethylglutaryl-coenzyme A (glutaryl-3-^{14}C) (stock, 7.4 μM unlabeled; HMG-CoA + 0.68 μM [^{14}C]HMG-CoA (4.5 μCi/μmol); final concentration 8.9 μM).

Resuspension buffer (70 μL) + microsomal solution (20 μL; 100 μg protein) + drug (10 μL) = 100 μL.

Incubation buffer (90 μL) + [^{14}C]HMG-CoA (10 μL) (final addition) = 100 μL.

Total volume of assay mix = 100 μL + 100 μL = 200 μL.

The assay solution was vortexed and incubated in a shaking water bath at 37 °C for 60 min. Termination of the reaction was accomplished with 30 μL of concentrated HCl. Conversion of the [^{14}C]mevalonic acid to the lactone form occurred in a water bath for 30 min at 37 °C. Conversion of [^{14}C]mevalonic acid to the lactone form occurred during refrigeration overnight. To each reaction tube was added DL-[2-^{3}H]mevalonic acid lactone (10000–15000 cpm + 200 μg of unlabeled mevalonolactone) as an internal standard to correct for incomplete recovery of [^{14}C]-mevalonate. After vortexing, an aliquot (50 μL) from the assay mix in each tube was put over a AG 1-X8 (200–400 mesh) formate form anion exchange resin column. The mevalonate was eluted with 3 × 750 μL of water into scintillation vials. Scintillation cocktail (Beckman Readi-Solv, 10 mL) was then added to each vial. The vials were vortexed and allowed to equilibrate for 1 h. Standards for the [^{14}C]HMG-CoA, [^{3}H]mevalonolactone, and acid-inactivated microsomes (blank) were also isolated by column separation in a Hewlett-Packard Model 3320 Tricarb scintillation spectrometer set for double label counting at maximum efficiency. Standards for [^{14}C]HMG-CoA, [^{3}H]mevalonolactone, and acid-inactivated microsomes (blank) were also isolated by TLC, scraped, and counted. Calculations were performed in the usual manner taking into consideration crossover of ^{3}H into the ^{14}C channel and visa versa, as well as dilution factors and specific activity of [^{14}C]HMG-CoA used. Reductase activity was expressed as picomole of [^{14}C]HMG-CoA converted to [^{14}C]mevalonic acid lactone/milligram of microsomal protein per minute. Compactin was used as a reference compound at concentrations of 10^{-9} and 10^{-7} M to determine the concentration at 50% inhibition from control value. Drugs were tested for their inhibitory characteristics at four concentrations run in triplicate. Statistical significance from control values was determined by using Dunnett's t test.

Molecular Modeling. Selected analogues were modeled by using an in-house modified version[17] of CAMSEQ-II[18] operating on an IBM 3083 machine. The structure of compactin was obtained from published[1b] X-ray data; the structure of pyrrole came from a compendium[20] of minimized structures. Coordinates for other groups were extracted from the library of fragments within CAMSEQ-II. Structures III and 8 were built to attaching the side chain containing the 4-hydroxypyran-2-one ring (coordinates for which were copied from the X-ray structure of compactin) to the benzene and pyrrole rings, respectively, and adding the other substituents. Side chains were rotated to remove steric contacts.

After CNDO/2 was employed to generate atomic charges, counterclockwise rotations (unless otherwise noted, from 0° to 180° by 10°) were performed using the SCAN module about θ, starting from the in-plane conformation shown in the structure at the top of Table IV (atoms A–B–C–D coplanar). The conformation of the 4-hydroxypyran-2-one ring was held fixed throughout these calculations. Steric and electrostatic energy terms were used. At each conformation of θ, the conformational flexibility of the 2- and 5-substituents was investigated (Table IV; column headed by "other rotations"), including energy evaluation, to insure that a low-energy conformer of θ was selected. Both the endo and exo isomers of the norbornenyl analogue 8t as well as the R and S isomers of 8e were modeled. The axial-attached isomer of 8cc proved to be sterically hindered and was not included. Figures 1 and 2 were generated by using the SAS-GRAPH program package.[21] In eq 1, the number in parentheses is the standard error of the regression coefficient, n is the number of compounds, r is the correlation coefficient, F is a significance test, and s is the standard error.

(20) SYBYL Standard Fragment Library, generously supplied by Tripos Associates, St. Louis, MO.

(21) SAS Institute, Inc. SAS/GRAPH User's Guide, Version 5 Edition; SAS Institute, Inc., Cary, NC, 1985.

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Registry No. 1 (R_1 = Ph), 768-03-6; 1 (R_1 = 4-F-C_6H_4), 51594-59-3; 1 (R_1 = 4-Ph-C_6H_4), 42575-11-1; 1 (R_1 = 4-Cl-C_6H_4), 7446-87-5; 1 (R_1 = 4-CH_3O-C_6H_4), 7448-86-4; 1 (R_1 = 3-F_3C-C_6H_4), 123184-14-5; 1 (R_1 = 3-CH_3O-C_6H_4), 51594-60-6; 1 (R_1 = 2-CH_3O-C_6H_4), 77942-10-0; 1 (R_1 = 2-naphthyl), 4452-06-6; 1 (R_1 = 1-naphthyl), 22422-69-1; 1 (R_1 = bicyclo[2.2.1]-hept-5-en-2-yl), 100234-78-4; 1 (R_1 = bicyclo[2.2.2]-oct-5-en-2-yl), 123184-15-6; 1 (R_1 = cyclohexyl), 2177-34-6; 1 (R_1 = Ph_2CH), 93021-71-7; 1 (R_1 = $CH(C_2H_5)_2$), 123184-16-7; 1 (R_1 = 2-F-C_6H_4), 89638-21-1; 1 (R_1 = 2,4-F_2-C_6H_3), 123184-17-8; 1 (R_1 = $CH(CH_3)_2$), 1606-47-9; 2 (R_2 = CH_3), 75-07-0; 2 (R_2 = $CH(CH_3)_2$), 78-84-2; 2 (R_2 = $CH(C_2H_5)_2$), 97-96-1; 2 (R_2 = cyclopropyl), 1489-69-6; 2 (R_2 = cyclobutyl), 2987-17-9; 2 (R_2 = cyclohexyl), 2043-61-0; 2 (R_2 = $C(CH_3)_3$), 630-19-3; 2 (R_2 = 4-F-C_6H_4), 459-57-4; 2 (R_2 = C_2H_5), 123-38-6; 3a, 583-05-1; 3b, 123183-95-9; 3c, 63472-37-7; 3d, 53842-12-9; 3e, 2108-54-5; 3f, 123183-96-0; 3g, 123183-97-1; 3h, 104562-48-3; 3i, 123183-98-2; 3j, 123263-79-6; 3k, 70353-45-6; 3l, 123183-99-3; 3m, 61771-79-7; 3n, 123184-00-9; 3o, 123184-01-0; 3p, 104568-68-5; 3q, 123184-02-1; 3r, 123184-03-2; 3s, 123184-04-3; 3t, 123184-05-4; 3u, 123184-06-5; 3v, 123184-07-6; 3w, 123184-08-7; 3x, 123184-09-8; 3y, 123184-10-1; 3z, 123184-11-2; 3aa, 123184-12-3; 3bb, 123184-13-4; 5a, 123184-20-3; 5b, 123184-21-4; 5c, 123184-22-5; 5d, 123184-23-6; 5e, 123184-89-4; 5f, 123184-24-7; 5g, 123184-25-8; 5h, 123184-26-9; 5i, 123184-27-0; 5j, 123184-28-1; 5k, 123184-29-2; 5l, 123184-30-5; 5m, 123184-31-6; 5n, 123184-32-7; 5o, 123184-33-8; 5p, 123184-34-9; 5q, 123184-35-0; 5r, 123184-36-1; 5s, 123184-37-2; 5t, 104568-69-6; 5u, 123184-88-3; 5v, 123184-38-3; 5w, 123184-39-4; 5x, 123184-40-7; 5y, 123184-41-8; 5z, 104568-91-4; 5aa, 104568-69-6; 5bb, 123184-42-9; 5cc, 123184-43-0; 5dd, 123184-44-1; 5ee, 123184-45-2; 5ff, 123184-46-3; 5gg, 123184-47-4; 5hh, 123184-48-5; 5ii, 123184-49-6; 5jj, 123184-50-9; 6a, 123184-51-0; 6b, 123184-52-1; 6c, 123184-53-2; 6d, 123184-54-3; 6e, 123184-55-4; 6f, 123184-56-5; 6g, 123184-57-6; 6h, 123184-58-7; 6i, 123184-59-8; 6j, 123184-60-1; 6k, 123184-61-2; 6l, 123184-62-3; 6m, 123184-63-4; 6n, 123184-64-5; 6o, 123184-65-6; 6p, 123184-66-7; 6q, 123184-67-8; 6r, 123184-68-9; 6s, 123184-69-0; 6t, 104568-70-9; 6u, 123184-70-3; 6v, 123184-71-4; 6w, 123184-72-5; 6x, 123184-73-6; 6y, 123184-74-7; 6z, 123184-75-8; 6aa, 123184-76-9; 6bb, 123184-77-0; 6cc, 123184-78-1; 6dd, 123184-79-2; 6ee, 123184-80-5; 6ff, 123184-81-6; 6gg, 123184-82-7; 6hh, 123184-83-8; 6ii, 123184-84-9; 6jj, 123184-85-0; 7a, 123184-90-7; 7b, 123184-91-8; 7c, 123184-92-9; 7d, 123184-93-0; 7e, 123184-94-1; 7f, 123184-95-2; 7g, 123184-96-3; 7h, 123184-97-4; 7i, 123184-98-5; 7j, 123184-99-6; 7l, 123185-00-2; 7m, 123185-01-3; 7o, 123185-02-4; 7q, 123185-03-5; 7r, 123185-04-6; 7s, 123185-05-7; 7t, 123185-06-8; 7u, 123185-07-9; 7w, 123185-08-0; 7x, 104568-71-0; 7y, 123185-09-1; 7z, 123185-10-4; 7aa, 123185-11-5; 7bb, 123185-12-6; 7cc, 123185-13-7; 7dd, 123185-14-8; 7ee, 123185-15-9; 7ff, 123185-16-0; 7gg, 123185-17-1; 7hh, 123185-18-2; 7ii, 123185-19-3; 7jj, 123185-20-6; 7kk, 123185-21-7; 7ll, 123185-22-8; 7mm, 123185-23-9; 7nn, 123185-24-0; 8a, 123185-25-1; 8b, 123185-26-2; 8c, 123185-27-3; 8d, 123185-28-4; 8e (stereoisomer 1), 123185-29-5; 8e (stereoisomer 2), 123185-49-9; 8f, 104568-74-3; 8g, 105356-37-4; 8h, 104568-81-2; 8i, 104568-78-7; 8j, 123185-30-8; 8k, 123185-31-9; 8l, 104568-80-1; 8m, 123185-32-0; 8n, 123185-33-1; 8o, 104568-77-6; 8p, 123185-34-2; 8q, 104568-83-4; 8r, 104568-82-3; 8s, 104568-79-8; 8t (stereoisomer 1), 123355-04-4; 8t (stereoisomer 2), 123283-97-6; 8u, 123185-35-3; 8v, 123185-36-4; 8w, 104568-85-6; 8x, 104568-73-2; 8y, 104568-76-5; 8z, 123185-37-5; 8aa, 104568-75-4; 8bb, 123185-38-6; 8cc, 123185-39-7; 8dd, 104568-92-5; 8ee, 123185-40-0; 8ff, 123185-41-1; 8gg, 123185-42-2; 8hh, 105356-38-5; 8ii, 123185-43-3; 8jj, 123185-44-4; 8kk, 123185-45-5; 8ll, 123185-46-6; 8mm, 123185-47-7; 8nn, 123185-48-8; $EtCOCH_2CO_2Me$, 30414-53-0; $CF_3COCH_2CO_2Me$, 83643-84-9; m-$FC_6H_4COCH_2Br$, 53631-18-8; $(CH_3)_2CHCH_2CO_2Me$, 42558-54-3; p-$FC_6H_4COCH_2Br$, 403-29-2; 2,6-$(MeO)_2C_6H_3COCH_2Br$, 123184-19-0; 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, 4568-71-2; 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride, 123184-18-9; 3-aminopropionitrile $^1/_2$-fumarate, 2079-89-2; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1H-pyrrol-1-yl]-2-ethanol, 123184-86-1; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1H-pyrrol-1-yl]-2-ethyl methanesulfonate, 123184-87-2; methyl acetoacetate, 105-45-3; cholesterol, 57-88-5.

Supplementary Material Available: CAMSEQ-II energies calculated for individual conformations of θ for compounds appearing in Table IV. The data are plotted in Figure 2. Also, a description of the format of a CAMSEQ-II MOL file, followed by MOL files giving x, y, z coordinates for the conformations of compounds I, III, and 8x used in the pharmacophore model (7 pages). Ordering information is given on any current masthead page.

Inhibitors of Cholesterol Biosynthesis. 2. 1,3,5-Trisubstituted [2-(Tetrahydro-4-hydroxy-2-oxopyran-6-yl)ethyl]pyrazoles

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A series of 1,3,5-trisubstituted pyrazole mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Since previous studies suggested that the 5-(4-fluorophenyl) and 3-(1-methylethyl) substituents afforded optimum potency, attention was focused on variations in position 1 of the pyrazole ring. Biological evaluation of analogues bearing a variety of 1-substituents suggested that, although most substituents were tolerated, none afforded an advantage over phenyl, which exhibited potency comparable to that of compactin in vitro.

We previously described a series of 2,5-disubstituted pyrrole mevalonolactones whose 3,5-dihydroxyheptanoic acid derivatives were shown to possess varying degrees of intrinsic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in vitro.[1] Structure–activity relationships (SAR) for this series of compounds were determined, and the preferred substituents in the 2- and 5-positions of the pyrrole nucleus were found to be 4-fluorophenyl and 1-methylethyl, respectively. This paper describes the synthesis and biological activity of a series of 1,3,5-trisubstituted pyrazole mevalonolactones[2] with

(1) Roth, B. D.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. Submitted to *J. Med. Chem.*

(2) During the course of this study, a series of trisubstituted pyrazole mevalonolactones were reported to inhibit HMG-CoA reductase by J. R. Wareing at Sandoz Pharmaceuticals Corp. U.S. Patent. 4613610.

0022-2623/90/1833-0031$02.50/0

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Registry No. 1 (R_1 = Ph), 768-03-6; 1 (R_1 = 4-F-C_6H_4), 51594-59-3; 1 (R_1 = 4-Ph-C_6H_4), 42575-11-1; 1 (R_1 = 4-Cl-C_6H_4), 7448-87-5; 1 (R_1 = 4-CH_3O-C_6H_4), 7448-86-4; 1 (R_1 = 3-F_3C-C_6H_4), 123184-14-5; 1 (R_1 = 3-CH_3O-C_6H_4), 51594-60-6; 1 (R_1 = 2-CH_3O-C_6H_4), 77942-10-0; 1 (R_1 = 2-naphthyl), 4452-06-6; 1 (R_1 = 1-naphthyl), 22422-69-1; 1 (R_1 = bicyclo[2.2.1]-hept-5-en-2-yl), 100234-78-4; 1 (R_1 = bicyclo[2.2.2]-oct-5-en-2-yl), 123184-15-6; 1 (R_1 = cyclohexyl), 2177-34-6; 1 (R_1 = Ph_2CH), 93021-71-7; 1 (R_1 = $CH(C_2H_5)_2$), 123184-16-7; 1 (R_1 = 2-F-C_6H_4), 89638-21-1; 1 (R_1 = 2,4-F_2-C_6H_3), 123184-17-8; 1 (R_1 = $CH(CH_3)_2$), 1606-47-9; 2 (R_2 = CH_3), 75-07-0; 2 (R_2 = $CH(CH_3)_2$), 78-84-2; 2 (R_2 = $CH(C_2H_5)_2$), 97-96-1; 2 (R_2 = cyclopropyl), 1489-69-6; 2 (R_2 = cyclobutyl), 2987-17-9; 2 (R_2 = cyclohexyl), 2043-61-0; 2 (R_2 = $C(CH_3)_3$), 630-19-3; 2 (R_2 = 4-F-C_6H_4), 459-57-4; 2 (R_2 = C_2H_5), 123-38-6; 3a, 583-05-1; 3b, 123183-95-9; 3c, 63472-37-7; 3d, 53842-12-9; 3e, 2108-54-5; 3f, 123183-96-0; 3g, 123183-97-1; 3h, 104562-48-3; 3i, 123183-98-2; 3j, 123263-79-6; 3k, 70353-45-6; 3l, 123183-99-3; 3m, 61771-79-7; 3n, 123184-00-9; 3o, 123184-01-0; 3p, 104568-68-5; 3q, 123184-02-1; 3r, 123184-03-2; 3s, 123184-04-3; 3t, 123184-05-4; 3u, 123184-06-5; 3v, 123184-07-6; 3w, 123184-08-7; 3x, 123184-09-8; 3y, 123184-10-1; 3z, 123184-11-2; 3aa, 123184-12-3; 3bb, 123184-13-4; 5a, 123184-20-3; 5b, 123184-21-4; 5c, 123184-22-5; 5d, 123184-23-6; 5e, 123184-89-4; 5f, 123184-24-7; 5g, 123184-25-8; 5h, 123184-26-9; 5i, 123184-27-0; 5j, 123184-28-1; 5k, 123184-29-2; 5l, 123184-30-5; 5m, 123184-31-6; 5n, 123184-32-7; 5o, 123184-33-8; 5p, 123184-34-9; 5q, 123184-35-0; 5r, 123184-36-1; 5s, 123184-37-2; 5t, 104568-69-6; 5u, 123184-88-3; 5v, 123184-38-3; 5w, 123184-39-4; 5x, 123184-40-7; 5y, 123184-41-8; 5z, 104568-91-4; 5aa, 104568-69-6; 5bb, 123184-42-9; 5cc, 123184-43-0; 5dd, 123184-44-1; 5ee, 123184-45-2; 5ff, 123184-46-3; 5gg, 123184-47-4; 5hh, 123184-48-5; 5ii, 123184-49-6; 5jj, 123184-50-9; 6a, 123184-51-0; 6b, 123184-52-1; 6c, 123184-53-2; 6d, 123184-54-3; 6e, 123184-55-4; 6f, 123184-56-5; 6g, 123184-57-6; 6h, 123184-58-7; 6i, 123184-59-8; 6j, 123184-60-1; 6k, 123184-61-2; 6l, 123184-62-3; 6m, 123184-63-4; 6n, 123184-64-5; 6o, 123184-65-6; 6p, 123184-66-7; 6q, 123184-67-8; 6r, 123184-68-9; 6s, 123184-69-0; 6t, 104568-70-9; 6u, 123184-70-3; 6v, 123184-71-4; 6w, 123184-72-5; 6x, 123184-73-6; 6y, 123184-74-7; 6z, 123184-75-8; 6aa, 123184-76-9; 6bb, 123184-77-0; 6cc, 123184-78-1; 6dd, 123184-79-2; 6ee, 123184-80-5; 6ff, 123184-81-6; 6gg, 123184-82-7; 6hh, 123184-83-8; 6ii, 123184-84-9; 6jj, 123184-85-0; 7a, 123184-90-7; 7b, 123184-91-8; 7c, 123184-92-9; 7d, 123184-93-0; 7e, 123184-94-1; 7f, 123184-95-2; 7g, 123184-96-3; 7h, 123184-97-4; 7i, 123184-98-5; 7j, 123184-99-6; 7l, 123185-00-2; 7m, 123185-01-3; 7o, 123185-02-4; 7q, 123185-03-5; 7r, 123185-04-6; 7s, 123185-05-7; 7t, 123185-06-8; 7u, 123185-07-9; 7w, 123185-08-0; 7x, 104568-71-0; 7y, 123185-09-1; 7z, 123185-10-4; 7aa, 123185-11-5; 7bb, 123185-12-6; 7cc, 123185-13-7; 7dd, 123185-14-8; 7ee, 123185-15-9; 7ff, 123185-16-0; 7gg, 123185-17-1; 7hh, 123185-18-2; 7ii, 123185-19-3; 7jj, 123185-20-6; 7kk, 123185-21-7; 7ll, 123185-22-8; 7mm, 123185-23-9; 7nn, 123185-24-0; 8a, 123185-25-1; 8b, 123185-26-2; 8c, 123185-27-3; 8d, 123185-28-4; 8e (stereoisomer 1), 123185-29-5; 8e (stereoisomer 2), 123185-49-9; 8f, 104568-74-3; 8g, 105356-37-4; 8h, 104568-81-2; 8i, 104568-78-7; 8j, 123185-30-8; 8k, 123185-31-9; 8l, 104568-80-1; 8m, 123185-32-0; 8n, 123185-33-1; 8o, 104568-77-6; 8p, 123185-34-2; 8q, 104568-83-4; 8r, 104568-82-3; 8s, 104568-79-8; 8t (stereoisomer 1), 123355-04-4; 8t (stereoisomer 2), 123283-97-6; 8u, 123185-35-3; 8v, 123185-36-4; 8w, 104568-85-6; 8x, 104568-73-2; 8y, 104568-76-5; 8z, 123185-37-5; 8aa, 104568-75-4; 8bb, 123185-38-6; 8cc, 123185-39-7; 8dd, 104568-92-5; 8ee, 123185-40-0; 8ff, 123185-41-1; 8gg, 123185-42-2; 8hh, 105356-38-5; 8ii, 123185-43-3; 8jj, 123185-44-4; 8kk, 123185-45-5; 8ll, 123185-46-6; 8mm, 123185-47-7; 8nn, 123185-48-8; $EtCOCH_2CO_2Me$, 30414-53-0; $CF_3COCH_2CO_2Me$, 83643-84-9; m-$FC_6H_4COCH_2Br$, 53631-18-8; $(CH_3)_2CHCH_2CO_2Me$, 42558-54-3; p-$FC_6H_4COCH_2Br$, 403-29-2; 2,6-$(MeO)_2C_6H_3COCH_2Br$, 123184-19-0; 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, 4568-71-2; 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride, 123184-18-9; 3-aminopropionitrile $^1/_2$-fumarate, 2079-89-2; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethanol, 123184-86-1; 2-[2-(4-fluorophenyl)-5-(1,1-dimethylethyl)-1*H*-pyrrol-1-yl]-2-ethyl methanesulfonate, 123184-87-2; methyl acetoacetate, 105-45-3; cholesterol, 57-88-5.

Supplementary Material Available: CAMSEQ-II energies calculated for individual conformations of θ for compounds appearing in Table IV. The data are plotted in Figure 2. Also, a description of the format of a CAMSEQ-II MOL file, followed by MOL files giving x, y, z coordinates for the conformations of compounds I, III, and 8x used in the pharmacophore model (7 pages). Ordering information is given on any current masthead page.

Inhibitors of Cholesterol Biosynthesis. 2. 1,3,5-Trisubstituted [2-(Tetrahydro-4-hydroxy-2-oxopyran-6-yl)ethyl]pyrazoles

D. R. Sliskovic,* B. D. Roth, M. W. Wilson, M. L. Hoefle, and R. S. Newton

Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received March 16, 1989*

A series of 1,3,5-trisubstituted pyrazole mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Since previous studies suggested that the 5-(4-fluorophenyl) and 3-(1-methylethyl) substituents afforded optimum potency, attention was focused on variations in position 1 of the pyrazole ring. Biological evaluation of analogues bearing a variety of 1-substituents suggested that, although most substituents were tolerated, none afforded an advantage over phenyl, which exhibited potency comparable to that of compactin in vitro.

We previously described a series of 2,5-disubstituted pyrrole mevalonolactones whose 3,5-dihydroxyheptanoic acid derivatives were shown to possess varying degrees of intrinsic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in vitro.[1] Structure–activity relationships (SAR) for this series of compounds were determined, and the preferred substituents in the 2- and 5-positions of the pyrrole nucleus were found to be 4-fluorophenyl and 1-methylethyl, respectively. This paper describes the synthesis and biological activity of a series of 1,3,5-trisubstituted pyrazole mevalonolactones[2] with

(1) Roth, B. D.; Hoefle, M. L.; Stratton, C. D.; Sliskovic, D. R.; Wilson, M. W.; Newton, R. S. Submitted to *J. Med. Chem.*

(2) During the course of this study, a series of trisubstituted pyrazole mevalonolactones were reported to inhibit HMG-CoA reductase by J. R. Wareing at Sandoz Pharmaceuticals Corp. U.S. Patent. 4613610.

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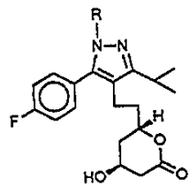
Table I. Physical Properties and in Vitro HMG-CoA Reductase Inhibitory Actives of Pyrazole Mevalonolactones I

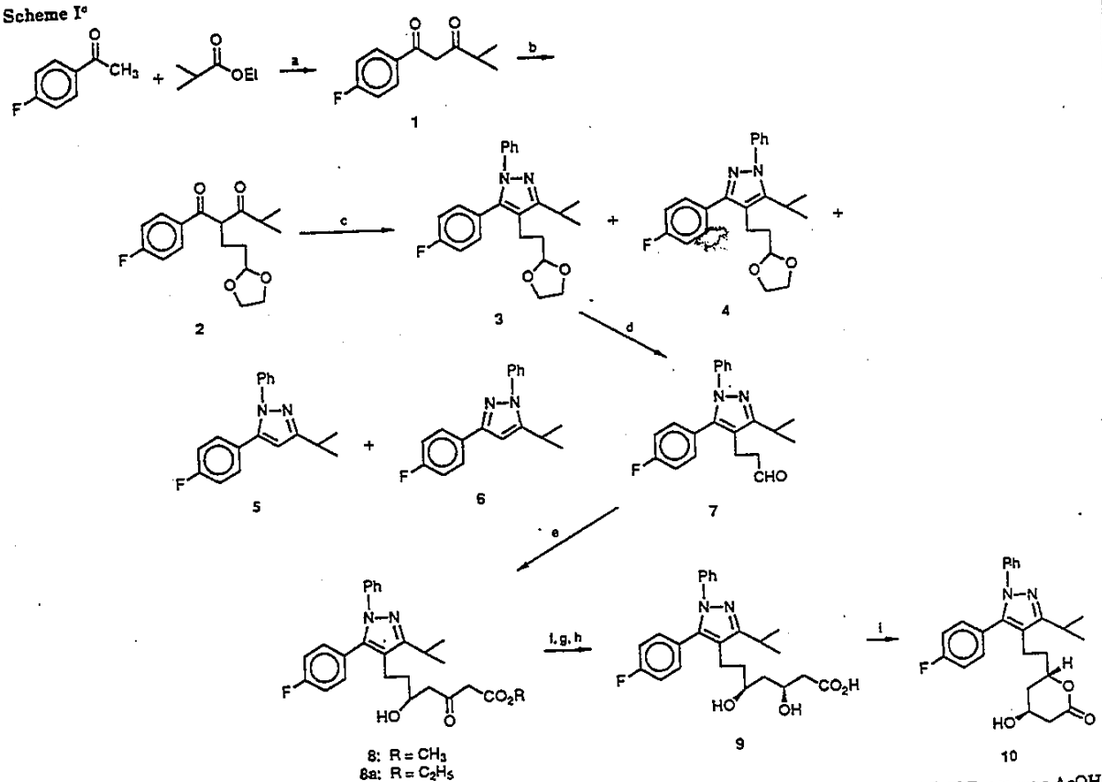


I

no.	R	mp, °C	formula[a]	method of prep	CSI IC_{50},[f,g] μM	rel (CSI) potency[b]
10	Ph	165–167	$C_{25}H_{27}FN_2O_3$	A, B	0.035	83.0
25	4-fluorophenyl	138–142	$C_{25}H_{26}F_2N_2O_3$	A	0.032	62.0
26	4-methylphenyl	152–153	$C_{26}H_{29}FN_2O_3$	A	0.040	49.0
27	4-tolylsulfonyl	foam	$C_{26}H_{29}FN_2O_5S$	B	0.660	4.5
28	4-methoxyphenyl	134–139	$C_{26}H_{29}FN_2O_4$,[c]	A	0.039	75.8
29	benzyl	145–148	$C_{26}H_{29}FN_2O_3$,[d]	A	0.158	12.6
30	1-naphthyl	75–81	$C_{29}H_{29}FN_2O_3$,[e]	B	0.234	19.6

[a] Analytical results are within ±0.4% of the theoretical values unless otherwise noted. [b] Potency of compactin arbitrarily assigned a value of 100, and the IC_{50} value of the test compound was compared with that of compactin determined simultaneously. [c] Anal. Calcd: C, 69.01. Found: C, 68.30. >98% pure by HPLC. [d] Anal. Calcd: H, 6.70. Found: H, 7.22, Calcd: N, 6.42. Found: N, 5.85. >98% pure by HPLC. [e] Anal. Calcd: C, 73.21. Found: C, 72.46. >98% pure by HPLC. [f] Cholesterol synthesis inhibition (CSI). Assays of each inhibitor concentration were performed in triplicate and the precision for compactin was 37%. See ref 1. [g] All compounds tested had a diastereomeric purity of >95% of the trans diastereomer as determined by HPLC and/or 200-MHz NMR.

Scheme I[a]



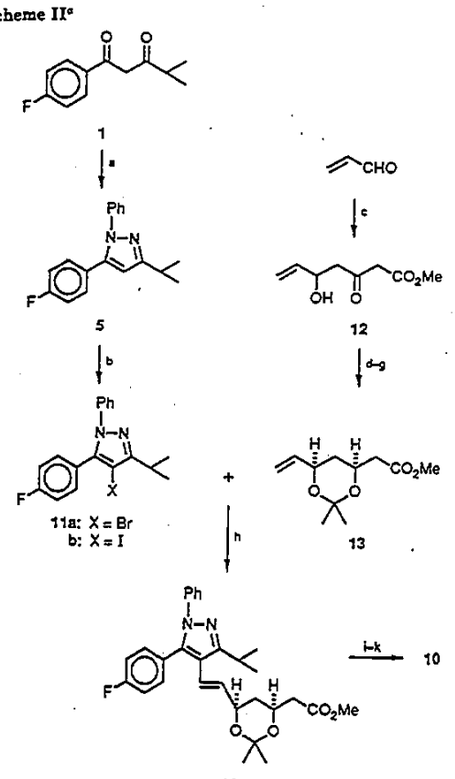
[a] (a) NaH, DMF, 80 °C; (b) NaH, DMF, NaI, $BrCH_2CH_2\overline{CHO(CH_2)O}$; (c) $PhNHNH_2$, AcOH, room temperature; (d) 70% aqueous AcOH, Δ; (e) $^-CH_2CO^-CHCO_2R$; (f) BR_3, air; (g) $NaBH_4$, −78 °C; (h) H_2O_2, ^-OH; (i) tol, Δ.

improved inhibitory potencies compared to the pyrrole mevalonolactones.

Chemistry

The target lactones, listed in Table I, were prepared by the general synthetic routes outlined in Schemes I and II. The general method (method A) employed for the construction of the pyrazole nucleus was condensation of a 1,3-dicarbonyl compound with a suitably substituted hydrazine. Two regioisomers can theoretically arise, but by

Scheme II[a]



[a] (a) $PhNHNH_2$, AcOH, room temperature; (b) NBS or NIS, DMF, 0 °C; (c) $^-CH_2CO^-CHCO_2Et$; (d) Bu_3B, air; (e) $NaBH_4$; (f) H_2O_2/OH^-, (g) $(CH_3)_2C(OCH_3)_2$, CSA, acetone; (h) $(Ph_3P)_2PdCl_2$, Et_3N, DMF, 70 °C; (i) H_2, Pd/C; (j) HCl, NaOH; (k) Tol, Δ.

judicial choice of solvent and reaction temperature, one regioisomer can predominate. Initial studies began with the incorporation of the preferred substituents (4-fluorophenyl and isopropyl) discovered in the SAR of the pyrrole mevalonolactones.[1] The requisite 1,3-diketone 1 was synthesized by a Claisen type acylation of 4-fluoroacetophenone with ethyl isobutyrate.[3] This product, which was almost completely enolized (86% by NMR), was alkylated with 2-(2-bromoethyl)-1,3-dioxolane[4] to give the C-alkylated 1,3-diketone 2 in 58% yield, together with a small amount of material presumed to be the O-alkylated product. Condensation with phenylhydrazine in acetic acid at room temperature afforded predominantly one regioisomer (~90%), tentatively assigned structure 3 in which the aryl groups exist in a 1,5-relationship (rather than 1,3). NMR studies[5] on 1,3- and 1,5-diphenylpyrazoles have shown that the chemical shifts of phenyl groups in the 1,3-regioisomer extend from δ 7.0 to 8.1 ppm. In our case, downfield resonances at δ 8.0 ppm were barely discernible. The majority of the aryl proton resonances were found in the region from δ 7.0 to 7.3 ppm which was in accordance with resonances published for 1,5-diphenylpyrazole. This regiochemistry was confirmed by an X-ray crystallographic analysis of the eventual target lactone derived from 3 (vide supra).[6] An ORTEP drawing of the solid-state conformation of compound 10 is shown in Figure 1. Increased amounts of the 1,3-regioisomer 4 were obtained by changing the reaction solvent to absolute ethanol or by raising the reaction temperature (regardless of solvent choice). Using either (4-chlorophenyl)hydrazine or (4-fluorophenyl)-hydrazine in absolute ethanol at reflux, the regioisomer ratio of pyrazoles obtained was 5:1 (1,5:1,3), this ratio was improved (~10:1) by changing solvent to acetic acid. Also isolated from this reaction was an oil later identified by NMR and independent synthesis[7] as a 5:1 mixture of pyrazole regioisomers 5 and 6 which was presumably derived from the O-alkylated material present from the previous reaction.

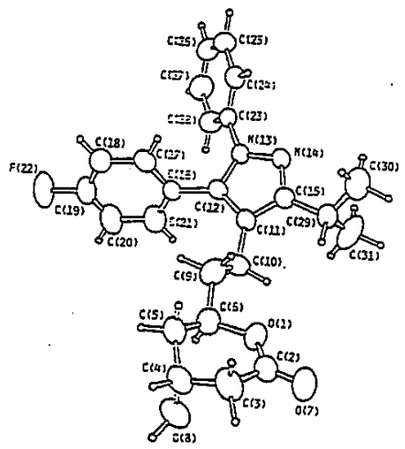


Figure 1. ORTEP view of lactone 10. Solid-state conformation and crystallographic atom numbering scheme; small circles denote hydrogen atoms.

Acidic hydrolysis of the acetal 3 provided aldehyde 7, which was condensed with the dianion of methyl acetoacetate.[8] Reduction of the resulting δ-hydroxy-β-keto ester 8 was achieved by the boron chelation method of Narasaka and Pai.[9] Thus, compound 8 was complexed with tri-*n*-butylborane prior to treatment with sodium borohydride. The resulting boronate ester was hydrolyzed with 30% hydrogen peroxide and base to give a mixture of syn (9) and anti 1,3-dihydroxy acids, which were lactonised in refluxing toluene with azeotropic removal of water to give predominantly the trans lactone 10 in good yield. HPLC analysis of the lactone 10 showed that the stereoselectivity achieved (3.3:1 trans:cis diastereomers) was not as high as that achieved in the pyrrole series (10:1 trans:cis).[1] No improvement in stereoselectivity was found on addition of an extra equivalent of *n*-Bu_3B, ruling out the possibility of competitive chelation with the pyrazole free nitrogen atom; thus the reason for this lack of stereoselectivity in the pyrazole series remains unclear. Excellent stereoselectivity (>20:1 trans:cis) was achieved by employing triethylborane as chelating agent with pivalic acid catalysis and methanol as cosolvent.[10]

An alternative route (Scheme II) was devised in which the key step was the palladium-catalyzed vinylation of a

(3) Levine, R.; Conroy, J. A.; Adams, J. T.; Hauser, C. R. *J. Am. Chem. Soc.* 1945, *67*, 1516.
(4) Buchi, G.; Wüest, H. *J. Org. Chem.* 1969, *34*, 1122.
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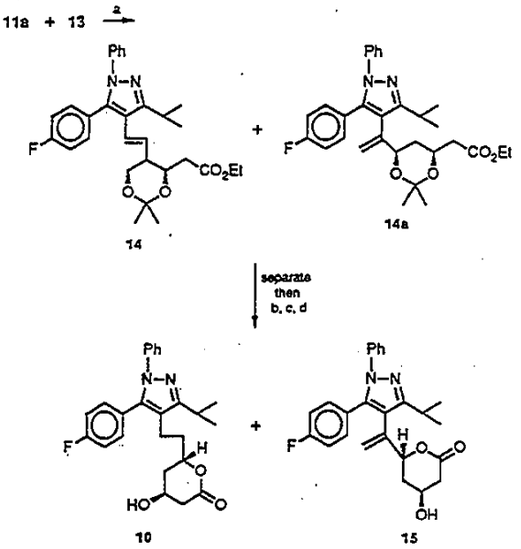
(10) Verhoeven, T. R. *Eur. Pat.* 0164, 049, 1985.

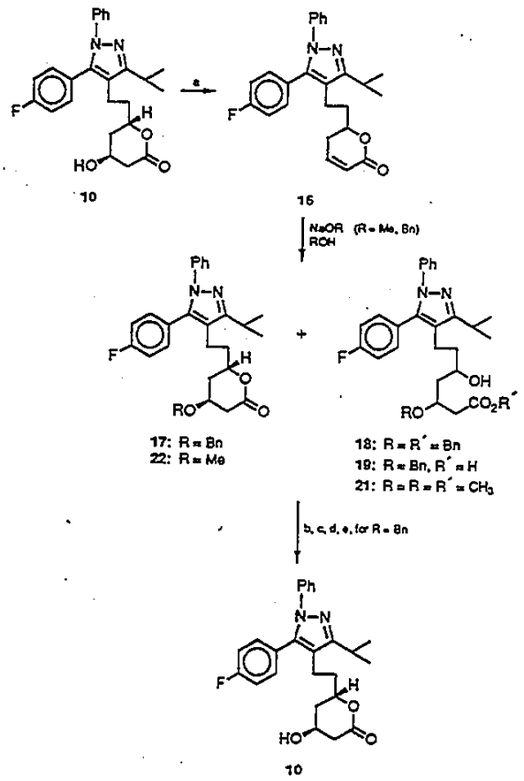
Scheme III



[a] (a) $(PPh_3)_2PdCl_2$, DMF, Et_3N; (b) H_2, Pd/C; (c) HCl, NaOH; (d) Tol, Δ, $-H_2O$.

halopyrazole (11a,b) with the intact lactone side chain (13).[11] This route had the advantages of being convergent and providing products of satisfactory stereochemical purity (method B). The heterocyclic halides 11a,b were prepared by condensation of 1,3-diketone 1 with phenylhydrazine in acetic acid at room temperature followed by halogenation of the resulting pyrazole 5 with either NBS or NIS in DMF at 0 °C. The alkene portion (13) was constructed via aldol condensation of acrolein with the dianion of methyl (or ethyl) acetoacetate,[12] reduction as before gave the diol, which was protected as the acetonide 13 (25:1 trans:cis diastereomers). Although treatment of 11a with 13 under the standard conditions described by Heck[11] did in fact provide a modest (50%) yield of 14, this reaction proved capricious. A variety of catalysts were employed (e.g., $(Ph_3P)_2PdCl_2$, $Pd(OAc)_2$, 10% Pd/C, polymer-supported catalysts, etc.), and it was concluded that 2–6 mol % of $(Ph_3P)_2PdCl_2$ was the preferred catalyst. A number of bases (e.g., tri-*n*-butylamine, diisopropylethylamine, and triethylamine) and solvents (e.g., DMF and acetonitrile) were examined, and the best yields were obtained with triethylamine and DMF as solvents. Cbanging the heterocyclic halide from bromide (11a) to iodide (11b) gave increased amounts of the dehalogenated pyrazole 5. Although it has been reported that use of a more hindered phosphine ligand on the catalyst reduces this side reaction, replacement of $(Ph_3P)_2PdCl_2$ with $[(o\text{-}CH_3Ph)_3P]_2PdCl_2$ provided no improvement in yield.[11] The 200-MHz NMR showed the formation of predominantly the trans alkene 14 (J_{trans} = 15 Hz). A minor product was produced by addition to the more substituted carbon atom of the double bond (Scheme III), giving the olefin 14a. This structure was confirmed by HETCOR NMR[13] on the resulting lactone 15. Catalytic reduction of olefin 14, removal of the protecting groups, and lactonization afforded lactone 10 as a mixture of diastereomers (64:1 trans:cis).

Scheme IV[a]

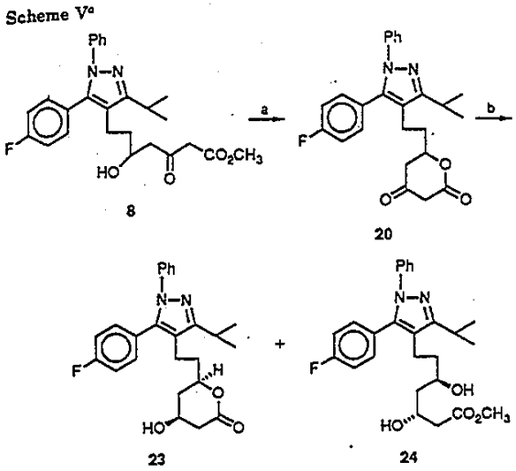


[a] (a) Ac_2O, DBU, CH_2Cl_2; (b) NaOH, (c) H^+; (d) H_2, Pd/C, EtOAc; (e) Tol, Δ.

In order to avoid the very low temperature reduction of compound 8 in Scheme I and the capricious nature of the Heck reaction shown in Scheme II, an alternative synthesis was devised in which the required 1,3-asymmetry was introduced by the stereospecific 1,4-conjugate addition of an alkoxide.[14] Thus, elimination of water from the mixture of lactone diastereomers 10 produced by borohydride reduction or from the cis lactone 23 obtained from the catalytic reduction of compound 20 produced the $\Delta^{\alpha,\beta}$-unsaturated lactone 16 in 68% yield (Scheme IV). Addition of sodium benzylate in benzyl alcohol afforded a mixture of products thought to consist mainly of compounds 17 and 18. After base hydrolysis the mixture was acidified to predominantly hydroxy acid 19. This material was then hydrogenated over 10% Pd/C and the resulting material lactonized to give compound 10 as a mixture of diastereomers (8:1 trans:cis by HPLC). In a similar fashion, sodium methoxide was added to lactone 16 to give, after base hydrolysis, acidification, and lactonization, the 4-methoxy lactone 22 as a mixture of diastereomers (7.4:1 trans:cis by HPLC). The cis diastereomer 23 was obtained as the predominant product by catalytic hydrogenation of ketone 20, which was prepared by base hydrolysis of compound 8 (Scheme V). Catalytic reduction of compound 20 gave, after chromatography, a mixture of ester 24 and lactone 23 (4:1 cis:trans diastereomers).

(11) Heck, R. F. *Org. React.* (*N.Y.*) 1982, *27*, 345.
(12) Brussani, G.; Ley, S. V.; Wright, J. L.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* 1986, 303.
(13) Benn, R.; Günther, H. *Angew. Chem., Int. Ed. Engl.* 1983, *22*, 350.
(14) Roth, B. D.; Roark, W. H. *Tetrahedron Lett.* 1988, 3255–58.

Scheme V[a]



[a] (a) NaOH then H^+; (b) 10% Ru-C, H_2, MeOH, room temperature.

Table II. In Vitro Inhibitory Potencies against HMG-CoA Reductase

no.	CSI IC_{50},[a,c] μM	rel potency[b]
15	17.8	0.17
20	10.0	0.32
22	3.16	1.00
23	0.7	4.40

[a] Cholesterol synthesis inhibition (CSI). Assays of each inhibitor concentration were performed in triplicate and the precision for compactin was 37%. See ref 1. [b] Potency of compactin arbitrarily assigned a value of 100, and the IC_{50} value of the test compound was compared with that of compactin determined simultaneously. See ref 1. [c] The diastereomeric purities of compounds 22 and 23 are indicated in the Experimental Section. Compound 15 had a diastereomeric purity of >95% of the trans diastereomer as indicated by 200-MHz NMR.

Biological Results

The target lactones and related compounds listed in Tables I and II were saponified to the hydroxy acids and tested for their ability to inhibit the enzyme HMG-CoA reductase by employing a crude liver homogenate derived from rats fed a chow diet containing 5% cholestyramine.[1,15] This screen was designated CSI (cholesterol synthesis inhibition screen). The biological activities are displayed in Tables I and II as an IC_{50} (i.e., the concentration needed to inhibit enzyme activity by 50%). Compactin was employed as the internal standard in each testing protocol.

The optimum distance between the lactone and the heterocyclic ring in the pyrrole series was achieved by a two-carbon bridging unit.[1] This feature was incorporated in all the pyrazole derivatives described here apart from compound 15, in which the pyrazole and lactone portions are separated by only one carbon atom. This compound is relatively inactive.

Modification of the lactone portion generally decreases the activity and confirms the strict structural requirements found by others.[16] Methyl ether 22 exhibited about $^1/_{100}$ potency of compactin whereas the racemic hydroxy compound 10 was nearly equipotent; if resolved, this compound would be expected to be more potent than compactin. The keto analogue 20 also exhibited low potency.[17] The cis lactone stereoisomer 23 (a 4:1 mixture of cis:trans diastereomers by HPLC) also displayed significantly reduced biological activity.[16] The residual biological activity was probably due to the presence of the trans diastereomer.

As previous studies suggested that the 5-(4-fluorophenyl) and 3-(1-methylethyl) substituents afforded optimum potency, we focused our attention on variations in position 1 of the pyrazole ring. A number of (para-substituted phenyl)hydrazines were employed, and it was demonstrated that in the limited series of compounds prepared, varying the electronic distribution in the phenyl ring did not, in general, have deleterious effects on in vitro potency. Electron-withdrawing, e.g., 25, and electron-donating, e.g., 26 and 28, groups were equally tolerated; however, compound 27, which has a hydrophilic electron-withdrawing group present, was considerably less potent. Replacement by naphthyl (e.g., 30) caused a significant decrease in potency as did replacement by an alkyl group, e.g., 29.

Conclusion

A small series of pyrazole mevalonolactones were prepared and evaluated for their ability to inhibit the enzyme HMG-CoA reductase in vitro. By focusing on compounds possessing the 5-(4-fluorophenyl)-3-(1-methylethyl) substitution found to be optimum in previous studies, a compound (10) was rapidly identified that was almost equipotent to compactin. Additional modification of the 1-phenyl ring of 10 did not improve activity in vitro.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were determined on a Nicolet MX-1 FT-IR spectrophotometer. Nuclear magnetic resonance spectra were determined on either a Varian EM-390 or a Varian XL-200 spectrometer. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses were determined on a Perkin-Elmer 240C elemental analyzer. HPLC analyses were performed on a Varian 5500 HPLC with a UV 200 detector (wavelength was 251 nm). The detailed protocol of the biological assay is described in ref 1.

1-(4-Fluorophenyl)-4-methyl-1,3-pentanedione (1). A mixture of 4-fluoroacetophenone (150 g, 1.09 mol) and ethyl isobutyrate (126 g, 1.09 mol) in dioxane (1.5 L) was added dropwise under a nitrogen atmosphere to a vigorously stirred suspension of hexane-washed sodium hydride (133 g, 58.8% NaH, 3.25 mol) in dioxane (3.0 L). Vigorous evolution of gas ensued, after which the mixture was heated to 80–90 °C for 4 h. The mixture was then allowed to cool to room temperature, after which it was poured into ice-cold 2 M hydrochloric acid (6 L) with vigorous stirring and extracted with ethyl acetate (4 × 1 L). The combined ethyl acetate extracts were washed with water (2 × 500 mL) and brine (2 × 500 mL) and dried ($MgSO_4$). The solution was filtered and the filtrate concentrated under vacuum. Distillation of the residue yielded compound 1: bp 100–110 °C/1 mm (116 g, 50%); ^1H NMR ($CDCl_3$) δ 1.25 (s, 3 H), 1.30 (s, 3 H), 2.60 (m, 1 H, J = 7 Hz), 6.1 (s, 1 H), 7.15 (m, 2 H), 7.9 (m, 2 H), and 16.2 (br s, 1 H) ppm. IR (thin film) 2973, 2825, 1653, 1603, 1578, 1509, 1462, 1240, 1160, 1089, 851, and 793 cm^{-1}. Anal. ($C_{12}H_{13}FO_2$) C, H, F.

2-[2-(1,3-Dioxolan-2-yl)ethyl]-1-(4-fluorophenyl)-4-methyl-1,3-pentanedione (2). To a suspension of hexane-washed sodium hydride (22.8 g, 58% NaH, 0.56 mol) in anhydrous dimethylformamide (DMF) (750 mL) was added dropwise, with

(15) Dugan, R.; Slakey, L. L.; Briedis, A. V.; Porter, J. W. *Arch. Biochem. Biophys.* 1972, *152*.
(16) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* 1985, *28*, 347.
(17) One possible explanation for this lack of activity may have been that during the biological assay procedure, base treatment of compound 20 may not have produced the open acid form. We thank the reviewer for this suggestion.

vigorous stirring under a nitrogen atmosphere, a solution of 1 (116 g, 0.56 mol) in anhydrous DMF (450 mL). Vigorous effervescence ensued. When gas evolution had ceased, sodium iodide (21.0 g, 0.14 mol) was added, followed by the dropwise addition of 2-(2-bromoethyl)-1,3-dioxolane[4] (100.9 g, 0.56 mol) in anhydrous DMF (450 mL). The resulting mixture was heated at 80–90 °C for 36 h after which it was cooled to room temperature and poured into ice–water (2 L). This was extracted with ethyl acetate (4 × 1 L), and the combined organic extracts were washed successively with water (500 mL) and brine (500 mL) and dried ($MgSO_4$). The solution was filtered and the filtrate was concentrated under vacuum. The residue was flash chromatographed on silica gel, eluting with 25% ethyl acetate–hexane to yield 2 (100 g, 58%); ^1H NMR ($CDCl_3$) δ 1.1 (s, 3 H), 1.15 (s, 3 H), 1.7 (m, 2 H), 2.2 (m, 2 H), 2.8 (m, 1 H), 3.9 (m, 4 H), 4.7 (t, 1 H), 4.9 (t, 1 H), 7.2 (m, 2 H), and 8.1 (m, 2 H) ppm; IR (thin film) 2972, 1723, 1676, 1600, 1509, 1411, 1237, 1160, and 1037 cm^{-1}. Anal. ($C_{17}H_{21}FO_4$) C, H, F.

4-[2-(1,3-Dioxolan-2-yl)ethyl]-5-(4-fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (3). To solution of 2 (104.75 g, 0.34 mol) in absolute ethanol under nitrogen (1 L) was added dropwise, with stirring, phenylhydrazine (40.45 g, 0.374 mol). When addition was complete, the solution was heated under reflux for 5 days[18] and then cooled to room temperature. The solution was concentrated under vacuum and chromatographed on silica gel. Elution with 15% ethyl acetate–hexane gave a yellow oil (9.7 g, R_f 0.55 (15% EtOAc–hexane)) identified by NMR and synthesis as a 5:1 mixture of regioisomers 5 and 6. Further elution gave a 10:1 regioisomer mixture of pyrazoles 3 and 4 (NMR shows two sets of isopropyl methyl groups at δ 1.4 and 1.2 ppm in a 10:1 ratio). This mixture solidified and was recrystallized (hexane) to give 3: mp 98–100 °C (hexane) (50.85 g, 40%); ^1H NMR ($CDCl_3$) δ 1.4 (s, 3 H), 1.35 (s, 3 H), 1.8 (m, 2 H), 2.7 (m, 2 H), 3.1 (t, 1 H), 3.9 (m, 4 H), 4.8 (t, 1 H), and 7.2 (m, 9 H) ppm; IR (KBr) 2950, 2900, 1596, 1566, 1511, 1440, 1377, 1227, 1158, 1143, 1058, 970, and 842 cm^{-1}. Anal. ($C_{23}H_{25}FN_2O_2$) C, H, N.

3(or 5)-(4-Fluorophenyl)-5(or 3)-(1-methylethyl)-1-phenyl-1*H*-pyrazoles (5 and 6). To a solution of 1 (1 g, 0.0048 mol) in absolute ethanol (10 mL) was added via a syringe, with stirring, phenylhydrazine (0.52 mL, 0.0053 mol). The solution was heated to reflux for 24 h and then cooled to room temperature. The solution was concentrated under vacuum and then chromatographed on silica gel. Elution with 5% ethyl acetate–hexane gave a yellow oil (1.1 g, R_f 0.24 (5% EtOAc–hexane)) identified by NMR as a 5:1 regioisomer mixture of 5 and 6. The oil solidified and was recrystallized (hexane) to give a 5:1 mixture of regioisomers: mp 67–70 °C (0.5 g, 37%); ^1H NMR ($CDCl_3$) δ 1.2 (d, 6 H, $(CH_3)_2CH$, regioisomer (6) (ht = 1)), 1.3 (d, 6 H, $(CH_3)_2CH$, regioisomer (5) (ht = 5), 3.1 (m, 1 H), 6.35 (s, 1 H, 4 H regioisomer (5) (ht = 5)), 6.5 (s, 1 H, 4 H regioisomer (6) (ht = 1)) and 6.9–7.4 (m, 9H) ppm; IR (KBr) 3450, 3053, 2964, 1594, 1510, 1440, 1374, 1302, 1222, 1164, 996, and 849 cm^{-1}. Anal. ($C_{18}H_{17}FN_2$) C, H, N.

5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole-4-propanal (7). A solution of 3 (50.85 g, 0.134 mol) in 70% aqueous acetic acid (1.0 L) was heated under reflux for 48 h with stirring. The solution was then cooled to room temperature and partitioned between ethyl acetate (1.0 L) and water (1.0 L). The phases were separated, and the aqueous phase was reextracted with ethyl acetate (1.0 L). The combined organic layer was washed successively with saturated sodium bicarbonate solution (250 mL), water (250 mL), and brine (250 mL). The ethyl acetate solution was dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was flash chromatographed on silica gel, eluting with 15% ethyl acetate–hexane. The eluted material solidified and was recrystallized (hexane) to give 7: mp 86–88 °C (hexane) (29.0 g, 65%); ^1H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.35 (s, 3 H), 2.4 (t, 2 H), 2.7 (t, 2 H), 3.05 (m, 1 H), 7.2–7.6 (m, 9 H), and 9.6 (s, 1 H) ppm. IR (KBr) 2961, 2869, 1728, 1609, 1598, 1498, 1439, 1376, 1334, 1224, 1159, 971, 840, and 767 cm^{-1}. Anal. ($C_{18}H_{17}FN_2O$) H, N; C: calcd, 69.21; found, 68.51.

(±)-Methyl 5-(4-Fluorophenyl)-δ-hydroxy-3-(1-methylethyl)-β-oxo-1-phenyl-1*H*-pyrazole-4-heptanoate (8). Methyl acetoacetate (11.48 mL, 0.106 mol) in anhydrous THF (100 mL) was added dropwise to a stirred suspension of sodium hydride (58.8% oil suspension, 4.56 g, 0.116 mol) in anhydrous THF (100 mL) at 0 °C under an N_2 atmosphere. When gas evolution was complete, a 2.6 M solution (40.9 mL, 0.106 mol) of *n*-butyllithium in hexane was added over 30 min. The resulting solution was stirred for an additional 60 min at 0 °C and then cooled to −78 °C (dry ice/acetone). This was then treated with a solution of 7 (23.8 g, 0.0709 mol) in anhydrous THF (100 mL) added dropwise over 60 min. The resulting orange solution was stirred 30 min at −78 °C and then at 0 °C for an additional 30 min before quenching with glacial acetic acid (35 mL) and 2 M aqueous HCl (70 mL) with vigorous stirring. The resulting mixture was then partitioned between diethyl ether (750 mL) and water (250 mL). After separation of phases, the aqueous layer was reextracted with diethyl ether (200 mL), and the combined organic extracts were washed successively with 0.2 M HCl (200 mL), water (200 mL), saturated sodium bicarbonate solution (3 × 150 mL), and brine (200 mL). The ether solution was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield a yellow oil, which was then flash chromatographed on silica gel. Elution with 40% ethyl acetate gave 8 (32.3 g, 84%): ^1H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.4 (s, 2 H), 1.45 (m, 2 H), 2.47 (d, 2 H), 2.7 (m, 2 H), 3.1 (m, 1 H), 3.6 (s, 3 H), 3.38 (s, 2 H), 3.9 (m, 1 H), and 6.8–7.2 (m, 9 H) ppm. The ethyl ester 8a was also synthesized in comparable yield with ethyl acetoacetate: ^1H NMR ($CDCl_3$) δ 1.27 (t, 3 H), 1.36 (s, 3 H), 1.40 (s, 3 H), 1.45 (m, 2 H), 2.6 (d, 2 H), 2.4–2.7 (m, 2 H), 3.1 (m, 1 H), 3.4 (s, 2 H), 3.9 (m, 1 H), 4.2 (q, 2 H), and 7.0–7.2 (m, 9 H) ppm; IR (thin film) 2965, 1743, 1714, 1654, 1599, 1559, 1512, 1500, 1374, 1227, 1160, and 844 cm^{-1}; HPLC indicated, 100% purity (retention time 23.2 min). Anal. ($C_{26}H_{29}FN_2O_4$) C, H; N: calcd, 6.19; found, 5.73.

(±)-*trans*-6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (10). **(i) Use of Tri-*n*-butylborane and Air Activation.** Through a THF (150 mL) solution of tri-*n*-butylborane (76.5 mL, 1 M, 0.076 mol) and 8 (31.48 g, 0.070 mol) was bubbled air (125 mL), and the solution was stirred at room temperature under a nitrogen atmosphere for 24 h. The solution was then cooled to −78 °C, and sodium borohydride (3.15 g, 0.0835 mol) was added in one portion. The mixture was allowed to warm to −20 °C over 2 h and then to 0 °C where it was stirred for 1 h. The reaction was then quenched by the addition of glacial acetic acid (14.6 mL, 0.205 mol) and water (17 mL). When gas evolution had ceased, 2 N sodium hydroxide (167 mL) was added followed by the dropwise addition of 30% hydrogen peroxide (25.7 mL, 0.25 mol) over 1 h. The resulting mixture was allowed to warm to room temperature overnight and then partitioned between ether (500 mL) and water (500 mL). The aqueous layer was separated and the ether layer was washed with 3 N NaOH (2 × 200 mL). The combined aqueous layers were then cooled to 0 °C and acidified with ice-cold 6 N HCl. This was then extracted with ethyl acetate (4 × 200 mL). The combined organic extracts were then washed with water (200 mL) and brine (2 × 200 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum to yield 9 (30 g, 95%) as a mixture of 3*R*,5*R*/3*S*,5*S* and 3*S*,5*R*/3*R*,5*S* racemates. This material was dissolved in toluene (500 mL) and heated under reflux with azeotropic removal of water for 3 h. The mixture was cooled to room temperature and concentrated in vacuo. The residue was flash chromatographed on silica gel, eluting with 75% ethyl acetate–hexane to produce 10 (16.6 g, 60%) as a colorless solid: mp 157–159 °C (5:1 cyclohexane:chloroform).

^1H NMR ($CDCl_3$) δ 1.3 (s, 3 H), 1.4 (s, 3 H), 1.6–1.9 (m, 4 H), 2.2 (br s, 1 H), 2.5–2.8 (m, 4 H), 3.1 (m, 1 H), 4.3 (m, 1 H), 4.6 (m, 1 H), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 3400, 2962, 2868, 1707, 1598, 1511, 1440, 1376, 1252, 1225, 1052, 972, 843, and 767 cm^{-1}.

HPLC (stationary phase, Altex C 18 column; mobile phase, 50:50 0.05 M citric acid (pH = 4.0)/CH_3CN) indicated a 3.3:1 mixture of trans (t_R = 13.1 min)/cis (t_R = 12.0 min) diastereomers. Anal. ($C_{26}H_{27}FN_2O_3$) C, H, N. The cis diastereomer was visible by NMR; the H6 and H4 protons appeared as a broad multiplet at δ 4.1 ppm.

(ii) Use of Triethylborane with Pivalic Acid Catalysis. To a room temperature solution of triethylborane (2.5 mL of a 1 M THF solution (0.002 14 mol)) under a nitrogen atmosphere

(18) Use of acetic acid as solvent greatly reduces reaction times.

was added, with stirring, a catalytic amount of pivalic acid (0.022 g, 0.000 21 mol). The resulting solution was stirred at room temperature for 1 h before a THF (7 mL) solution of 8a (1 g, 0.002 14 mol) was added dropwise. The resulting solution was stirred at room temperature for a further 1 h before cooling to −78 °C. Methanol (1 mL) was added followed by the addition of sodium borohydride (0.0893 g, 0.002 36 mol) in one portion. Vigorous gas evolution ensued. This mixture was stirred at −78 °C for 2.5 h. It was then poured into an excess of ice-cold 30% hydrogen peroxide (10 mL) and extracted with ethyl acetate. The organic layer was then washed extensively with water and brine, dried ($MgSO_4$), filtered, and evaporated to yield 1.0 g of the corresponding 1,3-diol (quantitative) as a 23:1 mixture of 3*R*,5*R*/3*S*,5*S*; and 3*S*,5*R*/3*R*,5*S* racemates. (HPLC indicated that the 3*R*,5*R*/3*S*,5*S* racemate had a retention time of 13.5 min and the 3*R*,5*R*/3*R*,5*S* racemate had a retention time of 11.7 min.)

5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (5). To a solution of 1 (10.6 g, 0.0509 mol) in glacial acetic acid (100 mL) was added at room temperature phenylhydrazine (6.04 g, 0.0559 mol). The mixture was stirred overnight at room temperature and then poured into ice-cold saturated aqueous sodium bicarbonate (200 mL). An oil precipitated, which then crystallized. These crystals were collected and redissolved in hexane. The hexane solution was washed with water (100 mL) and brine (100 mL) and then dried ($MgSO_4$). The solution was then concentrated to one-quarter of its original volume and cooled to yield 5 as colorless crystals: mp 70–72 °C (hexane) (12.0 g, 84%); ^1H NMR δ ($CDCl_3$) 1.34 (s, 3 H), 1.38 (s, 3 H), 3.1 (m, 1 H), 6.3 (s, 1 H), 6.9–7.3 (m, 9 H) ppm; IR (KBr) 3052, 2964, 1594, 1510, 1440, 1374, 1302, 1222, 1164, 1089, 995, and 849 cm^{-1}. Anal. ($C_{18}H_{17}FN_2$) C, H, N.

4-Bromo-5-(4-fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (11a). *N*-Bromosuccinimide (6.21 g, 0.0348 mol) was added to a solution of 5 (11.3 g, 0.0348 mol) in DMF (130 mL) at 0 °C under a nitrogen atmosphere. After 1 h, a solid was deposited, which was filtered and washed extensively with water. This solid was recrystallized from toluene to yield 11a: mp 126–128 °C (toluene) (8.1 g, 56%); ^1H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 3.1 (m, 1 H), 7.0–7.3 (m, 9 H); IR (KBr) 1593, 1551, 1496, 1376, 1304, 1227, 1160, 1109, 1036, 968, and 843 cm^{-1}. Anal. ($C_{18}H_{16}BrFN_2$) C, H, N.

5-(4-Fluorophenyl)-4-iodo-3-(1-methylethyl)-1-phenyl-1*H*-pyrazole (11b). *N*-Iodosuccinimide (4.81 g, 0.0214 mol) was added in one portion to a stirred solution of 5 (5.0 g, 0.0178 mol) in DMF (100 mL) cooled to 0 °C under a dry nitrogen atmosphere. The mixture was allowed to warm to room temperature overnight and then recooled to 0 °C before more *N*-iodosuccinimide (0.24 g, 0.0011 mol) was added. This was then allowed to warm to room temperature and then poured into water (500 mL). This aqueous mixture was extracted with diethyl ether (2 × 250 mL). The ether extracts were diluted with hexane (200 mL) and washed with water (100 mL), 10% aqueous sodium bisulfite (100 mL), and brine (100 mL) and dried ($MgSO_4$). Filtration and concentration afforded 11b (6.8 g, 94%) as orange/tan needles (mp 141–143 °C) (hexane): ^1H NMR ($CDCl_3$) δ 1.38 (s, 3 H), 1.42 (s, 3 H), 3.1 (m, 1 H), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 2929, 1600, 1542, 1500, 1460, 1427, 1373, 1298, 1229, 1159, 1028, 968, and 845 cm^{-1}. Anal. ($C_{18}H_{16}FIN_2$) C, H, N.

Methyl 5-hydroxy-3-oxo-6-heptenoate (12) was prepared as described by Ley et al.[12] Ethyl 5-hydroxy-3-oxo-6-heptenoate was prepared similarly in 94% yield: 12: ^1H NMR ($CDCl_3$) δ 1.2 (tr, 3 H), 2.78 (d, 2 H, 4-H, *J* = 6.3 Hz), 3.4 (s, 2 H, 2-H), 4.2 (q, 2 H), 4.6 (dt, 1 H, 5-H, *J* = 6.0, 6.3 Hz), 5.07–5.35 (m, 2 H, 7-H), and 5.88 (ddd, 1 H, 6-H, *J* = 16.3, 10.0, 6.0 Hz) ppm.

Methyl 6-Ethenyl-2,2-dimethyl-1,3-dioxane-4-acetate (13). Air (20 mL) was bubbled through a solution of triethylborane (64 mL, 1 M THF, 0.064 mol) and 12 (10 g, 0.058 mol) in anhydrous THF (50 mL) under a nitrogen atmosphere. The resulting solution was stirred overnight at room temperature and then cooled to −78 °C. Sodium borohydride (2.64 g, 0.0696 mol) was added in one portion, and the vigorously stirred suspension was allowed to warm slowly to 0 °C over 2 h. (Vigorous gas evolution was noticed at −50 °C.) The reaction was quenched by the dropwise addition of glacial acetic acid (15 mL) followed by addition of water (20 mL) and methanol (20 mL). After all the solid had been consumed, saturated aqueous sodium bicarbonate solution (50 mL) was added carefully, followed by the dropwise addition of 30% hydrogen peroxide (19.2 mL). This solution was stirred for 1 h and then poured into ether (800 mL). The organic phase was washed with water (2 × 160 mL) and brine (100 mL). It was dried ($MgSO_4$), filtered, and evaporated. The residue was flash chromatographed on silica gel, eluting with ethyl acetate–hexane (50:50), to give methyl 3,5-dihydroxy-6-heptenoate (7.05 g, 69%) as a mixture of 3*R*,5*R*/3*S*,5*S* and 3*S*,5*R*/3*R*,5*S* racemates, which was used in the subsequent step without further purification. This crude mixture (7.0 g, 0.04 mol) was dissolved in a mixture of dichloromethane (100 mL) and 2,2-dimethoxypropane (20 mL, 0.162 mol). A catalytic amount of camphorsulfonic acid (0.05 g) was added and the solution was stirred overnight at room temperature. Concentration and flash chromatography on silica gel (eluting with 25% ethyl acetate–hexane) of the resulting residue gave 13 (4.25 g, 50%) as a 25:1 mixture of 3*R*,5*R*/3*S*,5*S* and 3*S*,5*R*/3*R*,5*S* racemates (HPLC indicated that the 3*R*,5*R*/3*S*,5*S* racemate had a retention time of 8.5 min and the 3*S*,5*R*/3*R*,5*S* racemate had a retention time of 8.4 min): ^1H NMR ($CDCl_3$) δ 1.2–1.3 (m, 1 H, 5-H), 1.38 (s, 3 H), 1.45 (s, 3 H), 1.60 (m, 1 H, 5-H'), 2.36 (dd, 1 H, *J* = 14, 6 Hz), 2.56 (dd, 1 H, *J* = 14, 6 Hz), 3.6 (s, 3 H), 4.3–4.5 (m, 2 H, 4-H, 6-H), 5.1–5.3 (m, 2 H), 5.8 (m, 1 H) ppm; IR (thin film) 2994, 1743, 1439, 1382, 1316, 1261, 1203, 1170, 1099, 1001, and 926 cm^{-1}. Anal. ($C_{11}H_{18}O_4$) H; C: calcd, 61.66; found, 60.12.

(*E*)-Methyl 6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethenyl]-2,2-dimethyl-1,3-dioxane-4-acetate (14). A solution of 11a (1.07 g, 0.003 mol), 13 (1.1 g, 0.0051 mol), and bis(triphenylphopshine)palladium(II) chloride (0.042 g, 0.00006 mol, 2 mol %) in 6 mL of a 50:50 mixture of triethylamine and DMF was stirred and heated at reflux overnight under a nitrogen atmosphere. The solution was cooled to room temperature and diluted with ether (100 mL) and washed with water (100 mL), 2 M hydrochloric acid (50 mL), water (100 mL), saturated aqueous sodium bicarbonate (100 mL), and brine (500 mL). The organic extracts were dried ($MgSO_4$), filtered, and evaporated. The residue was flash chromatographed on silica gel, eluting with 10% ethyl acetate–hexane, to give 14 (0.74 g, 50%) as yellow crystals, mp 136–137 °C, together with small amounts of 5: ^1H NMR ($CDCl_3$) δ 1.25–1.6 (m, 14 H), 2.36 (dd, 1 H, *J* = 14, 6 Hz), 2.56 (dd, 1 H, *J* = 14, 6 Hz), 3.20 (m, 1 H), 3.7 (s, 3 H), 4.3 (m, 2 H), 5.7 (dd, 1 H, *J* = 15 Hz, 7 Hz), 6.23 (d, 1 H, *J* = 15 Hz), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 2914, 1739, 1663, 1597, 1546, 1510, 1441, 1379, 1276, 1225, 1160, 1078, 974, and 841 cm^{-1}; HPLC indicated a 59:1 mixture of 4*R*,6*R*/4*S*,6*S* and 4*S*,6*R*/4*R*,6*S* racemates (the 4*R*,6*R*/4*S*,6*S* racemate had a retention time of 12.57 min, and the 4*S*,6*R*/4*R*,6*S* racemate had a retention time of 13.87 min). Anal. ($C_{29}H_{33}FN_2O_4$) C, H, N.

(±)-*trans*-6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (10). A solution of 14 (0.63 g, 0.001 28 mol) in ethyl acetate (10 mL) was hydrogenated under a balloon of hydrogen gas with 10% palladium on charcoal as catalyst at 25 °C for 2 days. The catalyst was then removed by filtration through Celite, and the filtrate was concentrated and redissolved in 50:50 THF/1 M HCl (30 mL). This was stirred for 5 h at room temperature, and then 25% sodium hydroxide was added until the solution was basic (pH ~10). After stirring for 30 min, the mixture was diluted with water and extracted with ether. The aqueous solution was then acidified with 2 M hydrochloric acid and extracted with ethyl acetate. The organic extracts were then washed with brine and dried ($MgSO_4$). Filtration and concentration provided the crude dihydroxy acid, which was lactonized with azeotropic removal of water by refluxing in toluene for 3 h. The cooled solution was concentrated to ca. 10 mL and allowed to stand. Pure lactone 10 crystallized as a white solid (0.35 g, 65%) (mp 163–165 °C, 2× 165–167 °C): HPLC indicated a 64:1 mixture of trans (t_R = 13.4 min)/cis (t_R = 12.3 min) diastereomers. Anal. ($C_{25}H_{27}FN_2O_3$) C, H, N.

(±)-*trans*-6-[1-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethenyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (15). A mixture of crude 14 (34 g, 0.067 mol) and 10% Pd/C (1 g) in absolute EtOH (100 mL) was hydrogenated for 2 days at atmospheric pressure and room temperature. The catalyst was removed by filtration through Celite. After concentration, the filtrate residue was dissolved in 3:2:1 THF/3 M

HCl–MeOH (600 mL) and the mixture stirred for 3 days at room temperature. This was made alkaline (25% aqueous NaOH) and partitioned between ether and water. The aqueous layer was then acidified (2 M HCl) and extracted with ethyl acetate (2 × 250 mL). The combined organic extracts were then washed with brine (100 mL), dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in toluene and refluxed with azeotropic removal of water for 2 h. Concentration and flash chromatography on silica gel provided a first fraction identified as 15 (1.5 g, 5.3%; mp 157–158 °C) and a second fraction of 10 (6 g, 22%; mp 156–157 °C): ^1H NMR ($CDCl_3$) δ 1.3 (s, 6 H), 1.5 (m, 1 H), 1.7 (m, 1 H), 2.1 (br s, 1 H), 2.4 (m, 1 H), 2.7 (m, 1 H), 3.1 (m, 1 H), 4.1 (m, 1 H), 4.9 (dd, 1 H), 5.4 (d, 1 H), 5.7 (d, 1 H), and 7.0–7.4 (m, 9 H) ppm; IR (KBr) 2931, 1725, 1642, 1598, 1546, 1510, 1438, 1379, 1229, 1159, 1071, 1045, 975, 845, and 766 cm^{-1}. Anal. ($C_{25}H_{25}FN_2O_3$) C, H, N.

6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]-5,6-dihydro-2*H*-pyran-2-one (16). A solution of 10 (3.3:1 mixture of trans:cis isomers) (20 g, 0.0473 mol) was dissolved in anhydrous dichloromethane (50 mL) under a nitrogen atmosphere. Acetic anhydride (5.3 g, 0.052 mol) and DBU (15.8 g, 0.104 mol) were added dropwise to the solution. The reaction mixture was stirred overnight and then diluted with ether (150 mL) and washed with 2 M HCl (100 mL), saturated aqueous sodium bicarbonate solution (100 mL), and brine (100 mL), and dried ($MgSO_4$). Filtration and concentration gave a residue (17 g), which was passed through silica gel. Elution with hexane gave 16 (13 g, 68%) as a white solid (mp 89 °C (hexane)): ^1H NMR ($CDCl_3$) δ 1.36 (d, 6 H), 1.6–1.9 (m, 2 H), 2.2 (m, 2 H), 2.7 (m, 2 H), 3.0 (m, 1H), 4.3 (m, 1 H), 6.0 (dd, 1 H), 6.8 (m, 1 H), and 7.0–7.3 (m, 9 H) ppm; IR (KBr) 2961, 2868, 1723, 1596, 1562, 1511, 1439, 1376, 1336, 1248, 1159, 1094, 1043, 970, and 844 cm^{-1}. Anal. ($C_{25}H_{25}FN_2O_2$) C, H, N.

6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]dihydro-2*H*-pyran-2,4(3*H*)-dione (20). Ethyl acetoacetate (1.14 mL, 0.0089 mol) in anhydrous THF (15 mL) was added dropwise to a stirred suspension of hexane-washed sodium hydride (58.8% oil suspension) (0.225 g) in anhydrous THF (20 mL) at 0 °C under an N_2 atmosphere. When gas evolution was complete, a solution of *n*-butyllithium in hexane (3.9 mL, 0.0089 mol, 2.3 M) was added over 30 min. The resulting solution was stirred an additional 30 min at 0 °C and then cooled to −78 °C. This was then treated with a solution of 7 (2.0 g, 0.0059 mol) in anhydrous THF (15 mL). The resulting solution was stirred at −78 °C for an additional 40 min and then at 0 °C for 30 min. This was then poured into 25% aqueous NaOH (50 mL). The resulting mixture was then washed with ether (to remove starting aldehyde) and then acidified with ice-cold 6 M HCl. This was then extracted with ethyl acetate, the organic extract was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. Recrystallization from Et_2O–hexane (1:10) provided 20 (1.62 g, 65%): mp 141–143 °C; ^1H NMR ($CDCl_3$) δ 1.3 (d, 6 H), 1.6–1.9 (m, 2 H), 2.4 (m, 2 H), 2.8 (m, 2 H), 3.1 (m, 1 H), 3.3 (d, 2 H), 4.5 (m, 1 H), 7.1–7.3 (m, 9 H) ppm; IR (KBr) 2900, 1599, 1511, 1440, 1376, 1273, 1226, 1159, 842, and 766 cm^{-1}. Anal. ($C_{25}H_{25}N_2O_3F$) H, N; C: calcd, 71.41; found, 70.93.

Addition of Benzyl Alcohol to Compound 16. To a solution of 16 (6 g, 0.0148 mol) in benzyl alcohol (45 mL) at 0 °C was added sodium benzylate in benzyl alcohol (5.9 mL, 0.5 M). The reaction was allowed to warm to room temperature and then stirred for 24 h. The solution was then diluted with methanol and made alkaline (0.02 mol, 3 M NaOH). The resulting aqueous layer was washed with ether, acidified with 2 M HCl, and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried ($MgSO_4$). Filtration and concentration yielded a crude mixture of products (7.8 g) consisting mainly of the benzyl ether dihydroxy acid 19 and a small amount of lactone 17. This material was dissolved in ethyl acetate (30 mL) and 10% Pd/C (0.5 g) added. This was then hydrogenated at 1 atm of pressure for 2 days. The catalyst was then removed by filtration and the filtrate concentrated. The residue was dissolved in toluene (50 mL) and heated to reflux with azeotropic removal of water. The solution was cooled and the product (10) crystallized (3.8 g, 60%). HPLC showed a 8:1 trans:cis mixture of diastereomers.

Addition of Methanol to Compound 16. To a solution of compound 16 (1.1 g, 0.0027 mol) in methanol (25 mL) at room temperature under a nitrogen atmosphere was added sodium methoxide (0.017 g, 0.0003 mol). Reaction was almost instantaneous. TLC showed the formation of two products, the main product was presumably the ring opened methyl ether 21, the minor product was the lactone 22. This was then made alkaline with 25% NaOH and concentrated in vacuo. The residue was extracted with hexane and the remaining aqueous solution was acidified (0 °C, 12 N, HCl). The solution was then extracted with ethyl acetate and the organic solution was washed with water and brine and dried ($MgSO_4$). Filtration and concentration yielded crude product (1.1 g). This was dissolved in toluene (100 mL) and heated under reflux with azeotropic removal of water for 4 h. Flash chromatography on silica gel eluting with 40% ethyl acetate–hexane gave 6-[2-[5-(4-fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-methoxy-2*H*-pyran-2-one (22) (0.89 g, 75%): mp 86–88 °C; HPLC indicated a 7.4:1 mixture of trans (t_R = 23.9 min):cis (t_R = 21.8 min) diastereomers; ^1H NMR ($CDCl_3$) δ 1.25 (d, 6 H), 1.4–1.9 (m, 4 H), 2.4–2.6 (m, 4 H), 3.0 (m, 1 H), 3.2 (s, 3 H), 3.6 (m, 1 H), 4.3 (m, 1 H), 6.9–7.1 (m, 9 H) ppm; IR (KBr) 2958, 1744, 1595, 1565, 1511, 1439, 1376, 1253, 1224, 1157, 1098, 1071, and 840 cm^{-1}. Anal. ($C_{28}H_{29}FN_2O_3$) C, H, N.

(±)-*cis*-6-[2-[5-(4-Fluorophenyl)-3-(1-methylethyl)-1-phenyl-1*H*-pyrazol-4-yl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one (23). A methanolic solution (25 mL) of 20 (1 g, 0.0024 mol) was hydrogenated at atmospheric pressure and room temperature using 10% Ru/C as catalyst. This was stirred at room temperature for 5 days, filtered, and concentrated to yield 1.3 g of crude material. Flash chromatography on silica gel, eluting with 40% ethyl acetate–hexane provided a first fraction identified as 24 (0.55 g, 51%): mp 92–94 °C; ^1H NMR ($CDCl_3$) δ 1.37 (d, 6 H), 1.5 (m, 4 H), 2.4–2.7 (m, 4 H), 3.1 (m, 1 H), 3.7 (s, 3 H), 3.8 (m, 1 H), 4.2 (m, 1 H), and 7.0–7.2 (m, 9 H) ppm; IR (KBr) 2958, 2867, 1735, 1595, 1562, 1511, 1439, 1325, 1337, 1222, 1159, 1093, 983, and 840 cm^{-1}. Anal. ($C_{26}H_{31}FN_2O_4$) C, H, N.

A second fraction gave material identified as 23 (0.13 g, 13%): mp 145–147 °C; HPLC indicated a 4:1 mixture of cis (t_R = 10.51 min):trans (t_R = 11.41 min) diastereomers; ^1H NMR ($CDCl_3$) δ 1.3 (d, 6 H), 1.4–2.0 (m, 4 H), 2.3–2.9 (m, 4 H), 3.1 (m, 1 H), 4.1 (m, 2 H), and 7.0–7.2 (m, 4 H) ppm. Anal. ($C_{25}H_{27}FN_2O_3$) C, H, N.

The other diastereomer exhibits peaks at δ 4.5 ppm (H6′) and 4.3 ppm (H4′); IR (KBr) 3400, 2950, 1700, 1605, 1511, 1376, and 845 cm^{-1}.

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Supplementary Material Available: Preliminary X-ray crystallographic data for lactone 10 (4 pages). Ordering information is given on any current masthead page.

The solution was washed with saturated $NaHCO_3$ and evaporated to dryness. The residue was purified by chromatography on silica gel ($CHCl_3$–MeOH) to give the title compound (387 mg, 24%) after crystallization from petroleum ether: ^1H NMR ($CDCl_3$) δ 0.06 (s, 6 H, Me_2Si), 0.89 (s, 9 H, Me_3C), 3.66, 3.77 (A_2B_2, 4 H, $SiOCH_2CH_2O$), 5.25 (s, 2 H, NCH_2O), 5.27 [dd, J = 10.9, 1.1 Hz, 1 H, CH=C*H*(*Z*)H(*E*)], 5.98 [dd, J = 17.6, 1.1 Hz, 1 H, CH=CH(*Z*)*H*(*E*)], 6.42 (dd, J = 17.6, 10.9 Hz, 1 H, C*H*=CH_2), 7.41 (s, 1 H, 6-H), 9.59 (br, 1 H, NH).

1-[[2-[(*tert*-Butyldimethylsilyl)oxy]ethoxy]methyl]-6-(phenylthio)-5-vinyluracil. Following the general procedure for the preparation of 17–19, the title compound was prepared from the above compound with diphenyl disulfide as an electrophile: yield 46%; ^1H NMR ($CDCl_3$) δ 0.01 (s, 6 H, Me_2Si), 0.84 (s, 9 H, Me_3C), 3.63 (s, 4 H, $SiOCH_2CH_2O$), 5.33 [dd, J = 11.8, 2.0 Hz, 1 H, CH=C*H*(*Z*)H(*E*)], 5.61 (s, 2 H, NCH_2O), 6.33 [dd, J = 16.8, 2.0 Hz, 1 H, CH=CH(*Z*)*H*(*E*)], 6.71 (dd, J = 16.8, 11.8 Hz, 1 H, C*H*=CH_2), 7.15–7.30 (m, 5 H, SPh), 10.15 (br, 1 H, NH).

Following method A, 55 was prepared from the above compound.

1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)-5-vinyluracil (55): yield 41%; mp 100–103 °C (EtOAc–petroleum ether); UV (MeOH) λ_{max} 306 (ϵ 7600), 243 nm (ϵ 14 000); MS m/z 320 (M^+); ^1H NMR (Me_2SO-d_6) δ 3.35–3.52 (m, 4 H, $HOCH_2CH_2O$), 4.62 (t, J = 5.4 Hz, 1 H, OH), 5.22 [dd, J = 11.3, 2.2 Hz, 1 H, CH=C*H*(*Z*)H(*E*)], 5.48 (s, 2 H, NCH_2O), 6.21 [dd, J = 16.4, 2.2 Hz, 1 H, CH=CH(*Z*)*H*(*E*)], 6.63 (dd, J = 16.4, 11.3 Hz, 1 H, C*H*=CH_2), 7.23–7.40 (m, 5 H, SPh), 11.75 (br, 1 H, NH). Anal. ($C_{15}H_{16}N_2O_4S \cdot {}^1/_2H_2O$) C, H, N.

Antiviral Assay Procedures. The anti-HIV assays were based on the inhibition of the virus-induced cytopathic effect in MT-4 cells as previously described.[32] Briefly, MT-4 cells were suspended in culture medium at 2.5 × 10^5 cells/mL and infected with 1000 $CCID_{50}$ (50% cell culture infective dose) of HIV. Immediately after virus infection, 100 μL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4 (Table II) or 5 (Table I) day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[33] Cytotoxicity of the compounds was assessed in parallel with their antiviral activity. It was based on the viability of mock-infected host cells as determined by the MTT method.[33]

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Inhibitors of Cholesterol Biosynthesis. 3. Tetrahydro-4-hydroxy-6-[2-(1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-one Inhibitors of HMG-CoA Reductase. 2. Effects of Introducing Substituents at Positions Three and Four of the Pyrrole Nucleus

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A series of *trans*-tetrahydro-4-hydroxy-6-[2-(2,3,4,5-substituted-1*H*-pyrrol-1-yl)ethyl]-2*H*-pyran-2-ones and their dihydroxy acids were prepared and tested for their ability to inhibit the enzyme HMG-CoA reductase in vitro. Inhibitory potency was found to increase substantially when substituents were introduced into positions three and four of the pyrrole ring. A systematic exploration of structure–activity relationships at these two positions led to the identification of a compound ((+)-33, (+)-(4*R*)-trans-2-(4-fluorophenyl)-5-(1-methylethyl)-*N*,3-diphenyl-1-[(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl)ethyl]-1*H*-pyrrole-4-carboxamide) with five times the inhibitory potency of the fungal metabolite compactin.

Inhibition of HMG-CoA reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, has proven to be an effective means for lowering total and low-density lipoprotein (LDL) cholesterol in animal models and man.[1,2] The early reports describing the activity of the fungal metabolites compactin (mevastatin)[3] and mevinolin (lovastatin)[4] have been followed by a host of publications describing a large variety of natural[5] and synthetic inhibitors.[6] Previously, we disclosed a series of 1,2,5-trisubstituted-pyrrol-1-ylethylmevalonolactones which were found to be moderately potent inhibitors of HMGR in vitro.[7] By systematically altering the 2 and 5 substituents, maximal potency was obtained with the 2-(4-fluorophenyl)-5-isopropyl analogue (1). On the basis of those results, a molecular-modeling analysis led to the description of a pharmacophore model which characterized the size of the substituents at positions 2 and 5 and the conformation of the side chain. We have now discovered

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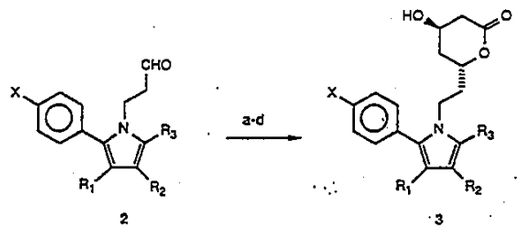
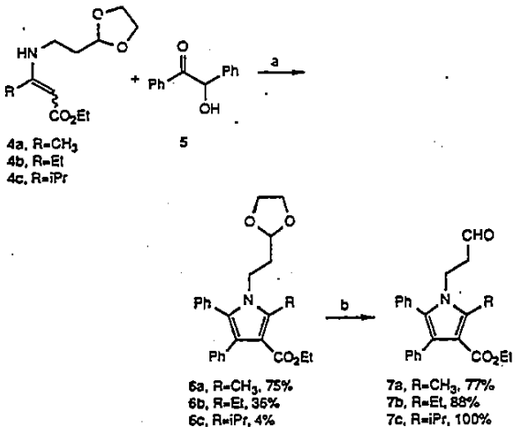
Scheme I[a]



[a] (a) $\overline{CH_2COCHCO_2ET}$, THF, −78 °C; (b) *n*-$Bu_3B$/$NaBH_4$, −78 °C; (c) H_2O_2, NaOH; (d) toluene, reflux.

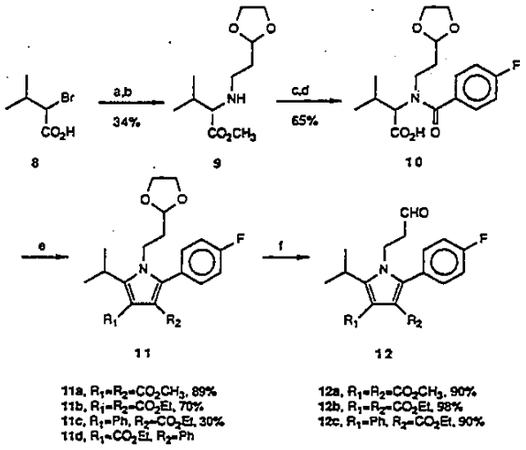
Scheme II. Method A[a]



[a] (a) $ZnCl_2$, EtOH, reflux; (b) *p*-TSA, acetone–water, reflux.

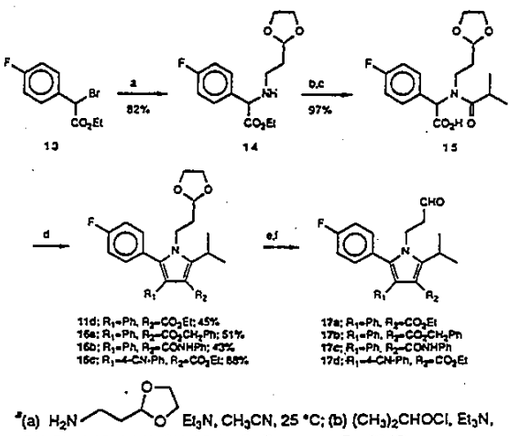
that the introduction of substituents into the 3 and 4 positions of the pyrrole ring results in significant improvements in potency at inhibiting HMGR in vitro. The results of these studies are described in this report.

Scheme III. Method B[a]

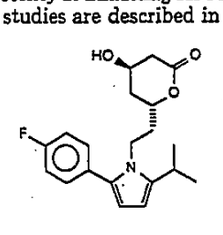


[a] (a) CH_3OH,DCC, DMAP; (b) H_2N (O) Et_3N, CH_3CN, reflux; (c) 4-F-Ph-COCl, Et_3N; (d) NaOH; (e) R_1—≡—R_2, Ac_2O, 90 °C; (f) *p*-TSA, acetone–water, reflux.

Scheme IV. Method C[a]



[a] (a) H_2N (O) Et_3N, CH_3CN, 25 °C; (b) $(CH_3)_2CHOCl$, Et_3N, CH_2Cl_2, 0 °C; (c) NaOH; (d) Ac_2O, R_1—≡—R_2, 90 °C; (e) HCl, EtOH, reflux; (f) *p*-TSA, acetone–water, reflux.



1

Chemistry

The general synthetic strategy employed was identical with that employed previously.[7] Thus, the pyrrole-3-propionaldehydes 2 were converted to the racemic trans

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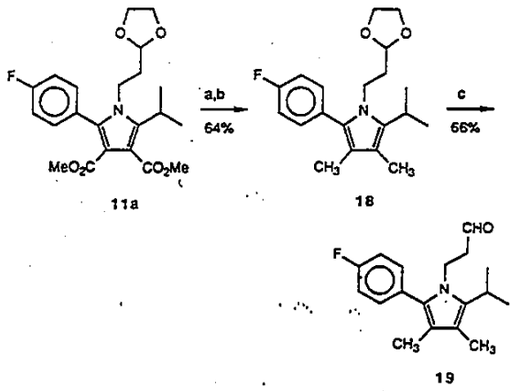
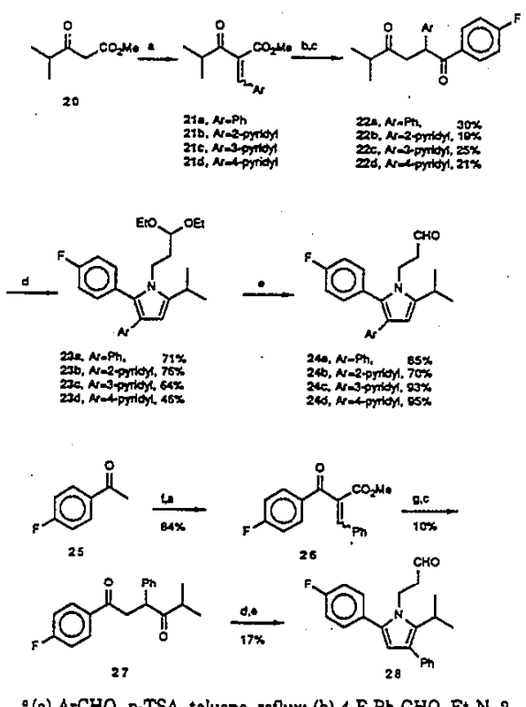
Scheme V. Method D[a]



[a] (a) $LiAlH_4$, ether–dichloromethane, reflux; (b) Et_3SiH, TFA–CH_2Cl_2, 0 °C; (c) *p*-TSA, acetone–water.

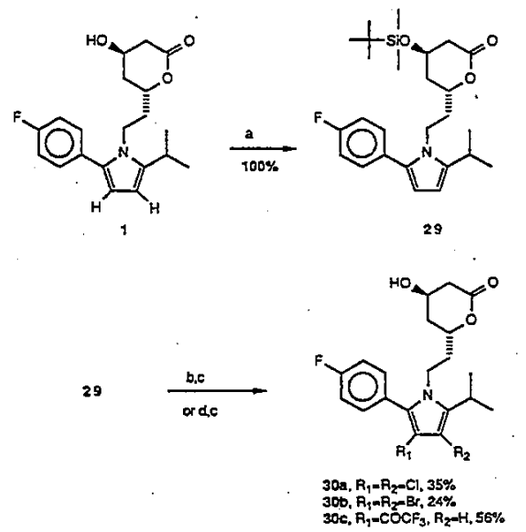
lactone stereoisomers **3** by (1) Weiler dianion condensation with ethyl acetoacetate, (2) stereoselective reduction to the syn-1,3-diol with tributylborane and sodium borohydride, (3) base hydrolysis, and (4) lactonization by refluxing in toluene with azeotropic removal of water (Scheme I). The requisite propionaldehydes **2** were prepared by several different synthetic routes. The less sterically hindered pentasubstituted pyrrole-3-propionaldehydes (**7a**, R = CH_3; **7b**, R = Et, Scheme II) could be prepared by $ZnCl_2$-catalyzed condensation of enamines **4a** and **4b** (prepared from 2-(2-aminoethyl)-1,3-dioxolane[8] and the requisite β-keto ester) with benzoin **5** (method A).[9] This reaction proved ineffective for the more sterically hindered pyrrole **7c**, containing the preferred 5-isopropyl substituent. The 5-isopropylpyrroles could be prepared in good yields, however, by the regioselective [3 + 2] cycloaddition of acetylenes with the amido acids **10** or **15** (Schemes III and IV).[10] Thus, reaction of ethyl phenylpropiolate with amido acid **10** in hot acetic anhydride afforded a 4:1 mixture of **11c** and **11d** (Scheme III, method B) from which **11c** crystallized in 30% yield. The reaction of **15** under identical conditions was regiospecific, producing **11d** as the sole product (Scheme IV, method C). The regiochemistry of compounds **11c** and **11d** were determined by comparison of their proton NMRs with that of the closely related **6c** ($(CH_3)_2CH$, occurs at δ 3.50 ppm in both **6c** and **11d**, but at δ 3.00 ppm in **11c**). As expected, the yield in this cycloaddition reaction was improved when more electron-deficient acetylenes were employed (compare **11a**, **11b**, and **16c** vs **11d** Scheme IV). The 3,4-dimethylpyrrole analogue **19** was prepared by reduction of diester **11a** to the corresponding diol with lithium aluminum hydride, followed by deoxygenation with triethylsilane and trifluoroacetic acid (Scheme V, method D).[11] The regioisomeric 3- and 4-arylpyrrole-3-propionaldehyde isomers **24a–d** and **28** were prepared by a Stetter reaction[12] of the appropriate aldehydes with the complementary α-benzylidene-β-keto esters (4-fluorobenzaldehyde with **21** and isobutyraldehyde with **26**, Scheme VI), followed by Paal–Knorr cyclization[13] with 3,3-diethoxy-1-amino-

Scheme VI. Method E[a]



[a] (a) ArCHO, *p*-TSA, toluene, reflux; (b) 4-F-Ph-CHO, Et_3N, 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride; (c) NaOH, CH_3OH, 25 °C; (d) $H_2NCH_2CH_2CH(OEt)_2$, *p*-TSA, toluene, reflux; (e) H_3O^+, (f) NaH, $(CH_3O)_2CO$; (g) $(CH_3)_2CHCHO$, Et_3N, 2-(2-hydroxyethyl)-3-methyl-4-benzylthiazolium chloride.

Scheme VII[a]



[a] (a) *t*-$BuMe_2SiCl$, imidazole, DMF, 25 °C, 18 h; (b) 2 equiv *N*-halosuccinimide, DMF, 0 °C; (c) *n*-Bu_4NF, HOAc, THF, 25 °C; (d) $(CF_3CO)_2O$, DMF, 0 °C.

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