Trends in Pharmacological Sciences

including TOXICOLOGICAL SCIENCES

New insights into the non-nicotinic regulation of adrenal medullary function

DURING THE PAST ten years, the most significant advances in our knowledge of how adrenal medullary catecholamine secretion is controlled have come from experiments using freshly isolated or cultured bovine chromaffin cells. Among the advances has been a fairly detailed understanding of the mechanisms coupling nicotinic stimulation of such cells with their exocytotic release of catecholamines[1]. Recently, however, there has been a notable increase in studies of non-nicotinic regulation of adrenal medullary function, and in many cases these have stemmed from the use of preparations other than bovine chromaffin cells. This change in emphasis was highlighted in a recent meeting on chromaffin cell biology*. Reported work on the functions of muscarinic and neuropeptide receptors is beginning to indicate the marked complexity of the control of catecholamine secretion.

Muscarinic responses

In most mammalian species, activation of both nicotinic and muscarinic acetylcholine receptors stimulate catecholamine secretion from the adrenal medulla. Bovine chromaffin cells, however, secrete catecholamines only in response to nicotinic stimulation[2]. This is not because they lack muscarinic receptors, since high-affinity binding sites for [^{3}H]quinuclidinyl benzilate are detectable in the bovine adrenal medulla[3]. Furthermore, cultured bovine adrenal medullary cells respond to muscarinic agonists in at least four ways – they exhibit increases in $^{45}Ca^{2+}$ efflux[4], in cGMP levels[4,5], in cytosolic free Ca^{2+} concentration[6] and in turnover of polyphosphoinositides[7] (Oka, Tokushima; Bunn, Melbourne).

5-HT$_{1A}$ receptors and the new anxiolytics

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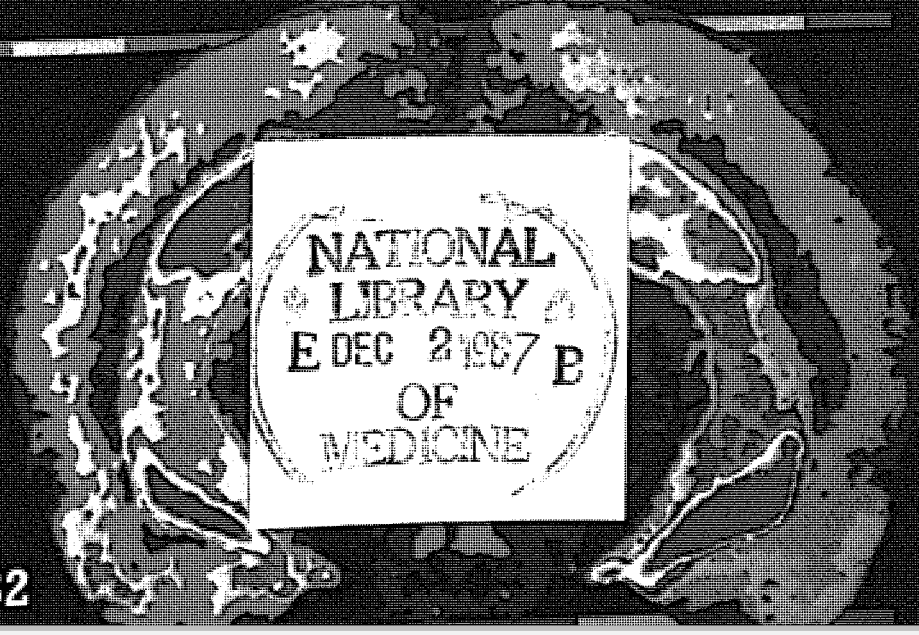

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Synthesis, SARs and therapeutic potential of HMG-CoA reductase inhibitors

Ta-Jyh Lee

Elevated plasma levels of low-density lipoprotein cholesterol is a major risk factor for the development of coronary heart disease, the leading cause of death and disability in Western countries. Because most cholesterol in the body is synthesized de novo, *the control of endogenous cholesterogenesis would be an attractive and potentially effective means of lowering plasma cholesterol levels. This approach has been validated by the recent discoveries of two novel fungal metabolites, mevastatin and lovastatin. These compounds are potent inhibitors of HMG-CoA reductase, which regulates the rate-limiting step in the biosynthetic pathway of cholesterol.* Ta-Jyh Lee *discusses the rationale for the design and synthesis of several potent inhibitors related to mevastatin and lovastatin, and the therapeutic potential of HMG-CoA reductase inhibitors.*

The atheromatous plaque or atheroma is formed in part from lipid deposits, primarily cholesteryl esters, in the inner walls of arteries. Growth of the atheroma leads to constriction of the arterial lumen and ultimately results in atherosclerosis and coronary heart disease (CHD), the leading cause of death and disability in Western countries.

Epidemiological evidence[1–3] strongly indicates that hypercholesterolemia – or more accurately, elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) – is a major risk factor for the development of CHD. These observations have stimulated intensive efforts directed towards the development of therapeutic agents for prevention and treatment of atherosclerosis based on the attenuation of plasma cholesterol levels (see Ref. 4).

Results of the recently completed Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT)[5,6] provide strong support for the rationale underlying this approach. This trial clearly demonstrated that the reduction of LDL-C through dietary modification and treatment with the bile acid sequesterant colestyramine, either alone or in combination, diminished the incidence of CHD morbidity and mortality in hypercholesterolemic men at high risk for CHD. Nevertheless, these measures often fail to lower elevated plasma LDL-C levels to the desired extent, particularly in patients with familial hypercholesterolemia. Because most cholesterol in the body is synthesized *de novo*, the control of endogenous cholesterogenesis would be an attractive and potentially more effective way to lower plasma cholesterol levels.

Cholesterol is synthesized from acetyl-CoA via a series of more than twenty enzymatic reactions. The major rate-limiting step in this pathway is regulated by the activity of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, EC1.1.1.34)[7], which catalyses the conversion of HMG-CoA to mevalonic acid (Fig. 1). Therefore, for several decades, this enzyme has been considered to be a prime target for pharmacological intervention for the control of endogenous cholesterogenesis. The practical realization of this goal has been advanced greatly by the recent discoveries of several novel fungal metabolites, which are potent inhibitors of HMG-CoA reductase.

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Inhibitory activities of mevastatin and lovastatin

Mevastatin (Fig. 2,**1a**, formerly known as ML-236B (Ref. 8) and Compactin[9]) was isolated independently by two different groups from the cultures of *Penicillium citrinum* and *Penicillium brevicompactum* and proved to be a remarkably potent HMG-CoA reductase inhibitor. Later, a closely related compound, lovastatin (Fig. 2,**1b**), formerly known as monacolin K (Refs 10, 11) and mevinolin[12] was isolated independently by two different groups from cultures of *Monacus ruber* and *Aspergillus terreus*. The subsequent isolation of a number of related compounds has been reported (see Ref. 13). Mevastatin and lovastatin are clearly the most extensively investigated members of this family of compounds.

The inhibition of HMG-CoA reductase by mevastatin and lovastatin is reversible and competitive with respect to HMG-CoA, the natural substrate. The K_i values of the dihydroxy acid forms of mevastatin and lovastatin (Fig. 2, **2a** and **2b**, which are the biologically active forms of mevastatin and lovastatin) are 1 nM and 0.6 nM, respectively. Neither compound affects other enzymes involved in cholesterol biosynthesis. In mammalian cells cultured in a medium containing LDL, the synthesis of other biologically important substances such as ubiquinone, dolichol and tRNA, which also are derived from mevalonate and re-

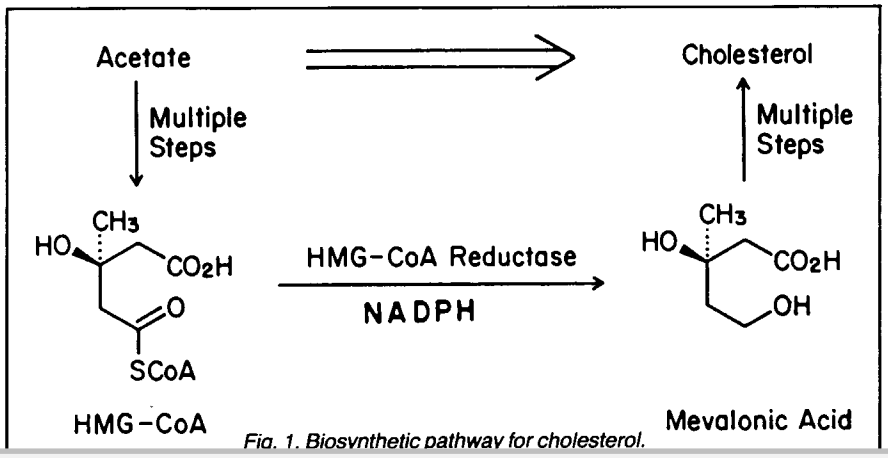


Fig. 1. Biosynthetic pathway for cholesterol.

quired for cell growth, is not affected even when the activity of HMG-CoA reductase is severely suppressed (up to 98%) by mevastatin[14,15].

These findings strongly suggest that mevastatin and lovastatin are specific inhibitors of HMG-CoA reductase. However, of greatest interest is the finding that these two natural products are highly effective hypocholesterolemic agents in several animal species[13] and humans[16–19]. Not surprisingly, after the disclosure of these initial findings, efforts directed toward the synthesis of mevastatin, lovastatin and related analogs were initiated in many laboratories (see Ref. 20).

Totally synthetic analogs of mevastatin

Before the discovery of lovastatin, an effort was initiated in our laboratories directed towards the development of totally synthetic HMG-CoA reductase inhibitors devoid of the structural complexities of mevastatin.

A model synthetic analog of mevastatin was formulated based largely on the following considerations. The structural similarity between the dihydroxy acid moiety of structure **2** and HMG-CoA or mevalonic acid is readily recognized. However, since the K_m value for HMG-CoA, the natural substrate of the enzyme, is approximately 10^{-5} M (about 10^4 weaker than the K_i values of mevastatin and lovastatin), the substituted polyhydronaphthyl moieties in mevastatin[21] and lovastatin must also play an important role in the inhibition of the enzyme.

Based on this line of reasoning and the known facts about the HMG-CoA reductase-catalysed reduction of HMG-CoA to mevalonic acid, our early model of a synthetic analog of structure **2** is represented by the generalized structure **3**. It consists of a dihydroxy acid moiety similar to that of structure **2a** and a lipophilic group linked by either a saturated or an unsaturated 2-carbon bridge. Thus, structure **4**, which is the corresponding lactone form of structure **3**, was the initial target. It should be noted that the stereochemical requirements at positions C-4 and C-6 of lactone **4** were not known at this point. Although the chiralities of two steric centers in the lactone ring of mevastatin (cf.

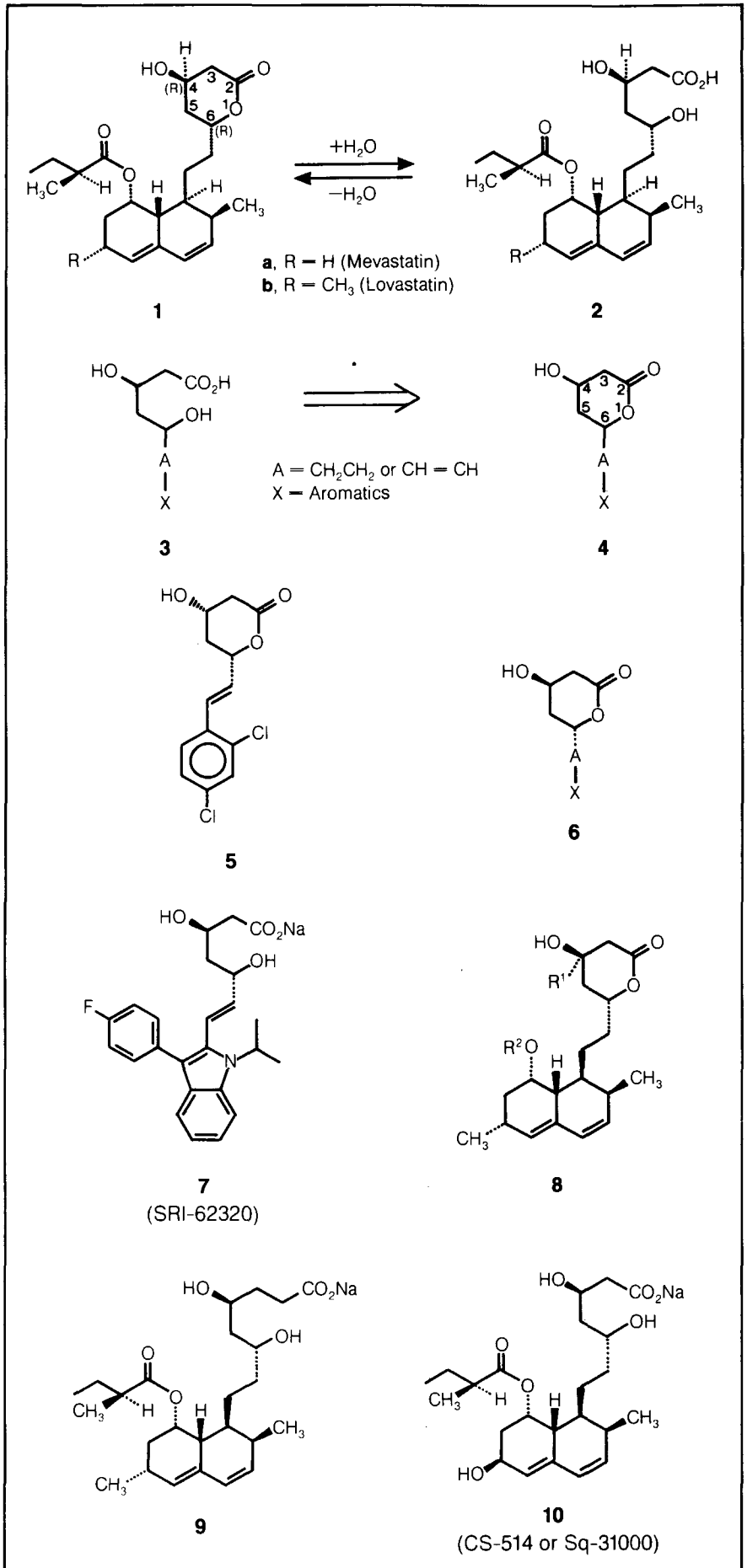
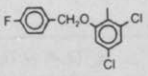
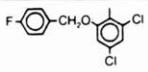
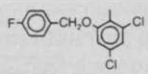
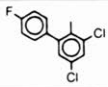
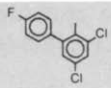
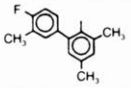
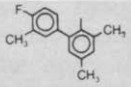


Fig. 2. Structures of mevastatin, lovastatin and the corresponding ring-opened dihydroxy acid forms, and other analogs.

TABLE I. *In-vitro* inhibitory activities of lactones **6** (Fig. 2) towards HMG-CoA reductase

Code	Optical activity	A	X	Relative potency*
a	±	t-CH=CH		0.08
a	+	t-CH=CH		0.16
b	±	t-CH=CH		7.5
c	±	CH_2CH_2		38
c	+	CH_2CH_2		58
d	±	t-CH=CH		100
d	+	t-CH=CH		280
e	+	t-CH=CH		289
f	+	CH_2CH_2		128

*All compounds in this table were tested after being converted to the sodium salts of the corresponding dihydroxy acids. The relative potency of the test compound was determined by comparing its IC_{50} value with that of mevastatin, which was tested simultaneously and arbitrarily assigned a relative potency value of 100.

structure **1a**) had been determined earlier[9], their critical importance to intrinsic inhibitory activity was yet to be ascertained. This issue was quickly resolved by determining that: (1) all activity resides in the *trans*-diastereomer **6a**(±) (i.e. the *cis*-diastereomer **5**(±) is inactive); and (2) only enantiomer **6a**(+) is active[22]. Similar results were observed subsequently in every compound series examined (see Table I), indicating that the chiralities of the two steric centers in structure **6** are critical in determining intrinsic HMG-CoA reductase inhibitory activity. Later, the finding that compound **6d**(+) possessed the same chirality in the lactone ring as that present in mevastatin was determined by X-ray crystallography[23] and further supported this conclusion.

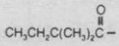
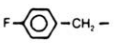
However, the weak intrinsic inhibitory potency of structure **6a**(+) needed to be optimized. Attachment of either an arylmethoxy group (benzyl ether series)[24] or an aryl moiety (biphenyl series)[23] at the 6-position of the 2,4-dichlorophenyl ring in compound **6a**(±) dramatically enhanced

introduction of a 4-fluoro group on the benzyl moiety induces a remarkable increase in potency. Later, this substitution proved to be equally useful in other series. Another significant contribution towards the improvement of potency was observed when compound **6b**(±) was hydrogenated to **6c**(±). Resolution of **6c**(±) yielded **6c**(+), which had an inhibitory potency approaching that of mevastatin.

Even more profound enhancement of potency was observed when suitable aryl groups were placed at the 6-position of the 2,4-dichlorophenyl ring in **6a**(+). For example, a 1750-fold increase in potency attended the formal conversion of compound **6a**(+) to **6d**(+) (Table I). In view of the toxicity ascribed to chlorinated biphenyls, it was deemed desirable to find a suitable replacement for the chloro groups in **6d**(+). The methyl group was considered to be a favorable bioisosteric replacement for the chloro group because of their comparable sizes and lipophilicities.

Furthermore, aromatic methyl groups are susceptible to bio-oxidation and the resultant metabolite(s) is expected to be more readily eliminated from the body. Indeed, replacement of the chloro-substituents in structure **6d**(+) with methyl groups and further refinement ultimately afforded compound **6e**(+), which has an inhibitory potency almost three times that of mevastatin. It is also noteworthy that a substantial decrease in potency occurs when structure **6e**(+) is reduced to **6f**(+). This result is contrary to earlier observations in the benzyl ether series and the prediction of an advantage in having a saturated or an unsaturated 2-carbon bridge connecting the lactone and the lipophilic groups remains precarious. Finally, the recent disclosure of research on compounds related to compound **7** (SRI-62320)[25] appears to be particularly interesting. As evidence of the therapeutic usefulness of HMG-CoA reductase inhibitors continues to mount, it is anticipated that research activities in search of new and novel inhibitors will be greatly intensified in the future.

TABLE II. *In-vitro* inhibitory activities of lactones **8** (Fig. 2) towards HMG-CoA reductase

Code	R^1	R^2	Relative potency*
a	H		0.4
b	H		254
c (MK-733)	H		622
d	H		119
e	CH_3		13

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