Novel Steroidal Inhibitors of Human Cytochrome $P450_{17\alpha}$ (17α-Hydroxylase-$C_{17,20}$-lyase): Potential Agents for the Treatment of Prostatic Cancer

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Received January 19, 1995®

Steroidal compounds having a 17-(3-pyridyl) substituent together with a 16,17-double bond have been synthesized, using a palladium-catalyzed cross-coupling reaction of a 17-enol triflate with diethyl(3-pyridyl)borane, which are potent inhibitors of human testicular 17α-hydroxylase-$C_{17,20}$-lyase. The requirement for these structural features is stringent: compounds having 2-pyridyl (**9**), 4-pyridyl (**10**), or 2-pyridylmethyl (**11**) substituents instead of the 3-pyridyl substituent were either poor inhibitors or noninhibitory. Reduction of the 16,17-double bond to give 17β-pyridyl derivatives diminished potency with 3-pyridyl substitution (**3** → **27**; IC_{50} for lyase, 2.9 → 23 nM) but increased it with a 4-pyridyl substituent present (**10** → **28**; IC_{50} 1 μM → 53 nM). In contrast, a variety of substitution patterns in rings A–C of the steroid skeleton afforded inhibitors having potencies similar to those most closely related structurally to the natural substrates pregnenolone and progesterone, respectively 17-(3-pyridyl)androsta-5,16-dien-3β-ol (**3**, $K_{i_{app}}$ < 1 nM; IC_{50} for lyase, 2.9 nM) and 17-(3-pyridyl)androsta-4,16-dien-3-one (**15**; IC_{50} for lyase, 2.1 nM). Thus compounds having variously aromatic ring A (**18**), saturated rings A/B (**21**, **22**), and oxygenated ring C (**26**) exhibited IC_{50} values for lyase (1.8–3.0 nM) falling within a 2-fold range. The most potent compounds are candidates for development as drugs for the treatment of hormone-dependent prostatic carcinoma.

Carcinoma of the prostate is now the most prevalent cancer in men in the USA. In 1993, 165 000 new cases were expected to be diagnosed, of which 35 000 will die of metastatic prostatic cancer.[1] The most widely accepted drug treatment is the use of GnRH agonists, which act by interfering with the production of testosterone by the testes and represent a medical alternative to orchiectomy.[2] However neither GnRH agonists nor orchiectomy deplete the synthesis of androgens through the adrenal route, and levels of testosterone and dihydrotestosterone in the prostate are respectively still 25% and 10% of pretreatment levels even after 3 months treatment with a GnRH agonist.[3] The importance of androgen synthesis by the adrenal route in maintaining tumor growth is suggested by the improved therapeutic benefit, both in terms of increase in progression-free survival time and survival advantage, seen in patients treated with the combination of GnRH agonist or orchiectomy with an antiandrogen, compared with those given GnRH agonist or orchiectomy alone.[4,5] It is proposed that the role of the antiandrogen is to counteract the stimulant action of residual androgens, synthesized through the adrenal route, on androgen receptors in the prostate cancer cells.

In principle, the effects of the combined therapy could be realized by a single drug which inhibits the enzyme steroidal 17α-hydroxylase-$C_{17,20}$-lyase. This enzyme is responsible for androgenic hormone biosynthesis which produces dehydroepiandrosterone and androstenedione, immediate precursors of testosterone, from their respective precursors pregnenolone and progesterone, in both testes and adrenals. The imidazole antifungal agent ketoconazole inhibits this enzyme when given in high doses to male patients and produces the symptoms of androgen suppression. This drug has been used to treat prostate cancer,[6] and although success has been reported in some studies,[7,8] it proved less promising in others.[9,10] The undesirable side effects, coupled with the inconvenience of the three times daily schedule which is dictated by its short half-life, limit its potential clinical usefulness. Nevertheless the clinical results obtained, coupled with a very recent report that careful scheduling of ketoconazole can produce prolonged responses in previously hormone-refractory prostate cancer,[11] lend credence to the selection of this enzyme target and impetus to the design and development of a more enzyme-selective, less toxic, and less metabolically labile inhibitor.

We report here on the synthesis and inhibitory activity toward the individual 17α-hydroxylase and $C_{17,20}$-lyase components of the target enzyme, obtained from human testis, of a variety of steroidal compounds having as their common structural feature a 17-(3-pyridyl) substituent together with a 16,17-double bond in the steroidal skeleton. We have previously explored nonsteroidal inhibitors containing a pyridyl residue, starting from the serendipitous discovery that certain esters of 4-pyridylacetic acid were effective inhibitors of the hydroxylase-lyase enzyme from rat testis,[12] findings which have in part been rationalized by crystallographic and molecular modeling studies.[13] More recently, esters of 3-pyridylacetic acid have been evaluated, using enzyme from human testis.[14]

The design concept used here was to consider how a pyridyl substituent could be incorporated into the actual steroid skeleton such that the pyridyl nitrogen lone pair would coordinate to the iron atom of the heme cofactor in the active site of the enzyme. The initial step of the *de novo* mechanism-based design approach was to postulate a complete catalytic cycle for the enzyme

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® Abstract published in *Advance ACS Abstracts,* May 15, 1995.

0022-2623/95/1838-2463$09.00/0

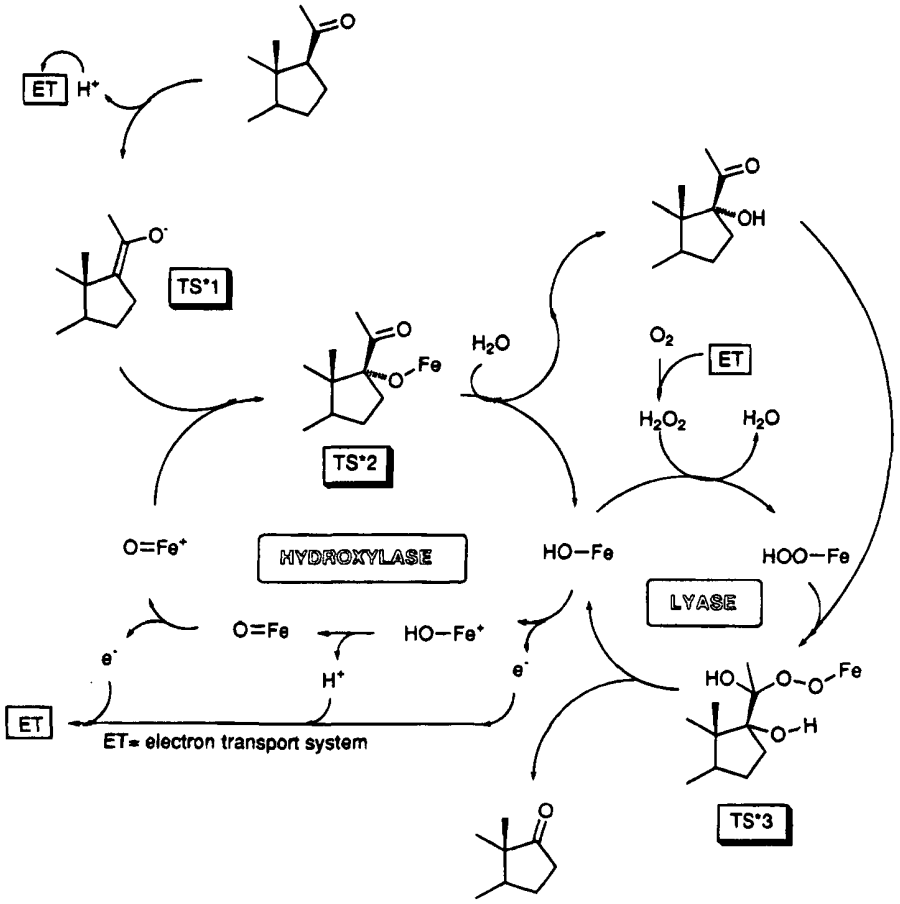
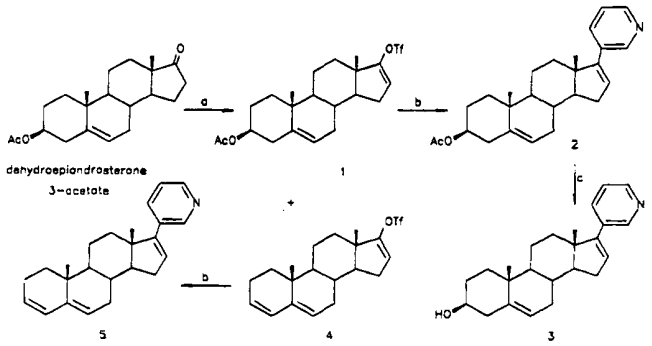


Figure 1. Postulated complete catalytic cycle for the 17α-hydroxylase-$C_{17,20}$-lyase enzyme. For clarity, only the steroid D-ring and the cofactor iron atom are shown.

(Figure 1) and then to consider the juxtaposition between the steroid D-ring and the heme cofactor from the putative transition state geometry. For this purpose, three-dimensional molecular models were constructed of the putative transition states using the Cochrane orbit molecular modeling system. From this analysis, it was postulated that a steroid incorporating a 16,17-double bond with the 17-position substituted by a 2-pyridyl group may inhibit the hydroxylase step and a 3-pyridyl derivative may inhibit the lyase step, while a 4-pyridyl analog should not inhibit either step. However, the enzyme may not tolerate an aromatic ring attached to the 17-position, and all three compounds may be inactive, even if the coordination geometry is correct.

The steroidal skeleton chosen for the first compound which was synthesized on the basis of this concept, namely the novel steroid **3**, was that of pregnenolone, which appears to be the preferred substrate for the hydroxylase activity of the human enzyme in the testis.[15] Alternative orientations of the pyridyl ring relative to the steroidal framework were explored by synthesizing the 2- (**9**) and 4- (**10**) pyridyl analogs, as was the effect of a spacer group between a 2-pyridyl residue and a C-17 (compound **11**). The second 17-(3-pyridyl) derivative synthesized was **15**, analogously related to progesterone, the alternative substrate for the hydroxylase activity of the target enzyme. Further molecules synthesized retained the ring D substitution pattern of **3** and **15** while further exemplifying the effect on enzyme inhibition of structural variations in rings

Scheme 1[a]



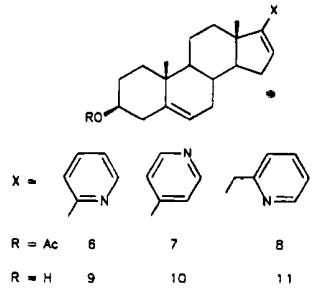
[a] (a) Tf_2O, base; (b) 3-PyBEt_2, $Pd(PPh_3)_2Cl_2$, THF, H_2O, Na_2CO_3; (c) NaOH, H_2O, MeOH.

A, B, and C. Finally, the effect of reducing the 16,17-double bond in **3** and **10** was explored.

Results

Chemistry. A general method for introducing the required 17-pyridyl 16,17-ene functionality into ring D was by palladium-catalyzed cross-coupling of steroidal 17-enol triflates with suitable pyridyl-containing nucleophilic coupling partners. For the synthesis of **3** (Scheme 1), dehydroepiandrosterone 3-acetate was converted into its 17-enol triflate **1** by base-catalyzed reaction with triflic anhydride in the presence of the hindered base 2,6-di-*tert*-butyl-4-methylpyridine. This reaction also produced the 3,5-diene **4** in 10% yield. The 3-pyridyl group was then introduced into the 17-position by reacting **1** with diethyl(3-pyridyl)borane in THF,

Chart 1

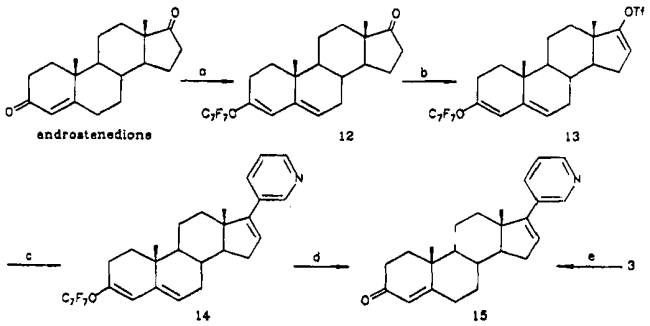


using bis(triphenylphosphine)palladium(II) chloride as catalyst (0.01 equiv) and aqueous Na_2CO_3 as nucleophilic activator. The reaction proceeded remarkably efficiently, without the potential side reactions of triflate hydrolysis or ethyl coupling, to give the acetate **2** in 84% isolated yield. From **4**, the 3-pyridyl derivative **5** was similarly obtained. The acetyl group of **2**, which was stable to the mildly basic conditions of the coupling reaction, was easily removed with aqueous methanolic NaOH to afford the target 3-pyridyl steroid **3**.

Although these coupling reactions were developed independently, the palladium-catalyzed cross-coupling of organoboron reagents with an enol triflate has been reported recently by Suzuki and co-workers.[16] Their reactions employed arylboronic acids and 9-alkyl-9-BBN reagents and the mild base K_3PO_4 as the nucleophilic activator under strictly anhydrous conditions. Our use of diethyl(3-pyridyl)borane was prompted by its commercial availability (it is also easily synthesized[17]) and its previous use in palladium-catalyzed cross-coupling reactions with aryl iodides.[18] Some features of our reaction compared with that of Suzuki are noteworthy. We found that the catalyst $Pd(PPh_3)_2Cl_2$ was superior to $Pd(PPh_3)_4$ and consistently gave better yields of coupled product. The catalyst could also be used at much lower levels, and even at 0.001 equiv, good yields were obtained with prolonged reaction times. Importantly our reaction did not require anhydrous conditions, and indeed an aqueous THF solvent system was employed. Our method of introducing the 17-pyridyl 16,17-ene functionality was more efficient and higher yielding than a previous route,[19,20] reaction of 3-pyridyllithium with a 17-keto steroid and dehydration of the resulting tertiary alcohol.

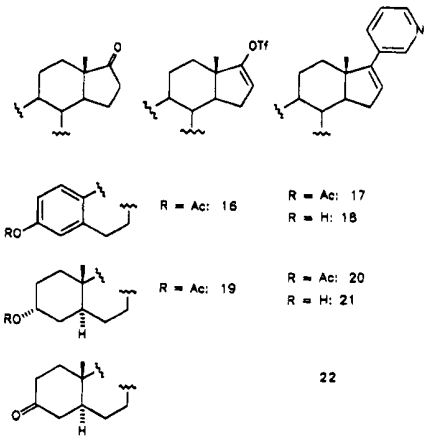
The 2-pyridyl (**6**), 4-pyridyl (**7**), and 2-picolyl (**8**) steroidal acetates (Chart 1) were synthesized similarly to **2** but employing different nucleophilic coupling partners and modifying the conditions accordingly. The reagents used to prepare **6** and **8** were 2-pyridyl- and 2-picolylzinc chloride, respectively. In the latter case the intermediate **8** was converted without isolation directly into **11** in good overall yield (79%). An attempt to prepare the 3-picolyl analog of **11** using 3-picolylzinc chloride was unsuccessful due to homocoupling of this reagent. In the synthesis of the 2- (**6**) and 4- (**7**) pyridyl steroid acetates, the novel palladium catalyst bromo(isopropenyl)bis(triphenylphosphine)palladium(II) was employed. Its use enabled the coupling reaction to be carried out at ambient temperature, thereby avoiding side reactions, and **6** was obtained in 74% yield from which hydrolysis gave the required 2-pyridyl analog **9**. The catalyst had been developed to enable low-temperature cross-coupling reactions for the stereoselective synthesis of (*E*)-4-hydroxytamoxifen[21,22] and was prepared from 2-bromopropene and tetrakis(triphenylphosphine)palladium(0) by a procedure analogous to that used to make benzylchlorobis(triphenylphosphine)-palladium(II).[23] When the coupling reaction was performed using 4-pyridylzinc chloride, prepared from 4-bromopyridine, only a low yield (18%) of the 4-pyridyl steroid acetate **7** was obtained. Instability of 4-halopyridines can restrict the use of 4-pyridylmagnesium and -zinc halides in palladium cross-coupling reactions, and diethyl(4-pyridyl)borane has been used as an alternative reagent.[24] Here, lithium trimethoxy(4-pyridyl)boronate, an intermediate in the synthesis[25] of 4-pyridylboronic acid, was the organoboron reagent used, and the coupled product thus obtained, **7**, was hydrolyzed directly to give the 4-pyridyl steroid **10** in 53% yield overall from **1**.

Scheme 2[a]



[a] (a) C_7F_8, CsF, DMF; (b) Tf_2O, base; (c) 3-PyBEt$_2$, $Pd(PPh_3)_2Cl_2$, THF, H_2O, Na_2CO_3; (d) HCl, H_2O, EtOH; (e) Al(O-*i*-Pr)$_3$.

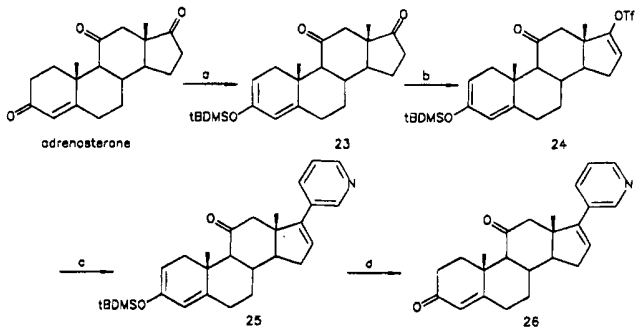
Chart 2



The preparation of **15**, starting from androstenedione (Scheme 2), required selective protection of the 3-keto function, to prevent the formation of a 3-dienol triflate.[26] Protection as the perfluorotolyl enol ether **12** by reaction with octafluorotoluene in the presence of cesium fluoride has proved to be a convenient one-step procedure.[27] The perfluoroaryl group was stable to the subsequent steps needed to insert the pyridyl substituent and was then cleaved by acidic hydrolysis. It was later found that **15** was more conveniently prepared directly from **3** by Oppenauer oxidation using cyclohexanone and aluminum isopropoxide.

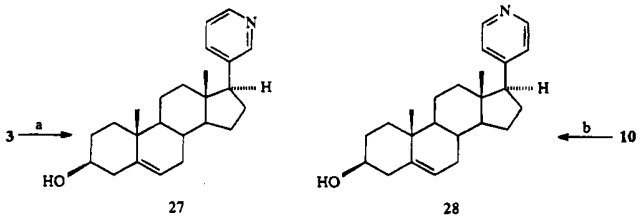
Several 3-pyridyl derivatives (**18**, **21**, **22**; Chart 2) exemplifying further structural variation in rings A and B were prepared using procedures analogous to those already described. Adrenosterone was the starting point for the synthesis of a ring-C-substituted variant, **26** (Scheme 3), which was prepared in good overall yield (60%). The formation of the *tert*-butyldimethylsilyl dienol ether **23** provided an alternative protecting

Scheme 3[a]



[a] (a) *t*-BDMSOTf, base; (b) $(i\text{-Pr})_2$NLi, $PhNTf_2$; (c) 3-PyBEt_2, $Pd(PPh_3)_2Cl_2$, THF, H_2O, Na_2CO_3; (d) Bu_4NF, THF, H_2O.

Scheme 4[a]



[a] (a) N_2H_4, AcOH, EtOH, air; (b) Red-Al, $ZnCl_2$, THF.

strategy for the 3-keto function. The chemical shifts for the two vinylic protons in this product were very similar to those previously reported for the silyl dienol ether formed from a testosterone derivative,[28] and the present product is therefore similarly formulated as the 2,4-dienol ether. In the following step, *N*-phenyltriflimide[29] was employed to prepare the enol triflate **24** since use of triflic anhydride resulted in desilylation and 3-dienol triflate formation. This also enabled selective formation of the 17-enol triflate without affecting the 11-keto function by preparing the intermediate lithium enolate under kinetic conditions at low temperature.

Lastly, analogs containing a saturated D-ring were prepared from the corresponding 16,17-ene compounds. Reduction of **3** using diimide, generated *in situ* from hydrazine hydrate, gave the 17β-(3-pyridyl) steroid **27** (Scheme 4). Reduction of the 16,17-double bond of the 4-pyridyl steroid (**10**) utilized the electron-withdrawing influence of the 4-pyridyl substituent under electrophilic activation by zinc chloride to achieve direct hydride reduction with Red-Al to produce the 17β-(4-pyridyl) steroid **28**. The β-orientation of the pyridyl ring in compounds **27** and **28** was confirmed by ^{1}H-NMR spectroscopy which showed an apparent triplet with a coupling constant of 10 Hz for the 17α-proton which is characteristic of 17β-substituted steroids.[20,30] Attempts at preparing the corresponding 17α-(4-pyridyl) analog, by either direct reduction of **10** or epimerization of **28**, were unsuccessful.

Inhibition of Human Testicular 17α-Hydroxylase and $C_{17,20}$-Lyase. Structure–Activity Relationships. We have identified as potent inhibitors of human testicular steroidal 17α-hydroxylase-$C_{17,20}$-lyase a variety of pyridyl steroids having as their common structural feature the 17-(3-pyridyl) 16,17-ene moiety (Table 1). Although it might be expected that the most potent compounds would be those (**3**, **15**) with structures most closely related to natural substrates, there was an unexpected tolerance for structural variation in this respect. Comparing **3** and **15** with analogs (**18**, **21**, **22**, **26**) synthesized from other naturally occurring steroid precursors, there was little variation (from 1.8 to 3.0 nM) in the IC_{50} values for inhibition of the lyase component. The absence of any functionality at the 3-position in the steroid skeleton leads to a modest drop in potency (compound **5**). The markedly lower potency of the acetoxy derivative **2** compared with **3** could reflect a limited bulk tolerance at the 3-position, as indicated by the total loss in activity for the much more sterically demanding perfluorotolyl derivative **14** of the potent inhibitor **15**. The stringent requirement for the 17-(3-pyridyl) 16,17-ene functionality for good inhibition was in marked contrast to the relative flexibility in relation to other features discussed and is reflected in the marked reduction, or abolition of activity, on relocating the pyridyl nitrogen (compounds **9**, **10**) or on reducing the 16,17-double bond of **3** to give the 17β-pyridyl derivative **27**. In contrast, reduction of the 4-pyridyl derivative **10** gave a product, **28**, with markedly improved inhibitory potency over its parent.

Table 1. Enzyme Inhibition Data

	IC_{50} (nM)[a]		IC_{50} (μM)[a]	
compound	$C_{17,20}$-lyase	17α-hydroxylase	aromatase	5α-reductase
2	17	18		
3	2.9	4	>20	>50
5	5.6	12.5		
9	76	270	>20	
10	1000	4000	>20	
11	>10 000	>10 000		
14	>10 000	>10 000		
15	2.1	2.8	1.8	10
18[b]	1.8	2.6		
21	2.5	4.3		
22	3	4.7		>50
26	2.9	13		
27	23	47		
28	53	160		
ketoconazole	26	65		

[a] The standard errors were usually <10% of the IC_{50} value. The concentration of enzyme in the assays for lyase/hydroxylase inhibition was estimated to be about 4–5 nM, except in the assays of **9**, **11**, **14**, and ketoconazole for which the concentration was ca. 25 and 10 nM for the lyase and hydroxylase assays, respectively. [b] Other biological activity: estrogen receptor binding affinity (estradiol = 100), 4.9.

The most inhibitory compounds in the present study were far more potent than any inhibitor of hydroxylase/lyase for which comparable data have been previously described. The $K_{i_{app}}$ for **3** was <1 nM, whereas the most potent inhibitor, also steroidal, reported to date is 17β-(cyclopropylamino)androst-5-en-3β-ol[31] with a $K_{i_{app}}$ of 90 nM. Another steroidal compound, 4-pregnen-3-one-20β-carboxaldehyde oxime has been developed as a combined inhibitor of this enzyme and testosterone 5α-reductase.[32] Though a potent inhibitor (K_i = 16 nM) of the reductase, it was much less inhibitory toward the rat hydroxylase/lyase, being comparable to ketoconazole. 17β-Ureido-substituted steroids with potent activity toward the rat hydroxylase/lyase enzyme have been described.[33,34] Though the data are presented in a way not easily comparable with the results of the present study, one of these compounds, 17β-ureido-1,4-androstadien-3-one, markedly suppressed testosterone levels and ablated androgen-dependent organs in the rat. Liarozole is a nonsteroidal imidazole derivative having activity toward the rat testicular enzyme very similar[35] to that of ketoconazole. No example among our previously mentioned[12,14] esters of 4- and 3-pyridylacetic acid compares in potency with the best of the present steroidal derivatives.

Other Biological Activities. While inhibition of other targets was not explored in detail in the present study, limited evaluations have been carried out (Table 1), particularly where such activity might be anticipated, from structural analogy with compounds known to interact with the target in question. Thus **15**, structurally related to androstenedione, a substrate for aromatase, was a moderate inhibitor of aromatase. Likewise the inhibition by **15** of testosterone 5α-reductase might reflect its structural resemblance to the natural substrate testosterone, whereas **22**, correspondingly related to the product 5α-dihydrotestosterone, was not an inhibitor. Notably, compound **3** inhibited neither aromatase nor testosterone 5α-reductase at the highest concentration tested, respectively 20 and 50 μM. Lastly, the estradiol-related analog **18** had an appreciable binding affinity for the estrogen receptor, 5% of that of estradiol itself.

Concluding Remarks

Two of the compounds described here, namely **2** (as a prodrug for **3**) and **15**, have been evaluated *in vivo* in the WHT mouse.[36] Each markedly reduced the weights of androgen-dependent organs, and **2** depressed testosterone to undetectable levels. The adrenals were unaffected, implying that **3** and **15**, unlike ketoconazole, do not inhibit enzymes in the pathway leading to corticosterone. This evidence for selective inhibition of testosterone biosynthesis, together with the further evidence for selectivity of action provided here for **3** in particular, makes **3** a strong candidate for further development as a potential drug for the treatment of prostatic carcinoma in humans.

Experimental Section

Chemical Methods. ^{1}H-NMR spectra (250 MHz) (internal Me_4Si = δ 0) were determined in $CDCl_3$ (unless otherwise indicated) using a Bruker AC 250 spectrometer. Infrared spectra were determined with a Perkin-Elmer 1720X spectrometer. Mass spectra (electron impact, 70 eV) were obtained by direct insertion with a VG 7070H spectrometer and VG 2235 data system. Melting points were determined with a Reichert micro hot stage apparatus and are uncorrected. Chromatography refers to column chromatography on silica gel (Merck Art. 15111) with the solvent indicated applied under positive pressure. Light petroleum refers to the fraction with bp 60–80 °C. 3-Pyridyl(diethyl)borane was purchased from Aldrich Chemical Co., Gillingham, Dorset, U.K. Elemental analyses were determined by CHN Analysis Ltd., South Wigston, Leicester, England.

3β-Acetoxyandrosta-5,16-dien-17-yl Trifluoromethanesulfonate (1) and Androsta-3,5,16-trien-17-yl Trifluoromethanesulfonate (4). To a stirred solution of dehydroepiandrosterone 3-acetate (24.8 g, 75 mmol) in dry CH_2Cl_2 (500 mL) containing 2,6-di-*tert*-butyl-4-methylpyridine (18.5 g, 90 mmol) was added trifluoromethanesulfonic anhydride (12.6 mL, 75 mmol). After 12 h the mixture was filtered, washed with water (50 mL), and dried ($MgSO_4$) and the solvent evaporated. Chromatography, on elution with CH_2Cl_2–light petroleum (1:6), gave first **4** (3.02 g, 10%) as an oil: ν_{max} for C=O str absent; ^{1}H NMR δ 0.99 (s, 3, H-18), 1.02 (s, 3, H-19), 5.39 (m, 1, H-6), 5.59 (m, 1, H-16), 5.62 (m, 1, H-3), 5.93 (dm, 1, J = 9.4 Hz, H-4); *m/z* 402 (M^+).

Further elution with CH_2Cl_2–light petroleum (1:3) afforded **1** (20.1 g, 58%): mp 75–76 °C (from hexane); ν_{max} 1734 cm^{-1} (C=O str); ^{1}H NMR δ 1.00 (s, 3, H-18), 1.06 (s, 3, H-19), 2.04 (s, CH_3CO), 4.59 (m, 1, H-3α), 5.39 (dm, 1, J = 4.9 Hz, H-6), 5.58 (m, 1, H-16); *m/z* 402 (M^+ – AcOH). Anal. ($C_{22}H_{29}O_5F_3S$) C, H, F, S.

3β-Acetoxy-17-(3-pyridyl)androsta-5,16-diene (2). Diethyl(3-pyridyl)borane (3.3 g, 23 mmol) was added to a stirred solution of **1** (6.94 g, 15 mmol) in THF (75 mL) containing bis(triphenylphosphine)palladium(II) chloride (0.105 g, 0.15 mmol). An aqueous solution of Na_2CO_3 (2 M, 30 mL) was then added and the stirred mixture heated at 80 °C for 1 h and then partitioned between Et_2O and H_2O. The organic phase was dried (Na_2CO_3), filtered through a short column of silica gel, and concentrated. Chromatography, on elution with Et_2O–light petroleum (1:2), afforded **2** (4.95 g, 84%): mp 144–145 °C (from hexane); ν_{max} 1732 cm^{-1} (C=O str); ^{1}H NMR δ 1.05 (s, 3, H-19), 1.08 (s, 3, H-18), 2.04 (s, 3, CH_3CO), 4.60 (m, 1, H-3α), 5.42 (dm, 1, J = 4.7 Hz, H-6), 5.99 (m, 1, H-16), 7.23 (dd, 1, $J_{5,4}$ = 8.1 Hz, $J_{5,6}$ = 3.9 Hz, pyridyl H-5), 7.65 (ddd, 1, $J_{4,2}$ = 2.0 Hz, $J_{4,6}$ = 1.6 Hz, pyridyl H-4), 8.46 (dd, 1, pyridyl H-6), 8.62 (d, 1, pyridyl H-2); *m/z* 392 (M^+ + H). Anal. ($C_{26}H_{33}NO_2$) C, H, N.

17-(3-Pyridyl)androsta-5,16-dien-3β-ol (3). To a solution of **2** (4.90 g, 12.5 mmol) in methanol (50 mL) was added 2.5 M NaOH (10 mL), and the mixture was stirred at 80 °C for 5 min and then allowed to cool, poured into water, neutralized with 1 M HCl, rebasified with saturated aqueous $NaHCO_3$, and extracted with hot toluene (3 × 100 mL). The toluene extracts were dried (Na_2CO_3) and concentrated. Chromatography, on elution with Et_2O–toluene (1:2), gave **3** (3.45 g, 79%): mp 228–229 °C (from toluene); ν_{max} 3351 (OH str); ^{1}H NMR δ 1.05 (s, 3, H-19), 1.07 (s, 3, H-18), 3.54 (m, 1, H-3α), 5.40 (dm, 1, J = 5.0 Hz, H-6), 5.99 (m, 1, H-16), 7.22 (dd, 1, pyridyl H-5), 7.65 (ddd, 1, pyridyl H-4), 8.46 (dd, 1, pyridyl H-6), 8.62 (d, 1, pyridyl H-2); *m/z* 349 (M^+). Anal. ($C_{24}H_{31}NO$) C, H, N.

17-(3-Pyridyl)androsta-3,5,16-triene (5). The method followed that described for **2** but used **4** (2.01 g, 5.0 mmol). Chromatography, on elution with CH_2Cl_2, gave **5** (1.39 g, 84%): mp 110–112 °C (from hexane); ^{1}H NMR δ 1.02 (s, 3, H-19), 1.07 (s, 3, H-18), 5.44 (m, 1, H-6), 5.61 (m, 1, H-3), 5.95 (dm, 1, J = 9.8 Hz, H-4), 6.01 (m, 1, H-16), 7.23 (dd, 1, pyridyl H-5), 7.66 (ddd, 1, pyridyl H-4), 8.46 (dd, 1, pyridyl H-6), 8.63 (d, 1, pyridyl H-2); *m/z* 331 (M^+). Anal. ($C_{24}H_{29}N$) H, N; C: calcd 86.96; found, 86.24.

3β-Acetoxy-17-(2-pyridyl)androsta-5,16-diene (6). To Et_2O (6 mL), at −18 °C, was added *n*-butyllithium (0.96 mL, 2.5 M solution in hexanes) followed dropwise by 2-bromopyridine (0.228 mL, 2.4 mmol) in Et_2O (2 mL). The resulting blood-red solution of 2-pyridyllithium was added dropwise to a solution of $ZnCl_2$ (382 mg, 2.8 mmol) in THF, cooled to −18 °C, and the orange-brown solution of 2-pyridylzinc chloride was stirred for a further 30 min. For the preparation of the palladium catalyst, a solution of tetrakis(triphenylphosphine)-palladium(0) (1.16 g, 1 mmol) in benzene (10 mL) was treated with 2-bromopropene (0.18 mL, 242 mg, 2 mmol) and the mixture stirred for 16 h at ambient temperature, whereupon the initially orange suspension became a yellow solution. The solvent was removed under vacuum, the residue was triturated with Et_2O, and the pale yellow product (0.70 g) bromo(isopropenyl)bis(triphenylphosphine)palladium(II) was recovered by filtration: ^{1}H NMR δ 0.81 (s, CH_3), 4.6 (m, C=CH_2), 7.2–7.8 (m, arom H).

To a solution of **1** (926 mg, 1 mmol) in THF (10 mL) containing the palladium catalyst (76 mg, ca. 0.1 mmol) was added the solution of 2-pyridylzinc chloride, and the mixture was stirred at ambient temperature. After 1 h, the mixture was partitioned between Et_2O and H_2O and the organic phase was dried (Na_2CO_3) and concentrated. Chromatography, on elution with Et_2O–light petroleum (1:4), gave **6** (0.583 g, 74%): mp 189–190 °C (from light petroleum); ν_{max} 1734 cm^{-1} (C=O str); ^{1}H NMR δ 1.09 (s, 3, H-19), 1.15 (s, 3, H-18), 2.04 (3, s, CH_3CO), 4.62 (m, 1, H-3α), 5.42 (dm, 1, H-6), 6.37 (m, 1, H-16), 7.09 (dd, 1, $J_{5,4}$ = 7.9 Hz, $J_{6,5}$ = 4.1 Hz, pyridyl H-5), 7.38 (d, 1, $J_{3,4}$ = 7.9 Hz, pyridyl H-3), 7.59 (t, J = 7.7 Hz, 1, pyridyl H-4), 8.55 (d, 1, pyridyl H-6); *m/z* 391 (M^+). Anal. ($C_{26}H_{33}NO_2$) C, H, N.

3β-Acetoxy-17-(4-pyridyl)androsta-5,16-diene (7). 4-Bromopyridine (4.5 g) was liberated from its hydrochloride (5 g, 26 mmol) using the procedure previously applied to 4-chloropyridine[37] but keeping solutions below 10 °C during concentration to prevent polymerization. The free base was twice concentrated from Et_2O (to remove residual $CHCl_3$), and then a solution of the freshly prepared 4-bromopyridine (1.58 g, 10

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