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\textsubscript{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 \rightarrow 27; IC\textsubscript{50} for lyase, 2.9 \rightarrow 23 \text{nM}) but increased it with a 4-pyridyl substituent present (10 \rightarrow 28; IC\textsubscript{50} 1 \mu M \rightarrow 53 \text{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, \text{Kiapp} < 1 \text{nM}; IC\textsubscript{50} for lyase, 2.9 \text{nM}) and 17-(3-pyridyl)androsta-4,16-dien-3-one (15; IC\textsubscript{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\textsubscript{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. 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. However neither GnRH agonists nor orchiectomy deplete the synthesis of androgens which act by interfering with the production of testosterone by the 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, and although success has been reported in some studies, it proved less promising in others. 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, 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\textsubscript{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, findings which have in part been rationalized by crystallographic and molecular modeling studies. More recently, esters of 3-pyridylacetic acid have been evaluated, using enzyme from human testis. 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 \textit{de novo} mechanism-based design approach was to postulate a complete catalytic cycle for the enzyme.
(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. 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 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.
using bis(triphenylphosphine)palladium(II) chloride as catalyst (0.01 equiv) and aqueous Na₂CO₃ 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. Their reactions employed aryloboronic acids and 9-alkyl-9-BBN reagents and the mild base K₂PO₄ 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) and its previous use in palladium-catalyzed cross-coupling reactions with aryl iodides. Some features of our reaction compared with that of Suzuki are noteworthy. We found that the catalyst Pd(PPh₃)₂Cl₂ was superior to Pd(PPh₃)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 function, to prevent the formation of a 3-dienol triflate, reaction 3, which was obtained in 74% yield from which hydrolysis gave the required 2-pyridyl analog 5. The preparation of the 2-pyridyl (6), 4-pyridyl (7), and 2-picolyl (8) steroid 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 and was prepared from 2-bromopropene and tetrakis(triphenylphosphine)palladium(0) by a procedure analogous to that used to make benzylchlorobis(triphenylphosphine)palladium(II). 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. Here, lithium trimethoxy(4-pyridyl)boronate, an intermediate in the synthesis 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.

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. Protection as the perfluorotoluene enol ether 13 by reaction with octafluorotoluene in the presence of cesium fluoride has proved to be a convenient one-step procedure. 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 cyclobexanone 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, (Scheme 3), which was prepared in good overall yield (60%). The formation of the tert-butyldimethylsilyl dienol ether 23 provided an alternative protecting

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**Chart 1**

![Chart 1](image)

**Scheme 2**

![Scheme 2](image)
strategy for the 3-keto function. The chemical shifts for the two vinyl protons in this product were very similar to those previously reported for the silyl dienol ether formed from a testosterone derivative, and the present product is therefore similarly formulated as the 2,4-dienol ether. In the following step, N-phenyltriflimide was employed to prepare the enol triflate 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 mild 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 1H-NMR spectroscopy which showed an apparent triplet with a coupling constant of 10 Hz for the 17α-proton which is characteristic of 17β-substituted steroidal,

Inhibition of Human Testicular 17α-Hydroxylase and 17α,20β-Lyase. Structure–Activity Relationships. We have identified as potent inhibitors of human testicular steroid 17α-hydroxylase-17α,20β-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 IC50 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.

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 KIC for 3 was <1 nM, whereas the most potent inhibitor, also steroidal, reported to date is 17β-cyclopentylaminomethanone-3β-ol with a KIC 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.

<table>
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<th>Compound</th>
<th>IC50 lyase (nM)</th>
<th>IC50 lyase (nM)</th>
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<tr>
<td>2</td>
<td>17</td>
<td>&gt;20</td>
</tr>
<tr>
<td>3</td>
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<td>28</td>
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<td>180</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>26</td>
<td>65</td>
</tr>
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</table>

* The standard errors were usually <10% of the IC50 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.

Other biological activity: estrogen receptor binding affinity (estradiol = 100, 4.9).
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 5a-reductase might reflect its structural resemblance to the natural substrate testosterone, whereas 22, correspondingly related to the product 5a-dihydrotestosterone, was not an inhibitor. Notably, compound 3 inhibited neither aromatase nor testosterone 5a-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. 1H-NMR spectra (250 MHz) (internal Me4Si = 0) were determined in CDCl3 (unless otherwise indicated) using a Bruker AC 250 spectrometer. Infrared spectra were determined with a Perkin-Elmer 1720X spectrophotometer. 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). Further elution with Et2O under positive pressure. Light petroleum refers to the fraction bp 60-80 °C. 3-Pyridyl[dibromo]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-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 NaHCO3, and extracted with hot toluene (3 x 100 mL). The toluene extracts were dried (Na2CO3) and concentrated. Chromatography, on elution with Et2O-toluene (1:2), gave 3 (3.04 g, 23 mmol, 74%) mp 189-190 °C (from light petroleum); 1H NMR δ 1.15 (s, 3, H-18), 1.63 (d, 1, J = 7.9 Hz, H-6), 3.70 (d, 1, J = 9.4 Hz, H-4); m/z 402 (M+). Anal. (C19H15NO2) C, H, N.

3β-Acetoxyandrosta-5,16-dien-3β-ol (3). To a solution of 2 (4.90 g, 12.5 mmol) in methanol (50 mL) containing bis(triphenylphosphine)palladium(II) chloride (0.155 g, 0.15 mmol), an aqueous solution of Na2CO3 (2 M, 30 mL) was added and the stirred mixture heated at 80 °C for 1 h and then partitioned between Et2O and H2O. The organic phase was dried (Na2CO3), filtered through a short column of silica gel, and then concentrated. Chromatography, on elution with Et2O-light petroleum (1:2), afforded 3 (4.95 g, 84%); mp 144-145 °C (from hexane); νmax 1732 cm-1 (C=O str); 1H NMR δ 1.05 (s, 3, H-19), 1.08 (s, 3, H-18), 2.94 (g, 3, CH2CO), 4.69 (m, 1, J = 4.1 Hz, H-6), 5.85 (m, 1, J = 9.4 Hz, H-4); m/z 402 (M+). Anal. (C19H15NO2) C, H, F, S.
mol/100 mL) in Et2O (5 mL) was added dropwise during 30 min to a mixture of n-butyllithium (2.5 M in hexanes, 4 mL, 10 mmol) and Et2O (20 mL) at -20 °C. Of the resulting solution of 4-pyridylzinc chloride (15 mmol, 4.5 mL) was added to a stirred solution of anhydrous ZnCl2 (681 mg, 5 mmol) in dry THF (25 mL). After 1 h at ambient temperature, 30 mL of the red solution of 4-pyridylzinc chloride was added to a solution of 1 (925 mg, 2 mmol) in THF containing bromoisopropenylbis(triarylphosphine)palladium(II) (see above; 75 mg, 0.1 mmol) and the mixture stirred at ambient temperature overnight. The resulting solution of H-16), 7.26 (d, 2, J = 6.0 Hz, pyridyl H-3, H-5), 8.50 (d, 2, pyridyl H-2, H-6); m/z 331 (M+ - AcOH). Anal. (C25H33NO3) C, H, N.

15-(Pyridyl)androsta-5,16-dien-3-ol (9). The method followed that described for 3 but used 6 (392 mg, 1 mmol), except that on completion of the reaction the product was extracted with Et2O followed by benzene and crystallized without prior chromatography giving 9 (273 mg, 78%): mp 206–207 °C (from benzene–light petroleum); δmax 1732 cm⁻¹ (C=O str); 'H NMR δ 1.08 (s, 3, H-18), 1.63 (s, 3, H-19), 3.56 (m, 1, H-3a), 5.42 (dm, 1, H-6), 6.18 (m, 1, H-16), 7.26 (d, 2, J = 6.0 Hz, pyridyl H-3, H-5), 8.55 (d, 1, J = 4.2 Hz, pyridyl H-6); m/z 349 (M⁺). Anal. (C26H35NO) C, H, N.

17-(4-Pyridyl)androsta-5,16-dien-17-ol (10). A solution of 4-bromopyridine (from the hydrochloride; 25 g, 129 mmol; 80%): mp 226–228 °C (from toluene); δmax 1768 cm⁻¹ (C=O str); 'H NMR δ 1.07 (s, 1, H-16), 1.24 (s, 1, H-18), 5.76 (s, 1, H-4), 5.99 (m, 1, H-16), 7.23 (dd, 1, pyridyl H-5), 7.64 (dd, 1, pyridyl H-6), 8.47 (dd, 1, pyridyl H-5), 8.63 (d, 1, pyridyl H-2); m/z 563 (M⁺). Anal. (C20H23NO) C, H, N.

17-(3-Pyridyl)androsta-5,16-dien-3-one (15). (a) From 14. To a solution of 14 (0.423 g, 0.75 mmol) in THF (5 mL) was added EtOH (5 mL) followed by 1 M HCl (5 mL), and the mixture was heated with stirring at 80 °C for 48 h. The mixture was then poured into H2O (20 mL), neutralized with 1 M NaOH, and extracted with Et2O (3 × 30 mL). To the ether extracts were combined, dried (Na2CO3), and concentrated. Chromatography, on elution with Et2O, gave 15 (185 mg, 71%): mp 148–150 °C (from Et2O); δmax 1674 cm⁻¹ (C=O str); 'H NMR δ 1.07 (s, 3, H-18), 1.24 (s, 3, H-19), 5.76 (s, 1, H-4), 5.99 (m, 1, H-16), 7.23 (dd, 1, pyridyl H-5), 7.64 (dd, 1, pyridyl H-6), 8.47 (dd, 1, pyridyl H-5), 8.62 (d, 1, pyridyl H-2); m/z 347 (M⁺). Anal. (C19H17NO) C, H, N.

(b) From 3, by Oppenauer Oxidation. From a solution of 3 (4.17 g, 12 mmol) in dry toluene (300 mL) and cyclohexanone (60 mL) was distilled off part of the solvent (80 mL) to eliminate moisture. After allowing to cool to 90 °C, Al(O-i-Pr)₃ (4.06 g, 20 mmol) was added and the mixture heated under reflux for 90 min and then allowed to cool, diluted with Et2O (200 mL), washed with aqueous trisodium citrate (15%, w/v), 2 × 30 mL), dried (Na2CO3), and concentrated. Chromatography, on elution with MeOH–toluene (1:5), afforded 13 (9.4%, identical to the product obtained by method a above. Anal. (C29H31NO) C, H, N; calcd, 8.09, found 7.67.

3-Acetoxyestra-1,3,5(10),16-tetraen-17-yl trifluoromethanesulfonate (16). The method followed that described for 1 but used estrone 3-acetate (4.69 g, 15 mmol). Chromatography, on elution with CH2Cl2–light petroleum (1:2), gave 16 (5.21 g, 78%) as a waxy solid; mp 206-207 °C (from benzene–light petroleum); δmax 1738 cm⁻¹ (C=O str); 'H NMR δ 1.00 (s, 3, H-18), 2.29 (s, 3, CH2O), 5.62 (m, 1, H-16), 6.81 (bs, 1, arom H-4), 6.85 (dd, 1, J = 8.5 Hz, J = 2.6 Hz, arom H-2), 7.26 (d, 1, arom H-1); m/z 445 (M⁺ + H).
17-(3-Pyridyl)-5-androst-16-en-3-ol (21). The method followed that described for 3 but used 20 (2.33 g, 5.9 mmol). Chromatography, on elution with MeOH—toluene (1:40), gave 21 (1.62 g, 78%): v max 198—199 C (from toluene); 1H NMR δ 0.84 (s, 3, H-19), 1.00 (s, 3, H-18), 4.06 (bs, 1, H-17), 5.97 (m, 1, H-16), 7.21 (dd, 1, pyridyl H-5), 7.64 (ddd, 1, pyridyl H-4), 8.45 (dd, 1, pyridyl H-5); 13C NMR (125 MHz) δ 14.9, 14.9, 130.4, 143.1, 157.3; m/z 299 (M+). Anal. (C23H31NO) C, H, N; C: calcd, 82.47; found, 82.00.

17-(3-Pyridyl)-5-androst-16-en-3-one (22). The method essentially followed method b for the formation of 15, from 3 by Oppenauer oxidation, but used crude 3 (1.05 g, 3.0 mmol). Chromatography, on elution with MeOH—toluene (1:40), gave 22 (0.90 g, 86%): v max 190—192 C (from toluene); 1H NMR δ 0.99 (s, 3, H-18), 1.18 (s, 3, H-19), 4.75 (dm, 1, H-2), 5.37 (app t, 1, J = 2.1 Hz, 1, H-4); m/z 273 (M+—C3H7). Anal. (C23H30NO) C, H, N; C: calcd, 82.47; found, 82.00.

17-(4-Pyridyl)-17-(3-Pyridyl)-androst-5-en-3-ol (28). To a suspension of 10 (699 mg, 2 mmol) in dry THF (10 mL) was added dropwise by syringe a solution of Red-Al (2.4 mL, 3.4 M in hexane, 8 mL) to give slightly impure 28 (672 mg, 96% which was purified by further recrystallization from toluene: mp 272—273 C; 1H NMR δ 0.47 (s, 3, H-18), 1.00 (s, 3, H-19), 2.66 (app t, 1, J = 9.6 Hz, H-17a), 3.54 (m, 1, H-3a), 5.07 (m, 1, H-16), 7.12 (d, 2, J = 6.0 Hz, pyridyl H-3, H-5), 8.48 (d, 2, pyridyl H-2, H-6). Anal. (C23H31NO) C, H, N; C: calcd, 82.47; found, 82.00.

Enzyme Preparation and Assay Procedure for the 17a-Hydroxylase-C17,20lyase Enzyme. The 17β-labeled compounds were obtained from NEN Products, Stevenage, Herts, U.K. The biochemical reagents were from Boehringer Mannheim, U.K., Lewes, East Sussex, U.K., or Sigma Chemical Co. Ltd., Poole, Dorset, U.K. The chemicals were of analytical grade.

A microsomal fraction was prepared by the method of Chasalow from human testes removed at orchietomy from previously untreated patients with cancer of the prostate. The microsomes were resuspended in 50 mM sodium phosphate buffer (pH 7.4)—glycerol (3:1) at the equivalent of 1 mM of fresh tissue and stored in liquid nitrogen until use.

The assay was based on that of Chasalow, and the assay mixture contained 5 mM 17β-labeled substrate (1—3 μCi/mmol), 250 μM NADPH, 10 mM D-glucose 6-phosphate, 1 mM MgCl2, 2 U/mL D-glucose 6-phosphate dehydrogenase, 0.1 mM dithiothreitol, 0.2 mM EDTA, 1% ethanol, 1% DMSO, 3% glycerol, and 95% 50 mM sodium phosphate buffer (pH 7.4). The test compounds were prepared in 50% DMSO, the controls receiving just 50% DMSO. The reaction was carried out at 37 °C. It was started by the addition of the microosomal preparation and stopped by the addition of 2 volumes of methanol—MeOH (1:1). The 17β-labeled steroids were stored at −20 °C until analysis. The reaction was linear with time, and the rate was proportional to the protein concentration under the conditions used (data not shown).

HPLC Analysis. (a) Hydroxylase Activity. For measurement of the hydroxylase activity, the substrate was progesterone and the unlabeled steroids added at the end of the assay were progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone, and etiocholanolone. 

The samples were injected onto a 10 cm Nucleosil 5μM C18 column fitted with an UpTight guard column filled with Nucleosil C18 packing. The mobile phase was 60% MeOH at a flow rate of 1 mL min−1. The effluent was monitored at 240 nm before being mixed with Ecoscint A containing 25% MeCN and monitored for 1H using a Berthold LB500C detector. Activity was measured as the production of 17α-hydroxyprogesterone.
240 nm before being mixed with Eosacost A containing 5% MeCN, 5% MeOH and monitored for H1 using a Berthod LB506C detector. Activity was measured as the production of androstenedione and testosterone.

Inhibitory Activity. For ease of dissolution, test compounds were first converted into their hydrochlorides. In a typical procedure, HCl gas was passed through a solution of the base in Et20 and the hydrochloride which precipitated was collected by filtration. The precipitate was washed with Et20 and thoroughly dried. Each compound was first converted into their hydrochlorides. In a typical procedure, HCl gas was passed through a solution of the base in Et20 and the hydrochloride which precipitated was collected by filtration. The precipitate was washed with Et20 and thoroughly dried.

The correlation coefficients were calculated by regression to the median effect equation of ch01.1:~~

\[
\text{IC}_{50} = \frac{K_{\text{app}}}{f_e} + 0.5 \times \text{enzyme concentration}
\]

Other Biological Activities. The reagents and conditions for the assays for inhibition of aromatase enzyme from the microsomal fraction of human placenta and of testosterone 5α-reductase from human benign prostatic tissue were as previously described. The estrogen receptor binding assay, using immature rat uterine cytosol, was a modification of that described by Wakeling and is described elsewhere.

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References

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