J. Med. Chem. **1998,** *41,* 902−912

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

Vincent C. O. Njar,† Katsuya Kato, Ivo P. Nnane, Dmitry N. Grigoryev, Brian J. Long, and Angela M. H. Brodie*

Department of Pharmacology & Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, Maryland 21201

Received August 21, 1997

A new synthetic route to a variety of novel Δ^{16}-17-azolyl steroids is described: it involves the nucleophilic vinylic "addition−elimination" substitution reaction of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**2**) and azolyl nucleophiles. Some of these novel Δ^{16}-17-azolyl steroids, **6**, **17**, **19**, and **27**−**29**, prepared in good overall yields, are very potent inhibitors of human and rat testicular $P450_{17\alpha}$. They are shown to be noncompetitive and appear to be slow-binding inhibitors of human $P450_{17\alpha}$. The most potent compounds are 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (**17**), 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,-16-diene (**19**), and 17-(1*H*-imidazol-1-yl)androsta-4,16-dien-3-one (**28**), with K_i values of 1.2, 1.4, and 1.9 nM, respectively, being 20−32 times more potent than ketoconazole (K_i = 38 nM). Spectroscopic studies with a modified form of human $P450_{17\alpha}$ indicate that the inhibition process involves binding of steroidal azole nitrogen to the heme iron of the enzyme. Furthermore, some of these potent $P450_{17\alpha}$ inhibitors (**27**−**29**) are also powerful inhibitors of steroid 5α-reductase, and others (**17** and **19**) appear to exhibit strong antiandrogenic activity in cultures of the LNCaP human prostatic cancer cell line. These novel compounds with impressive dual biological activities make them strong candidates for development as therapeutic agents for treatment of prostate cancer and other disease states which depend on androgens.

Introduction

The steroidal enzyme 17α-hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) is a cytochrome P450 monooxygenase complex that catalyzes the conversion of progesterone and pregnenolone into the androgens, androstenedione and dehydoepiandrosterone (DHEA), respectively,[1−3] the direct precursors of estrogens and testosterone. The mechanism of this reation has been studied in some detail.[4−6] The conversion of pregnenolone into DHEA is the major reaction of human testicular $P450_{17\alpha}$. Inhibition of this enzyme as a mechanism for inhibiting androgen biosynthesis could be a worthwhile therapeutic strategy for the treatment of androgen-dependent diseases; for example, prostatic cancer has recently gained attention.[7,8] Indeed, this strategy may be a promising alternative to the treatment of prostatic cancer patients with antiandrogens and LHRH analogues since not only testicular androgens will be inhibited but also adrenal and peripheral production of androgens. It should be noted that prostate cancer is the second leading cause of cancer-related mortality in men in the United States and Europe.[8]

Several categories of steroidal[9−20] and nonsteroidal[21−27] compounds which inhibit $P450_{17\alpha}$ have been reported. Of these compounds, only ketoconazole, an active imidazole fungicide, had been used in the treatment of patients with advanced prostatic cancer.[28,29] However, this compound inhibits several other steroidal P450 enzymes, is not a very potent inhibitor of $P450_{17\alpha}$, and causes significant side effects such as nausea, dry skin, asthenia, etc.[8] This highlights the need to design potent and specific inhibitors of $P450_{17\alpha}$. Given the significance of azole groupings of many drugs,[30] which are P450 enzyme inhibitors including aromatase,[31] we reasoned that introducing azole grouping at C-17 together with the 16-double bond of the androstane skeleton should yield specific and potent $P450_{17\alpha}$ inhibitors. With this design, it may be possible to produce substratelike compounds which not only interact with the steroid-binding site of the enzyme, thus introducing high specificity, but also provide a sixth ligand to the enzyme's heme iron resulting in tight binding. This paper describes the synthesis, testing, and characterization of novel Δ^{16}-17-azolyl steroids, some of which are very potent inhibitors of both human and rat testicular $P450_{17\alpha}$.

Results and Discussion

Chemistry. Although a variety of C-17 azole androstane steroids are known[32−35] and the related Δ^{16}-17 azole counterparts in which the azole is attached to the steroid nucleus through a carbon atom of the heterocycle have been prepared,[14,20,36] the isomeric compounds of this paper, in which the azole is attached to the steroid nucleus via a nitrogen of the azole, appear to constitute a new class of compounds. We have previously reported the synthesis of two of these compounds (**6** and **17**).[18] Further details of their biological activity are described here.

* To whom correspondence should be addressed.
† On leave from Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

S0022-2623(97)00568-2 CCC: $15.00
Published on Web 02/24/1998

We have now discovered a general method (vide infra) for introducing the desired Δ^{16}-17 azole functionality into ring D: a procedure which involves nucleophilic vinylic "addition−elimination" substitution reaction of a steroidal Δ^{16}-17-chloro-16-formyl compound (e.g., **2**) with suitable azole nucleophiles. The syntheses of these heretofore undescribed Δ^{16}-17-azole steroidal compounds are outlined in Schemes 1−3. The key intermediate of our synthesis, 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**2**), was prepared following the Vilsmeier−Haack reaction of the commercially available 3β-acetoxyandrost-5-en-17-one (**1**) with phosphorus oxychloride ($POCl_3$) and dimethylformamide (DMF) as reported by Siddiqui et al.[37] albeit in a higher yield (77% vs 62%). In addition, the related 16-deformylated compound, 3β-acetoxy-17-chloroandrosta-5,-16-diene (**3**), was as obtained in 11.4% yield; this product was not reported by Siddiqui et al.[37]

For the synthesis of 3β-hydroxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (**6**) (Scheme 1), treatment of the Δ^{16}-17-chloro-16-formyl compound **2** with sodium triazolate in DMF at 80 °C gave the expected 17-(1*H*-1,2,4-triazole) **4** in 89% yield. Decarbonylation[38] of **4** with in situ generated Rh(1,3-bis(diphenylphosphino)propane)$_2^+$-Cl^- catalyst [Rh(dppp)$_2^+Cl^-$] in refluxing xylenes gave compound **5** (87%), and following cleavage of the 3β-acetoxy group, the target 3β-hydroxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (**6**) was obtained in 90% isolated yield.

Based on the current status of knowledge[39] of nucleophilic vinylic substitution reaction of activated vinylic systems, it is plausible that the Δ^{16}-17-azole **4** is formed via the formal carbanion intermediate following addition of the azole grouping at C-17 and subsequent elimination of chloride ion. That the 16-formyl activating group was essential for this nucleophilic vinylic "addition−elimination" substitution reaction to take place was revealed by the observation that compound **3** (note that it is devoid of 16-CHO) when treated with sodium triazolate in DMF at 100 °C for 2 days was completely unchanged.

We had previously reported that the 17-chlorine of compound **2** was also easily displaced by methoxide ion (MeO^-) under mild reaction conditions.[18] Furthermore, formation of a minor product in which the pyrazole ring is attached to C-17 through a carbon atom (vide infra, see **22**) indicates that this novel nucleophilic "addition−elimination" substitution reaction could be successfully extended to carbon nucleophiles. These, together with other examples reported below, demonstrate the generality of the process, and we believe that similar results may be realized with a variety of nucleophiles under appropriate reaction conditions.

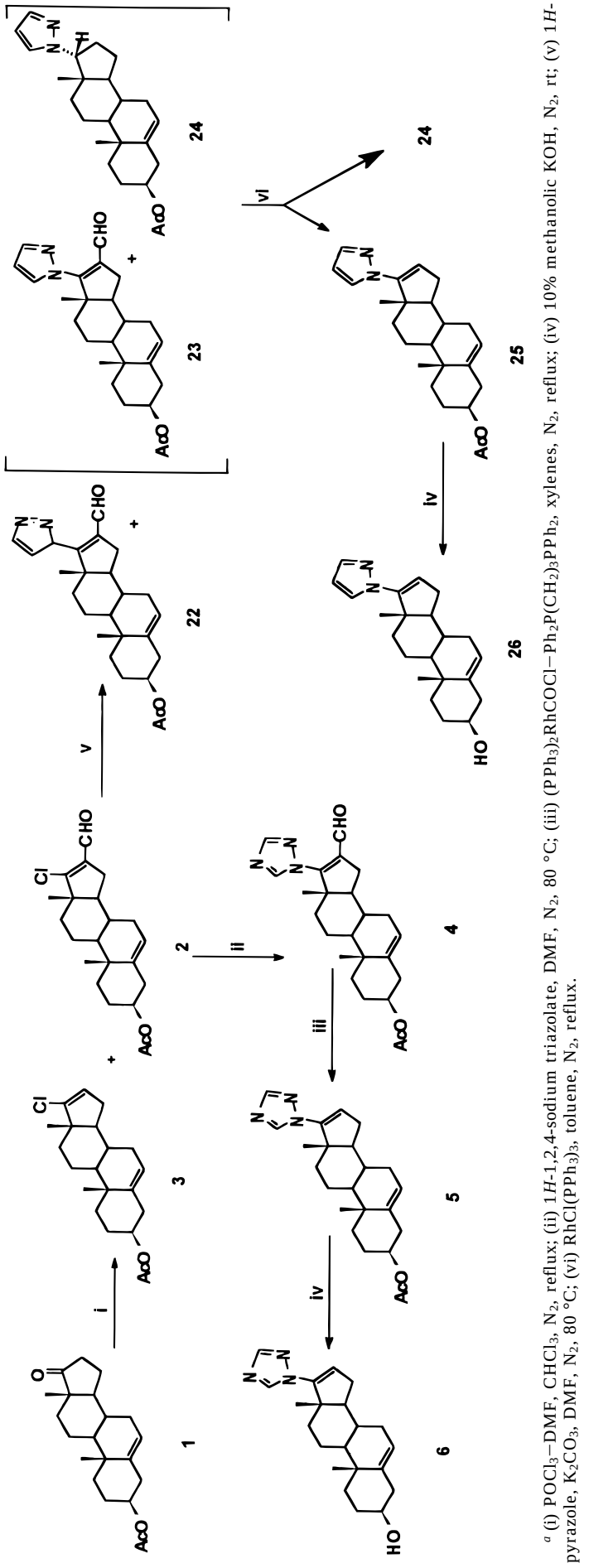
The other 3β-acetoxy-17-azoles: imidazole (**7**), 2*H*-1,2,3-triazole (**8**), 1*H*-1,2,3-triazole (**9**), 2*H*-tetrazole (**10**), 1*H*-tetrazole (**11**), 3*H*-pyrazole (**22**), and 1*H*-pyrazole (**23**) (Scheme 2), were prepared similarly to **4**, but employing different azole nucleophiles and modifying the conditions accordingly. In sharp contrast to the reaction of compound **2** with sodium triazolate described above, its reaction with sodium imidazolate gave a mixture of many intractable products, from which no pure compound could be isolated. To our delight when we treated compound **2** with imidazole in the presence of K_2CO_3[40] in DMF at 80 °C, we obtained the desired 3β-acetoxy-17-imidazole (**7**) in quantitative yield. Attempted decarbonylation of the 16-CHO group using tris(triphenylphosphine)rhodium chloride[40] (RhCl(P-$Ph_3)_3$, Wilkinson's catalyst) resulted in extremely low yield (ca. 8%) of the expected compound **12**, while the use of Rh(dppp)$_2^+Cl^-$ catalyst afforded **12** in 51% yield. We subsequently found that **7** when refluxed in benzonitrile with 10% palladium on activated charcoal[43] afforded **12** in 76% yield. Methanolysis of **12** gave the desired 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (**17**, 95%).

The reaction of **2** with 1*H*-1,2,3-triazole in the presence of K_2CO_3 in DMF gave a mixture of two regioisomers, which were separated by flash column chromatography (FCC) on silica gel into the less polar 2*H*-1,2,3-triazole (**8**) and the more polar 1*H*-1,2,3-triazole (**9**) derivatives in 28% and 62% isolated yields, respectively. These assignments are tentative and are based on data of chromatographic mobilities of 1,2,3-triazole regioisomers of established structure.[40] ^{1}H NMR measurements, in this case, did not distinguish between the two equivalent protons of the 2*H*-1,2,3-triazole moiety in **8** from the two unequivalent protons in **9**. While **8** was smoothly decarbonylated with Wilkinson's catalyst[41] in refluxing toluene to give compound **13** in 92% yield, the 1*H*-triazole **9** required the use of Rh(dppp)$_2^+Cl^-$ catalyst[38] in refluxing xylenes to afford **14** in 90% yield. These 1,2,3-triazole regioisomers, **13** and **14**, were each hydrolyzed to give the desired 3β-hydroxy-17-(2*H*-1,2,3-triazol-2-yl)androsta-5,16-diene (**18**) and 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (**19**), respectively, both in quantitative yields.

The synthesis of tetrazole derivatives (**10** and **11**) was first attempted by using K_2CO_3 as base, 1*H*-tetrazole, and compound **2**. This yielded a complex mixture from which no product could be isolated. However, when Li_2-CO_3[40] was used as the base, we obtained the less polar 2*H*-tetrazole (**10**) and the more polar 1*H*-tetrazole (**11**) compounds in 28% and 45% yields, respectively. Here again these assignments are tentative and are based on the same reason as stated above for the 1,2,3-triazole regioisomers (**8** and **9**). Compounds **10** and **11** were each converted separately into the desired tetrazole regioisomers, 3β-hydroxy-17-(2*H*-tetrazol-2-yl)androsta-5,16-diene (**20**) and 3β-hydroxy-17-(1*H*-tetrazol-1-yl)-androsta-5,16-diene (**21**), by decarbonylation at C-17 (Wilkinson's catalyst in refluxing toluene) to give **15** and **16**, respectively, followed by hydrolysis.

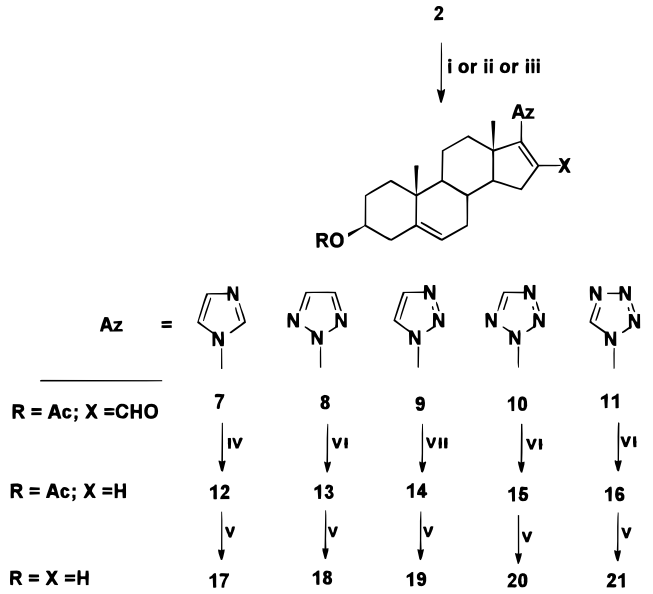
The reaction of **2** with 1*H*-pyrazole in the presence of K_2CO_3 was more eventful as it resulted in the formation of an unexpected compound (vide infra) together with two other products. This reaction resulted in a mixture of products, which following FCC gave first a less polar fraction, identified as 3β-acetoxy-17-(3*H*-pyrazol-3-yl)-16-formylandrosta-5,16-diene (**22**, 12%). Spectral and analytical data were consistent with the assigned structure of **22**. The major (ca. 63%) more polar fraction consisted of two compounds (^{1}H NMR evidence). This latter fraction resisted separation on various chromatographic systems, but following decarbonylation reaction (Wilkinson's catalyst, refluxing touluene) and separation by FCC the expected 3β-acetoxy-17-(1*H*-pyrazol-1-yl)-androsta-5,16-diene (**25**, 25% from **2**) and *unexpected* 3β-

Scheme 1[a]



[a] (i) $POCl_3$–DMF, $CHCl_3$, N_2, reflux; (ii) 1*H*-1,2,4-sodium triazolate, DMF, N_2, 80 °C; (iii) $(PPh_3)_2RhCOCl$–$Ph_2P(CH_2)_3PPh_2$, xylenes, N_2, reflux; (iv) 10% methanolic KOH, N_2, rt; (v) 1*H*-pyrazole, K_2CO_3, DMF, N_2, 80 °C; (vi) $RhCl(PPh_3)_3$, toluene, N_2, reflux.

Scheme 2[a]



[a] (i) Imidazole, K_2CO_3, DMF, N_2, 80 °C; (ii) 1*H*-1,2,3-triazole, K_2CO_3, DMF, N_2, 75 °C; (iii) 1*H*-tetrazole, Li_2CO_3, DMF, N_2, 80 °C; (iv) 10% Pd on activated charcoal, PhCN, reflux; (v) 10% methanolic KOH, N_2, rt; (vi) $RhCl(PPH_3)_3$, toluene, N_2, reflux; (vii) $(PPh_3)_2PCOCl-Ph_2P(CH_2)_3PPh_2$, xylenes, N_2, reflux.

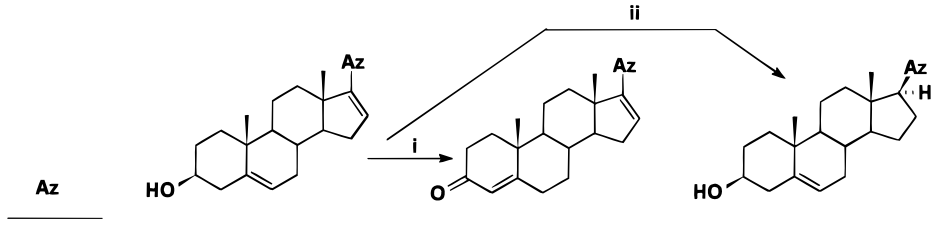
acetoxy-17α-(1*H*-pyrazol-1-yl)androst-5-ene (**24**) in 19% yield (note the cleavage of the 16-CHO group, reduction of Δ^{16}, and α-orientation of the 17-pyrazole group) were obtained. Hydrolysis of **25** gave the desired 3*β*-hydroxy-17-(1*H*-pyrazol-1-yl)androsta-5,16-diene (**26**) in quantitative yield. The formation of compound **24** is striking and unexpected. Although we are certain (^{1}H NMR evidence) that **24** was formed in the initial reaction (i.e., **2** + 1*H*-pyrazole + K_2CO_3), the mechanism of its formation is unclear at this time. Assignment of the α-configuration of the 17-pyrazole group of **24** is based on its ^{1}H NMR data compared with those of compounds of established structure. The C-18 protons of **24** appear as a singlet at *δ* 0.97. This is in good agreement with those reported[42,43] for the C-18 protons in 17α-acetyl steroids (*δ* 0.80−0.84). By this technique they are distinguishable from the isomeric 17*β*-acetyl derivatives in which the C-18 protons resonate at a relatively higher field (*δ* 0.60−0.67). In addition, the 17*β*-H of compound **24** appears as a doublet of doublet at *δ* 3.24 which is unlike 17α-H of 17*β*-substituted steroids which appears as a triplet. Further support for the assigned structure of **24** came from the ^{1}H NMR data of the related 17*β*-(1-methyl-3-pyrazolyl)-5-androstene-3*β*-acetate in which the C-18 protons appear as a singlet at *δ* 0.55[36] and also from ^{1}H NMR data of compounds **30** and **31** of this work (vide infra).

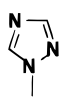
The Δ^4-3-oxo analogues **27**−**29** (Scheme 3) were each synthesized from compounds **6**, **17**, and **19**, respectively, by a modified Oppenauer oxidation[44] using *N*-methylpiperidone and aluminum isopropoxide. Finally, analogues containing a saturated D-ring, **30** and **31**, were each also prepared from the Δ^{16} compounds, **6** and **17**, respectively, by reduction with diimide.[13] The *β*-orientation of the azole ring in compounds **30** and **31** was established by ^{1}H NMR spectroscopy which showed a triplet with a coupling constant of 9.6 Hz for the 17α-H which is characteristic of 17*β*-substituted steroids.[42,43] There was no evidence for the formation of steroids with the α-stereochemistry at C-17 in these reduction reactions.

Following our identification of the potent inhibitors (vide infra) of $P450_{17\alpha}$, the overall four-step/five-step reaction sequence has been carried out routinely on the 5−10-g scale in good overall yield. For example, starting from **1**, the overall yields of two of our most potent compounds, **17** (four steps) and **28** (five steps), are 56% and 44%, respectively.

Enzyme Inhibition Studies. The source of enzyme used in the present study was human testicular microsomes that contained cytochrome $P450_{17\alpha}$ as well as cytochrome b_5. The enzyme reaction was monitored by determination of the release of C^3H_3COOH from [21-$^3H_3$]-17α-hydroxypregnenolone during cleavage of the C-21 side chain in the conversion to DHEA. This assay measures only the lyase activity of the $P450_{17\alpha}$ enzyme. We have previously reported[20] that this assay is comparable to the HPLC assay procedure (which utilizes [7-^{3}H]pregnenolone as substrate) and measures both the hydroxylase and lyase activities of the enzyme.

Scheme 3[a]



Az			
	6	27	30
	17	28	31
	19	29	-

[a] (i) Al(*i*-PrO)$_3$, 1-methyl-4-piperidone, toluene, reflux; (ii) N_2H_2, AcOH, EtOH, air, 80 °C.

Table 1. Inhibition of Human $P450_{17\alpha}$ by Δ^{16}-17-Azolyl Steroids

compound[a]	% inhibition[b]
6, 17-(1*H*-1,2,4-triazolyl)-	60
17, 17-(1*H*-imidazolyl)-	97
18, 17-(2*H*-1,2,3-triazolyl)-	NI[c]
19, 17-(1*H*-1,2,3-triazolyl)-	94
20, 17-(2*H*-tetrazolyl)-	NI[c]
21, 17-(1*H*-tetrazolyl)-	NI[c]
26, 17-(1*H*-pyrazolyl)-	40
for comparison	
ketoconazole	67

[a] Each inhibitor concentration was 150 nM. [b] Concentration of substrate, 17α-hydroxypregnenolone = 10 μM. [c] NI, no inhibition at concentration of 150 nM. All values are mean of two determinations.

To estimate the inhibitor potency of the novel compounds synthesized in the present work, the tritiated substrate, a NADPH-generating system, and microsomes were incubated at 34 °C in O_2 in the presence or absence of the inhibitor. The reaction was usually monitored for 60 min during which time the formation of [^{3}H]acetic acid and thus DHEA was linear. The percentage inhibition data for the initial target compounds of this study are presented in Table 1 and highlight that 2*H*-1,2,3-triazole (**18**) and the two tetrazole regioisomers (**20** and **21**) were noninhibitory, while the 1*H*-pyrazole (**26**) was a moderate inhibitor. By contrast the 1*H*-1,2,4-triazole (**6**), 1*H*-imidazole (**17**), and 1*H*-1,2,3-triazole (**19**) were potent inhibitors of the enzyme. Ketoconazole also showed strong inhibition. Given that these Δ^{16}-17-azole compounds of Table 1 are structurally similar (all possess the Δ^5-3β-ol functionality), the striking difference in the inhibitory properties observed may be due to the differences in their basicities, a property imposed by the inherent different electronic character of each of the azole heterocycles. In addition, the presence of a nitogen atom at either the 3′ or 4′ position seems important for potent inhibition of the enzyme.

Following the initial screening assays, compounds **6**, **17**, and **19** together with their corresponding Δ^4-3-one counterparts, **27**−**29**, respectively, were evaluated further to determine first their IC_{50} values and then their apparent K_i values (from Lineweaver−Burk plots, e.g., Figure 1). These values are presented in Table 2. All six 17-azoles are excellent noncompetitive inhibitors of $P450_{17\alpha}$ as shown in the example in Figure 1. The activities of two of the compounds (**6** and **17**) against the rat enzyme were cited in a report of our preliminary studies.[18] After careful evaluation where both the IC_{50} and K_i values were determined in repeated assays, as shown in Table 2, these two compounds were found to be potent inhibitors of the human enzyme and more potent for the rat enzyme than our earlier tests indicated. The nature of inhibition kinetics exhibited by these compounds was that in which the V_{max} was decreased but the apparent K_m was unchanged; i.e., the intercept on the horizontal axis is the same in the absence or presence of inhibitor. This is one of the two characteristics of a noncompetitive inhibitor and indicates destruction of the catalytic activity of the enzyme. The other is when binding of the inhibitor and (variable) substrate is not mutually exclusive.

There was no marked difference between the inhibitory potencies of the Δ^5-3β-ol azoles (**6**, **17**, and **19**) with those of the corresponding Δ^4-3-one compounds (**27**−**29**). Compounds **17**, **19**, and **28**, with K_i values of 1.2, 1.4, and 1.8 nM, respectively (K_m of the substrate 17α-hydroxypregnenolone was 530 nM), were the most potent inhibitors, and they are among the most potent inhibitors of human testicular microsomal $P450_{17\alpha}$ described to date. These compounds were considerably more potent as $P450_{17\alpha}$ inhibitors when compared in the same assay with ketoconazole (K_i = 38 nM). The requirement of a 16,17-double bond was also observed with these $P450_{17\alpha}$ inhibitors: 17β-(1*H*-1,2,4-triazolyl) and 17β-(1*H*-imidazolyl) compounds (**30** and **31**) each exhibited diminished potency compared to the corresponding parent Δ^{16} compounds, **6** and **17**, respectively

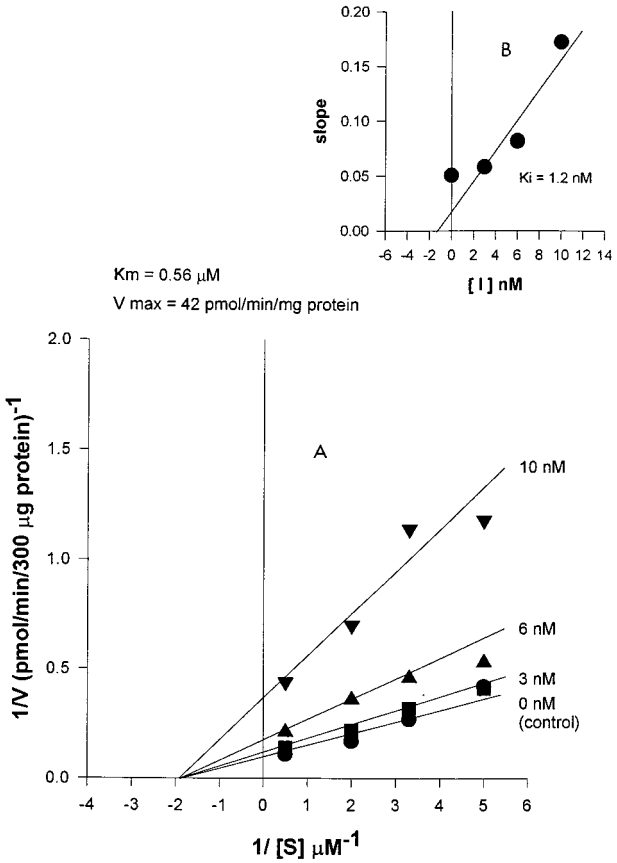


Figure 1. (A) Lineweaver−Burk analysis (1/*V* vs 1/[S]) of Δ^{16}-17-(1*H*-imidazole) **17** at 3, 6, and 10 nM. (B) Replot of the slopes of each reciprocal plot versus inhibitor concentration ([I]) to obtain the K_i value. The inhibition experiments with the other azoles (**6**, **19**, **27**−**29**) gave plots that were essentially the same as shown above.

Table 2. Inhibitory Potency of Δ^{16}-17-Azolyl Steroids toward Human and Rat $P450_{17\alpha}$ and Human Steroid 5α-Reductase

	human $P450_{17\alpha}$		rat $P450_{17\alpha}$	5α-reductase
compound	IC_{50} (nM)[a]	K_i (nM)[b,c]	IC_{50} (nM)[a]	IC_{50} (nM)[a]
6	90 ± 14	23	26 ± 13	∼160000
17	8 ± 1	1.2	9 ± 2	∼400000
19	13 ± 1	1.4	10 ± 0.4	∼10000
27	55 ± 11	41	11 ± 3	152 ± 10
28	7 ± 1	1.9	8 ± 0.7	142 ± 5
29	19 ± 1	8	9 ± 2	198 ± 33
for comparison				
ketoconazole	78 ± 3	38	209 ± 17	
finasteride				33 ± 2

[a] Mean ± SDM of at least two experiments. [b] K_i values were determined as described in the Experimental Section. [c] K_m for substrate, 17α-hydroxypregnenolone = 560 nM.

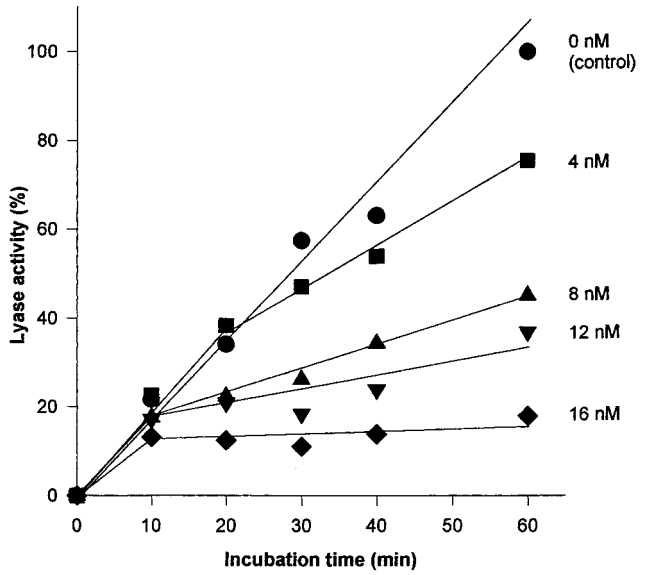


Figure 2. Progress curves for the inhibition of human testicular microsomal $P450_{17\alpha}$ by Δ^{16}-17-(1*H*-imidazole) **17** at different concentrations.

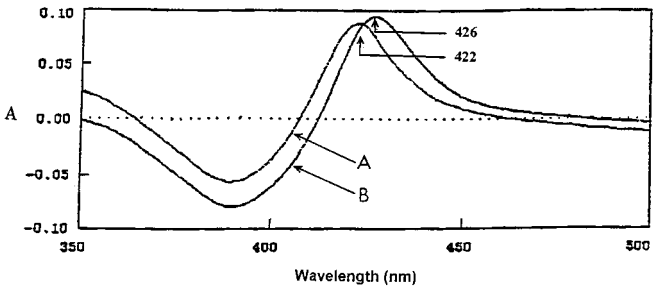


Figure 3. Difference absorption spectra. The experimental and reference cuvettes contained a modified form of $P450_{17\alpha}$ (P450 concentration 1.8 μM). Spectra show the effects of addition of 20 μM Δ^{16}-17-(1*H*-1,2,3-triazole) **6** (curve A) and 20 μM Δ^{16}-17-(1*H*-imidazole) **17** (curve B).

(**6** → **30**, IC_{50} 90 → 219 nM; **17** → **31**, IC_{50} 8 → 62 nM). A similar observation has been previously reported[13,14,20] for a number of Δ^{16}-17-heteroaryl $P450_{17\alpha}$ inhibitors.

When the lyase reaction was monitored in the presence of various concentrations of the imidazole **17**, a family of nonlinear progress curves were obtained in which the extent of inhibition increased with time (Figure 2). This suggests that **17** may be a slow-binding inhibitor.[45] Although the other potent inhibitors were not examined in this assay, it is likely that they may also behave in a similar fashion. Compound **17** appears to be the first example of a slow-binding inhibitor of cytochrome $P450_{17\alpha}$.

To investigate the mechanism of $P450_{17\alpha}$ inhibition further, the properties (chemical nature) of the complexes formed between the 1*H*-1,2,4-triazole **6** and imidazole **17** and a modified form of human $P450_{17\alpha}$[46] were studied using UV−vis difference spectroscopy following standard procedure.[47] Each of these compounds induced a type II difference spectrum (Figure 3), indicating coordination of a steroidal nitrogen (probably N-4 of the triazole ring or N-3 of the imidazole ring) to the heme iron of the cytochrome P450 enzyme, with formation of low-spin iron. The peak positions of the Soret maxima for the complexes with triazole **6** (422 nm) and imidazole **17** (426 nm) are in agreement with available data for the binding of nitrogen ligands to cytochrome P450 systems, resulting in complexes with Soret maxima at 421−430 nm.[48]

The inhibitory potency of (20*R*)- and (20*S*)-aziridinyl steroids has recently been reported, and this stems in part from the additional stabilization due to coordination of the heteroatom of their aziridinyl ring to the heme of rat $P450_{17\alpha}$.[15] The spectroscopic data described above suggest that this may also be the case for the Δ^{16}-17-azole steroids of this work. The ability of the steroidal azole nitrogen atom to coordinate with the heme of $P450_{17\alpha}$ indicates that C-17 and C-20 (the sites of enzymatic hydroxylations) can be positioned in close proximity to the heme center when these substratelike inhibitors are bound to the enzyme. Although it is not certain that these compounds bind in exactly the same manner as the natural substrates, their high binding affinities make a significantly different mode of binding unlikely. It should be noted that although two groups[13,14] have recently reported on 17-heteroaryl steroidal inhibitors of $P450_{17\alpha}$ and believe that the inhibitory property of their compounds is due (in part) to coordination of a heteroaryl atom to the heme iron of the enzyme complex, they are yet to provide evidence for this phenomenon.

Before evaluating these potent inhibitors in vivo in rodent models as potential therapeutic agents for the treatment of prostate cancer, we accessed the potency of these inhibitors also toward the rat testicular microsomal $P470_{17\alpha}$. A comparison was made between the inhibitory activity, expressed as IC_{50} values, displayed by the Δ^5-3β-ols **6**, **17**, and **19**; the Δ^4-3-one compounds **27**−**29**; and ketoconazole toward $P470_{17\alpha}$ located in human and rat testicular microsomes. The results are presented in Table 2 and show that whereas the potencies of **6**, **27**, and **29** each increased toward the rat enzyme by 3.5-, 5-, and 2-fold, respectively, potencies of **17**, **19**, and **28** were unchanged, while that for ketoconazole decreased by about 3-fold. Our most potent inhibitors, **17**, **19**, and **28** appear to be the first examples of inhibitors that are equipotent toward the human as well as the rat $P450_{17\alpha}$ enzymes. This finding suggests that results from preclinical in vivo studies with rats are likely to reflect the clinical situation.

Other Biological Activities. We had previously reported[11,12] that some $P450_{17\alpha}$ inhibitors were also potent inhibitors of 5α-reductase while others exhibited strong antiandrogenic activity.[17] Consequently, we first tested the potent $P450_{17\alpha}$ inhibitors of this work for 5α-reductase inhibition. The results are presented in Table 2 and highlight that the Δ^5-3β-ol compounds (**6**, **17**, and **19**) were poor inhibitors of the enzyme. By contrast, the corresponding Δ^4-3-one compounds (**27**−**29**) were potent inhibitors, being only about 4−6 times less potent than finasteride, a potent 5α-reductase inhibitor currently used in the treatment of benign prostatic hyperplasia. In addition, the abilities of compounds **17** and **19** to inhibit the androgen-stimulated growth of LNCaP human prostatic cancer cell were examined. As previously reported,[17] testosterone (0.1 nM) increased the growth of these LNCaP cells 6-fold compared to vehicle-treated cells and DHT (30 pM) stimulated proliferation 5-fold compared to control. The imidazole **17** was more effective than **19** in inhibiting the testosterone-stimulated growth of LNCaP cells, with 100% inhibition

occurring at 1 and 2.5 μM, respectively. Both compounds also inhibited DHT-induced cell growth with **17** again being more effective (100% inhibition at 2.5 and 5 μM, respectively). Since neither compound inhibited 5α-reductase nor was toxic to the cells in the concentration range 0.5−5.0 μM, these results suggest that their growth-inhibiting properties may be due to possible antiandrogenic effects.

Conclusion

We have discovered a method for the introduction of a variety of azolyl groups at the 17-carbon of a Δ^{16} steroid. This enabled us to synthesize several Δ^{16}-17-azolyl steroids of which **6**, **17**, **19**, and **27**−**29** proved to be powerful inhibitors of both human and rat testicular $P450_{17\alpha}$. In addition, we have shown that a nitrogen of **6** and **17** each coordinates to the enzyme's heme iron. Kinetic studies allowed us to classify these compounds as noncompetitive inhibitors of the enzyme. Unlike most previously described $P450_{17\alpha}$ inhibitors which show normal competitive or noncompetitive reversible kinetics, our most potent inhibitor, **17**, shows an apparent slow-binding behavior. Compounds **27**−**29** are also potent inhibitors of 5α-reductase, while **17** and **19** appear to possess strong antiandrogenic effects. These dual biological properties of some of these compounds increase their potential utility in the treatment of prostate cancer. Some of these inhibitors are currently undergoing further pharmacological study.

Experimental Section

General Methods. Melting points (mp) were determined with a Fischer-Johns melting point apparatus and are uncorrected. Proton magnetic resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ on a Mac NMR 5.3 300-MHz spectrometer (internal standard Me_4Si, δ 0), and high-resolution mass spectra (HRMS) were determined on a Kratos Aspect Systems instrument, EI mode. Elemental analyses were performed by Dr. R. N. Pandey of Guelph Chemical Laboratories Ltd., Guelph, Ontario, Canada. TLC was done on silica gel GHLF precoated plates (250 μm) purchased from Analtech, while flash column chromatography (FCC) was performed on silica gel (Merck grade 9385, 230−400 mesh, 60 Å) according to Still's method.[49] Petroleum ether refers to light petroleum, bp 40−60 °C.

Vilsmeier−Haack Reaction of 3*β*-Acetoxyandrost-5-en-17-one (1): 3*β*-Acetoxy-17-chloroandrosta-5,16-diene (2) and 3*β*-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (3). A solution of 3β-acetoxyandrost-5-en-17-one (**1**) (2 g, 6.6 mmol) in dry chloroform (40 mL) was added dropwise to a cold and stirred solution of phosphorus oxychloride (10 mL) and dimethylformamide (10 mL). The mixture was allowed to attain room temperature and then refluxed under N_2 for 5 h. It was then concentrated under reduced pressure and poured onto ice followed by extraction with a mixture of ether and EtOAc (8:2, v/v). The combined extracts were washed with brine and dried (Na_2SO_4), and solvent was removed to give a white solid (2.3 g). Analytical TLC [silica gel, petroleum ether/EtOAc (10:1)] revealed the presence of two compounds, both less polar than compound **1**. Purification by flash column chromatography [FCC, petroleum ether/EtOAc (15:1)] gave the title compounds **2** (0.24 g, 11.4%) and **3** (1.75 g, 77%). Analytical and spectroscopic data for **2** and **3** follow.

Compound 2: mp 160−162 °C; ^{1}H NMR δ 0.89 (3H, s, 18-Me), 1.06 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.61 (1H, m, 3α-H), 5.39 (1H, d, J = 4.8 Hz, 6-H), 5.63 (1H, d, J = 0.9 Hz, 16-H); HRMS calcd 348.1856 ($C_{21}H_{29}O_2Cl$), found 348.1766. Anal. ($C_{21}H_{29}O_2Cl$) C, H.

Compound 3: mp 163−165 °C; ^{1}H NMR δ 0.99 (3H, s, 18-Me), 1.07 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.60 (1H, m, 3α-H), 5.40 (1H, d, J = 4.8 Hz, 6-H), 9.99 (1H, s, 16-CHO); HRMS calcd 376.1805 ($C_{22}H_{29}O_3Cl$), found 376.1748. Anal. ($C_{22}H_{29}O_3Cl$) C, H.

3*β*-Acetoxy-17-(1*H*-1,2,4-triazol-1-yl)-16-formylandrosta-5,16-diene (4). A solution of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**2**; 0.6 g, 1.6 mmol) and sodium triazolate (436 mg, 4.79 mmol, 3 equiv) in dry DMF (10 mL) under N_2 was stirred at 78 °C for 30 min. After cooling to room temperature, the reaction mixture was poured onto ice−water (250 mL), and the resulting white precipitate was filtered, washed with water, and dried to give a white solid. This was crystallized from hexane/EtOAc to give the title compound **4** (580 mg, 89%): mp 160−162 °C; ^{1}H NMR δ 1.08 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.61 (1H, m, 3α-H), 5.42 (1H, d, J = 4.2 Hz, 6-H), 8.13 (1H, s, 3′-H), 8.42 (1H, s, 5′-H), 10.12 (1H, s, 16-CHO); HRMS calcd 409.2365 ($C_{24}H_{31}O_3N_3$), found 409.2348. Anal. ($C_{24}H_{31}O_3N_3$) C, H, N.

3*β*-Acetoxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (5). A mixture of bis(triphenylphosphine)rhodium(I) carbonyl chloride (338 mg, 0.489 mmol) and 1,3-bis(diphenylphosphino)propane (440 mg, 1.065 mmol) in dry xylenes (40 mL) was stirred at 80 °C under N_2 for 15 min when a fine yellow precipitate formed. 3β-Acetoxy-17-(1*H*-1,2,4-triazol-1-yl)-16-formylandrosta-5,16-diene (**4**; 2.0 g, 4.89 mmol) was added, and the mixture was refluxed under N_2 for 15 h, then cooled, and concentrated under reduced pressure. The crude product was dissolved in EtOAc (200 mL) and filtered through a 4-cm pad of silica gel (70−230 mesh). The silica gel was washed with EtOAc (2 × 200 mL), and the combined filtrates were evaporated to give the crude product. This was purified by FCC [petroleum ether/EtOAc/Et_3N (7.7:2:0.3)] to give the title compound **5** (1.63 g, 87.6%): mp 187−190 °C (from hexane/EtOAc); ^{1}H NMR δ 1.08 (3H, s, 18-Me), 1.10 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.62 (1H, m, 3α-H), 5.42 (1H, d, J = 4.5 Hz, 6-H), 5.96 (1H, s, 16-H), 7.99 (1H, s, 3′-H), 8.26 (1H, s, 5′-H); HRMS calcd 381.2416 ($C_{23}H_{31}O_2N_3$), found 381.2406. Anal. ($C_{23}H_{31}O_2N_3$) C, H, N.

3*β*-Hydroxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (6). Details of the synthesis of this compound were reported previously.[18]

3*β*-Acetoxy-17-(1*H*-imidazol-1-yl)-16-formylandrosta-5,16-diene (7). A mixture of compound **2** (500 mg, 1.329 mmol), imidazole (136 mg, 2.0 mmol), and K_2CO_3 (551 mg, 3.99 mmol) in dry DMF (10 mL) was heated at ca. 80 °C under N_2 for 2 h. After cooling to room temperature, the reaction mixture was poured onto ice-cold water (100 mL), and the resulting white precipitate was filtered, washed with water, and dried to give a white solid. This was titrated with a boiling mixture of hexane/EtOAc to give the title compound **7** (520 mg, 92%): mp 218−220 °C; ^{1}H NMR δ 1.08 (6H, s, 18- and 19-Me), 2.04 (3H, s, 3β-OAc), 4.61 (1H, m, 3α-H), 5.42 (1H, d, J = 4.8 Hz, 6-H), 7.11 (1H, s, 4′-H), 7.23 (1H, s, 5′-H), 7.63 (1H, s, 2′-H), 9.74 (1H, s, 16-CHO); HRMS calcd 408.2413 ($C_{25}H_{32}O_3N_2$), found 408.2426. Anal. ($C_{25}H_{32}O_3N_2$) C, H, N.

Reaction of 3*β*-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (2) with 1*H*-1,2,3-Triazole and K_2CO_3: 3*β*-Acetoxy-17-(2*H*-1,2,3-triazol-2-yl)-16-formylandrosta-5,-16-diene (8) and 3*β*-Acetoxy-17-(1*H*-1,2,3-triazol-1-yl)-16-formylandrosta-5,16-diene (9). A mixture of **2** (2 g, 5.23 mmol), 1*H*-1,2,3-triazole (552 mg, 7.98 mmol), and K_2CO_3 (2.20 g, 15.95 mmol) in dry DMF (40 mL) was heated at 80 °C under N_2 atmosphere for 2 h. After cooling to room temperature, the reaction mixture was poured onto ice−water (400 mL), and the resulting precipitate was filtered, washed with water, and dried to give a dirty-white solid. This was subjected to flash chromatography and, on elution with petroleum ether/EtOAc/Et_3N (6.7:3:0.3), gave first 3β-acetoxy-17-(2*H*-1,2,3-triazol-2-yl)-16-formylandrosta-5,16-diene (**8**; 684 mg, 28%): mp 145−148 °C; ^{1}H NMR δ 1.09 (3H, s, 18-Me), 1.26 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.61 (1H, m, 3α-H), 5.42 (1H, d, J = 4.2 Hz, 6-H), 7.85 (2H, s, 4′- and 5′-H), 10.55 (1H, s, 16-CHO). Anal. ($C_{24}H_{31}O_3N_3$) C, H, N. Further elution with petroleum ether/EtOAc/Et_3N (6:4:0.3) afforded 3β-acetoxy-17-(1*H*-1,2,3-triazol-1-yl)-16-formylandrosta-5,16-diene (**9**; 1.48 g, 62%): mp

215−217 °C; ^{1}H NMR δ 1.08 (3H, s, 18-Me), 1.18 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.63 (1H, m, 3α-H), 5.43 (1H, d, J = 4.2 Hz, 6-H), 7.85 (2H, s, 4′- and 5′-H), 9.94 (1H, s, 16-CHO). Anal. ($C_{24}H_{31}O_3N_3$) C, H, N.

3β-Acetoxy-17-(2*H*-tetrazol-2-yl)-16-formylandrosta-5,-16-diene (10) and 3β-Acetoxy-17-(1*H*-tetrazol-1-yl)-16-formylandrosta-5,16-diene (11). The method followed that described for compounds **8** and **9**, but using **2** (0.5 g, 1.329 mmol), 1*H*-tetrazole (187 mg, 1.59 mmol), and Li_2CO_3 (287 mg, 3.94 mmol) gave a crude product (520 mg). Flash column chromatography on elution with petroleum ether/EtOAc (5:1) gave first 3β-acetoxy-17-(2*H*-tetrazol-2-yl)-16-formylandrosta-5,16-diene (**10**; 92 mg, 28.2%): mp 170−172 °C dec; ^{1}H NMR δ 1.1 (3H, s, 18-Me), 1.29 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.62 (1H, m, 3α-H), 5.42 (1H, d, J = 4.2 Hz, 6-H), 8.68 (1H, s, 5′-H), 10.46 (1H, s, 16-CHO). Anal. ($C_{23}H_{30}O_3N_4$) C, H, N. Further elution with petroleum ether/EtOAc/Et_3N (7:3:0.3) afforded 3β-acetoxy-17-(1*H*-tetrazol-1-yl)-16-formylandrosta-5,16-diene (**11**; 146 mg, 44.6%): mp 196−198 °C dec; ^{1}H NMR δ 1.09 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.62 (1H, m, 3α-H), 5.43 (1H, d, J = 4.8 Hz, 6-H), 8.93 (1H, s, 5′-H), 9.92 (1H, s, 16-CHO). Anal. ($C_{23}H_{30}O_3N_4$) C, H, N.

3β-Acetoxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (12). A solution of 3β-acetoxy-17-(1*H*-imidazol-1-yl)-16-formylandrosta-5,16-diene (**7**; 4.0 g, 9.8 mmol) in dry benzonitrile (40 mL) was refluxed in the presence of 10% palladium on activated charcoal (2 g, i.e., 50% weight of the 16-formyl azole) for 3.5 h. After cooling to room temperature, the catalyst was removed by filtration through a Celite pad. The filtrate was evaporated, and the residue was purified by FCC [petroleum ether/EtOAc/Et_3N (6:4:0.3)] to give the title compound **12** (2.72 g, 73.2%): mp 138−140 °C; ^{1}H NMR δ 1.0 (3H, s, 18-Me), 1.07 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.60 (1H, m, 3α-H), 5.41 (1H, s, 6-H), 5.68 (1H, s, 16-H), 7.02 (1H, s, 4′-H), 7.08 (1H, s, 5′-H), 7.60 (1H, s, 2′-H); HRMS calcd 408.2413 ($C_{25}H_{32}O_3N_2$), found 408.2426. Anal. ($C_{25}H_{32}O_3N_2$) C, H, N.

3β-Acetoxy-17-(2*H*-1,2,3-triazol-2-yl)androsta-5,16-diene (13). A mixture of 3β-acetoxy-17-(2*H*-1,2,3-triazol-2-yl)-16-formylandrosta-5,16-diene (**8**; 140 mg, 0.342 mmol) in dry toluene (6 mL) and tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst; 332 mg, 0.351 mmol) was refluxed under N_2 for 5 h. After the mixture cooled to room temperature, EtOH (12 mL) was added, and on further cooling at approximately 0 °C, the yellow precipitate of bis(triphenylphosphine)carbonylchlororhodium (I) formed. Following filtration, the filtrate was concentrated to give the crude product. This was purified by FCC [petroleum ether/EtOAc (15:1)] to give the title compound **13** (120 mg, 92%): mp 154−155 °C; ^{1}H NMR δ 1.09 (3H, s, 18-Me), 1.14 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.60 (1H, m, 3α-H), 5.42 (1H, d, J = 4.2 Hz, 6-H), 6.17 (1H, br s, 16-H), 7.68 (2H, s, 4′- and 5′-H). Anal. ($C_{23}H_{31}O_2N_3$) C, H, N.

3β-Acetoxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (14). The method followed that described for compound **5** but using 3β-acetoxy-17-(1*H*-1,2,3-triazol-1-yl)-16-formylandrosta-5,16-diene (**9**; 2.0 g, 4.89 mmol). Purification of the crude product by FCC [petroleum ether/EtOAc/Et_3N (7.7:2:0.3)] gave the title compound **14** (1.67 g, 89.9%): mp 158−160 °C; ^{1}H NMR δ 1.09 (3H, s, 18-Me), 1.14 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.60 (1H, m, 3α-H), 5.40 (1H, d, J = 4.2 Hz, 6-H), 5.98 (1H, br s, 16-H), 7.73 (2H, s, 4′- and 5′-H). Anal. ($C_{23}H_{31}O_2N_3$) C, H, N.

3β-Acetoxy-17-(2*H*-tetrazol-2-yl)androsta-5,16-diene (15). The method followed that described for compound **13** but using 3β-acetoxy-17-(2*H*-tetrazol-2-yl)-16-formylandrosta-5,16-diene (**10**; 124 mg, 0.302 mmol). Purification of the crude product by FCC [petroleum ether/EtOAc (10:1)] gave the title compound **15** (61 mg, 52.8%): mp 155−156 °C; ^{1}H NMR δ 1.10 (3H, s, 18-Me), 1.17 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.62 (1H, m, 3α-H), 5.42 (1H, d, J = 4.85 Hz, 6-H), 6.46 (1H, s, 16-H), 8.52 (1H, s, 5′-H). Anal. ($C_{22}H_{30}O_2N_4$) C, H, N.

3β-Acetoxy-17-(1*H*-tetrazol-1-yl)androsta-5,16-diene (16). The method followed that described for **13** but using 3β-acetoxy-17-(1*H*-tetrazol-1-yl)-16-formylandrosta-5,16-diene (**11**; 140 mg, 0.3415 mmol). Purification of the crude product by FCC [petroleum ether/EtOAc (3:1)] gave the title compound **16** (45 mg, 34.5%): ^{1}H NMR δ 1.08 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.62 (1H, m, 3α-H), 5.42 (1H, d, J = 4.6 Hz, 6-H), 6.00 (1H, s, 16-H), 8.93 (1H, s, 5′-H). This compound was not particularly stable at room temperature (TLC evidence) and was therefore used for the subsequent reaction without further characterization.

3β-Hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (17). Details of the synthesis of this compound have been published previously.[18]

3β-Hydroxy-17-(2*H*-1,2,3-triazol-2-yl)androsta-5,16-diene (18). The method followed that described for compound **6** but using 3β-acetoxy-17-(2*H*-1,2,3-triazol-2-yl)androsta-5,-16-diene (**13**; 110 mg, 0.289 mmol). Purification of the crude product by FCC [petroleum ether/EtOAc (3:1)] gave the title compound **18** (95 mg, 97.1%) which was crystallized from hexane/EtOAc: mp 176−177 °C; ^{1}H NMR δ 1.09 (3H, s, 18-Me), 1.15 (3H, s, 19-Me), 3.54 (1H, m, 3α-H), 5.39 (1H, d, J = 5.1 Hz, 6-H), 6.17 (1H, s, 16-H), 7.68 (2H, s, 4′- and 5′-H). Anal. ($C_{21}H_{29}ON_3$) C, H, N.

3β-Hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (19). The method followed that described for compound **6** but using 3β-acetoxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,-16-diene (**14**; 1.5 g, 3.94 mmol). The product was recrystallized fron EtOAc/MeOH to give the title compound **19** (1.20 g, 90%): mp 220−224 °C; ^{1}H NMR δ 1.08 (3H, s, 18-Me), 1.14 (3H, s, 19-Me), 3.54 (1H, m, 3α-H), 5.39 (1H, d, J = 4.8 Hz, 6-H), 5.97 (1H, s, 16-H), 7.72 (2H, s, 4′- and 5′-H). Anal. ($C_{21}H_{29}ON_3$) C, H, N.

3β-Hydroxy-17-(2*H*-tetrazol-2-yl)androsta-5,16-diene (20). The method followed that described for compound **6** but using 3β-acetoxy-17-(2*H*-tetrazol-2-yl)androsta-5,16-diene (**15**; 51 mg, 0.134 mmol). Recrystallization of the product from hexane/EtOAc gave the title compound **20** (42 mg, 88%): mp 195−198 °C; ^{1}H NMR δ 1.09 (3H, s, 18-Me), 1.17 (3H, s, 19-Me), 3.55 (1H, m, 3α-H), 5.34 (1H, d, J = 5.2 Hz, 6-H), 6.46 (1H, s, 16-H), 8.53 (1H, s, 5′-H). Anal. ($C_{20}H_{28}ON_4$) C, H, N.

3β-Hydroxy-17-(1*H*-tetrazol-1-yl)androsta-5,16-diene (21). The method followed that described for **6** but using 3β-acetoxy-17-(1*H*-tetrazol-1-yl)androsta-5,16-diene (**16**; 36 mg, 0.094 mmol). Recrystallization of the product from hexane/EtOAc gave the title compound **21** (28 mg, 87.4%): mp 200−204 °C dec; ^{1}H NMR δ 1.06 (3H, s, 18-Me), 1.11 (3H, s, 19-Me), 3.54 (1H, m, 3α-H), 5.38 (1H, br s, 6-H), 6.13 (1H, s, 16-H), 8.73 (1H, s, 5′-H). Anal. ($C_{20}H_{28}ON_4$) C, H, N.

Reaction of Compound 2 with 1*H*-Pyrazole and K_2CO_3: 3β-Acetoxy-17-(3*H*-pyrazol-3-yl)-16-formylandrosta-5,16-diene (22), 3β-Acetoxy-17α-(1*H*-pyrazol-1-yl)-androst-5-ene (24), and 3β-Acetoxy-17-(1*H*-pyrazol-1-yl)-androsta-5,16-diene (25). Reaction of compound **2** (0.5 g, 1.329 mmol), pyrazole (136 mg, 1.994 mmol), and K_2CO_3 (551 mg, 3.99 mmol) as described for compound **7** and following FCC [petroleum ether/EtOAc (4:1)] gave first 3β-acetoxy-17-(3*H*-pyrazol-3-yl)-16-formylandrosta-5,16-diene (**22**; 63 mg, 12%): mp 165−168 °C; ^{1}H NMR δ 0.99 (3H, s, 18-Me), 1.07 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.60 (1H, m, 3α-H), 5.42 (1H, d, J = 4.8 Hz, 6-H), 6.38 (1H, s, 5′-H), 7.64 (1H, d, J = 1.5 Hz, 4′-H), 9.30 (1H, d, J = 2.4 Hz, 16-CHO). Anal. ($C_{25}H_{32}O_3N_2$) C, H, N. Further elution afforded a mixture (341 mg, approximately 2.5:1, determined by ^{1}H NMR) of 3β-acetoxy-17-(1*H*-pyrazol-1-yl)-16-formylandrosta-5,16-diene (**23**) and 3β-acetoxy-17α-(1*H*-pyrazol-1-yl)androst-5-ene (**24**). This mixture resisted separation by chromatography. The mixture (330 mg) was then subjected to the decarbonylation reaction as described for **13** to give a crude product (350 mg). Flash column chromatography on elution with petroleum ether/EtOAc (15:1) gave first 3β-acetoxy-17-(1*H*-pyrazol-1yl)androsta-5,16-diene (**25**; 123 mg, 25% from **2**): mp 159−161 °C; ^{1}H NMR δ 1.08 (3H, s, 18-Me), 1.11 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.62 (1H, m, 3α-H), 5.42 (1H, d, J = 5.1 Hz, 6-H), 5.77 (1H, s, 16-H), 6.32 (1H, s, 4′-H), 7.60 (1H, s, 3′-H), 7.63 (1H, d, J = 2.4 Hz, 5′-H). Anal. ($C_{24}H_{32}O_2N_2$) C, H, N. Further elution with petroleum ether/EtOAc (5:1) afforded

pure 3β-acetoxy-17α-(1*H*-pyrazol-1-yl)androst-5-ene (**24**; 93 mg, 19%): mp 238−240 °C; ^{1}H NMR δ 0.97 (3H, s, 18-Me), 1.08 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 3.24 (1H, dd, J_1 = 4.8 Hz, J_2 = 15.6 Hz, 17β-H), 4.62 (1H, m, 3α-H), 5.43 (1H, d, J = 5.1 Hz, 6-H), 6.45 (1H, s, 4′-H), 7.70 (1H, d, J = 2.4 Hz, 3′-H), 7.77 (1H, d, J = 4.8 Hz, 5′-H). Anal. ($C_{24}H_{34}O_2N_2$) C, H, N.

3β-Hydroxy-17-(1*H*-pyrazol-1-yl)androsta-5,16-diene (26). The method followed that described for **6** but using 3β-acetoxy-17-(1*H*-pyrazol-1-yl)androsta-5,16-diene (**25**; 100 mg, 0.263 mmol). Recrystallization of the product from hexane/EtOAc gave the title compound **26** (858 mg, 95.6%): mp 197−199 °C; ^{1}H NMR δ 1.06 (3H, s, 18-Me), 1.09 (3H, s, 19-Me), 3.56 (1H, m, 3α-H), 5.39 (1H, s, 6-H), 5.78 (1H, s, 16-H), 6.31 (1H, s, 4′-H), 7.59 (1H, s, 3′-H), 7.62 (1H, s, 5′-H). Anal. ($C_{22}H_{32}ON_2$) C, H, N.

17-(1*H*-1,2,4-Triazol-1-yl)androsta-4,16-dien-3-one (27). From a mixture of 3β-hydroxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (**6**; 250 mg, 0.7381 mmol), 1-methyl-4-piperidone (1.18 mL), and toluene (20 mL) was distilled off ca. 4 mL. Aluminum isopropoxide (253 mg, 1.241 mmol) was then added, and the mixture was refluxed under N_2 for 4 h. After cooling, the mixture was diluted with EtOAc (30 mL), washed successively with 5% aqueous $NaHCO_3$ (×3) and brine (×2), and then dried (Na_2SO_4). The solvent was evaporated, and the crude product was purified by FCC [CH_2Cl_2/EtOH (30:1)] to give the title compound **27** (200 mg, 80.5%): mp 247−250 °C; ^{1}H NMR δ 1.13 (3H, s, 18-Me), 1.24 (3H, s, 19-Me), 5.76 (1H, s, 16-H), 5.95 (1H, s, 4-H), 8.00 (1H, s, 3′-H), 8.26 (1H, s, 5′-H). Anal. ($C_{21}H_{27}ON_3$) C, H, N.

17-(1*H*-Imidazol-1-yl)androsta-4,16-dien-3-one (28). The method followed that described for **27** but using 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (**17**; 200 mg, 0.59 mmol). Purification of the crude product by FCC [CH_2Cl_2/EtOH (40:1)] afforded the title compound **28** (150 mg, 75.4%): mp 147−150 °C; ^{1}H NMR δ 1.03 (3H, s, 18-Me), 1.23 (3H, s, 19-Me), 5.69 (1H, s, 6-H), 5.76 (1H, s, 16-H), 7.10 (2H, br s, 4′- and 5′-H), 7.63 (1H, s, 2′-H). Anal. ($C_{22}H_{28}ON_2$) C, H, N.

17-(1*H*-1,2,3-Triazol-1-yl)androsta-4,16-dien-3-one (29). The method followed that described for **27** but using 3β-hydroxy-17-(1*H*-1,2,3-triazol-1-yl)androsta-5,16-diene (**19**; 400 mg, 1.18 mmol). Purification of the crude product by FCC [CH_2Cl_2/EtOH, (30:1)] afforded the title compound (358 mg, 90%): mp 118−120 °C; ^{1}H NMR δ 1.17 (3H, s, 18-Me), 1.25 (3H, s, 19-Me), 5.76 (1H, s, 6-H), 5.95 (1H, s, 16-H), 7.73 (1H, s, 5′-H), 7.74 (1H, s, 4′-H). Anal. ($C_{21}H_{27}ON_3$) C, H, N.

3β-Hydroxy-17β-(1*H*-1,2,4-triazol-1-yl)androst-5-ene (30). A mixture of **6** (200 mg, 0.590 mmol), hydrazine hydrate (0.57 mL, 1.77 mmol), and acetic acid (0.35 mL) in EtOH (20 mL) was heated at 80 °C while a stream of air was passed through the solution for 6 h. The reaction mixture was concentrated to ca. 10 mL, and after cooling, it was diluted with EtOAc (30 mL) followed by washing with saturated aqueous $NaHCO_3$ (10 mL × 2) and brine (10 mL × 2), drying (Na_2SO_4), and concentration to give a crude product (190 mg). This was purified by FCC [CH_2Cl_2/EtOH (30:1)] to give pure **30** (150 mg, 74.6%) as a white solid: mp 246−248 °C; ^{1}H NMR δ 0.56 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 3.53 (1H, m, 3α-H), 4.19 (1H, t, J = 9.6 Hz, 17α-H), 5.37 (1H, d, J = 5.2 Hz, 6-H), 7.93 (1H, s, 3′-H), 8.10 (1H, s, 5′-H). Anal. ($C_{21}H_{31}ON_3$) C, H, N.

3β-Hydroxy-17β-(1*H*-imidazol-1-yl)androst-5-ene (31). The method followed that described for **30** but using 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (**17**; 170 mg, 0.505 mmol) and after purification by FCC [CH_2Cl_2/EtOAc/Et_3N (7.7:2:0.3)] gave **31** (110 mg, 64.3%): mp 255−258 °C; ^{1}H NMR δ 0.58 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 3.53 (1H, m, 3α-H), 3.98 (1H, t, J = 9.8 Hz, 17α-H), 5.38 (1H, d, J = 5.4 Hz, 6-H), 6.96 (1H, s, 4′-H), 7.04 (1H, s, 5′-H), 7.54 (1H, s, 2′-H). Anal. ($C_{22}H_{32}ON_2$) C, H, N.

Enzyme Preparation and Assay Procedures. Microsomes were prepared from human testes (obtained from untreated prostatic cancer patients undergoing orchidectomy) and prostates and also from the testes of adult male Sprague−Dawley rats (Charles River Laboratories, weight 200−250 g), stored at −70 °C and used as previously described with some modifications.[11,12]

P450$_{17α}$ (Lyase) Assay. The 17α-lyase activity in incubations containing human or rat microsomes and the indicated candidate inhibitors was assessed by measuring the release of [^{3}H]acetic acid during the conversion of [21-$^3H_3$]-17α-hydroxypregnenolone to dehydroepiandrosterone. [21-$^3H_3$]-17α-Hydroxypregnenolone, with a specific activity of 13.61 μCi/μmol, was prepared in our laboratory as described by Akhtar et al.[4]

At the time of assay, microsomes were thawed and diluted in homogenization buffer (0.1 M sodium phosphate buffer, with dithiothreitol at 78 μM, pH 7.4) to a protein concentration of 0.5 μg/μL. The protein concentrations of microsomes were determined by the method of Lowry et al.[50] Incubations were carried out in a total volume of 1.01 mL. Sample tubes were first supplied with propylene glycol (approximately 10 μL), an ethanol solution containing the indicated candidate inhibitor, and an ethanol solution containing 300 000 dpm of [21-$^3H_3$]-17α-hydroxypregnenolone (13.61 μCi/μmol). The total substrate concentration in each tube was 10 μM. Control tubes received no inhibitor. The ethanol solutions were evaporated under air; then 750 μL of homogenization buffer and a NADPH-generating system (50 μL of homogenization buffer containing 6.5 mM $NADP^+$, 71 mM glucose-6-phosphate, 1.25 IU of glucose-6-phosphate dehydrogenase) were added to each tube, and the tubes were preincubated for 10 min at 34 °C. The reaction in each tube was initiated by adding either human testicular microsomes (300 μg of protein in 200 μL of homogenization buffer) or rat testicular microsomes (60 μg of protein in 200 μL of homogenization buffer), and the incubation was carried out for 60 min under oxygen with shaking in a water bath at 34 °C. At the end of the incubation, tubes were placed in an ice bath, supplied with 2 mL of chloroform, and vortexed. After standing at 4 °C for 20 min (or overnight), tubes were centrifuged at 4 °C for 15 min at 2000*g* and then 0.75 mL of the aqueous phase of each tube was placed into a fresh tube. To remove residual steroids which may remain after the chloroform extraction, 0.75 mL of charcoal solution (2.5 g of activated charcoal/100 mL of distilled water) was added to each tube and vigorously vortexed. After standing for 30 min (or overnight), the charcoal was pelleted by centrifugation at 2000*g* for 20 min at 4 °C. Finally, 0.75 mL of the supernatant was analyzed for tritium content by liquid scintillation spectrometry. To serve as background controls, two tubes in each experiment were placed on ice and supplied with chloroform before the addition of microsomes. In all experiments, the blank activity ranged from 1% to 5% of the control activity. IC_{50} values (vide supra) for inhibitors were calculated from the linear regression line in the plot of logit of lyase activity versus log of inhibitor concentration. K_i values were also determined from assays as described above. Each inhibitor was examined at three concentrations (for **6**, **27**, and ketoconazole at 50, 100, and 150 nM; for **17** at 3, 6, and 10 nM; for **19** at 5, 10, and 20 nM; for **28** at 3, 6, and 12 nM; for **29** at 10, 20, and 30 nM). Data from the various assays were used to obtain Lineweaver−Burk plots (e.g., Figure 1). From replots of slopes versus inhibitor concentration, K_i values were obtained (e.g., Figure 1B) and the K_m for 17α-hydroxypregnenolone (substrate) was determined (Table 2).

Spectral Studies. The UV−vis difference spectra were recorded using a Kontron Instuments U-930 spectrophotometer. A modified form of human P450$_{17α}$, which was expressed in *Escherichia coli* and purified as described by Imai et al.,[46] was diluted to 1.8 nmol of P450/mL in potassium phosphate buffer (50 nM), containing 20% glycerol (pH 7.25). At ambient temperature, the enzyme suspension was distributed between two 1-cm path length cuvettes (0.5 mL each) and a baseline was recorded from 350 to 500 nm. Difference spectra were then recorded at appropriate intervals following addition of the inhibitors (1 μL of a 10 mM solution in ethanol) to the experimental cuvette and 1 μL of ethanol to the reference cuvette. The concentration of inhibitors in the cuvette was 20 μM.

5α-Reductase Assay. The effects of these novel compounds and finasteride (a potent inhibitor of this enzyme) on human prostate 5α-reductase activity were evaluated as previously described[16,17] with some modifications. Ethanolic solutions of [7-^{3}H]testosterone (600 000 dpm), cold testosterone (4.8 ng), indicated inhibitors (0−200 nM), and propylene glycol (10 μL) were added to sample tubes in duplicate. The ethanol was evaporated to dryness under a gentle stream of air. The samples were reconstituted in phosphate buffer (0.1 M, pH 7.4, 400 μL) containing DTT (78 μM), and the NADPH-generating system (NADP, 6.5 mM; glucose-6-phosphate, 71 mM; glucose-6-phosphate dehydrogenase, 2.5 IU; in 100 μL of phosphate buffer) was added to each tube. The tubes were preincubated at 37 °C for 15 min. The enzymatic reactions were initiated by addition of human BPH microsomes (about 180 μg of microsomal protein in 500 μL of phosphate buffer) in a total volume of 1 mL, and the incubations were performed for 10 min under oxygen in a shaking water bath at 37 °C. The incubations were terminated by placing the sample tubes on ice and the addition of ether. Also, [^{14}C]DHT (3000 dpm) and cold DHT (50 μg) were added to each tube as an internal standard and visualization marker, respectively. The steroids were extracted with ether, separated by TLC (chloroform/ether, 80:20), and visualized by exposure to iodine vapor. The extracts were analyzed for ^{3}H and ^{14}C using a liquid scintillation counter. The percentage conversion of [7-^{3}H]testosterone to [^{3}H]dihydrotestosterone was calculated and used to determine 5α-reductase activity. IC_{50} values were determined from plots of 5α-reductase activity against four different concentrations of the inhibitor.

Growth Inhibition Studies of Human Prostate Cancer (LNCaP) Cells. The LNCaP human prostate cancer cell line was obtained from the American Type Culture Collection, Rockville, MD. The cells were grown in RPMI-1640 medium supplemented with 10% FBS. Three days prior to all experiments, cell were transferred to steroid-free medium as previously described.[17] To determine the effects of the Δ^{16}-17-azole compounds on cell growth, LNCaP cells (2×10^4) were plated into 24-well plates in steroid-free media. After ca. 12 h, the medium was aspirated and replaced with the same medium containing test compounds or vehicle. Medium was refreshed every 3 days, and after 9 days, the cells were counted using a Coulter counter.

Acknowledgment. We thank Mr. J. Neville Wright, Department of Biochemistry, University of Southampton, U.K., for obtaining the UV−vis difference spectra and Professor H. L. Holland, Department of Chemistry, Brock University, St. Catharines, Ontario, Canada, for his assistance in the aquisition of HRMS data. We are also grateful to Dr. A. Ayambem, Department of Chemistry, Virginia Tech, Blacksburg, VA, for acquisition of some of the ^{1}H NMR data. We also thank Dr. S. Jacobs, Department of Surgery, UMAB, and Dr. James Mohler, University of North Carolina, for supplying the tissues used in these experiments. This work was supported by NIH Grant CA-27440.

References

(1) Nakajin, S.; Hall, P. F. Microsomal Cytochrome P-450 from Neonatal Pig Testis: Two Enzymatic Activities of (17α-Hydroxylase and $C_{17,20}$-Lyase) Associated with One Protein. *Biochemistry* **1981**, *20*, 4037−4042.

(2) Nakajin, S.; Hall, P. F.; Onada, M. Testicular Microsomal Cytochrome P-450 for C_{21} Steroid Side-chain Cleavage: Spectral and Binding Studies. *J. Biol. Chem.* **1981**, *256*, 6134−6139.

(3) Hall, P. F. Cytochrome P-450 C_{21scc}: One Enzyme with Two Actions: Hydroxylase and Lyase. *J. Steroid Biochem. Mol. Biol.* **1991**, *40*, 527−532.

(4) Akhtar, M.; Corina, D. L.; Miller, S. L.; Shyadehi, A. Z.; Wright, J. N. Incorporation of Label from $^{18}O_2$ into Acetate During Side-chain Cleavage Catalyzed by Cytochrome $P450_{17\alpha}$ (17α-Hydroxylase-17,20-Lyase). *J. Chem. Soc., Perkin Trans. 1* **1994**, 263−267.

(5) Swinney, M.; Mak, A. Y. Androgen Formation by Cytochrome P 450 CYP 17: Solvent Isotope Effect and pL Studies Suggest a Role for Protons in the Regulation of Oxene Versus Peroxide Chemistry. *Biochemistry*, **1994**, *33*, 2185−2190.

(6) Akhtar, M.; Corina, D. L.; Miller, S. L.; Shyadehi, A. Z.; Wright, J. N. Mechanism of the Acyl-Carbon Cleavage and Related Reactions Catalyzed by Multifuntional P-450: Studies on Cytochrome $P450_{17\alpha}$. *Biochemistry* **1994**, *33*, 4410−4418.

(7) Bossche, H. V.; Moereels, H. 17α-Hydroxylase/17,20-Lyase. In *Design of Enzyme Inhibitors as Drugs*; Snadler, M., Smith, H. J., Eds.; Oxford University Press: Oxford, U.K., 1994; Vol. 2, pp 438−461.

(8) De Coster, R.; Wouters, W.; Bruynseels, J. P450-Dependent Enzymes as Targets for Prostate Cancer Therapy. *J. Steroid Biochem. Mol. Biol.* **1996**, *56*, 133−143.

(9) Arth, G. E.; Patchett, A. A.; Jefopoulus, T.; Bugianesi, R. L.; Peterson, L. H.; Ham, E. A.; Kuehl, F. A., Jr.; Brink, N. G. Steroidal Androgen Biosynthesis Inhibitors. *J. Med. Chem.* **1971**, *14*, 675−680.

(10) Angelastro, M. R.; Laughlin, M. E.; Schatzman, G. L.; Bey, P.; Blohm, T. R. 17β-(Cyclopropylamino)-androst-5-en-3β-ol, a Selective Mechanism-Based Inhibitor of Cytochrome $P450_{17\alpha}$(Steroid 17β-Hydroxylase/$C_{17,20}$Lyase). *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1571−1577.

(11) Li, J.; Li, Y.; Son, C.; Banks, P.; Brodie, A. 4-Pregnen-3-one-20β-carboxaldehyde: A Potent Inhibitor of 17α-Hydroxylase. *J. Steroid Biochem. Mol. Biol.* **1992,** *42*, 313−320.

(12) Li, J.; Li, Y.; Son, C.; Brodie, A. Inhibition of Androgen Synthesis by 22-Hydroximino-23,24-bisnor-4-cholen-3-one. *Prostate* **1995**, *26*, 140−150.

(13) Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. Novel Steroidal Inhibitors of Human Cytochrome $P450_{17\alpha}$ (17α-Hydroxylase-C17,20-lyase): Potential Agents for the Treatment of Prostatic Cancer. *J. Med. Chem.* **1995**, *38*, 2463−2471.

(14) Burkhart, J. P.; Gates, C. A.; Laughlin, M. E.; Resvick, R. J.; Peet, N. P. Inhibitors of Steroid $C_{17(20)}$ Lyase with C-17-Heteroaryl Steroids. *Bioorg. Med. Chem.* **1996**, *4*, 1411−1420.

(15) Njar, V. C. O.; Hector, M.; Hartmann, R. W. 20-Amino and 20,-21-Aziridinyl Pregnene Steroids: Development of Potent Inhibitors of 17α-Hydroxylase/C17,20-Lyase (P450 17). *Bioorg. Med. Chem.* **1996**, *4*, 1447−1453.

(16) Li, J. S.; Li, Y.; Son, Chong.; Brodie, A. Synthesis and Evaluation of Pregnane Derivatives as Inhibitors of Human Testicular 17α-Hydroxylase/C17,20-Lyase. *J. Med. Chem.* **1996**, *39*, 4335−4339.

(17) Klus, G. T.; Nakamura, J.; Li, J.; Ling, Y.; Son, C.; Kemppained, J. A.; Wilson, E. M.; Brodie, A. M. H. Growth Inhibition of Human Prostate Cells In Vitro by Novel Inhibitors of Androgen Synthesis. *Cancer Res.* **1996**, *56* (6), 4956−4964.

(18) Njar, V. C. O.; Klus, G. T.; Brodie, A. M. H. Nucleophilic Vinylic "Addition−Elimination" Substitution Reaction of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene: A Novel and General Route to 17-Substituted Steroids. Part 1-Synthesis of Novel 17-Azolyl-Δ^{16} Steroids; Inhibitors of 17α-Hydroxylase/17,20-Lyase (17α-Lyase). *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2777−2782.

(19) Njar, V. C. O.; Klus, G. T.; Hausa, H. J.; Brodie, A. M. H. Synthesis of Novel 21-Trifluopregnane Steroids: Inhibitors of 17α-Hydroxylase/17,20-Lyase (17α-Lyase). *Steroids* **1997**, *62*, 468−473.

(20) Ling, Y.; Li, J.; Liu, Y.; Kato, K.; Klus, G. T.; Brodie, A. 17-Imidazolyl, Pyrazolyl, Isoxaloyl Androstene Derivatives, Novel Steroidal Inhibitors of Human Cytochrome $C_{17,20}$-Lyase ($P450_{17\alpha}$). *J. Med. Chem.* **1997**, *40*, 3297−3304.

(21) Ayub, M.; Level, M. Inhibition of Testicular 17α-Hydroxylase and 17,20-Lyase but not 3-Hydroxysteroid Dehydrogenase-Isomerase or 17-Hydroxysteroid Oxidoreductase by Ketoconazole and Other Imidazole Drugs. *J. Steroid Biochem.* **1987**, *28*, 521−531.

(22) Vanden Bossche, H.; Willemsens, G.; Bellens, D.; Roels, J.; Janssen, P. A. From 14α-Demethylase Inhibitors in Fungal Cells to Androgen and Oestrogen Biosynthesis Inhibitors in Mammalian Cells. *Biochem. Soc. Trans.* **1990**, *18*, 10−13.

(23) McCague, R.; Rowlands, M. G.; Barrie, S. E.; Houghton, J. Inhibition of Enzymes of Estrogen and Androgen Biosynthesis by Esters of 4-Pyridylacetic Acid. *J. Med. Chem.* **1990**, *33*, 3050−3055.

(24) Sergejew, T.; Hartmann, R. W. Pyridyl Substituted Benzocycloalkenes: New Inhibitors of 17α-Hydroxylase/17,20-Lyase (P450 17α). *J. Enzymol. Inhib.* **1994**, *8*, 113−122.

(25) Wacher, G. A.; Hartmann, R. W.; Sergejew, T.; Grun, G. L.; Ledergerber, D. Tetrehydronaphthalenes: Influence of Heterocyclic Substituents on Inhibiton of Steroidogenic Enzymes P450 arom and P450 17. *J. Med. Chem.* **1996**, *39*, 834−841.

(26) Hartmann, R. W.; Wachter, G. A.; Sergejew, T.; Wurtz, R.; Duerkop, J. 4,5-Dihydro-3-(2-pyrazinyl)naphtho[1,2-c]pyrazole: A Potent and Selective Inhibitor of Steroid-17αHydroxylase-C17,20-Lyase (P450 17). *Arch. Pharm. (Weinheim)* **1995**, *328*, 573−575.

(27) Hartmann, R. W.; Frotscher, M.; Ledergerber, D.; Wachter, G. A.; Grun, G. L.; Sergejew, T. F. Synthesis and Evaluation of Azole-Substituted Tetrahydronaphthalenes as Inhibitors of P450 arom, P450 17, and P450 TxA_2. *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 251−261.
(28) Trachtenberg, J. Ketoconazole Therapy in Advanced Prostatic Cancer. *J. Urol.* **1984**, *132*, 61−63.
(29) Williams, G.; Kerle, D. J.; Doble, A.; Dunlop, H.; Smith, C.; Allen, J.; Yeo, T.; Blooms, S. R. Objective Responses to Ketoconazole Therapy in Patients with Relapsed Progressive Prostatic Cancer. *Br. J. Urol.* **1986**, *58*, 45−51.
(30) Vanden Bossche, H. Inhibitors of P450-Dependent Steroid Biosynthesis: From Research to Medical Treatment. *J. Steroid Biochem. Mol. Biol.* **1992,** *43*, 1003−1021.
(31) Cole, P. A.; Robinson, C. H. Mechanism and Inhibition of Cytochrome P-450 Aromatase. *J. Med. Chem.* **1990**, *33*, 2933−2942.
(32) Ronig, K. (Assignor to Searle Co.) 4-(Cyclopentanopolyhydrophenanthr-17-yl) Imidazoles and Derivatives Thereof. U.S. Patent 2,664,423, 1953; *Chem. Abstr.* **1953**, *49*, 7608e.
(33) Nambara, T.; Shimada, K.; Nemoto, T.; Goya, S. Synthesis of 14,15-Epoxyandrostan-17β-isoxazole and Pyrazoles. *Chem. Pharm. Bull.* **1970**, *18*, 1658−1662.
(34) Wolff, M. E.; Chang, H.-H.; Ho, W. Modified Cardenolides. V. Replacement of the C-17 Lactone Substituents by Alkylating Groups. *J. Med. Chem.* **1970**, *13*, 657−663.
(35) van Leuesn, D.; Batist, J. N. M.; Lei, J.; van Echten, E.; Brouwer, A. C.; van Leusen, A. M. Synthesis of 17-(2-Methoxy-3-oxazolin-4-yl)-, 17-(3-Oxazolin-4-yl)-, and 17-(2-ocazolin-4-ylidene)-Substituted Steroids and their Uses as Precursors of Corticosteroids. *J. Org. Chem.* **1994**, *59*, 5605−5657.
(36) Doorenbos, N. J.; Milewich, L. 17β-Isoxazolyl and 17β-Pyrazolyl Steroids from 3β-Hydroxy-21-formyl-pregn-5-en-20-one. Structural Assignments. *J. Org. Chem.* **1966**, *31*, 3193−3199.
(37) Siddiqui, A. U.; Maheshwar Roa, V. U.; Maimirani, M.; Siddiqui, A. H. Heterocyclic Steroids: Synthesis of Andrsteno[17,16-d]-pyrazoles and Androstenol[17,16-e]pyrimidines. *J. Heterocycl. Chem.* **1995**, *32*, 353−356.
(38) Meyer, M. D.; Kurse, L. I. Ergoline Synthons: Synthesis of 3,4-dihydro-6-methoxybenz[*cd*]indol-5(1*H*)-one (6-Methoxy-Uhles's Ketone) and 3,4-Dihydrobenz[*cd*]indol-5(1*H*)-one (Uhle's Ketone) via a Novel Decarbonylation of Indole-2-carboxylates. *J. Org. Chem.* **1984**, *49*, 3195−3199.
(39) March, J. *Advanced Organic Chemistry − Reactions, Mechanism and Structure*, 4th ed.; John Wiley & Sons: New York, 1992; pp 335−338 and references cited therein.
(40) Tasaka, A.; Tamura, N.; Matsushita, Y.; Kitazaki, T.; Hayashi, R.; Okonogi, K.; Itoh, K. Optically Active Antifungal Azoles. IV. Synthesis and Antifungal Activity of (2R,3R)-3-Azolyl-2-(substituted phenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols. *Chem Pharm. Bull.* **1995**, *43*, 432−440.
(41) Tadano, K.; Idogaki, Y.; Yamada, H.; Suami, T. Ortho Ester Claisen Rearrangements of Three 3-C-(Hydroxymethyl)methylene Derivatives of Hexofuranose: Stereoselective Introduction of a Quaternary Center on C-3 of D-*ribo*-, L-*lyxo*-, and D-*arabino*-Hexofuranose. *J. Org. Chem.* **1987**, *52*, 1202−1210.
(42) Wecher, W. J.; Murray, H. C. 11-Oxygenated Pregnenolones. II. Synthesis of 11α-Hydroxy-, 11β-Hydroxy- and 11-Oxopregnenolones and 11-Oxo-17α-pregnenolone. *J. Org. Chem.* **1963**, *28*, 755−760.
(43) Bridgeman, J. E.; Cherry, P. C.; Clegg, A. S.; Evans, J. M.; Ewart, E. R.; Kasal, A.; Kumar, V.; Meakins, G. D.; Morisawa, Y.; Richards, E. E.; Woodgate, P. D. Proton Magnetic Resonance Spectra of Ketones, Alcohols, and Acetates in the Androstane, Pregnane, and Estrane Series. *J. Chem. Soc. C* **1970**, 250−257.
(44) Raggio, M. L.; Watt, D. S. A Synthesis of Progesterone from Dehydroepiandrosterone. *J. Org. Chem.* **1976**, *41*, 1873−1875.
(45) Morrison, J. F.; Walsh, C. T. The Behavior and Significance of Slow-Binding Enzyme Inhibitors. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1988**, *61*, 201−301.
(46) Imai, T.; Globerman, H.; Gertner, J. M.; Kagawa, N.; Waterman, M. R. Expression and Purification of Functional Human 17α-Hydroxylase/17,20-Lyase (P450c17) in *Escherichia coli*. *J. Biol. Chem.* **1993**, *268*, 19681−19689.
(47) Jefcoate, C. R. Measurement of Substrate and Inhibitor Binding to Microsomal Cytochrome P-450 by Optical-Difference Spectroscopy. *Methods Enzymol.* **1978**, *52*, 258−279.
(48) Dawson, J. H.; Anderson, L. A.; Sono, M. Spectroscopic Investigations of Ferric Cytochrome P-450-CAM Ligand. *J. Biol. Chem.* **1982**, *257*, 3606−3617.
(49) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(50) Lowry, O. H.; Roseborough, N. S.; Farr, A. L.; Randall, R. S. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

JM970568R