17-Imidazolyl, Pyrazolyl, and Isoxazolyl Androstene Derivatives. Novel Steroidal Inhibitors of Human Cytochrome $C_{17,20}$-Lyase ($P450_{17\alpha}$)†

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We recently described a number of inhibitors of $P450_{17\alpha}$, the key enzyme of androgen biosynthesis. Here, we report the synthesis and activity of novel 17-imidazolyl, pyrazolyl, and isoxazolyl androstene derivatives as potential agents for the treatment of prostatic cancer. A number of 17-(4′-Imidazolyl) derivatives were prepared by condensing the corresponding 17-ketol acetate side chain with aldehyde and ammonium hydroxide. The 17β-(4′-imidazolyl) derivatives **(2a, 2e, 4a, 4c)** were found to be potent inhibitors of human testicular $P450_{17\alpha}$, with greater activity than ketoconazole. The juxtaposition between the imidazole ring and the steroid D ring appears to be important in contributing inhibitory properties. Compounds having a 17β-(2′-imidazolyl) ring **(9a, 10)** or a 20β-(2′-imidazolyl) ring **(12)**, instead of the 17β-(4′-imidazolyl) ring **(2a, 4a)**, are weak inhibitors. Among the 17-(4′-imidazolyl) derivatives, introduction of the 17α-hydroxy group **(4b)** and 16α,17α-epoxide group **(2d)** diminished potency **(2a → 2d**; lC_{50} 66 → 430 nM; **4a → 4b**; lC_{50} 58 → 1200 nM), while the 16,17 double bond increased the inhibitory activity by almost three times in the 5-en-3β-ol inhibitors (**2a → 2e**; lC_{50} 66 → 24 nM). There was virtually no difference in the inhibitory activity in the 4-en-3-one inhibitors **(4a → 4c**; IC_{50} 58 → 50 nM). The introduction of a methyl **(2b)** or phenyl group **(2c)** on the 2′-position of 4′-imidazolyl ring caused a dramatic decrease in the potency. As to modification of the A,B rings, the 3-acetate **(2f, 2g)** decreased the potency almost 3-fold compared with the 3-alcohol **(2e → 2f**, IC_{50} 24 → 75 nM; **2a → 2g**, 66 → 199 nM) and the conversion from the 5-en-3β-ol into the 4-en-3-one hardly affected the potency. As expected, **4c** was more potent than **2e** for the rat $P450_{17\alpha}$. 17-(3′-Pyrazolyl)- **(14b)** and 17-(5′-isoxazolyl)-androsta-5,16-dien-3β-ol **(15b)** were also potent inhibitors of $P450_{17\alpha}$, whereas the 17-(2′-imidazolyl) compound **(9b)** was one of the most potent inhibitor in this series. However, their 16-saturated counterparts **(9a**, **14a**, **15a)** were weak inhibitors. The 17β-(3′-isoxazolyl)- **(16)** and 17β-(5′-methyl-3′-oxazolyl)androst-5-en-3β-ol **(18)** were also inactive. The introduction of a methyl or phenyl group on the nitrogen of the pyrazolyl ring of **14b** [see **14c**, **14d**, and **14e]** also caused some loss of inhibition for $P450_{17\alpha}$. Compounds **2e**, **4a**, **4c**, **9b**, **14b**, **17a** and **17b** are among the most potent inhibitors of human $P450_{17\alpha}$ so far reported.

Inhibitors of the enzymes involved in the key steps of androgen synthesis have important uses in the treatment of prostatic diseases, such as benign prostatic hypertrophy (BPH) and prostatic cancer. $P450_{17\alpha}$ is responsible for androgenic hormone biosynthesis which produces dehydroepiandrosterone (DHEA) and androstenedione (A), the immediate precursors of testosterone (T), from their respective precursors pregnenolone and progesterone, in both testes and adrenals. A number of compounds which inhibit $P450_{17\alpha}$ have been described.[1−9] Of these compound, ketoconazole, an imidazole antifungal agent, is currently used in the treatment of patients with advanced prostatic cancer.[10,11] However, this compound inhibits several other P450 enzymes, is not a very strong inhibitor of $P450_{17\alpha}$, and is associated with significant side effects. Recently, finasteride,[12] an inhibitor of 5α-reductase (5α-R), the key enzyme in the synthesis of the more potent androgen dihydrotestosterone (DHT) from T, has been introduced as a new treatment for BPH. Although finasteride effectively reduces DHT levels in patients, testosterone levels are often increased.[13] In prostatic cancer, this can result in adverse effects by stimulating malignant growth of the prostate, since T will bind to the androgen receptor in the absence of DHT.

We have recently identified several compounds which inhibit both $P450_{17\alpha}$ and 5α-R.[14−16] Such compounds could block all androgen synthesis (T, DHT, and A) and be more effective alternatives or additions to orchiectomy in treating prostate cancer patients. In the present study, several more potent inhibitors of $P450_{17\alpha}$ were synthesized on the basis of observations that an azole moiety could act as a ligand to bind to the iron atom of the heme prosthetic group of the cytochrome P-450 enzyme and form a coordinated complex. Examples of these types of compounds are several potent aromatase inhibitors, such as fadrozole which is effective in breast cancer treatment.[17] Although the detailed mechanism of the 17α-hydroxylation and $C_{17,20}$-side-chain cleavage by $P450_{17\alpha}$ is presently unclear, based on our inhibitor studies, it appears that the C_{17} and C_{20} positions of the substrate when bound to the active site of the enzyme must be close to the heme group of the enzyme. Therefore, introduction of an imidazole group or other heterocyclic group with a nitrogen lone pair of electrons at these positions would coordinate to the iron atom of the heme in the active site of the enzyme.[18] Indeed, we have recently shown,[9] using spectroscopic

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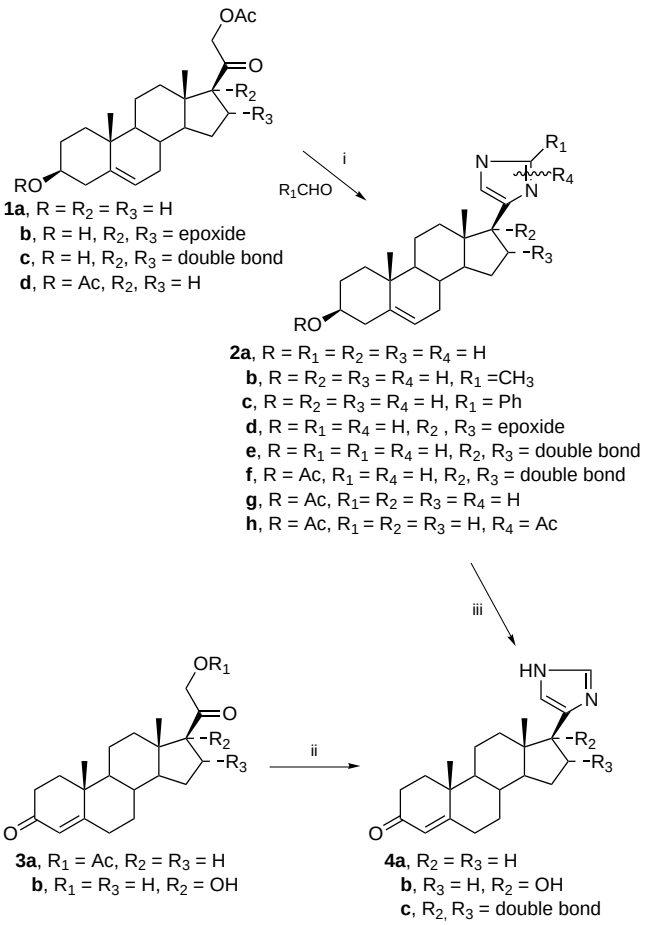
Scheme 1[a]



[a] (i) NH_4OH, $Cu(OAc)_2$, EtOH; (ii) HCHO, $Cu(OAc)_2$, EtOH; (iii), $Al(iPrO)_3$, cyclohexanone, toluene.

evidence, that two of our potent $P450_{17\alpha}$ inhibitors, i.e., 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene and the corresponding 17-triazole each coordinates (*via* an azole nitrogen) with the heme iron of the $P450_{17\alpha}$ enzyme complex. It should be noted that although two groups[6,7] have reported on 17-heteroaryl steroidal inhibitors of $P450_{17\alpha}$ and believe that the inhibitory property of their compounds are due (in part) to coordination of a heteroaryl atom to the heme iron of the enzyme complex, they have yet to provide evidence for this phenomenon. Using this rationale, we have designed and synthesized a new series of pregnene derivatives with imidazole, pyrazole, isoxazole, and oxazole groups substituted at the 17-position. This modification has turned out to be a most effective strategy for producing potent inhibitors of $P450_{17\alpha}$.

Results and Discussion

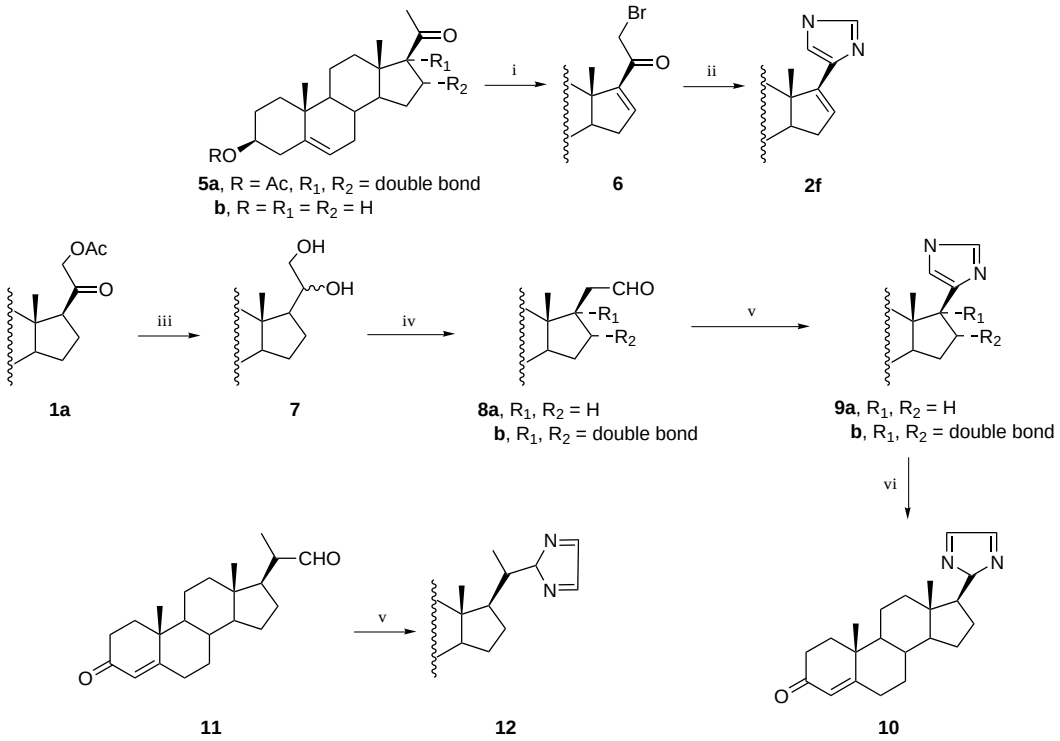
Chemistry. 4′-Imidazole **(2a)** was synthesized by the Weigenhagen method[19] reported in a U.S. patent in 1953:[20] 21-acetoxypregnenolone **(1a)** was condensed with formaldehyde and ammonium hydroxide in refluxing ethanol (see Scheme 1). However, the yield was poor (less than 10%) and the product was contaminated with starting material **(1a)** making it difficult to purify and requiring column chromatographic separation. The procedure was therefore modified by periodically replacing NH_3 and formaldehyde which evaporated during refluxing. In this way, the yield was raised to 30% and the pure product obtained simply by recrystallization. This improvement enables us to provide **2a** in gram amounts for further pharmacological study *in vivo.* ^1H NMR (in CD_3OD) of the two protons (2′- and 5′-H) of the imidazole ring appeared as a very flat signals at 6.8−7.3 and 7.5−8.0 ppm, which was probably due to tautomerism among imidazole forms. However, its hydrochloride salt showed a sharp peak at 7.39 and 8.81 ppm. Similarly, when **1a** was condensed with acetaldehyde or benzaldehyde, the 2′-methyl **(2b)** or 2′-phenyl **(2c)** compound was obtained. Compound **2d** was obtained from 16α,17α-epoxy-21-acetoxypregnenolone **(1b)**. Treatment of **1b** with $CrCl_2$ in acetic acid gave the 16-ene **1c**,[21] which was condensed with formaldehyde and ammonium hydroxide to give 16-en-17-(4′-imidazolyl) derivative **2e**. In the same way, the 3-acetate **1d** afforded **2g**, together with some hydrolyzed product 3β-ol **(2a)**, 21-acetoxy progesterone **(3a)** gave **4a**, and 17α,-21-diol **3b** gave **4b**. Acetylation of **2a** gave the diacetate **2h**, in which only one nitrogen of the imidazole ring was acetylated to give a single product.

Recently, Potter et al. reported that 17-(3′-pyridyl)-androsta-5,16-dien-3β-ol was also a potent inhibitor of human testicular $P450_{17\alpha}$.[6] We noted that they used the 3β-*O*-acetate form instead of the more potent free 3β-ol form for *in vivo* studies, possibly because it acts as a "prodrug" with better oral bioavailibility.[22] Synthesis of the 3-acetate derivative of **2e** was therefore attempted. As direct acetylation of **2e** could also acetylate the imidazole ring, as was shown for **2h**, the following approach was used (Scheme 2): 16-dehydroprogesterone 3-acetate **(5a)** was brominated with cupric bromide in THF[23] to give the 21-bromo derivative **(6)**, which proved to be more reactive than 21-acetoxy derivative **1c**. Condensation of 21-bromide **6** with fomaldehyde in ammonium hydroxide was complete in 1 h to give 3-acetate **2f**.

The 17β-(2′-imidazolyl) derivative **9a** was synthesized mainly by the method of Debus[19] (Scheme 2): reduction of the 21-acetoxy ketone **1a** with $LiA1H_4$ gave the diol **7**. Although it was expected that this reduction of the 20-keto group would result in a mixture of 20α- and 20β-alcohols, no attempt was made at separation. Diol **7** was cleaved with sodium periodate, to give the 20-aldehyde **8a**, which was condensed with glyoxal and ammonium hydroxide in methanol to give **9a**. Similarly, 16-en-20-aldehyde **8b** was prepared[24] and then condensed, to afford 16-en-17-(2′-immidazolyl) derivative **9b** albeit in a very low yield. Treated similarly, the 22-aldehyde **11** gave 20β-(2′-imidazole) **12**. Oppenauer oxidation of **9a** with cyclohexanone and aluminum isopropoxide in refluxing toluene gave the 4-en-3-one **10**. Similarly, **2e** was oxidized to give **4c**.

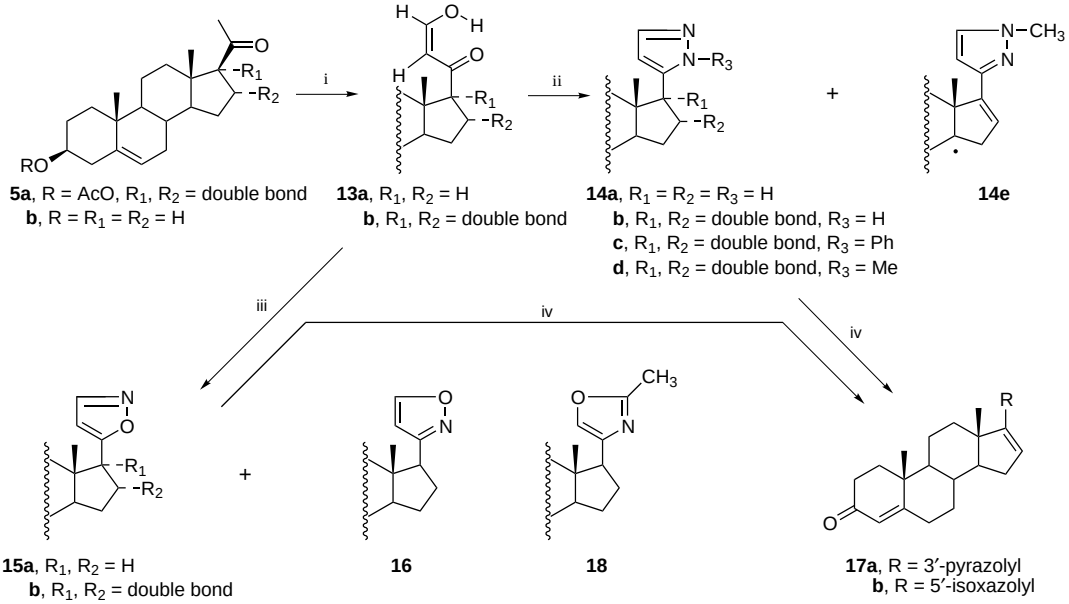
17-Pyrazole **14** and 17-isoxazole **15** and **16** were also synthesized (Scheme 3). The Claisen condensation of the 20-one **5b** with ethyl formate in NaOMe in benzene gave the 21-formyl derivative, which existed mainly in the keto−enol forms **(13a)**.[25] However, the Pelc procedure using pyridine gave a better quality and yield of the product **13a** and was therefore employed.[26] The keto−enol **13a** was condensed with hydrazine to give 17β-(3′-pyrazole) **14a**. While condensation with hydroxylamine hydrochloride in glacial acetic acid buffered with sodium acetate gave 17β-(5′-isoxazole) **15a**, in acetic acid alone, a mixture of **15a** and 17β-(3′-isoxazole) **16** was obtained. This mixture could not be separated

Scheme 2[a]



[a] (i) $CuBr_2$, THF; (ii) HCHO, NH_4OH, $Cu(OAc)_2$, EtOH; (iii) $LiAlH_4$, THF; (iv) $NAIO_4$, MeOH; (v) glyoxal, NH_4OH, $Cu(OAc)_2$, EtOH; (vi) $Al(iPrO)_3$, cyclohexanone, toluene.

Scheme 3[a]



[a] (i) HCOOEt, NaOMe, Py; (ii) NH_2NH_2 or $PhNHNH_2$; (iii) NH_2OH, HOAc/or NaOAc; (iv), $Al(iPrO)_3$, cyclohexanone, toluene.

by chromatography, and was treated with NaOMe. The 5′-isoxazole **15a** readily formed the α-cyano ketone,[26] while the 3′-isoxazole **16** remained unchanged and thus purified. The original base treatment procedure[25] did not work in our case and was therefore modified by us.

The Doorenbos's procedure for the condensation of 16-en-20-one **5a** with ethyl formate in benzene gave a very low yield of 21-formyl derivatives **13b**;[25] the procedure was improved by reaction in pyridine which gave a better yield of product. ^1H NMR demonstrated that the enol form **13b** was the main form present in $CDCl_3$, as shown by two doublets at δ 8.04 (d, $J = 3.9$ Hz, 22-H) and 5.79 (d, $J = 3.9$ Hz, 21-H), while the aldehyde peak at 9.80 ppm was hardly observed. Compound **13b** was treated with hydrazine or phenyl hydrazine to give 17-(3′-pyrazole) **14b** or 17-(l′-phenyl-5′-pyrazole) **14c** as reported.[25] When **13b** was condensed with methyl hydrazine, Doorenbos reported only the isolation of 17-(l′-methyl-5′-pyrazole) **14d**;[25] we were able to separate 9% of 17 -(l′-methyl-3′-pyrazole) **14e** from our reaction. The reaction of **13b** with hydroxylamine hydrochloride (either in MeOH, or in glacial acetic acid, or buffered with sodium acetate) yielded only the 5′-isoxazole **15b**, and the 3′-isoxazole was not found. This result shows that, because C_{20} is conjugated with the Δ^{16} double bond in **13b**, it is less prone to nucleophilic attack than is

the case with the saturated analogue **13a**. Both **14b** and **15b** were oxidized by Oppenauer oxidation separately to give the 4-en-3-one **17a** or **17b**.

17β-(2′-Methyl-4′-oxazole) **18** was prepared by following a similar procedure for the preparation of 2′-methylsteroidal(3,2-*d*)oxazoles reported by Ohta:[27] the 21-acetoxy ketone **1a** was refluxed with NH_4OAc in HOAc and the 3-acetate formed was hydrolyzed with $KHCO_3$ to give oxazole **18**.

Enzyme Assay. The potency of the pregnene derivatives as inhibitors of $P450_{17\alpha}$ was evaluated in human testicular microsomes. For most compounds, a radiometric assay was also used in which [21-^{3}H]-17α-hydroxyprenenolone was the substrate. Inhibition was assessed by measuring the release of [^{3}H]acetic acid during cleavage of the C-21 side chain in the conversion to DHEA. For some compounds, the conversion of radiolabeled pregnenolone to 17α-hydroxypregnenolone and DHEA by $P450_{17\alpha}$ was measured in the presence of several concentrations of test compounds, as described previously.[14] Reverse phase HPLC was employed to separate and measure the amount of substrate and metabolites. The activity of 17α-lyase was calculated from the conversion of pregnenolone to DHEA. Both assays gave comparable and reproducable results. The activity of the control reaction was approximately 0.5%, and the enzyme activity was linear with time for both assay procedures. In addition, the IC_{50} values of the inhibitors did not vary significantly at 90−300 μg of protein concentration of the testicular microsomes. IC_{50} assays were performed at least twice and up to four times on the more potent inhibitors. The mean values are shown in Table 1. Some biological effects of inhibitors **2a**, **2e**, **2g**, and **4a** have been reported.[29] As rodent models are most frequently used for *in vivo* studies, we also determined the extent of inhibition of the compounds on rat testicular microsomes. Although progesterone rather than pregnenolone is the preferred substrate for the rat enzyme,[28] [21-^{3}H]-17α-hydroxyprenenolone was used for these assays. Since many of the inhibitors are pregnenolone derivatives, their competition with this substrate may predict more accurately their *in vivo* activity in the rat.

Structure−Activity Relationships. The potencies of azole derivatives as inhibitors of the human and rat testicular $P450_{17\alpha}$ are listed in Table 1. However, the structure−activity relationships of these compounds are discussed with respect to the human testicular $P450_{17\alpha}$ enzyme. The compounds containing the 17-(4′-imidazolyl) ring **(2a** and **4a)** demonstrated strong inhibition of $P450_{17\alpha}$ (IC_{50} 66 and 58 nM). This suggests that the imidazolyl nitrogen lone pair of electrons at this position coordinates with the iron atom of the heme in the active site of the enzyme to produce effective inhibition. The introduction of the 16α,17α-epoxide **2d** (IC_{50} 430 nM) or the 17α-hydroxy group **(4b)** (IC_{50} 1200 nM) decreased inhibition dramatically. In contrast, introduction of a 16,17-double bond **(2e** and **4c**, IC_{50} 24 and 50 nM) retained or increased this inhibition. From our previous work,[16] the 20-oxime when conjugated with the 16,17-double bond increased the potency of inhibition more than 30-fold compared to its 16-saturated counterpart. Potter et al. also found that a 17-(3′-pyridyl) substituent together with a 16,17-double bond showed potent inhibition.[6]

Table 1. IC_{50} of Steroid Compounds on Human and Rat Testicular $C_{17,20}$-Lyase ($P450_{17\alpha}$)

	human			rat		
	inhibition		IC_{50},[a]	inhibition		IC_{50},[a]
compound	nM	%	nM	nM	%	nM
2a			66[b]			91
2b	400	26				4813
2c	400	21		1500	NI[c]	
2d			430	not tested		
2e			24[b]			49
2f			75	1500	92	
2g			199[b]	1500	55	
2h			100			5456
4a			58[b]			79
4b			1200	not tested		
4c			50[b]			14
9a	400	24		1500	19	
9b			21[b]	1500	65	
10	400	44		1500	25	
12			>30000	not tested		
14a	150	20		1500	92	
14b			42[b]			28
14c	150	11		1500	NI	
14d	150	12		1500	NI	
14e	150	25		1500	NI	
15a	150	21		1500	93	
15b			108[b]			84
16			1113	1500	54	
17a			59[b]			76
17b			39[b]			32
18	400	21		1500	NI	
ketoconazole			77			1067[b]

Microsomes of either human or rat testes were incubated with candidate inhibitors. Compounds were screened at 150 or 400 nM with human microsomes and 1500 nM with rat microsomes. IC_{50} values were determined over a range of six concentrations. Incubations were carried out at 34 °C with cofactors with [21-^{3}H]-17α-hydroxypregnenolone for 60 min, as described under methods. Following incubation with [21-^{3}H]-17α-hydroxypregnenolone, [^{3}H]acetic acid liberated during the cleavage reaction at the C-17 was measured in the aqueous phase after extraction of steroids with chloroform. This method was used to determine the IC_{50} values for all compounds, except **2f** and **4a**. These compounds were incubated with [7-^{3}H]pregnenolone and cofactors for 5 min and the substrate and products analyzed by HPLC. Both methods were used to determine the IC_{50} of **2e**, **2g**, and **16**. Results are average values of two determinations. [a] The variation in repeat values were usually <15% of the mean IC_{50} values. [b] Average values of three to five repeat experiments [c] NI no inhibition. [d] Compounds reported in ref 28.

As to modification of the imidazole ring, we found that the introduction of a methyl group at the 2′-position **(2b)** or the larger 2′-phenyl group **(2c)** decreased inhibitory activity. The finding that N-acetylation of the imidazolyl ring **(2h)** also caused some loss of activity is also not surprising, since the *N*-acetyl group results in a delocalization of the π electrons of the nitrogen and the acetyl or other bulky group on the imidazole ring might also hinder coordination with heme iron.

17β-(2′-Imidazole)s **9a** and **10** in which the steroid is bonded to the 2′-position of imidazole showed fairly poor potency in our assays. The 20β-(2′-imidazole) **12** did not show any activity, although its parent compound, the 20β-carboxaldehyde **11** was quite potent.[16] These results suggest that the juxtaposition between the imidazole ring and the steroid D ring is important. 17-(2′-Methyl-4′-oxazole) **18**, the bioisostere analog of **2b**, in which the N′-atom was substituted for the O-atom, was a less potent inhibitor, although the 5′-isoxazolyl **17b** was very potent. The lower potency of the 3-acetoxy derivatives **2g** and **2f**, compared with those of **2a** and **2e**, may reflect a limited bulk tolerance at the 3-position

but it still retained reasonable activity (IC_{50} 199 and 75 nM). However, compounds **2g** and **2f** might be useful as a "prodrug" of **2a** and **2e** for *in vivo* experiments.

17β-Pyrazole **(14a)** and isoxazole **(15a** and **16)** derivatives were moderate to weak inhibitors, but their 16-unsaturated counterparts **14b** and **17a**, **17b** as well as 16-unsaturated 17β-(2′-imidazole) **9b** were quite potent. The introduction of methyl or phenyl groups at the nitrogen of the pyrazolyl ring of **14b** (see **14c**, **14d**, and **14e)** showed moderate inhibition. More detailed studies on the type of inhibition of the more active inhibitors are in progress.

In summary, among the series of 17-imidazolyl, pyrazolyl, and isoxazolyl androstene derivatives synthesized, **2e**, **4a**, **4c**, **9b**, **14b**, **17a**, and **17b** were found to be potent inhibitors of human testicular $P450_{17\alpha}$ with greater activity than ketoconozole. These compounds were two to four times more potent as $P450_{17\alpha}$ inhibitors when compared in the same assay with ketoconazole (IC_{50} 77 nM in our assay system) which is currently in clinical use. Except for **9b**, these compounds showed strong inhibition of the rat $P450_{17\alpha}$ also and were several-fold more effective than ketoconazole. A number of these inhibitors are presently undergoing further pharmacological study.

Experimental Section

Synthetic Methods. ^{1}H NMR data (300 MHZ) (internal standard Me_4Si = δ 0) were recorded on a QE 300, NMR systems, General Electric Co., in $CDCl_3$ unless otherwise stated. Reactions were monitored by TLC on silica gel plates (Merck Type 60H) and visualized by dipping in 4% sulfuric acid in ethanol followed by heating at ca. 120−150 °C. Flash column chromatography was carried out on silica gel (Merck grade 9385, 230−400 mesh 60 Å) in the solvent systems indicated. LP refers to petroleum fractions of bp 35−60 °C. Solutions were dried using anhydrous Na_2SO_4. Melting points were measured on a Fischer-Johns melting point apparatus and are uncorrected.

17β-(4′-Imidazolyl)androst-5-en-3β-ol (2a). A solution of the 21-acetoxy ketone **1a** (1 g, 2.7 mmol), cupric acetate (2.5 g, 12.5 mmol), 28% aqueous ammonium hydroxide (20 mL) and 37% aqueous formaldehyde (3 mL, 37 mmol) in ethanol (50 mL) was refluxed for 6 h, while at 1, 2, 3, and 4 h an additional quantity of NH_4OH (10, 1, 1, and 1 mL) and formaldehyde (3.5, 1, 1, and 1 mL) were added and refluxing stopped at 6 h. Water (80 mL) was added and the solvent concentrated to about 40 mL and left overnight. The grey copper salt product was collected and suspended in 50% EtOH (100 mL) and heated to 50 °C while a stream of gaseous hydrogen sulfide was passed into the suspension for 1 h to precipitate copper sulfide and free the imidazole derivatives. The precipitate was collected and refluxed in EtOH (100 mL) for 30 min and then filtered. The combined filtrate was concentrated to give the crude product (400 mg) which was recrystallized from EtOH−H_2O to give pure **2a** (264 mg, 29%), mp 307−310 °C (lit[20] mp 298−300 °C).

HCl Salt. Four drops of concentrated HCl was added to a solution of **2a** (100 mg) in 2-propanol (12 mL) to give pH = 1 (litmus paper). After evaporation to dryness, the residue was recrystallized from MeOH−ether to give **2a**·HCl salt (80 mg, 72%), mp 270−275 °C, δ (CD_3OD) 8.81 (lH, S, 2′-H), 7.39 (lH, s, 5′-H), 5.36 (lH, d, J = 4.4 Hz, 6-H), 3.40 (lH, m, 3α-H), 2.78 (lH, t, J = 9.8 Hz, 17α-H), 1.02 (3H, s, 19-Me), 0.59 (3H, s, 18-Me). Anal. ($C_{22}H_{32}N_2O$·HCl) C, H, N.

Following the same or similar procedure, the following compounds were prepared:

17β-(2′-Methyl-4′-imidazolyl)androst-5-en-3β-ol (2b). The 21-acetoxy ketone **1a** (1 g, 2.7 mmol) and acetaldehyde (1 mL) gave the 2′-methyl compound **2b** (370 mg, 39%), mp 263−266 °C (253 °C shrinks) (from EtOH−CH_2Cl_2): δ (CD_3OD) 6.57 (lH, s, 5′-H), 5.35 (lH, d, J = 4.9 Hz, 6-H), 3.40 (lH, m, 3α-H), 2.57 (lH, t, J = 9.8 Hz, 17α-H), 2.30 (3H, s, 2′-Me), 1.01 (3H, s, 19-Me), 0.53 (3H, s, 18-Me). Anal. ($C_{23}H_{34}N_2O$) C, H, N.

17β-(2′-Phenyl-4′-imidazolyl)androst-5-en-3β-ol (2c). Compound **1a** (1 g) and benzaldehyde (4 mL) gave a crude product which was purified by flash chromatography. Elution with 3% MeOH−CH_2Cl_2 gave **2c** (240 mg, 22%), mp 237−240 °C (from acetone): δ (CD_3OD) 7.83 (2H, d, J = 7.3 Hz, phenyl-H), 7.40 (3H, m, phenyl-H), 6.85 (lH, s, 5′-H), 5.31 (lH, d, J = 4.4 Hz, 6-H), 3.40 (lH, m, 3α-H), 2.72 (IH, t, J = 10.1 Hz, 17α-H), 1.02 (3H, s, 19-Me), 0.61 (3H, s, 18-Me). Anal. ($C_{28}H_{36}N_2O$) C, H, N.

17β-(4′-Imidazolyl)-16α,17α-epoxyandrost-5-en-3β-ol (2d). From the 16,17-epoxide **1b** (3 g), the 17β-(4′-imidazolyl) epoxide **2d** was obtained as a white solid (1.2 g, 44%), mp 225−232 °C (from methanol): δ (CD_3OD) 7.69 (lH, s, 2′-H), 6.91 (lH, s, 5′-H), 5.35 (lH, d, J = 4.1 Hz, 6-H), 4.63 (lH, t, J = 9 Hz, 16-H), 3.41 (1H, m, 3-H), 1.01 (3H, s, 19-Me), 0.58 (3H, s, 18-Me). Anal. ($C_{22}H_{30}N_2O_2$·H_2O) C, H, N.

17-(4′-Imidazolyl)androsta-5,16-dien-3β-ol (2e). 20-Oxopregna-5,16-diene-3β,21-diol 21-acetate **(1c)**[21] (1.30 g, 3.5 mmol) gave a crude product (500 mg) which after two crystallizations yielded **2e** (160 mg, 11%), mp 313−318 °C (from EtOH-H_2O). Anal. ($C_{22}H_{30}N_2O$) C, H, N.

The salt **2e**·HCl, mp 300−306 °C (from EtOH−ether), which is very soluble in alcohol but slightly soluble in water was prepared as described above for **2a**·HCl: δ (CD_3OD) 8.89 (1H, s, 2′-H), 7.54 (lH, s, 4′-H), 6.32 (lH, s, 16-H), 5.39 (lH, s, 6-H), 3.40 (lH, m, 3α-H), 1.09 (3H, s, 19-Me), 1.03 (3H, s, 18-Me).

3β-Acetoxy-17-(4′-imidazolyl)androsta-5,16-diene (2f). A solution of 21-bromo-pregna-5,16-dien-3β-ol **(6)** (1 g, 2.3 mmol, prepared from **5a** with $CuBr_2$[23]), cupric acetate (2.5 g, 12.5 mmol), 28% aqueous ammonium hydroxide (20 mL), and 37% aqueous formaldehyde (3 mL, 37 mmol) was refluxed for 1 h. Worked up as described above for **2a**, the crude product obtained was then recrystallized from acetone−LP, to give **2f** (140 mg, 16%), mp 226−229 °C: δ 7.87−7.43 (lH, broad s, 2′-H), 7.22−6.87 (1H, broad s, 5′-H), 5.95 (lH, s, 16-H), 5.44 (H., d, J = 4.2 Hz, 6-H), 4.55 (H, m, 3α-H), 2.05 (3H, s, 3-OAc), 1.08 (3H, s, 19-Me), 1.00 (3H, s, 18-Me). Anal. ($C_{24}H_{32}N_2O_2$) C, H, N.

3β-Acetoxy-17β-(4′-imidazolyl)androst-5-ene (2g). Following the same procedure described above for **2a**, 3,21-diacetoxypregn-5-en-20-one **(1d)** (1 g, 2.4 mmol) gave the crude product which was purified by flash chromatography. Elution with 5% MeOH−CH_2Cl_2 yielded **2g** (250 mg, 27%), mp 210−213 °C (from acetone): δ 7.71 (lH, s, 2′-H), 6.84 (lH, s, 5′-H), 5.39 (lH, d, J = 4.4 Hz, 6-H), 4.60 (lH, m, 3α-H), 2.67 (lH, t, J = 9.3 Hz, 17α-H), 2.04 (3H, s, AcO), 1.02 (3H, s, 19-Me), 0.53 (3H, s, 18-Me). Anal. ($C_{24}H_{34}N_2O_2$) C, H, N. Further elution gave **2a** (200 mg, 24%), mp 304−307 °C.

17β-(*N*-Acetyl-4′-imidazolyl)-3β-acetoxyandrost-5-ene (2h). **2a** (100 mg) in pyridine (2 mL) and Ac_2O (0.5 mL) was heated at 110 °C for a few minutes until it became clear and then left at 25 °C for 14 h. Water was added, and the precipitate was collected and recrystallized from acetone−LP to give **2h** (45 mg, 40%), mp 206−209 °C: δ 8.05 (lH, s, 2′-H), 7.19 (lH, s, 5′-H), 5.39 (lH, s, 6-H), 4.61 (lH, m, 3β-H), 2.57 (3H, s, N-Ac), 2.04 (3H, s, 3-OAc), 1.02 (3H, s, 18-Me), 0.53 (3H, s, 19-Me). Anal. ($C_{26}H_{36}N_2O_3$) C, H, N.

17β-(4′-Imidazolyl)androst-4-en-3-one (4a). Following the procedure for the preparation of **2a**, deoxycorticosterone 21-acetate **(3a) (**1 g) gave crude product **4a** (0.45 g) which was recrystallized from methanol (0.15 g, 17%), mp 235−240 °C: δ 7.58 (lH, s, 2′-H), 6.82 (IH, s, 5′-H), 5.74 (lH, s, 4-H), 2.67 (lH, t, J = 9 Hz, 17α-H), 1.19 (3H, s, 19-Me), 0.5 (3H, s, 18-Me). Anal. ($C_{22}H_{30}N_2O$) C, H, N.

17α-Hydroxy-17β-(4′-imidazolyl)androst-4-en-3-one (4b). Following the procedure for the preparation of **2a**, 17a-hydroxydeoxycorticosterone **(3b) (**1 g) gave **4b** (0.36 g, 34%), mp 195−197 °C (from methanol): δ 7.61 (lH, s, 2′-H), 6.92 (lH, s, 5′-H), 5.74 (lH, s, 4-H), 1.19 (3H, s, 19-Me), 0.56 (3H, s, 18-Me). Anal. ($C_{22}H_{30}N_2O_2$) C, H, N.

17-(4′-Imidazolyl)androsta-4,16-dien-3-one (4c). A solution of the 16-ene **2e** (50 mg, 0.15 mmol) in toluene (1 0 mL) and cyclohexanone (1 mL) was heated, and part of the solvent

(3−4 mL) distilled off to eliminate any moisture. Aluminum isopropoxide (61 mg, 0.30 mmol) was added and the mixture refluxed for 3 h. Saturated sodium tartrate (3 mL) was added and the whole mixture steam distilled for 2 h, until no more toluene was distilled off. The remaining residue was extracted with EtOAc (3 × 50 mL). The combined EtOAc layer was evaporated, and the residue was subjected to flash chromatographic separation. Elution with 5 % MeOH−CH_2Cl_2 gave **4c** (35 mg, 70%), which was dissolved in MeOH (5 mL) and acidified to pH = 1 (pH paper) with 4 drops of concentrated HCI. After evaporation, the residue was recrystallized from MeOH−LP to give the **4c**·HCl salt (12 mg), mp 267−270 °C: δ (CD_3OD) 8.90 (lH, s, 2′-H), 7.55 (lH, s, 5′-H), 6.32 (lH, s, 16-H), 5.73 (lH, s, 4-H), 1.29 (3H, s, 19-Me), 1.06 (3H, s, 18-Me). Anal. ($C_{22}H_{28}N_2O$·HCl) C, H, N.

3β-Hydroxyandrost-5-ene-17β-carboxaldehyde (8a). A solution of the 21-acetoxy ketone **la** (2.25 g, 6 mmol) in THF (20 mL) was added dropwise to a stirring solution of $LiAlH_4$ (600 mg, 15.8 mmol) in THF (20 mL) at 25 °C and stirring continued for 30 min. Water was added carefully to destroy excess $LiAlH_4$. EtOAc (200 mL) was added, and the whole mixture was washed with 1 N HCI (400 mL × 3), followed by water (2 × 40 mL). After evaporation, the diol **7** (2 g) was obtained.

A solution of sodium periodate (2 g, 9.3 mmol) in water (20 mL) was added to the above diol **(7)** (2g, 6 mmol) in MeOH (100 mL) and the mixture stirred at 25 °C for 40 min. The precipitated sodium iodate was filtered off and the filtrate concentrated under reduced pressure below 45 °C to about 20 mL to yield the 17-al **8a** (1.8 g, 88%), mp 157−159 °C (lit.[30] mp 155−157 °C).

17β-(2′-Imidazolyl)-5-androsten-3β-ol (9a). The 17-al **8a** (1 g, 3.4 mmol), cupric acetate (2.0 g, 10 mmol), 28% aqueous ammonium hydroxide (20 mL), and 40% aqueous glyoxal (3 mL, 20.7 mmol) were refluxed for 6 h. At 1, 2, and 4 h, an additional amount of NH_4OH (each 5 mL) was added and refluxing stopped at 6 h. Water was added and the precipitate was collected, which was then extracted by refluxing with EtOH (2 × 50 mL). The combined filtrate was concentrated to 5−10 mL, and LP was added to give a precipitate which was recrystallized from MeOH−H_2O to yield colorless crystals **9a** (291 mg, 24%), mp 288−291 °C: δ (CD_3OD) 6.91 (2H, s , 4′,5′-H), 5.35 (lH, d, J = 3.9 Hz, 6-H), 3.40 (lH, m, 3α-H), 2.76 (lH, t, J = 9.3 Hz, 17α-H), 1.01 (3H, s, 19-Me), 0.53 (3H, s, 18-Me). Anal. ($C_{22}H_{32}N_2O$) C, H, N.

17-(2′-Imidazolyl)androsta-5,16-dien-3β-ol (9b). A solution of 16-en-17-al **8b** (1 g, 3.3 mmol) (prepared from **lc** by the procedure described above for **8a**[24]), cupric acetate (1.0 g, 5 mmol), 40% aqueous glyoxal (1 mL, 8.7 mmol), and 28% aqueous NH_4OH (6 mL) in ethanol (50 mL) was refluxed for 12 h, while at 1, 2, 3, 4, 6, and 10 h an additional quantity of NH_4OH (each 1.2 mL) and 40% aqueous glyoxal (each 0.5 mL) were added and the refluxing stopped at 12 h. Workup as described above for **2a** gave a crude product which was subjected to flash chromatographic separation, eluted with 5% MeOH−CH_2Cl_2, to give **9b** (60 mg, 5% yield), mp 272−275 °C (from acetone): δ 7.06 (2H, s, 4′,5′-H), 6.17 (IH, s, 16-H), 5.40 (lH, d, J = 4.9 Hz, 6-H), 3.55 (lH, m, 3α-H), 1.25 (3H, s, 19-Me), 1.08 (3H, s, 18-Me). Anal. ($C_{22}H_{30}N_2O$) C, H, N.

17β-(2′-Imidazolyl)androst-4-en-3-one (10). Following the same procedure described above for **4c**, the 3-ol **9a** (260 mg) was oxidized with cyclohexanone (1 mL) and aluminum isopropoxide (250 mg) in toluene (25 mL). The crude product obtained was recrystallized from EtOH−H_2O, decolorized with charcoal, to give **10** (169 mg, 65%), mp 256−9 °C: δ (CD_3OD) 6.92 (2H, s, 4′,5′-H), 5.71 (lH, s, 4-H), 2.78 (lH, t, J = 9.8 Hz, 17α-H), 1.22 (3H, s, 19-Me), 0.58 (3H, s, 18-Me). Anal. ($C_{22}H_{30}N_2O$) C, H, N.

20β-(2′-Imidazolyl)pregn-4-en-3-one (12). Following a similar procedure to that used for the preparation of **9a**, 3-oxopregn-4-en-3-e-20β-carboxaldehyde (**11**) (1 g) gave **12** (0.24 g, 22%), mp 289−294 °C (dec) (from MeOH): δ 6.92 (2H, s, 4′, 5′-H), 5.72 (lH, s, 4-H), 2.90 (lH, m, 20-H), 1.32 (3H, d, J = 7.5 Hz, 21-H), 1.19 (3H, s, 19-Me), 0.79 (3H, s, 18-Me). Anal. ($C_{24}H_{34}N_2O$) C, H, N.

21-Formyl-3β-hydroxypregn-5-en-20-one (13a) was prepared according to the method of Pelc et al.[26] in 69% yield, mp 149−152 °C (lit.[25] mp 111−115 °C or lit[26] mp 154−157 °C): **17β-(3′-pyrazolyl)-androst-5-en-3β-ol (14a)** (41% yield, mp 222−227 °C, lit.[25] mp 220−6 °C or lit.[26] 233−235 °C) and **17β-(5′-isoxazolyl)-5-androsten-3β-ol (15a)** (13% yield, mp 198−201 °C, lit[25] mp 198−202 °C) were prepared according to ref 25.

17β-(3′-Isoxazolyl)-5-androsten-3β-ol (16). A mixture of **15a** and **16** (100 mg) [prepared from β-keto aldehyde **13a** with hydroxylamine hydrochloride in acetic acid[25]] was dissolved in THF (5 mL), NaOMe (250 mg) was added, and the mixture was stirred for 1.5 h. Water (20 mL) was added and the whole mixture extracted with CH_2Cl_2. The organic layer was evaporated and the residue recrystallized from acetone−LP to give 3′-isoxazole **16** (59 mg, 45%, mp 203 −205 °C (lit.[25] mp 205.5 −207 °C).

21-Formyl-3β-hydroxypregna-5,16-dien-20-one (13b). To a stirred and cooled (ice bath) mixture of sodium methoxide (2 g, 6 mmol) and ethyl formate (8 mL, 543 mmol) (dried over phosphorus pentoxide and distilled) was added dropwise a solution of 16-en-20-one **5a** (2 g, 5.6 mmol) in pyridine (20 mL). After being stirred for 1 h, the solution was poured into water (250 mL) containing concentrated HCI (70 mL). The precipitate which was collected and washed with water gave **13b** (1.5 g, 75%), mp 118−123 °C. ^{1}H NMR showed that the impure β-keto aldehyde existed mainly in the enol form **(13b)**: δ 8.04 (IH, d, J = 3.9 Hz, 22-H), 6.71 (lH, s, 16-H), 5.79 (IH, d, J = 3.9 Hz, 21-H), 5.37 (lH, s, 6-H), 3.54 (lH, m, 3α-H), 1.06 (3H, s, 19-Me), 1.00 (3H, s, 18-Me). This product was used directly in the following reaction. A portion was recrystallized from acetone−LP, mp 234−238 °C.

17-(3′-Pyrazolyl)androsta-5,16-dien-3β-ol (14b). The β-keto aldehyde **13b** (1.5 g, 4.4 mmol) and anhydrous hydrazine (0.75 mL) in MeOH (50 mL) were refluxed under N_2 for 3 h. Water was added and the precipitate (900 mg) collected. Three recrystallizations from MeOH gave pure **14b** (45 mg, 3%), mp 216−220 °C (lit.[26] mp 222−223.5 °C).

17-(2′-Phenyl-3′-pyrazolyl)androsta-5,16-dien-3β-ol (14c). The β-keto aldehyde **13b** (1g, 2.9 mmol) and phenylhydrazine (0.40 mL) in MeOH (30 mL) were refluxed for 3 h. Water was added and the mixture extracted with EtOAc. The organic layer was evaporated and the residue chromatographed, to give on elution with 10% acetone−LP **14c** (116 mg, 9.6%), mp 208−210 °C (EtOH) (lit.[26] mp 208.5−210 °C).

17-(1′-Methyl-5′-pyrazolyl)- and 17-(1′-Methyl-3′-pyrazolyl)androsta-5,16-dien-3β-ols (14d and 14e). The β-keto aldehyde **13b** (1 g, 2.9 mmol) and methylhydrazine (400 mg, 8.7 mmol) in methanol (30 mL) were refluxed for 2 h under an atmosphere of N_2. After the solution was cooled to 25 °C, water (10 mL) was added dropwise, the yellow precipitate (800 mg) was collected, and two crystallizations from ethanol gave the pure 5′-pyrazole **14d** (220 mg, 21%), mp 251−254 °C (lit.[26] mp 242−244 °C). The mother liquor (550 mg) was flash chromatographed and eluted with 50% ethyl acetate−LP to give 3′-pyrazole **14e** (90 mg, 8.7%) [mp 224−226 °C (from acetone): δ 7.26 (lH, s, 5′-H), 6.26 (lH, d, J = 1.9 Hz, 16-H), 6.08 (IH, s, 4′-H), 5.38 (lH, d, J = 4.9 Hz, 6-H), 3.88 (3H, s, 1′-Me), 3.53 (lH, m, 3α-H), 1.07 (3H, s, 19-Me), 1.03 (3H, s, 18-Me), Anal. ($C_{23}H_{32}N_2O$) C, H, N] and **14d** (160 mg, 16%), mp 251−254 °C (from EtOH).

17-(5′-Isoxazolyl)androsta-5,16-dien-3β-ol (15b). To a benzene (5 mL) solution of **13b** (700 mg, 2.04 mmol) were added glacial acetic acid (5 mL) and a solution of hydroxylamine hydrochloride (200 mg, 2.88 mmol) and sodium acetate (100 mg) in water (0.5 mL). This mixture was made homogeneous by the addition of ethanol (3 mL) and refluxed for 4 h. After cooling, water (150 mL) was added and the mixture extracted with CH_2Cl_2. The organic layer was evaporated, and the residue was chromatographed, eluting with 20% acetone−LP, to afford **15b** (500 mg, 72%), mp 191−193 °C (from acetone−LP): δ 8.19 (lH, s, 3′-H), 6.45 (lH, s, 16-H), 6.18 (lH, s, 4′-H), 5.38 (lH, s, 6-H), 3.54 (lH, m, 3α-H), 1.07 (3H, s, 19-Me), 1.00 (3H, s, 18-Me). Anal. ($C_{22}H_{29}NO_2$) C, H, N.

17-(3′-Pyrazolyl)androsta-4,16-dien-3-one (17a). Following the procedure for the preparation of **4c**, Oppenauer

oxidation of **14b** (350 mg, 1.04 mmol) with cyclohexanone (5 mL) and aluminum isopropoxide (461 mg, 2.26 mmol) in toluene (60 mL) afforded a crude product, which was chromatographed and eluted with 2% MeOH−CH_2Cl_2, to give the 4-en-3-one **17a** (200 mg, 58%), mp 167−171 °C (155 °C shrinks) (from acetone-LP): δ 7.54 (lH, s, 5′-H), 6.33 (lH, s, 16-H), 6.05 (lH, (1H, s, 4′-H), 5.76 (1H, s, 4-H), 1.24 (3H, s, 19-Me), 1.04 (3H, s, 18-Me). Anal. ($C_{22}H_{28}N_2O$) C, H, N.

17-(5′-Isoxazolyl)androsta-4,16-dien-3-one (17b). Following the procedure for the preparation of **17a**, Oppenauer oxidation of **15b** (300 mg, 0.88 mmol) with cyclohexanone (5 mL) and aluminum isopropoxide (400 mg, 1.96 nunol) in toluene (50 mL) afforded the title compound **17b** (125 mg, 42%), mp 184−186 °C (from acetone−LP): δ 8.19 (lH, d, J = 1.4 Hz, 3′-H), 6.44 (lH, s, 16-H), 6.19 (lH, s, 4′-H), 5.76 (lH, s, 4-H), 1.24 (3H, s, 19-Me), 1.03 (3H, s, 18-Me). Anal. ($C_{22}H_{27}NO_2$) C, H, N.

17-(2′-Methyl-4′-oxazolyl)androst-5-en-3β-ol (18). The 21-acetoxy ketone **la** (500 mg, 1.34 mmol) and ammonium acetate (500 mg, 6.5 mmol) in glacial acetic acid (15 mL) were refluxed for 8 h. Water (50 mL) was added and the pale brown precipitate collected, washed with water, and dried. The crude product (350 mg) in MeOH (15 mL) was combined with $KHCO_3$ (500 mg) in water (5 mL), the mixture was refluxed for 4 h, H_2O was added, and the precipitate (216 mg) was collected and recrystallized from acetone to give **18** (125 mg, 26%), mp 181−183 °C: δ 7.20 (lH, s, 5′-H), 5.31 (lH, d, J = 5.4 Hz, 6-H), 3.49 (lH, m, 3α-H), 2.48 (lH, t, J = 9.8 Hz, 17α-H), 2.37 (3H, s, 2′-Me), 0.96 (3H, s, 19-Me), 0.48 (3H, s, 18-Me). Anal. ($C_{23}H_{33}NO_2$) C, H, N.

Enzyme Preparation and Assay for Testicular $C_{17,20}$-Lyase. [7-^{3}H]Pregnenolone (25 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and checked for purity and/or purified by TLC or HPLC prior to use. [21-^{3}H]-17α-Hydroxypregnenolone (13.61 μCi/μmol) was prepared in our laboratory as described by Akhtar et al.[31] Ketoconazole was purchased from Sigma Chemical Co. (St.Louis, MO). Scintillation cocktail 3a70B was purchased from RPI Corp. (Mount Prospect, IL).

Testicular Microsomes. The previously reported procedure[14] was followed to prepare microsomes from human testes (obtained from untreated prostatic cancer patients undergoing orchidectomy). Microsomes from the testes of adult Sprague−Dawley rats (Charles River Laboratories, weight 200−250 g) were prepared as previoulsy described.[16] The microsomes were stored at −70 °C until assayed. Just before use, the thawed microsomes were diluted with 0.1 M phosphate buffer to appropriate concentrations. The protein concentration of microsomes used in each assay was determined by the method of Lowry et al.[32]

Measurement of $C_{17,20}$-Lyase Activity. (1) Radiometric Assay. The substrate for this assay was [21-^{3}H]-17α-hydroxypregnenolone (13.61 μCi/μmol). Each tube contained 300 000 dpm and a total concentration of 10 μM 17α-hydroxypregnenolone. The cofactors (NADP 65 μM; glucose 6-phosphate 0.71 mM; glucose-6-phosphate dehydrogenase 0. 13 IU in 50 μL of phosphate buffer) and different concentrations of the test compounds in phosphate buffer (pH 7.4, total volume 1 mL) were added and tubes preincubated for 10 min at 34 °C. The reaction was initiated by the addition of the microsomes (approximately 90 μg of protein) and the incubation carried out for 60 min under oxygen at 34 °C. Chloroform was then added to extract the steroids and an aliquot (0.75 mL) of aqueous phase removed and mixed with an equal volume of 2.5% charcoal suspension. After vortexing, the tubes were allowed to stand for at least 30 min then centrifuged, an aliquot of the supernatant was removed, and the tritium concentration was measured by liquid scintillation counting. The results are listed in Table 1. IC_{50} values were calculated from the linear regression line of the plot of log of lyase activity versus log of four to five inhibitor concentrations. The results were obtained from duplicate sets of experiments and were repeated at least once and several times for the more active inhibitors.

(2) Product Isolation Assay. The assay was performed as previously[16] by incubating human testicular microsomes (approximately 90 μg of protein) with [7-^{3}H]-pregnenolone (5 × 10^4 dpm, 400 nM), the NADPH generating system as above, and different concentrations of the test compounds in phosphate buffer (pH 7.4, total volume 1 mL) under oxygen for 5 min at 34 °C. Authentic steroid markers and ^{14}C-labeled pregnenolone, 17α-hydroxypregnenolone, and DHEA were added to correct for procedural losses. The steroids were extracted with ether and then separated by HPLC using a NOVA-PAK, C_{18} reverse phase column and eluting with acetonitrile/methanol/water (30:10:60), and the radioactivity was measured in each fraction collected. The lyase was determined from the percentage conversion of [7-^{3}H]pregnenolone to DHEA (the conversion of substrate to androstenediol and testosterone under the experimental conditions was negligible).

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