Synthesis and Evaluation of Pregnane Derivatives as Inhibitors of Human Testicular 17α-Hydroxylase/$C_{17,20}$-Lyase[†,‡]

Ji-song Li, Yan Li, Chong Son, and Angela M. H. Brodie*

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

Received March 28, 1996[⊗]

The pregnene derivatives with modifications at the 17,20-side chain and D-ring were synthesized and evaluated as inhibitors of human testicular 17α-hydroxylase/$C_{17,20}$-lyase. The results demonstrate that compounds which have 20-substituents with moderate to strong dipole properties, such as 20-oxime (**3**, **20**), 20*β*-ol (**24**, **30**), and 20*β*-carboxaldehyde (**27**), are potent inhibitors of this enzyme complex. The 20-substituents with hydrophobic property were devoid of inhibitory activity, e.g., the dimethylhydrazones **8** and **9**. The 16-ene together with 20-oxime (**20**) showed the most potent inhibition of this enzyme complex, whereas 17(20)-ene modification as in 17(20)-ene-20-carbonitrile (**14**) did not increase activity in comparison to the 20*β*-carbonitrile (**16**). The bioisostere of **27** with 20-aza (**19**) also reduced the inhibitory activity. The results showed that isomeric configurations at the 20-position of some steroidal compounds are important factors which influence the potency of the inhibition significantly (e.g., 20*β*-ols **24** and **30** were 3−5-fold more potent than 20α-ols **23** and **29**). As expected, some compounds based on the pregn-5-en-3*β*-ol skeleton, which is similar to the natural substrate of human testicular 17α-hydroxylase/$C_{17,20}$-lyase in A- and B-rings, showed more potent inhibition than similar compounds which are based on the pregn-4-en-3-one skeleton (e.g., **23**−**25** compared to **29**−**31**). These results suggest that A- and B-rings make significant contributions to the binding of these steroidal compounds to the 17α-hydroxylase and $C_{17,20}$-lyase. In comparison to ketoconazole, a nonsteroidal inhibitor of 17α-hydroxylase and $C_{17,20}$-lyase which has been used in the treatment of prostatic cancer, the steroidal compounds **20**, **24**, and **27** demonstrate more potent inhibition for this enzyme complex. These inhibitors warrant further investigation in biological systems. The structural features of these compounds may serve as leads in the design of new inhibitors.

Androgen synthesis is mediated by the steroidal 17α-hydroxylase/$C_{17,20}$-lyase (cytochrome P-$450_{17\alpha}$), which catalyzes the conversion of C_{21} precursors (pregnenolone and progesterone) to the related C_{19} steroids (dehydroepiandrosterone and androstenedione) in the testes and adrenals, and 5α-reductase, which catalyzes the conversion of testosterone to dihydrotestosterone in the prostate. Effective inhibitors of these enzymes could be useful in the treatment of problems associated with androgen excess in women and in the treatment of androgen-sensitive prostatic cancer in men. A number of steroidal and nonsteroidal compounds which inhibit 17α-hydroxylase/$C_{17,20}$-lyase have been described.[1−10] Ketoconazole, an imidazole antifungal agent, has been used to inhibit testosterone biosynthesis in the treatment of patients with advanced prostatic cancer.[11,12] However, clinical use of this agent was limited due to its significant side effects caused by nonselective inhibition of several other cytochrome P-450 enzymes.[13]

Recent efforts in our laboratory have been applied to the development of selective and more potent inhibitors of 17α-hydroxylase/$C_{17,20}$-lyase. We hypothesized that modifications of enzyme substrates with different functional substituents at or close to the positions which may interact with the enzyme's active site (i.e., the 20-position for the $C_{17,20}$-lyase) would result in selective and potent inhibitors of this enzyme. A number of 20-substituted pregnene derivatives were therefore synthesized and evaluated as inhibitors. We have reported that one pregnene derivative, 3-oxo-4-pregnene-20*β*-carboxaldehyde,[14] and its aldoxime[15] demonstrated potent inhibition of rat testicular 17α-hydroxylase/$C_{17,20}$-lyase and human prostatic 5α-reductase. The present study describes the synthesis and evaluation of a further series of pregnene derivatives with modification mainly at the 17,20-side chain and D-ring as inhibitors of human testicular 17α-hydroxylase/$C_{17,20}$-lyase.

Results and Discussion

Chemistry. The synthetic approaches to prepare a variety of pregnene derivatives are described in Scheme 1. The sulfur ylide (dimethylsulfonium methylide)[16] was used to convert the 20-ketone of pregnenolone 3-acetate (**1a**; Scheme 1) to the (20*R*)-20,22-epoxide **2**. The dimethylsulfonium methylide solution was prepared from trimethylsulfonium iodide and sodium hydride in DMSO at 0 °C. The reaction was carried out at room temperature for 12 h. Under these conditions, the stereoisomer (20*R*)-**2** was obtained as the main product. The selectivity of this reaction is believed to be due to the high reactivity of the dimethylsulfonium methylide.[16,17] This sulfur ylide is unstable and may readily attack the less hindered α-side of 20-ketone to form the (20*R*)-epoxide. The configuration of the 20-epoxide was confirmed by the two oxirane proton signals

[†] This work was supported by NIH Grant CA 27440.

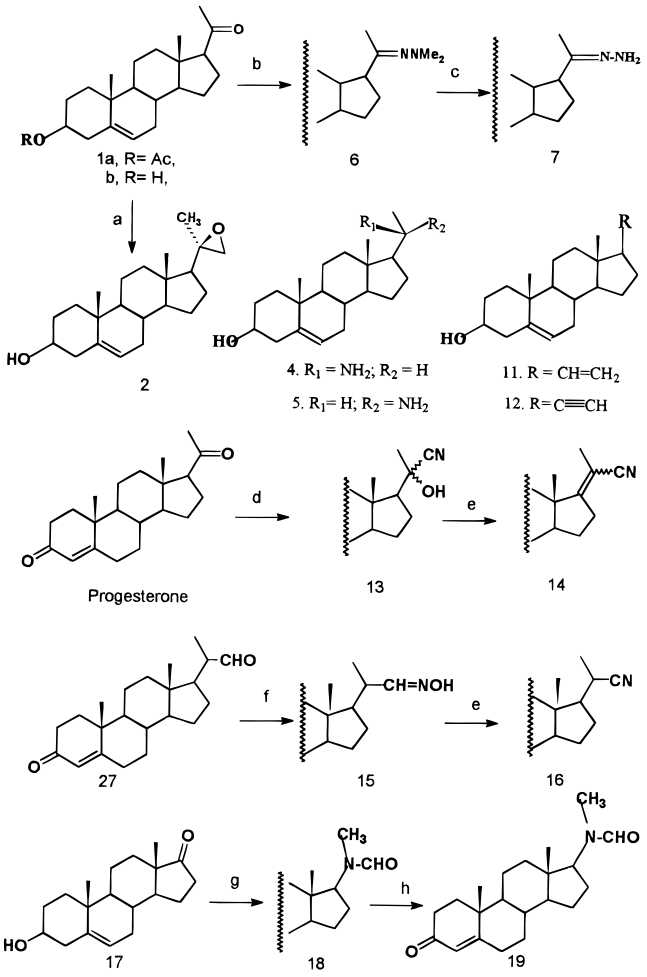
[‡] Abbreviations: DHEA, dehydroepiandrosterone; A, androstenedione; T, testosterone; THF, tetrahydrofuran.

* To whom correspondence should be addressed.

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1996.

S0022-2623(96)00245-2 CCC: $12.00

Scheme 1[a]



[a] (a) Me_3SI, NaH; (b) NH_2NMe_2; (c) NH_2NH_2; (d) $Me_2C(OH)CN$; (e) CH_3SO_2Cl, Py; (f) NH_2OH, NaOAc, 0 °C; (g) MeNHCHO, HCOOH; (h) $Al(O\text{-}i\text{-}Pr)_3$, cyclohexanone.

in its ^{1}H-NMR spectrum (ppm: 2.33 doublet and 2.50 doublet), which agrees with the reported data for the 3-acetate of **2**.[18]

A mixture of 20β-amine **4** and 20α-amine **5** was prepared according to the procedures of van de Woude and van Hove[19] by reduction of 20-oxime **3** with sodium propanol. The isomers were separated from the mixture by preparative TLC (silica gel) eluted with chloroform containing 30% methanol and 1% trimethylamine.

To synthesize the 20-hydrazone **7**, the exchange reaction[20] of dimethylhydrazone **6** with anhydrous hydrazine was used to avoid the formation of azines[21] as impurities. The 20-*N*,*N*-dimethylhydrazone **8** was prepared from the reaction of 16-ene pregnenolone **25** with *N*,*N*-dimethylhydrazine. Under a similar but much milder condition, the 22-dimethylhydrazone **9** was selectively synthesized from 3-oxo-4-pregnene-20β-carboxaldehyde (**27**) to avoid the reaction of 3-ketone with dimethylhydrazine. The compounds with 20-double- or 20-triple-bond properties, 20-ene **11** and 20-yne **12**, were synthesized by following the procedures of Krubiner et al.[22]

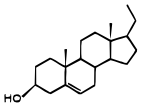
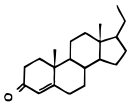
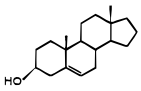
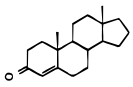
To synthesize the 17,20-ene-20-carbonitrile **14**, the 20-cyanohydrin **13** (prepared from progesterone, see Scheme 1)[23] was dehydrated with methanesulfonyl chloride in pyridine followed by separation with preparative TLC to provide (**14**). Under a similar condition, 20β-carboxaldoxime **15** was converted to 20β-carbonitrile **16**.

The procedure of Counsell et al.[24] was followed to prepare *N*-methyl-17β-formamidoandrost-5-en-3β-ol (**18**) from dehydroepiandrosterone (**17**) and *N*-methylformamide under reductive amination in formic acid. Oppenauer oxidation of **18** provided *N*-methyl-17β-formamidoandrost-4-en-3-one (**19**). Several commercially available compounds were purchased and tested for structure−activity relationship purposes (Table 1).

Enzyme Assay. Human testicular microsomes were employed to evaluate the potency of pregnene derivatives as inhibitors of 17α-hydroxylase/$C_{17,20}$-lyase. Pregnenolone is the natural substrate for human testicular 17α-hydroxylase/$C_{17,20}$-lyase[25] rather than progesterone. Although 17α-hydroxypregnenolone is the intermediate substrate converted to DHEA by $C_{17,20}$-lyase, it has been reported that most of the 17α-hydroxypregnenolone is converted without being released from the enzyme's binding site.[26] Our previous results also agree with this observation.[15] Therefore, pregnenolone was used for measuring the activity of this enzyme complex. Thus, the conversion of radiolabeled pregnenolone to 17α-hydroxypregnenolone and DHEA by 17α-hydroxylase/$C_{17,20}$-lyase was measured with four to five concentrations of test compounds. Reverse phase HPLC was used to separate and measure the amount of recovered substrate and metabolites. The activity of 17α-hydroxylase was calculated from the conversion of pregnenolone to 17α-hydroxypregnenolone and DHEA, and the activity of $C_{17,20}$-lyase was calculated based on the conversion of pregnenolone to DHEA.

The potency of inhibition (IC_{50}, 50% inhibition compared to control value) of all tested compounds is listed in Table 1. Compounds containing 20-oxime (**3**, **20**) and 20β-ol (**24**, **30**) demonstrated potent inhibition to both the 17α-hydroxylase and $C_{17,20}$-lyase activities, which suggests the existence of strong dipole−dipole interactions between the 20-moieties of these inhibitors and the enzyme's active site. The 20β-carboxaldehyde **27** also showed potent inhibition of this enzyme complex. This compound may benefit from the moderate dipole properties of its 20-substituent group. However, the 20-hydrazone **7** and 20-amines **4** and **5** showed poor potency in this assay. A possible reason is that the amine groups of these compounds will be ionized under physiologic conditions. The ionized moieties may not be preferred by the enzyme. Amine substituents have been used to design effective inhibitors of cytochrome $P\text{-}450_{scc}$.[27] However, 10β-amine estrenedione was a poor inhibitor of aromatase, another cytochrome P-450 enzyme.[28] The *N*,*N*-dimethylhydrazones at the 20-position (**6**, **8**) or the 22-position (**9**) were devoid of inhibitory activity for the 17α-hydroxylase and $C_{17,20}$-lyase, which implies that hydrophobic interactions between these substituents and the enzyme's active site are not favored. Similar effects can be seen in 20-ene **11** and 20-yne **12** which showed poor inhibition of this enzyme complex. In the modifications of the D-ring, we found that the most important factor which contributes to the inhibition of this enzyme complex is the 16,17-ene conjugated with 20-oxime. Compared with 20-oxime **3**, the 16-ene-20-oxime **20** showed 35-fold greater potency in inhibition. However, the 17,20-ene did not contribute to inhibitory activity (compare **14** to **16**). The introduction of 17α-bromo (compound **21**) showed only weak inhibition. The 3-acetate **26** was found to be a weaker

Table 1. IC_{50} of Steroid Compounds for the Inhibition of Human Testicular 17α-Hydroxylase/$C_{17,20}$-Lyase[a]

base structure	compd	substituents	IC_{50}[b] (μM) 17α-hydroxylase	IC_{50}[b] (μM) $C_{17,20}$-lyase
pregn-5-en-3β-ol 	**2**	(20*R*)-20,22-epoxide	0.73	0.72
	3*	20-one oxime	0.53	0.57
	4	20β-amine	89.8	43.9
	5	20α-amine	9.8	8.0
	6	20-one-*N*,*N*-dimethylhydrazone	NI[c]	NI
	7	20-one hydrazone	9.98	4.90
	8	16-en-20-one *N*,*N*-dimethylhydrazone	43.7	35.0
	11	20-ene	2.88	3.71
	12	20-yne	3.56	3.95
	20*	16-en-20-one oxime	0.016	0.016
	21*	17α-bromo-20-one	1.30	0.97
	22*	16α,17α-epoxy-20-one	0.44	0.68
	23*	20α-ol	0.72	0.51
	24*	20β-ol	0.18	0.19
	25*	16-en-20-one	0.51	0.49
	26*	16-en-20-one 3-acetate	1.89	1.71
pregn-4-en-3-one 	**9**	20β-carboxaldehyde *N*,*N*-dimethylhydrazone	NI	NI
	14	17(20)-ene-20-carbonitrile	1.22	0.89
	15	20β-carboxaldoxime	5.98	6.97
	16	20β-carbonitrile	1.10	0.75
	27*	20β-carboxaldehyde	0.23	0.16
	28*	16α,17α-epoxy-20-one	0.48	0.80
	29*	20α-ol	2.84	1.43
	30*	20β-ol	0.49	0.24
	31*	16-en-20-one	1.77	1.70
androst-5-en-3β-ol 	**18**	*N*-methyl-17β-formamide	0.29	0.28
	32*	17-one oxime	13.5	10.7
	33*	17-one hydrazone	6.48	3.78
androst-4-en-3-one 	**19**	*N*-methyl-17β-formamide	0.80	0.75
	34*	17α-ethynyl-17β-ol	NI	NI
ketoconazole			0.86	0.92

[a] The assay procedures are described in the Experimental Section. The [7-^{3}H]pregnenolone (400 nM) was used as substrate for the incubation. Compounds marked with an asterisk were purchased from Steraloids Inc. (Wilton, NH). All other compounds were synthesized in our laboratory. [b] IC_{50} refers to the inhibitor concentration which produced 50% inhibition of the enzyme activity. The correlation coefficients $r > 95\%$ for all tested compounds. [c] NI refers to no inhibition.

inhibitor than the 3-hydroxy compound **25**, which suggests that a free 3-hydroxy group is important for inhibitors to bind to this enzyme.

Introducing a 20-aza feature into the potent inhibitor **27** as a bioisostere modification was expected to achieve potent inhibition of this enzyme, since this modification has been successfully applied to steroid inhibitor design.[29] However, the 20-aza compound **19** showed approximately 4-fold lower inhibition compared to **27**.

It is of interest that isomeric configurations at the 20-position of some compounds are important factors which give significant influence to the inhibitory effects of these compounds. For example, the 20β-ols **24** and **30** are 3−6-fold stronger inhibitors than their 20α-isomers **23** and **29**. These effects reflected the steric requirements of the enzyme's active site which prefers specific configuration of steroidal compounds in this area.

As expected, some compounds based on the pregn-5-en-3β-ol skeleton (Δ^5), which are similar to the natural substrate pregnenolone, showed more potent inhibition compared to the similar compounds which are based on the pregn-4-en-3-one skeleton (Δ^4) (**23**−**25** compared to **29**−**31**). However, D-ring modifications may reduce this effect, as in the comparison of 16,17-epoxides **22** and **28**. The Δ^5-**28** was only slightly more potent than the Δ^4-**22**. These results suggest that A- and B-rings of some steroidal compounds may make significant contributions to their binding to the 17α-hydroxylase/$C_{17,20}$-lyase, but the structures of the D-ring may influence this effect.

The results shown in Table 1 also suggest the importance of the 17β-two-carbon side chain in the binding of steroidal compounds to the enzyme. Androstene derivative 17-oxime **32** showed very poor inhibition, whereas pregnene derivative 20-oxime **3** caused potent inhibition of this enzyme.

As both activities of 17α-hydroxylase and $C_{17,20}$-lyase are reported to be regulated by a single microsomal protein,[30] we do not expect to be able to achieve selective inhibitors for the $C_{17,20}$-lyase step. Even though some compounds did show slightly more potent inhibition of lyase activity, most compounds listed in Table 1 had similar potency for both 17α-hydroxylase and $C_{17,20}$-lyase activities. These results suggest that inhibition of $C_{17,20}$-lyase activity by these compounds is mainly derived from their inhibition of 17α-hydroxylase activity.

In comparison to ketoconazole, a nonsteroidal inhibitor, the 16-ene-20-oxime **20**, the 20β-ol **24**, and the (20*R*)-carboxaldehyde **27** are much more potent inhibitors (Table 1). The IC_{50} of the most potent inhibitor **20** is only 16 nM, which is about 50-fold more potent than

ketoconazole. The structural features of these compounds may serve as leads for the design of new inhibitors.

Experimental Section

Synthetic Method. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The identity and purity of compounds were determined by their ^{1}H-NMR spectra (QE 300, NMR Systems, General Electric Co.) with $CDCl_3$ as solvent, MS spectra (HP 59970 MS Chemstation, Hewlett-Packard Co.), and elemental analysis (Galbraith Labs, Inc., Knoxville, TN). Chemicals were purchased from either Sigmal Chemical Co. (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI) except those indicated. Silica gel TLC plates were from J.T. Baker Inc. (Philipsburg, NJ).

20′(*R*)-Spiro[oxirane-2,20′-pregn-5′-en]-3′β-ol (2). A suspension of sodium hydride (168 mg, 80%, 5.6 mmol), anhydrous DMSO (3 mL), and THF (3 mL) was stirred for 5 min and then cooled to 0 °C in an ice bath. Trimethylsulfonium iodide (0.58 g, 2.8 mmol) was added, and the mixture was stirred for 2 h. Pregnenolone acetate (**1a**; Scheme 1) (0.5 g, 1.4 mmol, in DMSO (4 mL) and THF (2 mL)) wad added dropwise into the reaction mixture, and the ice bath was removed. The reaction was continued for 12 h at room temperature. Water (5 mL) was then added, and the mixture was stirred for another 2 h. The reaction mixture was poured onto ice (50 g), and the product was extracted with dichloromethane. After the removal of solvent, the residue was recrystallized from acetone and then methanol to give white crystals of **2** (200 mg, 43%) with mp 160−62 °C. ^{1}H-NMR: δ 5.34 (1H, d, 6-H), 3.52 (1H, m, 3α-H), 2.33 (1H, d, CH_2O), 2.50 (1H, d, CH_2O), 1.38 (3H, s, 21-CH_3), 1.02 (3H, s, 19-CH_3), 0.82 (3H, s, 18-CH_3). MS: *m/e* 331 ($M^+ + 1$). Anal. ($C_{22}H_{34}O_2 \cdot H_2O$) C, H.

20-Oxo-5-pregnen-3β-ol *N,N*-Dimethylhydrazone (6) and 20-Oxo-5-pregnen-3β-ol Hydrazone (7). Following the Willey and Chang procedure,[31] pregnenolone (**1b**; Scheme 1) was reacted with 1,1-dimethylhydrazine to afford compound **6** at close to quantitative yield, mp 180−2 °C (lit.[31] mp 166 °C). ^{1}H-NMR ($CDCl_3$): δ 5.37 (1H, dd, 6-H), 3.52 (1H, m, 3α-H), 2.43 (6H, s, N-$(CH_3)_2$), 1.94 (3H, s, 21-CH_3), 1.01 (3H, s, 19-CH_3), 0.61 (3H, s, 18-CH_3).

Anhydrous hydrazine (1.02 g, 32 mmol) was added to a solution of the dimethylhydrazone **6** (358 mg, 1 mmol) in anhydrous ethanol (1 mL). The mixture was heated to reflux and maintained for 10 h. After cooling to room temperature, the mixture was poured into ice−water and the precipitate collected to give **7** (320 mg, 96%), mp 234−6 °C (from ethanol) (lit.[32] mp 205−15 °C). ^{1}H-NMR: δ 5.36 (1H, d, 6-H), 4.94 (2H, br s, NH_2), 3.53 (1H, m, 3α-H), 1.76 (3H, s, 21-CH_3), 1.01 (3H, s, 19-CH_3), 0.59 (3H, s, 18-CH_3).

20-Oxo-5,16-pregnadien-3β-ol *N,N*-Dimethylhydrazone (8). The mixture containing 16-ene pregnenolone (1 g, 3.2 mmol), 1,1-dimethylhydrazine (2 mL), ethanol (4 mL), and acetic acid (2 drops) was heated to reflux and then stirred overnight at room temperature. Water (30 mL) was added; the precipitate was collected by filtration and dried in vacuo to give 1 g of crude product. Recrystallization, first from methanol and then ethanol, gave 0.68 g (60%) of pure product with mp 167−9 °C. ^{1}H-NMR: δ 6.03 (1H, m, 16-H), 5.37 (1H, d, 6-H), 3.52 (1H, m, 3α-H), 2.48 (6H, s, N-$(CH_3)_2$), 2.02 (3H, s, 21-CH_3), 1.05 (3H, s, 19-CH_3), 0.97 (3H, s, 18-CH_3). Anal. ($C_{23}H_{36}NO$) C, H, N.

3-Oxo-4-pregnene-20β-carboxaldehyde 22-*N,N*-Dimethylhydrazone (9). The mixture containing 3-oxo-4-pregnene-20β-carboxaldehyde (**27**) (210 mg, 0.63 mmol), 1,1-dimethylhydrazine (60 μL, 0.79 mmol), and ethanol (4 mL) was stirred at room temperature for 3 h. Additional dimethylhydrazine (20 μL) was added, and the reaction was continued for another 1 h. Water (25 mL) was then added. The precipitate was collected by filtration and dried in vacuo to give 190 mg of crude product **9**. Recrystallization from methanol gave 68 mg (28.7%) of pure compound with mp 115−6 °C. ^{1}H-NMR: δ 6.51 (1H, d, 22-H), 5.72 (1H, s, 4-H), 2.69 (6H, s, N-$(CH_3)_2$), 1.18 (3H, s, 19-CH_3), 1.08 (3H, d, 21-CH_3), 0.75 (3H, s, 18-CH_3). Anal. ($C_{24}H_{38}N_2O$) C, H, N.

3-Oxo-4,17(20)-pregnadiene-20-carbonitrile (14). The mixture containing 20ξ-cyano-20ξ-hydroxy-4-pregnen-3-one (**13**; Scheme 1)[23] (0.4 g, 1.3 mmol), methanesulfonyl chloride (1 mL), and pyridine (5 mL) was stirred at room temperature for 5 h and then at 80 °C for 1 h. Water (20 mL) was added, and the precipitate was collected. The crude product was chromatographed on preparative silica gel TLC (developed with hexane:ethyl acetate, 1:1). Compound **14** was obtained as a white solid, 80 mg (19%), mp 195−6 °C (lit.[33] mp 203−5 °C). ^{1}H-NMR: δ 5.75 (1H, s, 4-H), 1.93 (3H, s, 21-CH_3), 1.20 (3H, s, 19-CH_3), 0.96 (3H, s, 18-CH_3).

3-Oxo-4-pregnene-20β-carbonitrile (16). The mixture of 22-oxime **15**[15] (400 mg, 1.23 mmol), methanesulfonyl chloride (0.5 mL), pyridine (0.5 mL), and methylene chloride (2 mL) was stirred and refluxed for 1.5 h. Water (1 mL) was added to destory the excess methanesulfonyl chloride. After removal of methylene chloride by concentration, water (20 mL) was added to the residue. The precipitates were collected and recrystallized from methanol to give **16** as needle crystals (0.18 g, 47.6%), mp 197−8 °C. ^{1}H-NMR: δ 5.73 (1H, s, 4-H), 2.66 (1H, m, 20-H), 1.35 (3H, d, 21-CH_3), 1.19 (3H, s, 19-CH_3), 0.78 (3H, s, 18-CH_3). Anal. ($C_{22}H_{31}NO$) C, H, N.

***N*-Methyl-17β-formamidoandrost-5-en-3β-ol (18) and *N*-Methyl-17β-formamidoandrost-4-en-3-one (19).** Following the procedure of Counsell et al.,[24] **18** was prepared from dehydroepiandrosterone (**17**) by reacting with *N*-methylformamide under reductive amination in formic acid, mp 225−7 °C (lit.[24] mp 218−20 °C). ^{1}H-NMR: 8.16 (1H, s, CHO), 5.35 (1H, d, 6-H), 3.53 (1H, m, 3-H), 2.91 (3H, s, 21-CH_3), 1.02 (3H, s, 19-CH_3), 0.74 (3H, s, 18-CH_3).

The solution of compound **18** (0.5 g, 1.5 mmol) in anhydrous toluene (20 mL) was heated to 80 °C. After ∼3 mL of toluene had distilled, cyclohexanone (6 mL) was added. Toluene (2 mL) was further removed; then aluminum isopropoxide (0.9 g) in toluene (6 mL) was added. The mixture was refluxed for 1 h. After cooling to room temperature, the mixture was washed with 2 N HCl twice and then steam distilled. The residue was recrystallized from ethyl acetate, and 120 mg (24%) of white solid was obtained, mp 143−4 °C. ^{1}H-NMR: 8.16 (1H, s, CHO), 5.74 (1H, s, 4-H), 2.91 (3H, s, N-CH_3), 1.20 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3). Anal. ($C_{21}H_{31}NO_2$) C, H, N.

The 20β-amino-5-pregnen-3β-ol (**4**) and 20α-amino-5-pregnen-3β-ol (**5**) were prepared from the procedure of van De Woude and van Hove;[19] 5,20-pregnadien-3β-ol (**11**) and 5-pregnen-20-yn-3β-ol (**12**) were prepared according to the procedures of Krubiner et al.[22] The structures of these compounds were confirmed by comparison of their melting points and ^{1}H-NMR spectra with the literature.

Biological Methods. [7-^{3}H]Pregnenolone (25 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and checked for purity and purified by TLC or HPLC prior to use when necessary. Ketoconazole was purchased from Sigma Chemical Co. Scintillation cocktail 3a70B was purchased from RPI Corp. (Mount Prospect, IL).

Human Testicular Microsomes. The previously reported procedure[14] was followed to prepare testicular microsomes from human testes (obtained from untreated prostatic cancer patients undergoing therapeutic orchidectomy in the University of Maryland Hospital and Veterans Hospital). 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.[34]

Measurement of Enzyme Activity: 17α-Hydroxylase/$C_{17,20}$-Lyase. Human testicular microsomes (ca. 90 μg of protein) were incubated with [7-^{3}H]pregnenolone (400 nM, 5 × 10^4 dpm), NADPH-generating system (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) under oxygen for 5 min at 34 °C. Authentic steroid markers and C^{14}-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). The radioactivity was measured in each fraction collected. The 17α-hydroxylase activity was determined from the percentage conversion of [7-^{3}H]pregnenolone to the total amount of 17α-hydroxypregnenolone and DHEA. The $C_{17,20}$-lyase activity was determined from the percentage conversion of pregnenolone to DHEA (the conversion of substrate to androstenediol and testosterone under the experimental conditions was negligible). The IC_{50} was calculated using a computer program for a nonlinear regression method (dose−effect analysis developed by J. Chou and T.-C. Chou, distributed by Elsevier-Biosoft (TM), 68 Hills Rd, Cambridge, U.K.). The correlation coefficients for the line fitting were greater than 95% for the compounds tested. The results were obtained from duplicate sets of experiments at four to five inhibitor concentrations and were repeated at least once. The results are listed in Table 1.

The kinetic study for this enzyme complex in our laboratory indicated that the K_m of 17α-hydroxylase was 102 nM and V_{max} was 0.0165 nmol/min/40 μg of protein and the K_m of the $C_{17,20}$-lyase was 61 nM and V_{max} was 0.0059 nmol/min/40 μg of protein.

Acknowledgment. We are grateful to Dr. Patrick Callery for his helpful discussion and for use of the ^{1}H-NMR, MS, and IR in the Department of Biomedicinal Chemistry, School of Pharmacy, UMAB. We thank Dr. Stephen Jacobs, Department of Surgery, UMAB, for supplying the tissues used in these biological experiments. Special thanks are due to Prof. Yangzhi Ling for his advice and assistance in preparing the manuscript.

References

(1) 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 $P450_{17α}$(Steroid 17α-hydroxylase/$C_{17,20}$Lyase). *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1571−1577.

(2) 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.

(3) Nakajin, S.; Takahashi, K.; Shinoda, M. Inhibitory effect and Interaction of Stanozolol with Pig Testicular Cytochrome P-450 (17α-hydroxylase/C17,20-Lyase). *Chem. Pharm. Bull. (Tokyo)* **1989**, *37*, 1855−1858.

(4) Nakajin, S.; Takahashi, K.; Shinoda, M. Inhibitory Effect and Spectral Changes on Pig Testicular Cytochrome P-450 (17α-hydroxylase/$C_{17,20}$-lyase) by 20β-Hydroxy-C21-steroids. *Yakugaku Zasshi* **1988**, *108*, 1188−1195.

(5) Jarman, M.; Barrie, S. E.; Deadman, J. J.; Houghton, J.; McCague, R. Hydroxyperfluoroazobenzenes: Novel Inhibitors of Enzymes of Androgen Biosynthesis. *J. Med. Chem.* **1990**, *33*, 2452−2455.

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

(7) Barrie, S. E.; Rowlands, M. G.; Foster, A. B.; Jarman, M. Inhibition of 17α-hydroxylase/$C_{17,20}$Lyase by bifluranol and its analogues. *J. Steroid Biochem.* **1989**, *33*, 1191−1195.

(8) Barrie, S. E.; Potter, G. A.; Goddard, P. M.; Haynes, B. P.; Dowsett, M.; Jarman, M. Pharmacology of Novel Steroidal Inhibitors of Cytochrome $P450_{17α}$ (17α-Hydroxylase/$C_{17,20}$ Lyase. *J. Steroid Biochem. Mol. Biol.* **1994**, *50*, 267−273.

(9) Rowlands, M. G.; Barrie, S. E.; Chan, F.; Houghton, J.; Jarman, M.; McCague, R.; Potter, G. A. Esters of 3-Pyridylacetic Acid that Combine Potent Inhibition of 17α-Hydroxylase/$C_{17,20}$-Lyase (Cytochrome $P450_{17α}$) with Resistance to Esterase hydrolysis. *J. Med. Chem.* **1995**, *38*, 4191−4197.

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

(11) Trachtenberg, J. Ketoconazole Therapy in Advanced Prostatic Cancer. *J. Urol.* **1984**, *132*, 61−63.

(12) Williams, G.; Kerle, D. J.; Doble, A.; Dunlop, H.; Smith, C.; Allen, J.; Yeo, T.; Bloom, S. R. Objective Responses to Ketoconazole Therapy in Patients with Relapsed Progressive Prostate Cancer. *Br. J. Urol.* **1986**, *58*, 45−51.

(13) Lake-bakaar, G.; Scheuer, P. J.; Sherlock, S. Hepatic Reactions Associated with Ketoconazole in the United Kingdom. *Br. Med. J.* **1987**, *294*, 419−422.

(14) Li, J.; Li, Y.; Son, C.; Banks, P.; Brodie, A. 4-Pregnene-3-one-20β-carboxaldehyde: a Potent Inhibitor of 17α-hydroxylase/$C_{17,20}$-Lyase and of 5α-Reductase. *J. Steroid Biochem. Mol. Biol.* **1992**, *42*, 313−320.

(15) 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.

(16) Corey, E. J.; Chaykovsky, M. Dimethyloxosulfonium Methylide ($(CH_3)_2SOCH_2$) and Dimethylsulfonium Methylide ($(CH_3)_2SCH_2$). Formation and Application to Organic Synthesis. *J. Am. Chem. Soc.* **1965**, *87*, 1353−1364.

(17) Cook, C. E.; Corley, R. C.; Wall, M. E. Steroids LXXVI (1). Stereospecific Formation of α- and β-epoxides in the Reaction of Dimethylsulfonium Methylide and Dimethylsulfoxonium Methylide with Dihydrotestosterone (2). *Tetrahedron Lett.* **1965**, 891−895.

(18) Sydykov, Z. S.; Segal, G. M. Synthesis of (20R)-3β-Acetoxy-Δ^5-Bisnorcholenal. *Izv. Akad. Nauk. SSSR, Ser. Khim.* **1975**, *11*, 2581−2584.

(19) van de Woude, G.; van Hove, L. Isolation and Identification of the Amines Resulting from Chemical and Catalytic Reduction of 20-Hydroxyimino-steroids (Restatement). *Bull. Soc. Chim. Belges* **1967**, *76*, 566−578.

(20) Newkome, G. R.; Fishel, D. L. Synthesis of Simple Hydrazones of Carbonyl Compounds by an Exchange Reaction. *J. Org. Chem.* **1966**, *31*, 677−681.

(21) Blout, E. R.; Gofstein, R. M. The Absorption Spectra of Certain Aldazines. *J. Am. Chem. Soc.* **1945**, *67*, 13.

(22) Krubiner, A. M.; Gottfried, N.; Oliveto, E. P. The Synthesis of 17-deoxy-17α- and 17β-20-pregnynes and -20-pregnenes. *J. Org. Chem.* **1969**, *34*, 3502−3505.

(23) Ercoli, A.; de Ruggieri, P. Corticosteroids, I. Conversion of Progesterone into Deoxycorticosterone Acetate. *Gazz. Chim. Ital.* **1954**, *84*, 312.

(24) Counsell, R. E.; Klimstra, P. O.; Ranney, R. E. Hypocholesterolemic Agents. III. N-methyl-N-(dialkylamino)alkyl-17β-Aminoandrost-5-en-3β-ol derivatives. *J. Med. Pharm. Chem.* **1962**, *5*, 1224−1234.

(25) Yanaihara, T.; Troen, P. Studies of the Human Testis. I. Biosynthetic Pathways for Androgen Formation in Human Testicular Tissue *in Vitro*. *J. Clin. Endocrinol. Metab.* **1972**, *34*, 783−792.

(26) Kominami, S.; Inoue, S.; Higuchi, A.; Takemori, S. Steroidgenesis in liposomal system containing adrenal microsomal Cytocrome P-450 electron Transfer Components. *Biochim. Biophys. Acta* **1989**, *985*, 293−299.

(27) Sheets, J. J.; Vickery, L. E. C-22-Substituted Steroid Derivatives as Substrate Analogues and Inhibitors of Cytochrome $P-450_{scc}$. *J. Biol. Chem.* **1983**, *258*, 1720−1725.

(28) Lovett, J. A.; Darby, M. V.; Counsell, R. E. Synthesis and Evaluation of 19-Aza- and 19-Aminoandrostenedione Analogues as Potential Aromatase Inhibitors. *J. Med. Chem.* **1984**, *27*, 734−740.

(29) Kohen, F.; Ranade, V. V.; Counsell, R. E. Hypocholesterolemic Agents. 9. C-20 Epimeric 22,25-Diazacholisterols. *J. Med. Chem.* **1972**, *15*, 1129−1131.

(30) Nakajin, S.; Shively, J. E.; Yuan, P.-M.; Hall, P. F. Microsomal Cytochrome P-450 from Neonatal Pig Testis: Activities (17α-Hydroxylase and $C_{17,20}$-Lyase) Associated with one Protein. *Biochemistry* **1981**, *20*, 4037−4042.

(31) Wiley, R. H.; Chang, S. H. Steroid Dimethylhydrazones. *J. Med. Chem.* **1963**, *6*, 610−611.

(32) Barton, D. H.; O'Brien, R. E.; Sternhell, S. A new Reaction of Hydrazones. *J. Chem. Soc.* **1962**, 470−476.

(33) Freerksen, R. W.; Gaggio, M. L.; Thoms, C. A.; Watt, D. S. Hydroxylation of αβ-Unsaturated Nitriles and Esters in Steroid Systems. *J. Org. Chem.* **1979**, *44*, 702−710.

(34) 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.

JM960245F