



Synthesis and In Vitro Activity of some Epimeric 20 α -Hydroxy, 20-Oxime and Aziridine Pregnene Derivatives as Inhibitors of Human 17 α -Hydroxylase/C_{17,20}-Lyase and 5 α -Reductase

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Abstract—Some epimeric 20-hydroxy, 20-oxime, 16 α , 17 α -, 17,20- and 20,21-aziridine derivatives of progesterone were synthesized and evaluated as inhibitors of human 17 α -hydroxylase/C_{17,20}-lyase (P450_{17 α}) and 5 α -reductase (5 α -R). The reduction of 16-dehydropregnenolone acetate (**3a**) was reinvestigated. NaBH₄ in the presence of CeCl₃ gave better stereoselectivity for 20 β -ol [20 α /20 β -OH (**4 α** /**4 β**) = 1/2.7] than LTBAH or the Meerwein–Ponndorf method reported; reduction with Zn in HOAc formed exclusively 20 α -ol (**4 α b**). The 20 α - and 20 β -hydroxy-4,16-pregnadien-3-one (**9 α**) and (**9 β**) were synthesized from the alcohols **4 α b** and **4 β b**. Several 20-oxime pregnadienes and 16 α ,17 α -, 17,20- and 20,21-aziridinyl-5-pregnene derivatives were also synthesized. LiAlH₄ reduction of the 16-en-20-oxime (**12b**) yielded 20 (*R*)-(**13a**) and 20(*S*)-17 α ,20-aziridine (**13b**) and 20(*R*)-17 β ,20-aziridine (**14a**). Several compounds inhibited the human P450_{17 α} with greater potency than ketoconazole. The 5 α -R enzyme assay showed that while (**9 α**) did not have any activity, (**9 β**) and (**3b**) were potent 5 α -reductase (IC₅₀ = 21 and 31 nM) inhibitors with activities similar to finasteride. The 20-oximes (**17a**) and (**17b**) were potent dual inhibitors for both 5 α -R (IC₅₀ = 63 and 115 nM, compared to 33 nM for finasteride) and P450_{17 α} (IC₅₀ = 43 and 25 nM, compared to 78 nM for ketoconazole). © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Androgens have been implicated in the development and progression of common disorders of the prostate, most notably, benign prostatic hypertrophy (BPH) and prostatic cancer. Two important enzymes in the biosynthesis of androgens are 17 α -hydroxylase/C_{17,20}-lyase (P450_{17 α}), which regulates an early step in the biosynthesis of testosterone (T) and other androgens in both the

testes and adrenal gland, and 5 α -reductase (5 α -R), which converts testosterone to the more potent androgen, dihydrotestosterone (DHT), in the prostate. Inhibitors of these enzymes have uses in the treatment of prostatic diseases. Recently, we and others have described a number of compounds that inhibit P450_{17 α} .^{1–11} Of these compounds, ketoconazole, an imidazole antifungal agent, is currently used to inhibit testosterone synthesis in patients with advanced prostatic cancer.^{12,13} However, this compound, which inhibits a number of other P450 enzymes, is not a very potent inhibitor of P450_{17 α} and is associated with significant side effects. Finasteride, an inhibitor of 5 α -R, has recently been introduced as a new treatment for BPH.¹⁴ Finasteride only reduces DHT levels by ~70% in these patients, but testosterone levels are often increased.¹⁵ Although this is not a problem for patients with BPH, it could result in growth stimulation of prostate cancers, since testosterone may bind to the androgen receptor, in the absence of DHT. Several recent studies have demonstrated the

Abbreviations: DHEA: dehydroisoandrosterone; A: androstenedione; T: testosterone; P450_{17 α} : 17 α -hydroxylase/C_{17,20}-lyase; 5 α -R: 5 α -reductase

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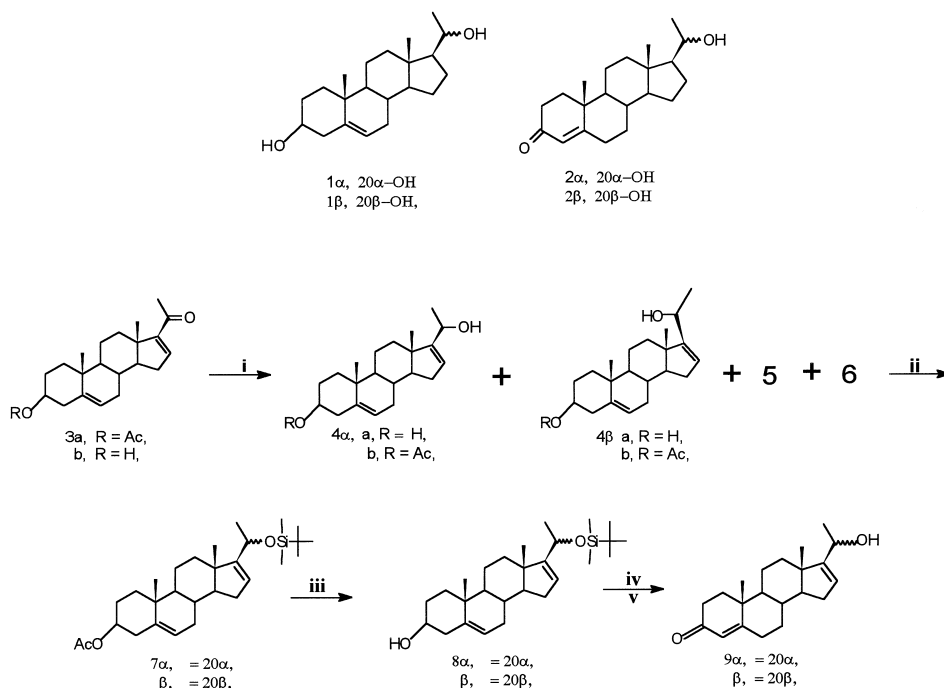
presence of mutations in the androgen receptor, which may be activated by anti-androgens. Clinical trials using an LHRH analogue (leuprolide) that inhibits testicular androgen production have demonstrated increased survival of patients when used in combination with radiation.¹⁶ These studies provide evidence of the value of hormonal therapy at least in patients with local recurrent disease. Therefore, the development of new types of potent enzyme inhibitors that inhibit P450_{17 α} , as well as the 5 α -R, could be effective in the treatment of prostatic cancer by achieving total androgen blockade.

Recent studies in our laboratory have identified several compounds that inhibit both P450_{17 α} and 5 α -R.^{8,9} Such compounds could block all androgen biosynthesis (T, DHT and androstenedione) and could be more effective as alternatives or additions to orchidectomy in treating prostate cancer patients. We previously reported that epimeric 20-hydroxy pregnane derivative (**1 α** and **1 β** ; **2 α** and **2 β**) demonstrate mild to moderate inhibition of the P450_{17 α} ¹⁰ (Scheme 1). 5,16-Pregnadien-3-ol-20-oxime (**12a**) is a potent inhibitor (IC₅₀ = 73 nM) for P450_{17 α} but showed no activity against 5 α -R. From our experience,¹⁰ the introduction of a 16,17-double bond into the steroid nucleus enhances the activity of the compound as a P450_{17 α} inhibitor, whereas the basic steroid 4-en-3-one structure is better than the 5-en-3 β -ol for activity against 5 α -R. We now report the activity of some epimeric

20-hydroxy, 20-oxime pregnadienes and also aziridine derivatives, as androgen synthesis inhibitors. Some of these compounds are also very potent dual inhibitors of P450_{17 α} and 5 α -R.

Chemistry. We have examined the reduction of 16-DPA(**3a**) by several reagents and solvent combinations. The 3 β ,20 β -diol (**4 β a**) was prepared by the Meerwein–Pondroff reduction of 16-DPA as described by Marker et al.¹⁷ The reduction product actually consisted of 20 α - and 20 β -epimers in a ratio of 1:1.14, as determined by the integration of their 18-methyl signal.²¹ Pure 20 β -ol (**4 β a**) was obtained after several crystallizations.

Because the 3-acetate group was needed as a protecting group to synthesize the 4-en-3-one derivative (**9**) (Scheme 1), the reduction time with aluminum isopropoxide was reduced. This led only to the formation of the hydrolysis product 3-ol (**3b**) (due to the ester exchange in 2-propanol) together with 20-ol (**4 α a** and **4 β a**). When 16-DPA was reduced with LTBAH,¹⁸ allylic 20-ol (**4 α b** and **4 β b**) with 20 α /20 β ratio of 1.41/1 was formed together with 16-saturated products (34%), pregnenolone 3-acetate (**5**) and its 20 β -ol (**6**). Reduction with sodium borohydride in methanol in the presence of cerium chloride¹⁹ gave exclusively the allylic alcohol (**4 α b** and **4 β b**) in a ratio of 1/2.7, from which pure 20 β -ol-3-acetate (**4 β b**) was obtained by recrystallization. The



Scheme 1. (i) CeCl₃, NaBH₄, MeOH; or Zn, HOAc; or LTBAH, THF; or Al(*i*-PrO)₃; (ii) *t*-BuMe₂SiCl, imidazole, DMF; (iii) KOH, MeOH; (iv) PTS, acetone; (v) Al(*i*-PrO)₃, cyclohexanone, toluene. (5) Pregnenolone 3-acetate; (6) pregnen-3 β ,20 β -diol.

20 β -hydroxy group was then protected as the *t*-butyldimethylsilyl ether (**7 β**).²⁰ After selective hydrolysis of the 3-acetate group in base, the 3-ol (**8 β**) was converted to the 4-en-3-one by Oppenauer oxidation with cyclohexanone in toluene. Cleavage of the 20-silyl ether using the conventional method with fluoride anion²⁰ was ineffective. This was successfully performed with PTS in acetone to give the pure 20 β -ol (**9 β**).

Synthesis of the 20 α -ol counterpart was attempted using LiAlH₄ in ether or THF. The reaction produced a molecular complex of the epimeric 20 α - and 20 β -diols which was difficult to separate, together with considerable 16-saturated product.^{21,22} Ercoli and Ruggieri²³ reported that treatment of 16-DPA with zinc and acetic acid reduced predominantly the 16,17 double bond. The allylic 20 α -ol (**4 α**) was obtained from the nonketonic residue by acetylation and chromatography. Complete conversion of the 16-DPA was achieved by doubling the amount of zinc and extending the reaction time. Surprisingly, the 20 α -ol (**4 α b**) was obtained as the sole product as evidenced by its 18-methyl signal at 0.9 ppm and a clean doublet for the C-21 methyl group seen at 1.33 ppm ($J=6.4-4$ Hz).²¹ The 16-ene group must play an important part in this stereoselective reduction, as its 16-saturated counterpart pregnenolone, was not reduced under the same conditions. Hydrolysis in base gave 3 β ,20 α -diol (**4 α a**). The 20 α -hydroxy-4,16-pregnadiene-3-one (**9 α**) was prepared from the 3-acetate (**4 α b**), following the same procedure described above for **4 β b** (Scheme 1).

The 20-oxime (**10**) was reduced with LiAlH₄ in refluxing TEF, and yielded mainly the epimeric 20,21 ε -aziridines (**11**), which consisted of about 1:1 epimers.²⁴ As the mixture (**11**) showed only moderate activity against human P450_{17 α} (see below), no further effort was made to separate the individual isomers. The reduction of 16-en-20-oxime (**12b**) with LiAlH₄ yielded 17 α ,20-aziridine (**13**) and 17 β ,20-aziridine (**14**). NMR studies showed that 17 α ,20-aziridine (**13**) actually consisted of a mixture of two isomers (**13a** and **13b**), with an approximate ratio of 1/2, which were separated by repeated chromatography. Similar findings with the $\Delta^{4,6}$ oxime steroid have been reported.²⁵ In the major isomers 20(*S*)-methyl-(**13b**), NOEs are observed from the C-13 methyl protons to both H-20 (0.9%) and the C-20 methyl proton (4.6%), suggesting that C-20 is on the β -face of the steroid. The relative sizes of these NOE suggest that the C-20 methyl is oriented towards the C-13 methyl, or *S* stereochemistry at C-20. The reciprocal experiment involving irradiation of the C-20 methyl protons was consistent with the above conclusions. In the minor isomer 20(*R*)-methyl-(**13a**), a substantial (5.2%) NOE is observed from the C-13 methyl protons to H-20, but no NOE could be observed to the C-20 methyl protons.

These findings are consistent with a β -orientation for C-20 and *R* stereochemistry at C-20. In 20(*R*)-17 β ,20-aziridine (**14a**), no NOE could be observed from the C-13 methyl proton to H-20, and only a relatively small (2.1%) NOE was observed to the C-20 methyl protons. The NOE observed from the C-20 methyl protons to the C-13 methyl protons was likewise relatively small. These data are consistent with an α -orientation of C-20 and *R* stereochemistry at C-20. The 20(*S*)-isomer (**14b**), which was previously synthesized by the hydrogenation of the corresponding azirine,²⁶ was not found. The ¹³C NMR of compounds **13a**, **13b**, and **14a** were assigned by COSY and HSQC experiments on an AMX500 spectrometer. The procedure of Drefohl²⁷ was followed to synthesize the 16 α ,17 α -aziridine (**16**). 16-DPA (**3a**) reacted with free methoxylamine* and gave the Michael adduct 16 α -methoxyamine (**15**),^{27a} which was then treated with base to give 16 α , 17 α -aziridine.^{27b} (*It is important to prepare free methoxylamine base from its HCl salt and distill out first, otherwise 20-methoxime (**12d**) will be invariably obtained.) Because the 21-methyl signal of (**16**) appeared as a low broad singlet overlapped with other proton signals and was hardly observed, COSY and HSQC experiments were carried out to confirm the structure. The ¹³C NMR of compound **15** and **16** are assigned.

Because 5,16-pregnadien-3-ol-20-oxime (**12a**) demonstrated potent inhibition of P450_{17 α} , derivatives were synthesized. Oppenauer oxidation of **12a** in toluene with cyclohexanone and aluminum isopropoxide gave the 4-en-3-one (**17a**). Acetylation of **17a** yielded the oxime acetate (**17b**); similarly, 3-acetate (**12b**) gave the diacetate (**12c**). Although 16-DPA reacted with methoxylamine to give the Michael addition product, 16 α -methoxylamino 20-methoxime (**12d**) was obtained when reacted with methoxylamine hydrochloride. Dehydroprogesterone (**18**) was treated with hydroxylamine, and produced 3(*E*)-(**19a**) and 3(*Z*)-oxime (**19b**) which were separated by chromatography. The determination of the configuration of the *Z* and *E* isomers was based on their 4-H chemical shift. In the 3(*Z*)-oxime (**19b**), the 4-H is shift downfield (6.47 ppm) compares to the 4-H in 3(*E*)-oxime (**19a**) (5.77 ppm). Apparently, in the 3(*Z*)-oxime isomer, the 4 vinyl proton is close to the electron-withdrawing oxygen atom of the oxime group, which makes it less shielding compared to the 4 vinyl proton in the 3(*E*)-isomer. This assignment is in agreement with cases reported in the literature.²⁸

Synthesis of the 21-nor oxime was also explored (Scheme 3). 16 α , 17 α -Epoxy (**20**) was treated with CrCl₂ to form the 16-ene (**21**).²⁹ Reduction with LiAlH₄ gave the diol (**22**), which was cleaved with sodium periodate to give the 17-aldehyde (**23**). Condensation with hydroxylamine gave the 21-nor oxime (**24**). Oppenauer oxidation gave the corresponding 4-en-3-one (**25**).

Inhibitor studies

Human testicular microsomes were employed to evaluate the potency of pregnene derivatives as inhibitors of P450_{17 α} as previously described.^{8–11} Two methods were used to measure IC₅₀ values and both methods gave similar results. For some compounds, the radiometric assay was used in which [21³H]-17 α -hydroxy-pregnenolone was the substrate for the human enzyme. Inhibition was assessed by measuring the release of [³H]-acetic acid during cleavage of the C-21 side chain in the conversion to DHEA. Thus, the IC₅₀ values assayed by this method are for C_{17,21}-lyase. For other compounds, the IC₅₀ values were determined using product isolation. Conversion of radiolabeled pregnenolone to 17 α -hydroxy-pregnenolone and DHEA by P450_{17 α} was measured in the presence of several concentrations of candidate inhibitors. Reverse-phase HPLC was employed to separate and measure the amount of substrate and metabolites.⁹ IC₅₀ assays were performed at least twice and up to four times for the more potent inhibitors. The mean values are shown in Table 1. As rodent models are most frequently used for in vivo studies, we determined inhibition of the conversion of [21³H]-17 α -hydroxy-pregnenolone by the compounds in rat testicular microsomes.

Inhibition of the 5 α -R in human prostatic tissue was measured from the conversion of radiolabeled testosterone to DHT following purification and isolation by TLC using an assay similar to that reported earlier.⁸

Structure–activity relationships

The inhibitory activities of the 20-hydroxy epimers are compared in Table 1. In the 5-pregnene-3 β -ol series, the 20 β -ol (**1 β**) was found previously to be a more potent inhibitor of P450_{17 α} (IC₅₀ = 180/190 nM) than the 20 α -ol (**1 α**) epimer (720/510 nM). As expected, both showed no activity against 5 α -R. However, 20 α -ol of 4-en-3-one (**2 α**) is a potent 5 α -R inhibitor (13.8 nM) and is stronger than its 20 β -ol epimer (**2 β**) (90 nM). The introduction of the 16,17-double bond gave the 20 α -ol (**4 α a**) and 20 β -ol (**4 β a**), which had about equal activity for P450_{17 α} (250/220 nM). While 20 β -hydroxy-4,16-pregnadien-3-ol (**9 β**) is a potent 5 α -R inhibitor (21 nM) comparable to finasteride (33 nM), its epimer 20 α -ol (**9 α**) showed no activity at all. The marked influence on the activity of the isomeric configuration at the C-20 position, together with the 16,17-ene suggest that this area interacts with the active site of the enzyme which has strict steric requirements. Because the 20 β -ol (**9 β**) could be metabolized to the 20-one in vivo, dehydropregesterone (**18**) was also tested. Compound **18** was found to be a relatively weak inhibitor of P450_{17 α} , but was a moderately good inhibitor of 5 α -R (96 nM). Progesterone is known to be a

potent 5 α -R inhibitor, but its rapid metabolism in the body and lack of oral activity, detracts from its value as a therapeutic agent.³⁰ However, because **9 β** and **18** both have a 16,17-double bond, their 17 β -acetyl side chain should be more difficult to degrade in vivo.

Pregnenolone-20-oxime (**10**) showed moderate potency (530/570 nM), while the 16-en-20-oxime (**12a**) is a good P450_{17 α} inhibitor (73 nM) and its 4-en-3-one derivative, 16-dehydropregesterone-20-oxime (**17a**), not only showed greater activity (43 nM) against human P450_{17 α} , but also showed potent activity against 5 α -R (63 nM). It was quite unexpected to find that (**17a**) is similar in potency to its 5-en-3-ol (**12a**) counterpart as human P450_{17 α} enzyme prefers pregnenolone (i.e., 5-en-3-ol) rather than progesterone (i.e., 4-en-3-one) as substrate. Comparison of the weak activity of 16-dehydropregesterone (**18**) with its 20-oxime (**17a**) (43 nM), and the activity of 16-dehydropregnenolone (**3b**) (510/490 nM) with its 20-oxime (**12a**) (73 nM), shows that the introduction of a 20-oxime group markedly increased the inhibitory effect for P450_{17 α} .

Acetylation of the 20-oxime (**17b**) increased the inhibitory activity against human P450_{17 α} and maintained activity against 5 α -reductase, compared to **17a**. The compound **12d**, the 20-methoxy of the oxime (**12a**) had no activity at all. The 21-nor oxime (**24**) had little inhibition for P450_{17 α} , suggesting that 20-methyl group is also critical for maintaining activity.

The synthesis of 16,17 α -(**16**), 17,20-(**13** and **14**), and 20,21-aziridindyl (**11**) derivatives originated from the idea that the aziridine nitrogen might show strong coordination to the heme iron of the enzyme.³¹ Additionally, the highly reactive aziridine ring might react covalently at the active site of the enzyme, resulting in irreversible enzyme inhibition, as was shown for inhibition of aromatase by a 10 β -aziridinyl steroid.³¹ However, the results were rather disappointing, as the 20,21-aziridine (**11**) showed only weak inhibition for the human P450_{17 α} . Njar et al. recently reported that 20(S)- and 20(R)-20,21-aziridine were potent inhibitors of the rat testicular P450_{17 α} enzyme.³² Indeed, we did find that epimeric 20,21 ϵ -aziridine (**11**) is a more potent inhibitor of rat enzyme (IC₅₀ = 184 nM, ketoconazole 2096 mM).

In summary, we have found that **17a** and **17b** are potent 5 α -R inhibitors, and are also very potent human P450_{17 α} inhibitors. We believe that 16-dehydropregesterone-20-oxime (**17a**) is the most potent dual inhibitor of these enzymes. Other potent inhibitors of 5 α -R were **2 α** , **3b**, and **9 β** . Recently, Potter et al. reported that the most potent inhibitor of human P450_{17 α} in their series was 17-(3-pyridyl) androst-5,16-diene-3-ol.⁷

Table 1. IC₅₀ of steroid inhibitors of human and rat testicular P450 17 α -hydroxylase/C_{17,20}-lyase and human 5 α -reductase

	Human			Rat	
	17 α -Hydroxylase/lyase ^a C _{17,20} -lyase		5 α -Reductase	C _{17,20} -lyase	
	% Inhibit. at 150 nM	IC ₅₀ (nM)	IC ₅₀ (nM)	(1500 nM, %)	IC ₅₀ (nM)
1α	16.4	720/510 ^{a,b}	NI ^c	21.1	
1β	13.4	180/190 ^{a,b}	NI	26.6	
2α	8.4	1547/788 ^a	13.8	37.9	
2β	23.3	204/143 ^a	90.2	73.7	300
3b	7.2	510/490 ^{a,b}	31.3	22.3	
4αa	2.0	250/220 ^a	NT ^d	4.8	
4βa	0.4	255/215 ^a	NT	8.7	
9α	15.5	Weak ^a	NI	58.0	
9β	16.8	NI ^a	21	80.6	
10	10.5	10	NT	55.0	
11	19.4	Weak ^a	NT	98.6	184
12a	80.3	73	NT	63.1	1300
12b	61.1	67	NI	57.3	2754
12c	73.1	51	NI	63.0	
12d	0.4	NI	NT	NI	
13a	34.8	339	NT	63.5	
13b	37.3	372	NT	72.3	
14b	6.5	NI	NT	24.5	
15	7.0	NI	NT	NI	
16	41.9	162	NT	24.5	
17a	76.7	43	63	91.7	48
17b	83.8	25	115	96.3	54
18	12.4	Weak	95.8	64.0	
19a	46.0	196	1270	67.7	1100
19b	79.7	75	Weak	59.2	1300
24	25.7	Weak	NT	33.8	
25	19.6	Weak	NT	41.1	
Ketoconazole	69.5	78	NI	65.3	900
Finasteride	NI	NI	33		

^aValues are for 17 α -hydroxylase/C_{17,20}-lyase. Compounds **1 α** –**11** were incubated with [³H] pregnenolone and cofactors for 5 min and products analyzed by HPLC. Inhibition of testicular 17 α -hydroxylase/lyase was determined from the conversion radiolabeled pregnenolone to DHEA in the presence of the test compounds. All other assays were performed by incubation with [21³H] 17 α -hydroxy-pregnenolone for 60 min at 34 °C with cofactors and test compounds. C_{17,20}-lyase activity was determined by measuring [³H] acetic acid liberated during cleavage at the C-17. Compounds were screened at 150 nM with human testicular microsomes and at 1500 nM with rat microsomes. IC₅₀ values were determined over a range of six concentrations. Values are averages of 3–5 repeat experiments. The variation in repeat values were usually <15% of the mean IC₅₀ values. Inhibition of 5 α -reductase was determined from the conversion of testosterone to DHT in the presence of test compounds. Human prostatic, microsomes were incubated with [³H] testosterone and cofactors for 10 min at 37 °C. The DHT produced was purified by TLC.

^bResults previously reported in ref. 10.

^cNI, no inhibition.

^dNT, not tested.

However, **17a** is also a potent inhibitor of 5 α -R. As the IC₅₀ of ketoconazole in their assay was 65/26 nM, its potency appears to be similar to **17a**. However, **17a** is also a potent inhibitor of 5 α -R, whereas the former showed no activity against this enzyme. These dual inhibitory activities of **17 α** and **17 β** suggests that they have potential for the treatment of prostate cancer. Further studies are under investigation in biological systems.

Experimental

Chemical methods. ¹H NMR data (300 MHz) (internal Me₄Si = δ 0) (QE 300, NMR systems, General Electric Co.) were recorded in CDCl₃ unless otherwise stated. Reactions were monitored by TLC on silica gel plates (Merck Type 601-1), and components 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 bp 35–60°C. Solutions were dried using anhydrous Na₂SO₄. Melting points were measured on a Fischer–Johns Melting Point apparatus and are uncorrected.

Reduction of 16-dehydropregnenolone-3-acetate (16-DPA) (3a)

Aluminum isopropoxide. 16-DPA (0.5 g, 1.4 mmol) was reduced with aluminum isopropoxide (2.5 g, 12.38 mmol) and dry iso-propanol (70 mL) as reported.¹⁷ The crude product (450 mg) obtained consisted of the 20ε-hydroxy epimer, (4αa and 4βa) (20α/20β, 1/1.14) as determined by integration of their 18-methyl signal at 0.91 and 0.87 ppm, respectively. Recrystallization from MeOH, and then EtOAc-LP, gave pure 20β-ol (4βa) (102 mg, 20%) as fine needle crystals, mp 168–170°C (lit. 169–171°C,¹⁷ or 169.5–171.5°C).²¹

When the reaction was refluxed for only 3 h, worked-up as above, and the residue flash chromatographed on silica gel, elution with 15% acetone-LP, gave 16-dehydropregnenolone (3b) (210 mg), mp 209–211°C (acetone/LP) (lit.²⁸ 212–214°C), and 20ε-ol (4αa and 4βa) (230 mg), mp 162–168°C (from MeOH).

Lithium tri-*t*-butoxyaluminum hydride (LTBAH). A solution of 1 M LTBAH in THF (3 mL, 3 mmol) was added dropwise to a stirred solution of 16-DPA (356 mg, 1 mmol) in dry THF (10 mL) in an ice-bath and then stirred for 16 h. CH₂Cl₂ (100 mL) was added and the whole mixture was washed with 5% HCl followed with water. After evaporation of the organic layer, the residue was flash chromatographed on silica gel, eluted with 7.5% acetone-LP, to give pregnenolone-3-acetate (5) (50 mg, 14%), mp 144–147°C (acetone), (lit.^{23a} 146–147°C) and 5-pregnene-3β,20β-diol (6) (70 mg, 20%), mp 160–164°C (from ether) (lit.³³ mp 160–164°C), and 20ε-ol (4b) (20α/β, 1.41/1) (180 mg, 51%), two crystallizations from acetone-LP, gave pure 20β-ol (4βb) (15 mg), mp 144–147°C (lit.^{23b} 145–146°C).

Sodium borohydride in the presence of cerium trichloride. A solution of 16-DPA (356 mg, 1 mmol) in CH₂Cl₂ (3 mL) was combined with a solution of CeCl₃·7H₂O (372 mg, 1 mmol) in methanol (8 mL); NaBH₄ (60 mg, 1.5 mmol) was added at once in one portion. The mixture was stirred at 25°C for 15 min, then worked-up as above, to give 20ε-ol (4αb and 4βb) (310 mg) (20α/20β 1/2.7). Recrystallization from acetone-LP gave pure 20β-ol (4βb) (143 mg, 40%), mp 144–147°C, (lit.^{23b} 145–146°C). ¹H NMR δ: 5.64 (3H, s, 16-H), 5.39 (1H, d, *J*=4.9 Hz, 6-H), 4.61 (1H, m, 3-H), 4.38 (1H, m, 20-H), 2.03 (3H, s, 3-OAc), 1.37 (3H, d, *J*=6.3 Hz, 20-Me), 1.06 (3H, s, 19-Me), 0.87 (3H, s, 18-Me).

Zn in glacial HOAc. 16-DPA (1 g) and zinc dust (5 g), in glacial acetic acid (50 mL), was stirred vigorously at 25°C until no starting material was present on TLC (16 h). The whole mixture was filtered and the Zn precipitate washed with CH₂Cl₂ (200 mL). The filtrate was washed with water, 5% aqueous KHCO₃ and then water. Evaporation of the solvent gave a solid residue (0.98 g), ¹H NMR showed that it was solely 20α-ol (4αb). Recrystallization from acetone-LP gave (4αb), (607 mg, 60%), mp 125–129°C. Part of the sample (50 mg) was recrystallized again, to give an analytical sample (35 mg), mp 130–132°C (lit.²³ mp 98–99°C). ¹H NMR δ: 5.66 (1H, s, 16-H), 5.39 (1H, d, *J*=4.9 Hz, 6-H), 4.61 (1H, m, 3-H), 4.36 (1H, m, 20-H), 2.04 (3H, s, 3-OAc), 1.33 (3H, d, *J*=6.4 Hz, 20-Me), 1.06 (3H, s, 19-Me), 0.91 (3H, s, 18-Me). Anal. (C₂₃H₃₄O₃)C,H.

5,16-Pregadiene-3β,20α-diol (4αa). The 3-acetate (4αb) (300 mg) was stirred in 0.5 M KOH/MeOH (10 mL) at 25°C for 1 h. CH₂Cl₂ (100 mL) was added and the mixture was washed with water. After evaporation of the solvent, the residue was immediately recrystallized from acetone-LP, to give 4αa (105 mg, 35%), mp 185–186°C (lit.²¹ 186.5–188.5°C).

20α-[(*tert*-Butyldimethylsilyl)oxy]-(7α) and 20β-[(*tert*-butyldimethylsilyl)oxy]-5,16-pregadiene-3β-ol-3-acetate (7β). To a solution of 20α-ol (4αb) (2.0 g, 5.56 mmol) in DMF (25 mL) were added *tert*-butyldimethylsilyl chloride (1.68 g, 11.2 mmol) and imidazole (1.51 g, 22.2 mmol) and the mixture stirred at 25°C for 14 h (TLC). The reaction mixture was diluted with ether and washed with water. After evaporation, the residue was recrystallized from Et₂O-acetone, to give (7α) (1.20 g, 46%), mp 130–132°C. ¹H NMR δ: 5.58 (1H, s, 16-H), 5.39 (1H, d, *J*=4.9 Hz, 6-H), 4.60 (1H, m, 3a-H), 4.31 (1H, m, 20-H), 2.04 (3H, s, 3-OAc), 1.25 (3H, d, *J*=6.3 Hz, 20-Me), 1.06 (3H, s, 19-Me), 0.90 (9H, s, -CMe₃), 0.88 (3H, s, 18-Me), 0.05, 0.03 (each 3H, s, SiMe₂). Anal. (C₂₉H₄₈O₃Si) C,H. Following the procedure above, the 20β-ol (4βb) (2.0 g, 5.56 mmol), was converted to the silyl ether (7β) (1.8 g) which was obtained as an oil and used directly in the next reaction.

20α-[(*tert*-Butyldimethylsilyl)oxy]-(8α) and 20β-[(*tert*-butyldimethylsilyl)oxy]-5,16-pregadiene-3β-ol (8β). The 3-acetate (7β) (oil, 1.8 g) was stirred in 0.5 M KOH-MeOH (30 mL) for 1 h. Ether (200 mL) was added and the whole mixture was washed with water. After evaporation, the residue was recrystallized from Et₂O-LP to give (8β) (1.2 g, 66%), mp 120–122°C. ¹H NMR δ: 5.53 (1H, s, 16-H), 5.35 (1H, d, *J*=4.2 Hz, 6-H), 4.40 (1H, m, 20-H), 3.49 (1H, m, 3-H), 1.25 (3H, d, *J*=6.4 Hz, 20-Me), 1.02 (3H, s, 19-Me), 0.87 (12H, s, 18-Me, -CMe₃) 0.03, 0.02 (each 3H, s, SiMe₂). Anal. (C₂₇H₄₅O₂Si) C,H.

Following the procedure described above, the 3-acetate (**7α**) (1 g) was converted to (**8α**) (0.65 g, 71%), mp 126–128 °C (from MeOH). ¹H NMR δ: 5.36 (1H, s, 16-H), 5.35 (1H, d, *J* = 4.9 Hz, 6-H), 4.30 (1H, m, 20-H), 3.50 (1H, m, 3-H), 1.23 (3H, d, *J* = 6.3 Hz, 20-Me), 1.02 (3H, s, 19-Me), 0.87 (9H, s, -CMe₃), 0.85 (3H, s, 18-Me), 0.03, 0.01 (each 3H, s, SiMe₂). Anal. (C₂₇H₄₅O₂Si) C, H.

20α-Hydroxy-(9α) and 20β-hydroxy-4,16-pregnadiene-3-one (9β). From a solution of 20α-silyl ether (**8α**) (500 mg, 1.16 mmol) in toluene (50 mL) and cyclohexanone (3 mL), part of the solvent (10 mL) was distilled off to eliminate the moisture. Aluminum isopropoxide (204 mg, 1.0 mmol) was added and the mixture refluxed for 3 h or until TLC showed that the reaction was complete. Saturated sodium tartrate (30 mL) was added and the mixture steam distilled until no more toluene and cyclohexanone could be collected. The remaining residue was extracted with EtOAc. After evaporation of the organic layer, the residue was taken up with acetone (20 mL) and treated with *p*-toluenesulfonic acid (300 mg) for 14 h at 25 °C. CH₂Cl₂ (200 mL) was added and the mixture washed with water. After evaporating the solvent, the residue was flash chromatographed on silica gel. Elution with 15% acetone/LP, gave the 20α-ol (**9α**) (190 mg, 41%), mp 146–149 °C (Et₂O-LP). ¹H NMR δ: 5.74 (1H, s, 4-H), 5.32 (1H, m, 16-H), 4.78 (1H, d, 8.5 Hz, 20-H), 1.74 (3H, d, *J* = 6.8 Hz, 20-Me), 1.21 (3H, s, 19-Me), 1.00 (3H, s, 18-Me), 0.79 (1H, s, -OH). Anal. (C₂₁H₃₀O₂) C, H.

Following the procedure described above, 20β-silyl ether (**8β**) (500 mg) gave 4-en-3-one (**9β**) (201 mg, 55%), mp 179–180 °C (acetone-LP). ¹H NMR δ: 5.74 (1H, s, 4H), 5.64 (1H, m, 16-H), 4.38 (1H, brs, 20-H), 1.37 (1H, d, *J* = 6.3 Hz, 20-Me), 1.22 (3H, s, 19-Me), 0.89 (3H, s, 18-Me). Anal. (C₂₁H₃₀O₂) C, H.

20,21ε-Aziridinyl-5-pregnene-3β-ol (11). Following the same procedure described below for **13**, the 20-oxime (**10**)³⁴ (300 mg, 0.9 mmol) was reduced with LiAlH₄ (500 mg, 13.2 mmol). The crude product was chromatographed on silica gel and eluted with 5% MeOH-CH₂Cl₂, to give **11** (140 mg, 49%), mp 210–216 °C (from acetone-LP). (lit.²⁴ 20β,21-aziridine, mp 199–202 °C; 20α,21-aziridine, mp 195–198 °C.) Anal. (C₂₁H₃₃ON) C, H, N.

3β-Acetoxy-5,16-pregnadiene-20-oxime acetate (12c). Following the same procedure described below for **17b**, the 3-acetate (**12b**)³⁴ (100 mg) was acetylated, to give the oxime acetate (**12c**) (80 mg, 78%), mp 194–196 °C. (From acetone/LP) (lit.³⁵ mp, 195–196 °C.)

3β-Hydroxy-5,16-pregnadiene-20-methoxime (12d). The 16-en-20-one (**3b**) (100 mg, 0.32 mmol), methoxylamine

hydrochloride (40 mg, 0.48 mmol), ethanol (1 mL) and pyridine (0.3 mL) was refluxed for 1 h. Water was added and the precipitate was collected, recrystallized from acetone-LP to afford (**12d**) (35 mg, 32%), mp 136–139 °C. ¹H NMR δ: 6.00 (1H, s, 16-H), 5.36 (1H, s, 6-H), 3.88 (3H, s, -OMe), 2.53 (1H, m, 3α-H), 1.93 (3H, s, 20-Me), 1.05 (3H, s, 19-Me), 0.97 (3H, s, 18-Me), anal. (C₂₂H₃₃O₂N) C, H, N.

20(R)-17α,20-Aziridinyl-(13a), 20(S)-17 α,20-aziridinyl-(13b), and 20(R)-17β,20-aziridinyl-5-pregnene-3β-ol (14a). A solution of the 20-oxime (**12b**)³⁴ (1.5 g, 4.0 mmol) in THF (50 mL) was added to a stirring solution of LiAlH₄ (2.0 g, 40 mmol) in THF (100 mL) containing *N*-methyl-*N*-butylamine (0.5 mL) cooled in ice-water and under nitrogen. The mixture was then refluxed under nitrogen for 2 days (TLC monitor). The excess LiAlH₄ was decomposed by adding aqueous THF (150 mL). The precipitated inorganic salt was filtered and washed with 5% MeOH-EtOAc (200 mL). After the solvents were evaporated, the residue was flash chromatographed, eluted with 25% acetone-LP, and gave a mixture of **13a** and **13b** (340 mg, 27%) in a ratio of 1:2 as determined by their 18-methyl signal, mp 191–195 °C (from acetone-LP), and then **14a** (290 mg, 23%), mp 186–189 °C (from acetone). ¹H NMR (CD₃OD) δ: 5.36 (1H, s, 6-H), 3.38 (1H, m, 3α-H), 1.33 (3H, d, *J* = 6.3 Hz, 20-Me), 1.02 (3H, s, 19-Me), 0.95 (3H, s, 18-Me). Anal. (C₂₁H₃₃O₂N) C, H, N.

The mixture of **13a** and **13b** (170 mg) was twice rechromatographed, eluted with 3% and 5% MeOH/-CH₂Cl₂, to give pure 20(R)-(**13a**) (20 mg, 1.6%), mp 197–201 °C (from ether) (¹H NMR (CD₃OD) δ: 5.35 (1H, d, *J* = 3.9 Hz, 6-H), 3.30 (1H, m, 3α-H), 1.19 (3H, d, *J* = 5.4 Hz, 20-Me), 1.03 (3H, s, 19-Me), 0.85 (3H, s, 18-Me)), and then 20 (S)-(**13b**) (90 mg, 14%), mp 186–189 °C (from acetone). ¹H NMR δ 5.35 (1H, s, 6-H), 3.40 (1H, m, 3α-H), 1.26 (3H, d, *J* = 5.9 Hz, 20-Me), 1.02 (3H, s, 19-Me), 0.94 (3H, s, 18-Me). Anal. (C₂₁H₃₃O₂N) C, H, N.

The ¹³C NMR of compounds **13a**, **13b**, and **14a** were assigned by COSY and HSQC experiments on an AMX500 spectrometer.

3β-Hydroxy-16α-methoxyaminopregna-5,16-dien-20-one (15) (mp 136–140 °C, lit.^{27a} mp 140–142 °C) and **16α,17α-aziridinyl-3β-hydroxy-5-pregnen-20-one (16)** (mp 185–187 °C, lit.^{27b} mp 186–188 °C). These were prepared according to ref. 27 compound **15**: ¹H NMR δ 5.35 (1H, d, *J* = 2.9 Hz, 6-H), 4.14 (1H, m, 16β-H), 3.53 (1H, m, 3α-H), 3.49 (3H, s, OMe), 2.48 (1H, d, *J* = 7.4 Hz, 17α-H), 2.17 (3H, s, 21-Me), 1.01 (3H, s, 19-Me), 0.69 (3H, s, 18-Me). Compound **16**: ¹H NMR δ 5.33 (1H, d, *J* = 4.9 Hz, 6-H), 3.52 (1H, m, 3α-H), 2.82

(1H, s, 16 β -H), 2.12 (3H, broad s, 21-Me), 1.05 (3H, s, 19-Me), 1.03 (3H, s, 18-Me).

20-Hydroximino-4,16-pregnadien-3-one (17a). Following the same procedure described above for **9 α** , the 20-oxime 3 β -ol (**12a**) (300 mg, 0.9 mmol) was oxidized with cyclohexanone (3 mL) and aluminum isopropoxide (303 mg, 1.5 mmol) in toluene (50 mL) for 6 h. The crude product obtained was flash chromatographed and eluted with 1% MeOH-CH₂Cl₂, to give 4-en-3-one (**17a**) (165 mg, 55%), mp 254–259 °C (MeOH-H₂O). ¹H NMR δ : 6.05 (1H, s, 16-H), 5.74 (1H, s, 4-H), 2.00 (3H, s, 20-Me), 1.21 (3H, s, 19-Me), 0.97 (3H, s, 18-Me). Anal. (C₂₁H₂₉ON) C, H, N.

4,16-Pregnadiene-3,20-dione-20-oxime acetate (17b). The oxime (**17a**) (200 mg, 0.61 mmol) was dissolved in pyridine (5 mL) and acetic anhydride (0.5 mL) and left overnight. Water (200 mL) was added and the precipitate was recrystallized from EtOH, to give the acetate (**17b**) (140 mg, 62%), mp 199–203 °C. ¹H NMR δ : 6.30 (1H, s, 16-H), 5.74 (1H, s, 4-H), 2.23 (3H, s, 0Ac), 2.10 (3H, s, 20-Me), 1.22 (3H, s, 19-Me), 1.03 (3H, s, 18-Me). Anal. (C₂₃H₃₁O₃N) C, H, N.

3(E), 20-Dioximido-(19a) and 3(Z), 20-dioximido-4,16-pregnadiene (19b). 16-Dehydropregesterone (**3b**) (100 mg, 0.29 mmol) and hydroxylamine hydrochloride (84 mg, 1.2 mmol) were refluxed in ethanol (5 mL) for 4 h. Water was added the crude mixture of **17a** and **17b** (100 mg) was collected. The mixture was separated by flash chromatography on silica gel and eluted with 25–30% ethyl acetate-LP. The first fraction collected contained 3(E), 20-dioxime (**19a**) (35 mg, 32%), mp 136–139 °C (from EtOH). ¹H NMR δ : 6.05 (1H, s, 16-H), 5.77 (1H, s, 4-H), 1.99 (3H, s, 20-Me), 1.09 (3H, s, 19-Me), 0.96 (3H, s, 18-Me). Anal. (C₂₁H₃₀O₂N₂) C, H, N. The second fraction collected contained 3(Z), 20-dioxime (**19b**) (40 mg, 36.5%), mp 225–227 °C (from EtOH). ¹H NMR δ : 6.47 (1H, s, 4-H), 6.05 (1H, t, 16-H), 1.99 (3H, s, 20-Me), 1.13 (3H, s, 19-Me), 0.96 (3H, s, 18-Me). Anal. (C₂₁H₃₀O₂N₂·1.5H₂O) C, H, N. These two isomers underwent isomerization during recrystallization in ethanol.

Androsta-5,16-dien-3 β -ol-17-carboxaldehyde (23). To a stirring solution of 21-acetoxy (**21**) (prepared from 16 α , 17 α -epoxide (**20**))²⁹ (0.5 g, 1.34 mmol) in THF (20 mL), a solution of LiAlH₄ (200 mg, 15.8 mmol) in THF (10 mL) was added dropwise at 25 °C and then stirring continued for 1 h. EtOAc (150 mL) was added and the whole mixture was washed with 1 N HCl (3 $\times$ 20 mL), and then with water. After evaporation of the solvent, the residue diol (**22**) (0.4 g) was dissolved in MeOH (30 mL), and a solution of sodium periodate (360 mg, 1.68 mmol) in water (5 mL) was added and the mixture stirred at

25 °C for 30 min. Water (200 mL) was added and the precipitate was recrystallized from MeOH, to give the 17-al (**23**) (300 mg, 75%), mp 163–168 °C. ¹H NMR δ : 9.72 (1H, s, CHO), 6.82 (1H, s, 16-H), 5.36 (1H, d, *J* = 4.8 Hz, 5-H), 3.50 (1H, m, 3 α -H), 1.06 (3H, s, 19-Me), 0.74 (3H, s, 18-Me). Anal. (C₂₀H₂₈O₂) C, H.

Androsta-5,16-dien-3 β -ol-20-oxime (24). Following the procedure for the preparation of **19**, the 17-al (**23**) (750 mg, 2.5 mmol) gave oxime (**24**) (250 mg, 32%), mp 196–202 °C. ¹H NMR δ : 7.82 (1H, s, CH = N-), 6.75 (1H, s, exchangeable OH in D₂O), 6.02 (1H, s, 16-H), 5.37 (1H, d, 4.9 Hz, 6-H), 3.70 (1H, m, 3 α -H), 1.05 (3H, s, 19-Me), 0.95 (3H, s, 18-Me). Anal. (C₂₀H₂₉O₂N) C, H, N.

Androsta-4,16-dien-3-one-20-oxime (25). Following the procedure described above for (**9 β**), the 3 β -ol (**24**) (200 mg, 0.63 mmol) was oxidized with cyclohexanone (6 mL), aluminum isopropoxide (400 mg, 1.96 mmol) in toluene (100 mL) for 4 h. The crude product obtained was chromatographed, eluted with 1% MeOH-CH₂Cl₂, to give 3-one (**25**), (100 mg, 50%), mp 265–268 °C (from acetone). ¹H NMR δ : 7.82 (1H, s, CH = N-), 6.87 (1H, s, exchangeable in D₂O, -OH), 6.01 (1H, s, 16-H), 5.74 (1H, s, 4-H), 1.22 (3H, s, 19-Me), 0.97 (3H, s, 18-Me). Anal. (C₂₀H₂₇O₂N) C, H, N.

Enzyme assays. [7-³H]-Pregnenolone (25 Ci/mmol), [7-³H]-testosterone, [4-¹⁴C]-DHT, and [4-¹⁴C]-DHEA were purchased from New England Nuclear Corp. (Boston, MA) and checked for purity and/or purified by TLC or HPLC prior to use. The [21-³H]17 α -hydroxypregnenolone was prepared in our lab as described previously.¹¹ Ketoconazole was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Finasteride was a gift from Merck Co. Scintillation cocktail 3a70B was purchased from RPI Corp. (Mount Prospect, IL, USA).

Testicular microsomes. The previously reported procedure⁹ was followed to prepare testicular microsomes. Testes were obtained from untreated prostatic cancer patients undergoing orchidectomy in the University of Maryland Hospital and the University of North Carolina Hospital. Testes were also obtained from normal adult rats (200 g bw Sprague–Dawley, Charles River). 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.³⁶

Human prostate microsomes. Microsomes from prostatic tissues from patients with benign prostatic hypertrophy (BPH) were prepared as above and stored at –70 °C. Just before use, the microsomes were diluted with 0.1 M

phosphate buffer to the appropriate concentrations. The protein concentrations were determined as above.³⁶

Measurement of 17 α -hydroxylase/C_{17,20}-lyase activity.

(1) *Radiometric assay of C_{17,20}-lyase activity*: in this assay, [21³H] 17 α -hydroxypregnenolone (13.61 μ Ci/ μ mol) was employed as the substrate. Each tube contained 300,000 dpm, a total concentration of 10 μ M 17 α -hydroxypregnenolone, various concentrations of the test compounds in phosphate buffer (pH 7.4, total volume 1 mL) and the NADPH generating system (NADP 65 μ M; glucose-6-phosphate 0.71 mM; glucose-6-phosphate dehydrogenase 0.13 IU in 50 μ L phosphate buffer). The tubes were preincubated for 10 min at 34°C. The reaction was initiated by the addition of the microsomes (approx. 90 μ g protein) and the incubation carried out for 60 min under oxygen at 34°C. Chloroform was then added to extract the steroids, an aliquot (0.75 mL) of aqueous phase was 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 and then centrifuged. An aliquot of the supernatant was removed and the tritium concentration measured by liquid scintillation counting. The results are listed in Table 1. IC₅₀ or 50% inhibition values were calculated from the linear regression line of the plot of logit of C_{17,20}-lyase activity versus log of four to five inhibitor concentrations. Ketoconazole was also incubated as an internal control for each compound and for comparison of the extent of inhibition. The results were obtained from duplicate sets of experiments and were repeated at least once and several times for the more active inhibitors.¹¹

(2) *17 α -Hydroxylase/C_{17,20}-lyase activity*: human testicular microsomes were incubated with [7-³H]-pregnenolone (400 nM, 5 $\times$ 120⁴ dpm), and the NADPH generating system above, for 5 min at 34°C under oxygen. Authentic steroid markers and C¹⁴-labeled pregnenolone, 17 α -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, C₁₈ reverse phase column and eluting with acetonitrile/methanol/water (30/10/60). The radioactivity was measured in each fraction collected, as previously described.¹⁰ The 17 α -hydroxylase/C_{17,20}-lyase activity was determined from the percentage conversion of [7-³H]-pregnenolone to the total amount of 17 α -hydroxypregnenolone and DHEA. The C_{17,20}-lyase activity was determined from the percentage conversion of 17-hydroxypregnenolone to DHEA (the conversion of substrate to androstenediol and testosterone under the experimental conditions was negligible). The results are listed in Table 1. IC₅₀ values were calculated from the dose-response curves for inhibition of this enzyme complex at four to five inhibitor concentrations. The

results were obtained from duplicate sets of experiments and were repeated at least once.

Measurement of 5 α -reductase activity. [7-³H]Testosterone (15–60 nM, 6 $\times$ 10⁵ dpm) was preincubated with different concentrations of the test compounds or finasteride in 0.85 mL, phosphate buffer (pH 7.4) for 10 min at 37°C. Then, human prostatic microsomes (approximately 160 μ g protein in 100 μ L phosphate buffer) and the NADPH generating system (NADP 650 μ M; glucose-6-phosphate 7.1 mM; glucose-6-phosphate dehydrogenase 1.25 IU in 50 μ L phosphate buffer) were added. The mixture was incubated under oxygen for 10 min at 37°C. Steroids [¹⁴C]-labeled (T, A, and DHT) and authentic markers (T, A, DHT, 5 α -androstane-3 α ,17 β -diol, and -3 β ,17 β -diol) were added after incubation. The steroids were extracted with ether and separated by TLC (chloroform:ether, 80:20). The DHT and the 3-diols were located by their markers after exposure of the plate to iodine vapor. Results were calculated from the percentage conversion of [7-³H]-testosterone to [³H]-DHT, and corrected for procedural losses by the recovery of [4¹⁴C]DHT (the conversion of DHT to 3-diols was negligible under these experimental conditions).

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