Synthesis and Evaluation of 17α-Aliphatic Heterocycle-Substituted Steroidal Inhibitors of 17α-Hydroxylase/C17−20-Lyase (P450 17)

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In the search for potent inhibitors of P450 17, the key enzyme in androgen biosynthesis, a series of steroidal inhibitors were synthesized and tested toward rat and human P450 17. Small aliphatic heterocycles (aziridine, oxirane, thiirane, diaziridine, diazirine, azetidine) were introduced into the 17β-position of anstrost-5-en-3β-ol. After identifying that aziridine is the most suitable functional group to coordinate with the heme iron, modifications of the steroidal skeleton were performed for further optimization. A wide range of inhibitory potencies toward P450 17 were found for the 21 test compounds. The most potent inhibitors toward the human and rat enzyme were aziridine compounds **3** (IC_{50} rat: 0.21 μM, K_i = 3 nM; IC_{50} human: 0.54 μM, K_i = 8 nM), **5** (IC_{50} rat: 0.43 μM, K_i = 7 nM; IC_{50} human: 0.29 μM, K_i = 4 nM), and **8** (21*R*:21*S* = 1:1; IC_{50} rat: 0.53 μM, K_i = 9 nM; IC_{50} human: 0.40 μM, K_i = 6 nM) which were more potent than the reference ketoconazole (IC_{50} rat: 67 μM; IC_{50} human: 0.74 μM). The inhibitory potency depends markedly on the stereochemistry at C20 of the inhibitors. This effect is more pronounced for the rat enzyme. Tested for selectivity, the highly potent inhibitors show poor inhibitory activity toward P450 arom, P450 scc, P450 TxA_2, and 5α-reductase. Tested for in vivo activity, **3** and **8** (0.019 mmol/kg) decreased the plasma testosterone concentration in rats by 81% and 84% after 2 h.

Introduction

Androgens play a critical role in the development and maintenance of sexual characteristics in human males. However, they are also growth factors for such severe and widespread diseases as benign prostatic hyperplasia (BPH) and prostatic cancer.[1,2] After lung cancer, prostatic cancer is the second leading cause of cancer death. Studies of the National Cancer Institute (NCI) in the United States show that the number of new cases of prostatic cancer increased dramatically between 1971 and 1993 (white population 127%, black population 82%). These data show the need for a new therapeutic concept, as an alternative to the treatment with antihormones, GnRH analogues, or orchiectomy. A promising strategy could be the selective inhibition of androgen biosynthesis.[3,4] The appropriate target is 17α-hydroxylase/C17−20-lyase (P450 17, Cyp 17),[5−7] a key enzyme in the androgen biosynthetic pathway. P450 17 is a cytochrome P450 enzyme (monooxygenase), which catalyzes hydroxylation of progesterone and pregnenolone in the 17α-position as well as cleavage of the C17−C20 bond to yield androstenedione and DHEA (dehydroepiandrosterone; Chart 1).[8−11]

These steroids are weak androgens which subsequently are converted by other enzymes (17βHSD, 3βHSD, 5α-reductase) to the more potent androgens testosterone and DHT (dihydrotestosterone). While GnRH analogues and orchiectomy do not affect the androgen biosynthesis in the adrenals, inhibition of P450 17 blocks synthesis in both tissues, testes and adrenals, decreasing plasma levels of testosterone and DHT. For that reason, P450 17 has attracted attention as a therapeutic target, and attempts were made to obtain specific steroidal[12−19] as well as nonsteroidal[1,4,18−32] inhibitors. The first inhibitors were unsuitable for therapeutic application because of side effects caused by poor selectivity of the inhibitor (ketoconazole)[33] or because of poor in vivo activity (liarozole).[34] Recently, some steroidal[12−17,35,36] (**I** (abiraterone),[12] **II**,[15] **III**[17]) and nonsteroidal[23,25,26,28,31,32] (**IV**,[31] **V**[28] (YM116)) (Chart 2) inhibitors have been developed and show high in vitro and in vivo potency. Common feature of these and most other steroidal inhibitors is a N-containing heterocycle at the 17-position (mostly imidazole or pyridine), which is capable of forming a coordinate bond with the heme iron of the prosthetic group of the enzyme.

The concept of drug design presented in this paper combines the steroidal structure of the high-affinity substrates pregnenolone and progesterone with heteroatom-containing functional groups for complexation with the heme iron. A series of small aliphatic heterocycles is introduced in the 17β-position of the steroidal skeleton. After identification of the most suitable functional group, alterations of the steroid skeleton are performed for further optimization. We report here on the synthesis of 21 steroidal inhibitors and their inhibitory activity toward human and rat P450 17. Selectivity (P450 arom, P450 scc, P450 TxA_2, 5α-reductase) and in vivo activity (SD-rats) of selected compounds are also presented.

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Chart 1. Catalytic Reaction of P450 17

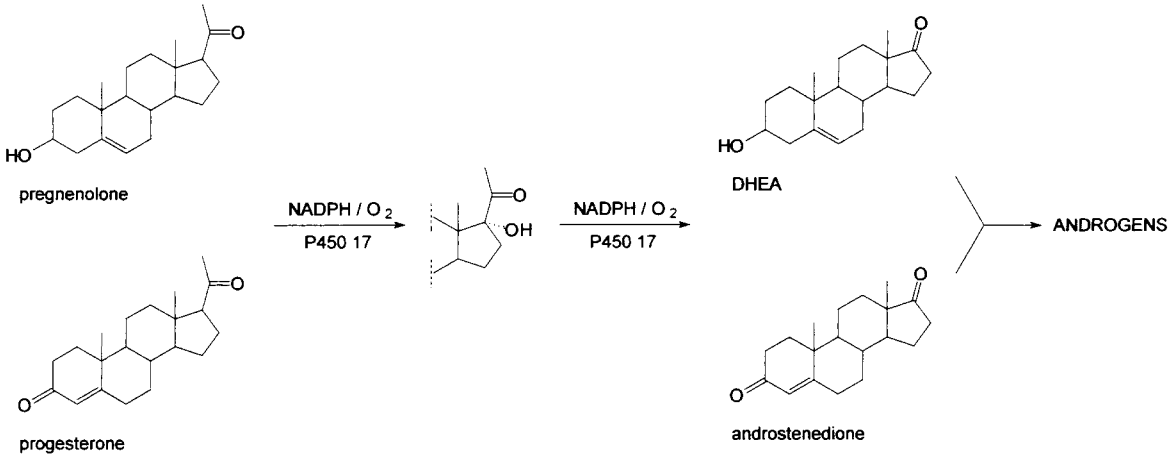
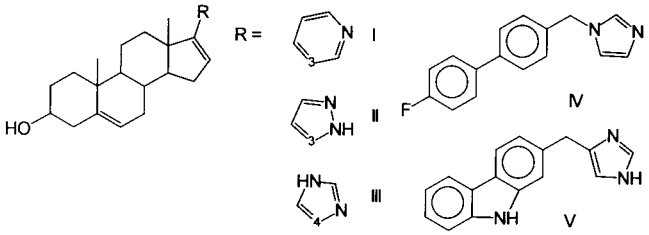
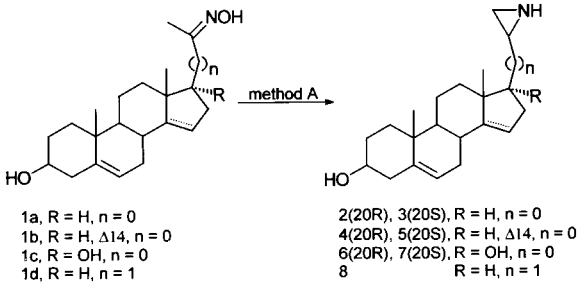


Chart 2. Selected Inhibitors of P450 17



Scheme 1[a]



[a] Reagents: (Method A) $LiAlH_4$, $CH_3NHC(CH_3)_3$.

Chemistry

Starting from the corresponding oximes (Scheme 1), the aziridines **4**−**8** were obtained via the same method as described for **2** and **3**.[37] For all compounds, a 1:1 diastereomeric mixture (20*R*:20*S*) was obtained, which was separated for **4**−**7** by FCC (flash column chromatography) and HPLC. The stereochemistry at C20 was assigned and the presence of the 17α-OH group was confirmed by comparison of the ^{1}H and ^{13}C NMR data with those of **2** and **3**. In the case of the 20*S* compounds, the 18-methyl singlet is shifted to higher field compared to the corresponding 20*R* diastereomers.[14,37] In the case of the 1:1 diastereomeric mixture **8** separation of the isomers was not possible.

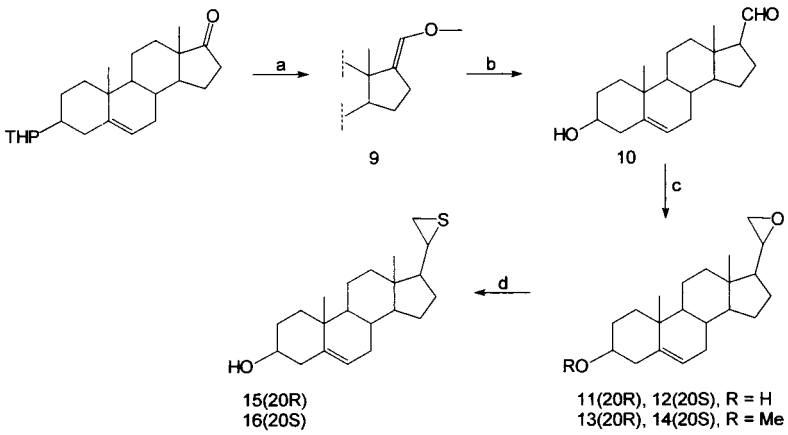
The oxirane compounds **11**−**14** (Scheme 2) were synthesized according to a method for steroidal 10*β*-oxiranyl compounds[38] by reaction of trimethylsulfonium iodide and NaH with an aldehyde.[39] Compound **10** was obtained according to Danishefsky.[40] In contrast to the literature method, introduction of a protecting group (THP) as well as isolation of compound **9** (*E*:*Z* = 3:4, determined by ^{1}H NMR, C20 vinyl proton) was necessary. The crude product obtained from the reaction with aldehyde **10** was a mixture of the 17*β*- and 17α-isomer (4:1, determined by ^{1}H NMR, C17 proton), from which the former was separated by chromatography. Ring closure led to a 3:1 diastereomeric mixture (20*R*:20*S*) of the 3*β*-ols **11**/**12** (yield 62%) and the corresponding 3*β*-methyl ethers **13**/**14** (yield 16%). The 3*β*-methoxy and 3*β*-hydroxy derivatives were separated by FCC. The diastereomers were purified by HPLC. Elucidation of the stereochemistry was not possible by ^{1}H NMR, but X-ray analysis of **13** revealed the *R* configuration at C20. With the diastereomeric mixture **11**/**12** as the starting material, a 1:3 mixture of the thiiranes **15**/**16** (20*R*:20*S*) (Scheme 2) was obtained by the reaction reported by Chan and Finkebine.[41] This reaction proceeds with inversion of the configuration at C20. The diastereomers were purified by HPLC, and their configuration was elucidated by X-ray analysis of **15**.

The direct synthesis of the diaziridine **18** (Scheme 3) by reaction of pregnenolone with chloramine T or hydroxylamine-*O*-sulfonic acid failed. Thus, the more reactive imine **17** was synthesized, which upon reaction with hydroxylamine-*O*-sulfonic acid gave the diaziridine **18**. Interestingly, the ^{1}H NMR signals of the C18 protons are split. This indicates that **18** is a mixture of two conformers, which is most probably caused by the configuration stability of the ring nitrogens. Treatment of **18** with Br_2 led to the diazirine **19**, which shows, in the ^{1}H NMR spectrum, sharp singlets for all CH_3 groups.

The starting point for the synthesis of the azetidine **22** (Scheme 4) was a mixture of compounds **2** and **3.** Deamination with acetic acid and $NaNO_2$ and subsequent acetylation of the 3*β*-hydroxy group led to **20**.[42] Treatment with chlorosulfonyl isocyanate[43,44] and reaction of the resulting chlorosulfonylazetidinone intermediate with Na_2SO_3[45] resulted in the lactam **21**, which was determined to be a 2:1 diastereomeric mixture (C20 atom ^{1}H NMR: singlet of 18-methyl group). Reduction using diisobutylaluminum hydride led to a 1:1 diastereomeric mixture of **22**. The azetidine has poor stability, and no efforts were made to separate the isomers and elucidate their stereochemistry.

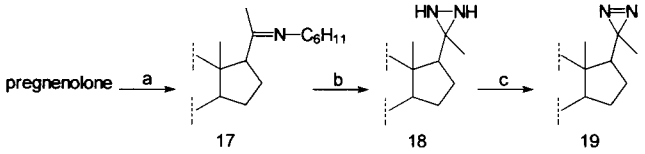
Selected compounds (**2**, **3**, **8**, **11**, **12**) were transformed into their progesterone derivatives **23**,[14] **24**,[14] and **25**−**27** by a modified Oppenauer oxidation[46] via *N*-methylpiperidone and aluminum isopropoxide as reagents (Scheme 5). In the case of compound **25,** a 1:1 mixture

Scheme 2[a]



[a] Reagents: (a) $CH_3OCH_2PPh_3Cl$, NaH; (b) 1. $HClO_4$, 2. PPTS; (c) NaH, $S(CH_3)_3I$; (d) Ph_3PS, picric acid.

Scheme 3[a]



[a] Reagents: (a) $C_6H_{11}NH_2$, F_3CCOOH; (b) H_2NOSO_3H, MeOH, NH_3 (satd); (c) Br_2, NEt_3.

of diastereomers was obtained, which could not be separarated by HPLC. The isomers **26** and **27** were isolated by HPLC.

Biological Results

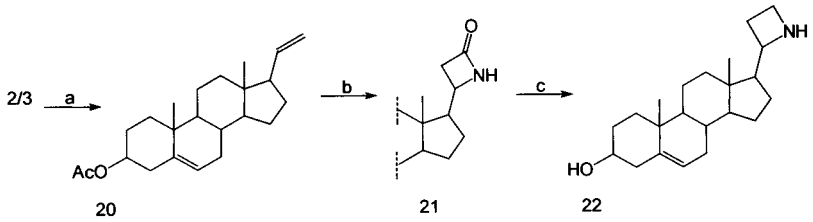
The inhibitory activity toward the P450 17 rat and human enzyme was tested in an experimental system comprising testicular microsomes and progesterone in large excess as substrate. Reverse-phase HPLC with UV detection was used to quantify the products. In contrast to the rat enzyme, 16α- and 17α-hydroxyprogesterone are formed with the human enzyme (ratio 3:10), and no products of lyase activity are found as previously reported.[47]

The IC_{50} values in Table 1 show that the aziridine-substituted compounds are very potent inhibitors of P450 17. Compound **3** exhibits IC_{50} values in the nanomolar range for both enzymes (0.21 μM rat and 0.54 μM human) and K_i values of 3 and 8 nM, respectively (competitive Lineweaver−Burk plot; data not shown). A strong sterochemical dependency was observed for the rat enzyme. The potencies of the 20*R* isomer **2** and the 20*S* isomer **3** differ by a factor of 162, whereas in the human enzyme surprisingly this factor is only 3. Similar differences can be seen with the other aziridine diastereomers (**4**/**5**, **6**/**7**, **23**/**24**). Exchange of the aziridine ring in the 17β-position by other three-membered heterocycles decreases inhibitory activity (oxirane, thiirane, diaziridine, diazirine). This effect does not depend on the species and is more pronounced for the rat enzyme. Besides the aziridines, only diaziridine **18** is a more active inhibitor for the rat enzyme than ketoconazole. As seen with compounds **2**/**3**, the 20*S* isomer **12** of oxiranes **11**/**12** is more potent than the 20*R* isomer **11**. In the case of the human enzyme, aziridine **3** is about 3−4 times more active than oxirane **12** (20*S* isomer) and thiirane **15** (20*R* isomer). Interestingly, in the case of the thiiranes, *R* configuration at C20 results in a higher inhibitory potency. The azetidine derivative **24** shows poor inhibition for both enzymes, which might be due to degradation of the substance under the assay conditions. Introduction of a OH group in the 17α-position of aziridines **2** and **3** causes a dramatic loss of activity (compounds **6** and **7**). This result was unexpected, since 17α-hydroxypregnenolone is also a substrate of P450 17. As expected, the *S*-configurated diastereomer is more potent toward the rat enzyme than the *R* isomer. A Δ14-double bond (**4**/**5**) increases inhibitory activity for the human enzyme. Introduction of a methylene spacer in the 17β-side chain (**8**) does not noticeably change activity toward the rat enzyme but increases activity toward the human enzyme. In the case of the aziridines, exchange of the pregnenolone skeleton by progesterone (**2**, **3**, **8** → **23**−**25**) results in a moderate decrease of activity toward both enzymes. In the case of the oxiranes, the progesterone compounds **26** and **27** as well as the 3β-OMe pregnenolones **13** and **14** showed a strong decrease of inhibitory activity compared to the corresponding pregnenolones (**11**, **12**).

The mode of inhibition of the aziridine derivatives was studied using UV−vis difference spectroscopy experiments. A characteristic type II difference spectrum was induced (data not given) indicating coordination of the steroidal aziridine nitrogen to the heme iron of P450 17.[48] This effect was not reversed by high excess of progesterone. To elucidate whether the inhibitor binds covalently to the enzyme, enzyme activity was determined after various time intervals, following preincubation of the aziridine with enzyme and removal of the inhibitor. A recovery of the enzyme activity of 25% after 5 min and up to 75% after 90 min was observed, indicating a reversible binding mode. A slow-binding mechanism[49] could be excluded.

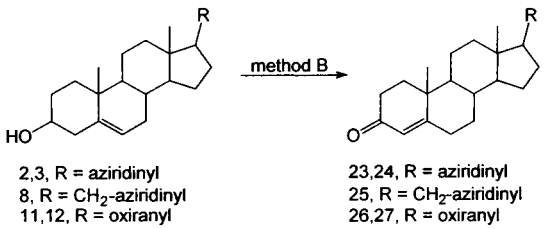
To evaluate the selectivity of the P450 17 inhibition, compounds **3** and **8** were tested for inhibition of P450 scc, P450 arom, and P450 TxA_2 because inhibition of these enzymes could cause side effects. P450 scc catalyzes the first step in steroid hormone biosynthesis, and inhibition of P450 scc would affect all steroid hormones. Inhibition of P450 arom and P450 TxA_2 might increase testosterone concentration and interfere with thrombocyte aggregation, respectively. Analyses were done by recently described procedures (P450 scc,[50] P450 arom,[50]

Scheme 4[a]



[a] Reagents: (a) HNO_2, HOAc; (b) 1. ClO_2SNCO, 2. Na_2SO_3 (25%), KOH (10%); (c) DibaH.

Scheme 5[a]



[a] Reagents: (Method B) $Al(OCH(CH_3)_2)_3$, *N*-methylpiperidone.

P450 TxA_2[51]). Compound **3** shows weak inhibition of P450 arom and P450 scc and stronger inhibition of P450 TxA_2, whereas compound **8** exhibits moderate inhibitory effects toward P450 TxA_2 and P450 arom as well as marked inhibition of P450 scc (IC_{50} P450 TxA_2: **3,** 4 μM; **8**, 35 μM; dazoxiben, 1.1 μM; IC_{50} P450 arom **3**, >50 μM; **8**, 7.1 μM; cyclohexylaminoglutethimide,[52] 0.15 μM; IC_{50} P450 scc: **3**, >25 μM; **8**, approximately 1 μM).

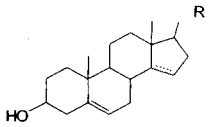
Selected compounds (**3**, **24**, **25**) were tested for inhibition of 5α-reductase type I and II isozymes. The DU 145 cell line and prostate homogenate of BPH patients were used as sources for type I and II isozyme, respectively, and the analyses were performed by recently described[53,54] procedures. As 5α-reductase transforms testosterone to the more potent androgen dihydrotestosterone, a dual inhibition of both 5α-reductase and P450 17 could lead to a more effective blockade of androgen biosynthesis. All tested compounds show weak inhibition toward type II isozyme (IC_{50} values: **3**, 10 μM; **24**, 16 μM; **25**, 9 μM). Only compound **25** exhibits a relatively strong inhibition of type I isozyme (IC_{50}: 1.6 μM).

The two most potent inhibitors of the human and rat enzyme (compound **3** and the diastereomeric mixture **8**) were evaluated for reduction of plasma testosterone concentration in male Sprague−Dawley rats. The compounds were administered intraperitoneally at equimolar doses comparable to 10 mg/kg body weight of ketoconazole. Each group consisted of 7−8 rats. Blood samples were taken by cardiac puncture after 2 and 6 h. Testosterone concentrations were determined by RIA using [^{125}I]testosterone. Ketoconazole does not significantly reduce the testosterone plasma level after 2 and 6 h. In contrast, **3** and **8** show strong reduction after 2 h of 81% and 84%, respectively. After 6 h no significant effect was observed.

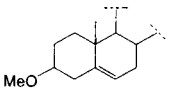
Discussion and Conclusion

The biological data shows that the aziridine group forms a strong coordinate bond with the heme iron, leading to extremely potent inhibitors of the human and rat enzyme (**3**, **5**, **8**). The inhibitory activities of the aziridine compounds depend markedly on the stereochemistry at C20. Interestingly this effect is more pronounced for the rat enzyme, reflecting more stringent spatial requirements. To elucidate why the *S* configuration results in stronger inhibition, conformational analyses were performed with **2**−**5**. In the lowes-energy conformation of **2** and **3**, the aziridine ring is located in a coplanar fashion to the steroid. This conformation is almost identical with the X-ray structure of the 17β-oxiranyl and 17β-thiiranyl analogues **13** and **15**. In the 20*S* compound **3** the aziridine nitrogen is located in the α-position, in a direction where the heme iron is supposed to be, while in the 20*R* diastereomer **2** the nitrogen is in the opposite (β) position. As expected from this model, introduction of a double bond in position 16 (compounds **4** and **5**) changes the inhibitory activity of **2** and **3** only slightly. Insertion of a methylene spacer between the steroidal moiety and the aziridine ring does not alter inhibitory potency strongly (**2**/**3** → **8**). For the rat enzyme, the most potent inhibitors **3**, **5**, and **8** have comparable activity to the powerful steroidal inhibitor **II** (Chart 2; IC_{50}: 0.18 μM in our test system). In case of the human enzyme, compounds **3**, **5**, and **8** are slightly less potent than compound **III** (IC_{50}: 0.04 μM in our test system) and compound **I**, which is described to be more potent than ketoconazole by a factor of 9.[12] Concerning the mode of inhibition of the aziridines, it has been shown that they are reversible inhibitors, which form a tight coordinate bond with the heme iron, leading to a very slow dissociation from the enzyme. The lower activities of the compounds bearing other heterocycles are obviously a result of the chemical nature of the heteroatoms because force-field calculations (conformational analysis, data not shown) reveal that all C20 derivatives have very similar low-energy conformations and the coordinating heteroatom is located almost in the same position in space as it is in compound **3**. Interestingly, the results obtained with analogous steroidal inhibitors of P450 arom are different: (19*R*)-aziridinylandrostenedione inhibits aromatase in a slow-binding manner,[55] whereas the (19*R*)-oxiranyl and thiiranyl analogues, for which the K_i values are lower than that of the aziridine, are reversible competitive inhibitors of the enzyme.[56] Presently no explantion for these findings is obvious. Alterations of the steroidal skeleton of aziridine **3** lead only to slight changes in the biological activity (compounds **4**, **5**, **23**, **24**). In contrast, introduction of the 17α-hydroxy group causes a loss of activity, although it has no influence on the spatial position of the aziridine ring (conformational analysis, data not shown). This observation supports the model of a bilobal hydrophobic pocket converging on the heme group. This has been supported by molecular modeling and site-directed mutagenesis.[7,57] In this model, the A-ring of the substrate should be capable of occupying

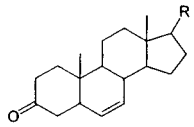
Table 1. Inhibition of the P450 17 Rat and Human Enzyme by Pregnenolone- and Progesterone-type Compounds



2-8, 11, 12, 15-19, 22



13, 14



23-27

compd	R	stereo-chemistry	IC_{50} μM [a] [K_i value] rat enzyme [b]	IC50 μM [a] [K_i value] human enzyme [c]
2		20R	34[d]	1.52
3		20S	0.21[d] [3 nM]	0.54 [8 nM]
4		20R	9.67	0.97
5	(Δ14)	20S	0.43 [7 nM]	0.29 [3 nM]
6		20R	> 125	> 2.5
7	OH	20S	32	> 2.5
8		1:1[e]	0.53 [9 nM]	0.40 [6 nM]
11		20R	n.i.	> 2.5
12		20S	70	1.25
13		20R	n.i.	n.i.
14		20S	> 125	n.i.
15		20R	> 125	1.8
16		20S	n.i.	> 2.5
18			16	> 2.5
19			> 125	> 2.5
22		1:1[e]	> 125	n.i.
23		20R	34[d]	> 2.5
24		20S	0.42[d]	1.52
25		1:1[e]	1.32	1.8
26		20R	n.d.	n.i.
27		20S	n.d.	> 2.5
KTZ[f]			67	0.74

[a] Concentration of inhibitor required to give 50% inhibition. [b]Rat testicular microsomes, concentration of progesterone (substrate) 25 μM. [c]Human testicular microsomes, concentration of progesterone (substrate) 25 μM. The given values are mean values of at least two experiments. The deviations were within ±5%. [d]See ref 14. ni, no inhibition (at 125 μM for rat enzyme and 2.5 μM for human enzyme, respectively); nd, not determined. [e]1:1 Diastereomeric mixture was tested. [f]KTZ, ketoconazole.

two different positions. It is located in the smaller lobe for 17α-hydroxylation. After this enzymatic step, the A-ring moves to the larger lobe for C17−20-lyase reaction. As a consequence of this shift, C20 is positioned closer to the heme iron enabling attack of the ferriperoxo species.[57] The decrease in activity observed with compound **3** upon hydroxylation (compound **7**) is due to the less pronounced interaction of the aziridine N with the heme iron because no type II difference spectrum could be obtained (data not given). This might be an indication that the steroidal inhibitor is now located in the larger lobe. Provided the model is correct that one heme is responsible for hydroxylase and lyase activity, the consequence of this is that a selective inhibition of the two activities is not possible by heme-coordinating inhibitors. Moreover selective inhibition of lyase activity seems not to be necessary from the clinical point of view. In trials with the P450 17 inhibitors ketoconazole[33] and liarozole,[34] it has been shown that the androgen plasma concentration was reduced without glucocorticoid plasma levels being affected. For this reason, it can be concluded that the aziridine compounds **3** and **8**, which have shown much higher in vivo activity than ketoconazole, might be promising drug candidates for the treatment of androgen-dependent diseases.

Experimental Section

Chemical Methods. Melting points were measured on a Kofler melting point Thermopan apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 398 infrared spectrometer as KBr pellets. ^{1}H NMR spectra were recorded on a Bruker AM-400 (400 MHz), ^{13}C NMR spectra on a Bruker AM-400 (120 MHz). Chemical shifts are given in parts per million. TMS was used as internal standard. All *J* values are given in Hz. Purity was checked by GC−MS on a HP G1800A GCD system. Mass spectra were recorded on a HP 1074 A (GCD) spectrometer (Hewlett-Packard). Elemental analyses were performed at the Inorganic Chemistry Department, University of the Saarland. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was performed using silica gel 60 (50−200 μm), flash column chromatography (FCC) using silica gel 60 (40−63 μm), HPLC using a semipreparative RP-18 column (16 mm × 250 mm, particle size 5 μm, Nucleosil-C18). Reaction progress was determined by TLC analysis on ALUGRAM SIL G/UV_{254} (Macherey-Nagel).

Method A: General Procedure for Synthesis of Aziridinyl Compounds 2,[14] 3,[14] and 4−8. The reaction was performed under N_2 atmosphere in a dry apparatus and solvents. To a suspension of 338 mg $LiAlH_4$ (8 mmol) and catalytic amounts of *N*-methyl-*N*-butylamine in 3 mL THF was dropped 0.81 mmol of the steroidal oxime dissolved in 9 mL THF over 40 min at 0 °C. The reaction mixture was refluxed for 16 h. To destroy the excess $LiAlH_4$, small amounts of water were added to the reaction mixture at 0 °C. The mixture was filtered and washed twice with ethyl acetate. The organic phase was washed twice with water and brine and dried over $MgSO_4$. The compounds were purified by FCC or HPLC (RP-18, 4 mm × 250 mm, Eurospher 100-C18, particle size 5 μm, MeOH, flow rate 1.5 mL/min).

(20*R*)-21-Iminopregna-5,14-dien-3β-ol (4): purification FCC (CH_2Cl_2:EtOH 9:1); HPLC t_R = 10.8 min; yield 30%, white solid; mp 135−40 °C. ^{1}H NMR ($CDCl_3$): δ 1.06 (s, C18-Me, 3H); 1.03 (s, C19-Me, 3H); 3.53 (m, C3αH, 1H); 5.19 (s, C15 =CH−, 1H); 5.40 (s, C6, =CH−, 1H). IR (KBr) cm^{-1}: ν_{max} 3400, 3300, 2950, 1470/60/40, 1380, 1060/50, 800. GC−MS: *m*/*z* 313 (M^+), 298, 270, 263, 237, 211, 194, 171. Anal. ($C_{21}H_{31}NO$) C, H, N.

(20*S*)-21-Iminopregna-5,14-dien-3β-ol (5): purification FCC (CH_2Cl_2:EtOH 9:1); HPLC t_R = 11.9 min; yield 30%, white solid; mp 145−50 °C. ^{1}H NMR ($CDCl_3$): δ 1.01 (s, C18-Me, 3H); 1.04 (s, C19-Me, 3H); 3.53 (m, C3αH, 1H); 5.17 (s, C15 =CH−, 1H); 5.40 (s, C6, =CH−, 1H). IR (KBr) cm^{-1}: ν_{max} 3400, 3300, 2950, 1470/60/40, 1380, 1060/50, 800. GC−MS: *m*/*z* 313 (M^+), 298, 270, 263,,237, 211, 194, 171. Anal. ($C_{21}H_{31}NO$) C, H, N.

(20*R*)-21-Iminopregn-5-ene-3β,17α-diol (6): purification FCC (CH_2Cl_2:EtOH 9:1); yield 42%, white solid; mp 192−5 °C. ^{1}H NMR ($CDCl_3$): δ 1.031 (s, C18-Me, 3H); 0.85 (s, C19-Me, 3H); 3.53 (m, C3αH, 1H); 5.35 (d, C6, =CH−, 1H, *J* = 5.4 Hz). ^{13}C NMR ($CDCl_3$): δ 141.4 (C5); 120.5 (C6); 79.4 (C17); 70.1

(C3); 23.8 (C16); 20.2 (C11); 19.3 (C19); 15.2 (C18). IR (KBr) cm^{-1}: ν_{max} 3500−3300, 2950, 1470/60/40, 1385, 1055. Anal. ($C_{21}H_{33}NO_2$) C, H, N.

(20*S*)-21-Iminopregn-5-ene-3*β*,17α-diol (7): purification FCC (CH_2Cl_2:EtOH 9:1); yield 42%, white solid; mp > 200 °C (degradation). ^1H NMR ($CDCl_3$): δ 1.028 (s, C18-Me, 3H); 0.85 (s, C19-Me, 3H); 3.53 (m, C3αH, 1H); 5.40 (s, C6, =CH−, 1H). ^{13}C NMR ($CDCl_3$): δ 141.4 (C5); 120.5 (C6); 79.4 (C17); 70.1 (C3); 23.8 (C16); 20.2 (C11); 19.3 (C19); 14.6 (C18). IR (KBr) cm^{-1}: ν_{max} 3500−3300, 2950, 1470/60/40, 1385, 1055. Anal. ($C_{21}H_{33}NO_2$) C, H, N.

(21*R*,21*S*)-22-Iminopregn-5-en-3*β*-ol (8): purification FCC (CH_2Cl_2:MeOH 9:1); yield 37%, white solid (1:1 21*R*:21*S* diastereomeric mixture). ^1H NMR ($CDCl_3$): δ 0.59 (s, C18-Me, 1.5H); 0.60 (s, C18-Me, 1.5H); 1.02 (s, C19-Me, 3H); 3.53 (m, C3αH, 1H); 5.35 (d, C6, =CH−, 1H, ^3J = 5.32 Hz). IR (KBr) cm^{-1}: ν_{max} 3400−3300, 2950, 1470/60/40, 1370/60/50, 1055. GC−MS: *m/e* 329 (M^+), 314, 279. Anal. ($C_{21}H_{33}NO_2$) C, H, N.

Method B: General Procedure for Synthesis of Compounds 23,[14] 24,[14] and 25−27. *N*-Methylpiperidone (3.38 mL, 29 mmol) was added to a solution of the steroid (0.31 mmol) in 10 mL dry toluene. The mixture was heated under reflux until 1−2 mL toluene was collected in a Dean−Stark trap. Aluminum isopropoxide (112 mg, 0.56 mmol) was added and the mixture was refluxed for 4 h. Aluminum isopropoxide (44.7 mg, 0.22 mmol) was added once again and refluxing was continued for 2 h. The mixture was cooled to room temperature and diluted with 20 mL of diethyl ether. The reaction mixture was washed with water and brine and was dried over Na_2SO_4. The solvent was evaporated and the crude product was purified by FCC.

(21*R*,21*S*)-22-Iminopregn-4-en-3-one (25): purification FCC (CH_2Cl_2:MeOH 4:1); yield 40%, white solid (1:1 21*R*:21*S* diastereomeric mixture). ^1H NMR ($CDCl_3$): δ 0.62 (s, C18-Me, 1.5H); 0.63 (s, C18-Me, 1.5H); 1.19 (s, C19-Me, 3H); 5.73 (s, C4, =CH−, 1H). IR (KBr) cm^{-1}: ν_{max} 3600−3400, 2950, 1680, 1620. MS: *m/e* 328 (M^+), 313, 280, 223. Anal. ($C_{22}H_{33}NO$) C, H, N.

(20*R*)-17*β*-Oxiranylpregn-4-en-3-one (26): purification FCC (CH_2Cl_2:EtOH 7:1); yield 51%, white solid; mp 144−7 °C. ^1H NMR ($CDCl_3$): δ 0.87 (s, C18-Me, 3H); 1.20 (s, C19-Me, 3H); 2.42 (dd, oxirane-CH, 1H, *J* = 2.8 Hz, 5.28 Hz); 2.71 (dd, oxirane-CH, 1H, *J* = 3.96 Hz, 4.88 Hz); 2.87 (ddd, oxirane-CH, 1H, *J* = 2.87 Hz, 4.00 Hz, 6.64 Hz); 5.73 (s, C4, =CH−, 1H). IR (KBr) cm^{-1}: ν_{max} 2950, 1680, 1620, 1430, 1380, 1330, 1230, 1190, 910, 860. Anal. ($C_{21}H_{30}O_2$) C, H.

(20*S*)-17*β*-Oxiranylpregn-4-en-3-one (27): purification FCC (CH_2Cl_2:EtOH 7:1); yield 49%, white solid; mp 149−54 °C. ^1H NMR ($CDCl_3$): δ 0.83 (s, C18-Me, 3H); 1.20 (s, C19-Me, 3H); 2.47 (m, oxirane-CH, 1H); 2.75 (dd, oxirane-CH, 1H, *J* = 4.96 Hz, 4.88 Hz); 2.81 (m, oxirane-CH, 1H); 5.73 (s, C4, =CH−, 1H). IR (KBr) cm^{-1}: ν_{max} 2950, 1680, 1620, 1430, 1380, 1330, 1230, 1190, 910, 860. Anal. ($C_{21}H_{30}O_2$) C, H.

General Procedure for Synthesis of Compounds 11−14. A suspension of 0.25 g NaH (6.16 mmol, 60%) was washed twice with THF and dried under a N_2 stream. 3.7 mL of dry DMSO was added and the suspension was heated to 80 °C for 45 min. The resulting solution was cooled to room temperature and 5 mL of THF was added. At −5 °C 1.02 g trimethylsulfonium iodide (6.4 mmol) in 20 mL of DMSO was added slowly with a syringe. After 3 min stirring, 330 mg (1.09 mmol) of **10** in 20 mL THF was added. The reaction mixture was stirred at −5 °C for 1 and 19 h at room temperature. The organic phase was washed with 5% $NaHCO_3$ and extracted with CH_2Cl_2. After washing with water and brine the organic phase was dried over $MgSO_4$. A 3:1 diastereomeric mixture of compounds **11**/**12** and **13**/**14** was obtained. The compounds were isolated and purified by FCC (CH_2Cl_2:EtOAc 5:1) and HPLC (RP-18, 16 mm × 250 mm, Nucleosil-C18, particle size 5 μm, acetonitrile/H_2O).

(20*R*)-17*β*-Oxiranylpregn-5-en-3*β*-ol (11): HPLC (acetonitrile/H_2O:40/60, flow rate 16 mL/min) t_R = 13.4 min; yield 46%, white solid; mp 158−60 °C. ^1H NMR ($CDCl_3$): δ 0.79 (s, C18-Me, 3H); 1.02 (s, C19-Me, 3H); 2.43 (dd, oxirane-CH, 1H, *J* = 3.08 Hz, *J* = 5.32 Hz); 2.71 (t, oxirane-CH, 1H, *J* = 4.4 Hz); 2.89 (m, oxirane-CH, 1H); 3.06 (m, C3αH, 1H); 5.35 (d, C6, =CH−, 1H, *J* = 4.8 Hz). IR (KBr) cm^{-1}: ν_{max} 3400−3300, 3015, 2950, 1470/50/30, 1060, 860. Anal. ($C_{21}H_{32}O_2$) C, H.

(20*S*)-17*β*-Oxiranylpregn-5-en-3*β*-ol (12): HPLC (acetonitrile/H_2O:40/60, flow rate 16 mL/min) t_R = 14.7 min; yield 16%, white solid; mp 165−8 °C. ^1H NMR ($CDCl_3$): δ 0.84 (s, C18-Me, 3H); 1.03 (s, C19-Me, 3H); 2.42 (dd, oxirane-CH, 1H, *J* = 2.64 Hz; *J* = 5.28 Hz); 2.70 (dd, oxirane-CH, 1H, *J* = 4 Hz; *J* = 5.28 Hz); 2.87 (m, oxirane-CH, 1H); 3.53 (m, C3αH, 1H); 5.34 (d, C6, =CH−, 1H, *J* = 5 Hz). IR (KBr) cm^{-1}: ν_{max} 3400−3300, 3015, 2950, 1470/50/30, 1060, 860. Anal. ($C_{21}H_{32}O_2$) C, H.

3*β*-Methoxy-(20*R*)-17*β*-oxiranylpregn-5-ene (13): HPLC (acetonitrile/H_2O:25/75, flow rate 24 mL/min) t_R = 13.2 min; yield 12%, white solid; mp 131−3 °C. ^1H NMR ($CDCl_3$): δ 0.81 (s, C18-Me, 3H); 1.02 (s, C19-Me, 3H); 2.48 (dd, oxirane-CH, 1H, *J* = 2.64 Hz; *J* = 5.32 Hz); 2.77 (dd, oxirane-CH, 1H, *J* = 5.32 Hz); 2.83 (m, oxirane-CH, 1H); 3.06 (m, C3αH, 1H); 3.35 (s, CH_3−O, 1H); 5.36 (d, C4, =CH−, 1H, *J* = 4.8 Hz). IR (KBr) cm^{-1}: ν_{max} 3030, 2950, 1465/50/30, 1380. MS: *m/z* 331 (M^+), 313, 288, 271. Anal. ($C_{22}H_{34}O_2$) C, H.

3*β*-Methoxy-(20*S*)-17*β*-oxiranylpregn-5-ene (14): HPLC (acetonitrile/H_2O:25/75, flow rate 24 mL/min) t_R = 15.0 min; yield 4%, white solid; mp 165−7 °C. ^1H NMR ($CDCl_3$): δ 0.84 (s, C18-Me, 3H); 1.02 (s, C19-Me, 3H); 2.41 (dd, oxirane-CH, 1H, *J* = 2.64 Hz; *J* = 5.28 Hz); 2.70 (dd, oxirane-CH, 1H, *J* = 5.28 Hz); 2.87 (m, oxirane-CH, 1H); 3.06 (m, C3αH, 1H); 3.35 (s, CH_3−O, 1H); 5.35 (d, C4, =CH−, 1H, *J* = 5 Hz). IR (KBr) cm^{-1}: ν_{max} 3030, 2950, 1465/50/30, 1380, 1095. MS: *m/z* 331 (M^+), 313, 288, 271. Anal. ($C_{22}H_{34}O_2$) C, H.

(20*R*)-17*β*-Thiiranylpregn-5-en-3*β*-ol (15) and (20*S*)-17*β*-Thiiranylpregn-5-en-3*β*-ol (16). Under a N_2 atmosphere, 890 mg triphenylphosphine sulfide (3 mmol) and 690 mg picric acid (3 mmol) were added to a solution of 95 mg (0.3 mmol) of a mixture of **11**/**12** in 50 mL toluene. The mixture was heated at 80 °C under N_2 for 5 h. The reaction mixture was cooled, diluted with ether (40 mL) and washed four times with 10% aqueous $NaHCO_3$ to remove picric acid. The organic layer was washed with water and brine, dried over Na_2SO_4 and evaporated in vacuo. The crude product was isolated and purified by FCC (CH_2Cl_2:EtOAc 5:1) and HPLC (RP-18, 16 mm × 250 mm, Nucleosil-C18, particle size 5 μm acetonitrile/H_2O:40/60, flow rate 16 mL/min) to give **15** in 21% yield and **16** in 62% yield. Compound **15**: t_R = 45.1 min); white solid; mp 158−60 °C. ^1H NMR ($CDCl_3$): δ 0.79 (s, C18-Me, 3H); 1.00 (s, C19-Me, 3H); 2.14 (d, thiirane-CH, 1H, *J* = 5.76 Hz); 2.45 (d, thiirane-CH, 1H, *J* = 6.2 Hz); 2.76 (m, thiirane-CH, 1H); 3.49 (m, C3αH, 1H); 5.32 (d, C6, =CH−, 1H, *J* = 4 Hz). IR (KBr) cm^{-1}: ν_{max} 3450−3400, 2950, 1380, 1060, 960, 850, 810. GC−MS: *m/z* 333 (M^+), 301, 299, 282. Compound **16**: t_R = 39.3 min; white solid; mp 159−63 °C. ^1H NMR ($CDCl_3$): δ 0.84 (s, C18-Me, 3H); 1.01 (s, C19-Me, 3H); 2.29 (d, thiirane-CH, 1H *J* = 5.2 Hz); 2.40 (d, thiirane-CH, 1H, *J* = 5.72 Hz); 2.70 (m, thiirane-CH, 1H); 3.50 (m, C3αH, 1H); 5.32 (d, C6, =CH−, 1H, *J* = 4.8 Hz). IR (KBr) cm^{-1}: ν_{max} 3450−3400, 2950, 1380, 1060, 960, 850, 810. GC−MS: *m/z* 333 (M^+), 301, 299, 282.

20-Cyclohexyliminopregn-5-en-3*β*-ol (17). 500 mg of pregnenolone (1.58 mmol) was dissolved in 9 mL of cyclohexylamine and catalytic amounts of triflouroacetic acid and refluxed under N_2 for 20 h. The excess cyclohexylamine was evaporated in vacuo and the resulting white solid was dried under reduced pressure (0.01 Torr): yield 98%, white solid; mp 137−8 °C. ^1H NMR ($CDCl_3$): δ 0.58 (s, C18-Me, 3H); 1.00 (s, C19-Me, 3H); 1.79 (s, C21-Me, 3H); 3.52 (m, C3αH, 1H); 5.34 (s, C6, =CH−, 1H). IR (KBr) cm^{-1}: ν_{max} 3300, 2950, 1660, 1450 1065. Anal. ($C_{27}H_{43}NO$) C, H, N.

17*β*-Diaziridinyl-20-methylandrost-5-en-3*β*-ol (18). To 200 mg of **17** (0.5 mmol) in dry 10 mL MeOH was added 1.5 mL of NH_3-saturated MeOH. Over 10 min 87 mg hydroxylamine-*O*-sulfonic acid (0.77 mmol) was added to the solution. The reaction mixture was stirred for 24 h and allowed to stand for additional 48 h at room temperature. The reaction mixture was poured onto ice and extracted trice with 50 mL ethyl

acetate. The combined organic phases were washed with water and brine, dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by FCC (CH_2Cl_2:EtOAc 25:1): yield 21%, white solid; mp 137−41 °C. ^{1}H NMR ($CDCl_3$): δ 0.73 (s, C18-Me, 0.9H); 0.82(s, C18-Me, 2.1H); 1.01 (s, C19-Me, 3H), 1.10 (d, C21-Me, 3H, J = 9.28 Hz); 2.23−2.29 (m, 17αH, 1H); 3.53 (m, C3αH, 1H); 5.34 (s, C6, ═CH−, 1H). ^{13}C NMR ($CDCl_3$) δ 138,2 (C-5); 118,8 (C-6); 69,1 (C-3); 54,2; 52,5; 51,7; 47,4; 40,4; 39,7; 36,6; 34,7; 33,9; 29,2; 29,1; 29,0; 25,9; 21,2; 18,5; 18,3 (C19); 16,8 (C18); 10,6 (C21). IR (KBr) cm^{-1}: ν_{max} 3400, 3240, 2950, 1460/40, 1390, 1170, 1070. Anal. ($C_{21}H_{34}N_2O\cdot0.5H_2O$) C, H, N.

17β-Diazirinyl-20-methylandrost-5-en-3β-ol (19). In 18 mL $CHCl_3$ 496 mg of **18** (1.5 mmol) was dissolved and mixed with 0.41 mL triethylamine (3 mmol). Over a period of 10 min, 0.47 g Br_2 (3 mmol) in 5 mL $CHCl_3$, was added at 0 °C. The reaction mixture was poured into water and evaporated in vacuo. The crude product was diluted in 6 mL of acetone and 2.25 g NaI (1.5 mmol) was added. The reaction mixture was stirred 16 h at room temperature before it was poured onto ice and extracted two times with ethyl acetate (50 mL). The combined organic phases were washed with aqueous $Na_2S_2O_3$ solution, water and brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by FCC (toluene: ether 4:1): yield 38%, white solid; mp 125−8 °C. ^{1}H NMR ($CDCl_3$): δ 0.86 (s, C18-Me, 3H); 1.00 (s, C21-Me, 3H); 1.02 (s, C19-Me, 3H); 3.51 (m, C3αH, 1H); 5.33 (d, C6, ═CH−, 1H, J = 5 Hz). ^{13}C NMR ($CDCl_3$): 140.9 (C-5); 121.3 (C-6); 71.7 (C-3); 55.9 (C-17); 53.8; 50.1; 43.8; 42.3; 38.7; 37.2; 36.5 (C-10); 316; 31.5; 31.3; 25.8; 24.0; 23.5; 21.3 (C-21); 20.7; 19.3 (C-19); 13.1 (C-18). IR (KBr) cm^{-1}: ν_{max} 3500−3450, 2950, 1598, 1450/40, 1390/80/70, 1065. UV (EtOH)/nm: λ_{max} 352, 239, 204. Anal. ($C_{21}H_{32}N_2O$) C, H, N.

3β-Acetoxy-17β-(azetidinyl-2-one)androst-5-ene (21). 50 μL of chlorosulfonyl isocyanate (0.57 mmol) was added to 125 mg of **20** (0.44 mmol) in 5 mL of toluene. The reaction mixture was heated at 50 °C for 24 h and cooled to room temperature, poured onto ice and extracted with toluene and CH_2Cl_2. The combined organic phases were washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. The resulting chlorosulfonylazetidinone intermediate was diluted in 1 mL ether and dropped to 2 mL 25% aqueous Na_2SO_3 and 1 mL ether. The reaction mixture was stirred for 60 min at room temperature. During the reaction, the solution was kept at neutral pH by addition of 10% aqueous KOH. The reaction mixture was poured onto ice and extracted with ethyl acetate and ether. The combined organic phases were washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by FCC (CH_2Cl_2:EtOAc 2:1). A 2:1 diastereomeric mixture was obtained: yield 36%, white solid. ^{1}H NMR ($CDCl_3$): δ 0.67(s, C18-Me, 2H); 0.72(s, C18-Me, 1H); 1.02 (s, C19-Me, 3H); 2.03 (s, CH_3-acetyl, 3H); 2.66/2.68 (ddd, azetidinone, 1H, J = 15.04 Hz, 2.64 Hz, 1.32 Hz); 3.02/3.04 (ddd, azetidinone, 1H, J = 14.6 Hz, 4.84 Hz, 2.2 Hz); 3.62/3.57 (m, azetidinone, 1H); 4.61 (m, C3αH, 1H); 5.37 (d, C6 = CH−, 1H, J = 4.8 Hz); 5.84/6.03 (s, amide-H, 1H). IR (KBr) cm^{-1}: ν_{max} 3200, 2950, 1760, 1750, 1730, 1465/50/40, 1380/60, 1030. MS: *m/e* 386 (M^+), 313, 310, 285, 282.

(20*R*,20*S*)-17β-Azetidinylpregn-5-en-3β-ol (22). To a solution of 190 mg **21** (0.49 mmol) in 5 mL THF was added 2.5 mL DibaH (20% in toluene, 3.5 mmol) dropwise. The reaction mixture was refluxed for 3 h. After the further addition of 2 mL DibaH (20% in toluene, 2.8 mmol), the mixture was refluxed for additional 21 h, cooled to room temperature and filtred. Water was added, and the solution was extracted twice with CH_2Cl_2 . The combined organic phases were washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by FCC (CH_2Cl_2:EtOAc 1:1). A 1:1 diastereomeric mixture was obtained: yield 62%, pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 0.71 (s, C18-Me, 1.5H); 0.78 (s, C18-Me, 1.5H); 1.01 (s, C19-Me, 3H); 2.26 (m, azetidine, 1H); 2,37 (dd, azetidine, 1H, J = 15.9 Hz, 9.28 Hz) 2.50 (d, azetidine, 0.5H, J = 15 Hz); 2.79 (dd, azetidine, 0.5H, J = 16.3 Hz, 3.2 Hz); 3.19 (t, azetidine, 0.5H, J = 10 Hz); 3.18 (dt, azetidine, 0.5H, J = 9.28 Hz, 3.2 Hz); 3.53 (m, C3αH, 1H); 5.34 (d, C6, ═CH−, 1H, J = 5 Hz). IR (KBr) cm^{-1}: ν_{max} 3300, 2950, 1740, 1440, 1380, 1050, 730. Anal. ($C_{22}H_{35}NO$) C, H, N.

Biological Methods. 1. Enzyme Preparations. The enzymes were prepared according to previously described methods: human and rat testicular P450 17,[21] human placental P450 arom,[50] bovine adrenal P450 scc,[50] and 5α-reductase type II.[53] For the P450 TxA_2 assay citrated human whole blood was used.[51]

2. Enzyme Assays. The following enzyme assays were performed as described previously: rat P450 17,[21] human P450 17,[31] P450 arom,[50] P450 TxA_2,[51] P450 scc,[50] DU145 cells (5α-reductase type I),[54] and 5α-reductase and type II.[53]

K_i and K_m values were determined according to Lee and Wilson.[58] Inhibitor concentrations were between the IC_{40} and IC_{70} value of the compound, substrate concentrations between 1.25 and 20 μM. The incubation time was 15 min. All other parameters were identical to the regular P450 17 assay. UV difference spectroscopy experiments were performed as previously described.[14]

3. Determination of Plasma Testosterone Concentration. Tests were performed with adult male Sprague−Dawley rats (each group consisted of 7−8 animals). All compounds were dissolved in a mixture of olive oil and benzyl alcohol (95:5) and administered once intraperitoneally at equimolar doses to 10 mg/kg ketoconazole (0.019 mmol/kg). Blood samples were taken by cardiac puncture under diethyl ether anesthesia after 2 and 6 h. Plasma testosterone values were determined by a commercially available RIA and are reported in ng/mL plasma.

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Supporting Information Available: X-ray structure and crystallographic data for **13** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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