Synthesis and Evaluation of Novel Steroidal Oxime Inhibitors of P450 17 (17α-Hydroxylase/C17−20-Lyase) and 5α-Reductase Types 1 and 2

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17α-Hydroxylase/C17−20-lyase (P450 17, CYP 17) and 5α-reductase are the key enzymes in androgen biosynthesis and targets for the treatment of prostate cancer and benign prostatic hyperplasia. In the search of inhibitors for both enzymes, 23 pregnenolone- or progesterone-based steroids were synthesized bearing an oxime group connected directly or via a spacer to the steroidal D-ring. Tested for inhibition of human and rat P450 17, some pregnenolone (**9**, **11**, **14**) and a series of progesterone compounds (**17**−**20**) turned out to be highly active inhibitors of the human enzyme. The most active compound was *Z*-21-hydroxyiminopregna-5,17(20)-dien-3*β*-ol (**9**) showing K_i values of 44 and 3.4 nM for the human and rat enzymes, respectively, and a type II UV-difference spectrum indicating a coordinate bond between the oxime group and the heme iron. In contrast to the pregnenolones which showed no inhibition of 5α-reductase isozymes 1 and 2, the progesterones **16**, **17**, **20**, **21**, and **23** showed marked inhibition, especially toward the type 2 enzyme. Compounds **17** and **20** were identified as potent dual inhibitors of both P450 17 and 5α-reductase. Tested for selectivity, the most potent P450 17 inhibitors **9**, **10**, and **14** showed no or only marginal inhibition of P450 arom, P450 scc, and P450 TxA_2. Selected compounds were tested for inhibition of the target enzymes using whole-cell assays. Compounds **9**−**11** strongly inhibited P450 17 being coexpressed with NADPH−P450 reductase in *E. coli* cells, and **16**, **20**, and **23** markedly inhibited 5α-reductase expressed in HEK 293 cells. Tested for in vivo activity, **9** (0.019 mmol/kg) decreased the plasma testosterone concentration in rats after 2 and 6 h by 57% and 44%.

Introduction

Two enzymes involved in androgen biosynthesis are targets for the treatment of prostatic diseases: 17α-hydroxylase/C17−20-lyase (P450 17, CYP 17) and 5α-reductase (Chart 1).[1,2] P450 17 catalyzes the 17α-hydroxylation of pregnenolone and progesterone (P) and the subsequent cleavage of the C20,21-acetyl group to yield the corresponding androgen.[3] The antimycotic ketoconazole, which is not only an inhibitor of fungal 14α-demethylase but also of human P450 17,[4] has already been successfully used in the treatment of prostate cancer (PC) in men.[5] Because of its side effects, some of which are related to its low selectivity, it is not commonly accepted for wide use.[6] A number of steroidal[1,2,7−12] and nonsteroidal[1,2,13−25] inhibitors of P450 17 have been described as potential drugs for the treatment of PC. All of them contain a functional group, mostly a nitrogen-bearing heterocycle, capable to form a coordinate bond with the heme iron of the enzyme. Regarding steroidal compounds, a 3-pyridyl group and a 4-imidazolyl group in position 17 lead to potent inhibitors I and II (Chart 2),[7,10] the former of which is in clinical trial.[2]

5α-Reductase catalyzes the conversion of testosterone (T) to the more potent androgen dihydrotestosterone (DHT).[26] High concentrations of the latter are associated with benign prostatic hyperplasia (BPH),[27] prostatic cancer,[28] and diseases such as male pattern baldness.[29] There are two isozymes (types 1 and 2) with different tissue distributions.[30] The most currently used 5α-reductase inhibitor in BPH treatment is finasteride, a steroidal compound inhibiting mainly isozyme 2. The long-term pharmacotherapy with finasteride generally has been well-tolerated.[31] Nevertheless, its limited activity and its side effects[32] have caused us and others to look for new classes of steroidal[33−35] and nonsteroidal[36−45] inhibitors.

Chart 1. Target Enzymes

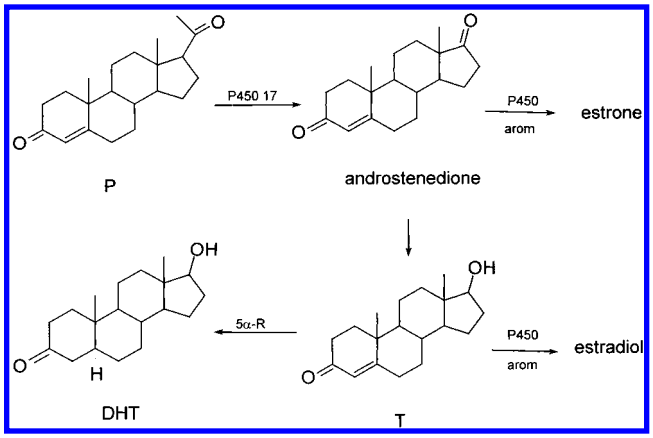
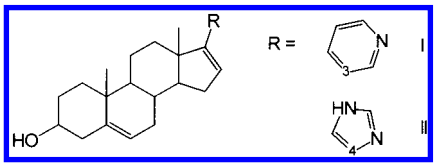


Chart 2. Selected Inhibitors of P450 17



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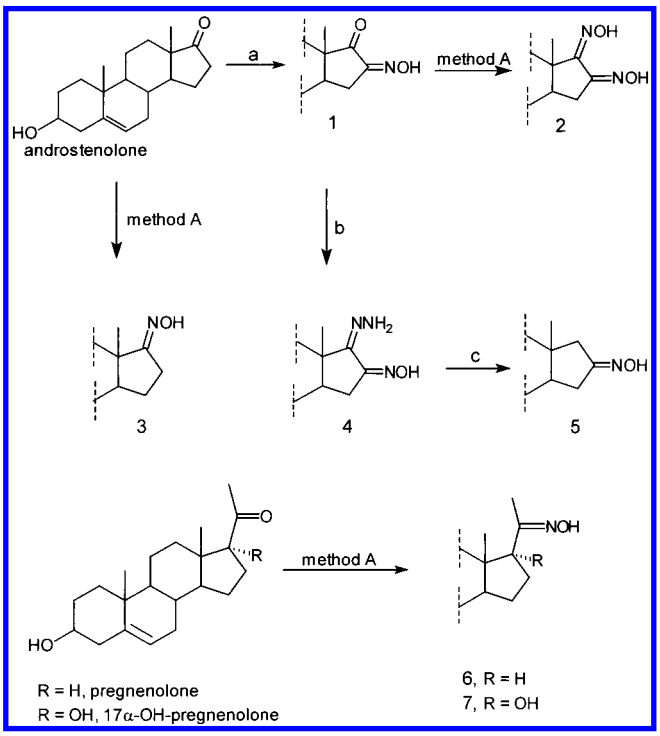
Scheme 1[a]



[a] Reagents and conditions: Method A: NH_2OH, NaOAc; (a) *n*-amyl nitrite, K; (b) $NH_2NH_2 \cdot H_2O$, CH_3COOH; (c) KOH.

As it cannot be expected that P450 17 inhibition leads to a complete blockade of androgen formation, it might be advantageous to additionally inhibit 5α-reductase. A dual inhibitor of both enzymes, however, must not inhibit aromatase, P450 arom, another P450 enzyme necessary for the metabolism of testosterone (Chart 1). Several compounds which inhibit both P450 17 and 5α-reductase have already been reported.[1,2,11]

There are few nonsteroidal[22] and steroidal[1,2,11] oximes described in the literature, some of which are dual inhibitors of P450 17 and 5α-reductase.[2,11] In this study we report on the synthesis of 23 steroidal pregnenolone- and progesterone-based oximes and the evaluation of their biological activity. Inhibition of the target enzymes (P450 17 rat and human; human 5α-reductase isozymes 1 and 2) using in vitro as well as cellular assays was determined as well as selectivity toward other P450 enzymes (P450 scc, P450 arom, P450 TxA_2). In vivo P450 17 inhibition of compound **9** was measured in rats.

Chemistry

Compounds **1, 4,** and **5** (Scheme 1) were synthesized as previously described.[46,47] The oximes **2, 3, 6,**[8] **7** (Scheme 1), **10,**[11,48] **11, 12, 13** (Scheme 2), and **15** (Scheme 3) were obtained by reaction of the corresponding steroidal ketones; the oximes **8, 9** (Scheme 2), and **14** (Scheme 3) were obtained by reaction of the corresponding steroidal aldehydes with NH_2OH. The synthesis of oximes **1**−**7, 14,** and **15** (Schemes 1−3) started from the pregnenolone derivatives; the synthesis of compounds **8**−**13** started from the corresponding 3β-protected steroids, which gave after cleavage of the protecting group the desired pregnenolone derivatives.

Introduction of a Δ14-double bond, Δ16-double bond, or Δ14,Δ16-double bond system into the D-ring was performed following the described route (Scheme 2). Starting from 3β-acetoxyandrost-5-en-17-one, the triflate **8c** was synthesized.[7] This triflate was transferred with ethyl vinyl ether and catalytic amounts of tetra(triphenylphosphine)palladium.[49] Two products were obtained resulting from the α- or β-attack of the palladium intermediate on the double bond. Subsequent β-elimination and hydrolysis with $HClO_4$ yielded **8b** and **10b** in a ratio of 2:9 which were separated by liquid chromatography (LC). ^1H NMR data show that **8b** is a 1:1 mixture of the *E*- and *Z*-isomers. Treatment of **10b** with NH_2OH and deprotection yielded the oxime **10**. **8b** was converted in the same way into a mixture of the acetylated oximes **8a** and **9a**. At this stage the *E*- and *Z*-isomers were separated by LC. Using ^1H NMR−NOESY technique, **8a** was classified as the *E*-isomer and **9a** as the *Z*-isomer. In the spectra of **8a** a coupling of the C20 proton and the C18 methyl protons was observed, while the C21 proton showed a signal with the C16 methylene group. Deprotection led to the oximes **8** and **9**, respectively. The Δ14,Δ16-ketone **11b** was obtained according to the method of Solo and Singh.[50] After protection of the Δ5-double bond by addition of Br_2, the allylic 14-position was brominated (AIBN, NBS). Treatment of the tribromide according to Finkelstein gave the Δ14,Δ16-ketone **11b**. Treatment of **11b** with NH_2OH and deprotection of **11a** gave the oxime **11**. For the preparation of compound **12** the method of Templeton and Yan[51] was applied, treating **11b** with tri-*n*-butyltin hydride under irradiation (sunbeam lamps). TLC monitoring revealed the formation of a side product, which was determined by ^1H NMR as the 17α-isomer of the desired Δ14-ketone. The ratio between the β- and α-isomers was 11:2. After LC separation, each isomer was transferred into the corresponding oxime **12** and **13**, respectively. The protected oxime **13a** was not isolated but directly deprotected to **13**.

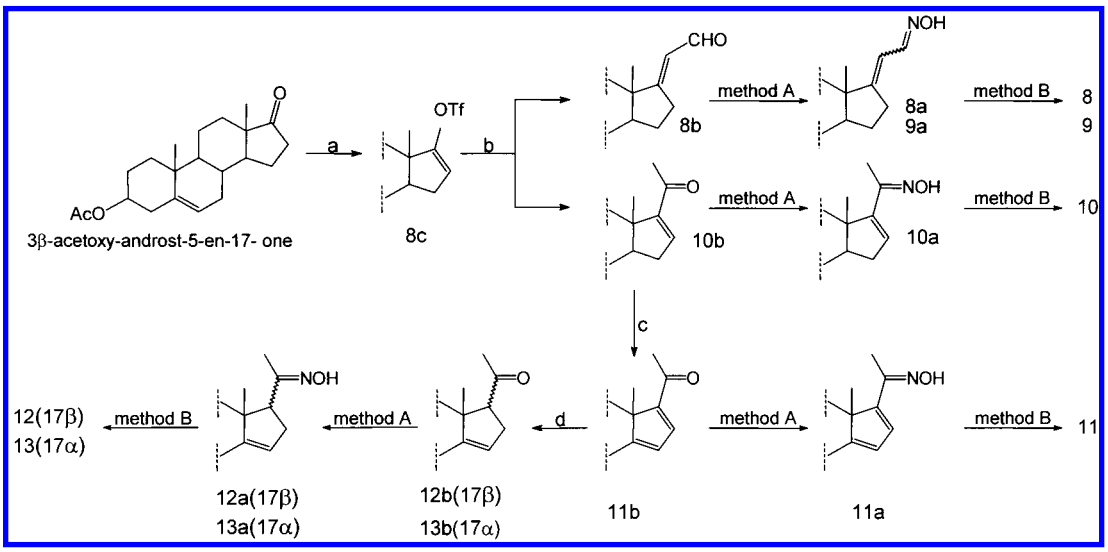
A methylene spacer was introduced into the 17β-side chain by the following synthetic route (Scheme 3). 3β-THP-androst-5-en-17-one was transferred to the nitrile **14c** using Wittig−Horner reaction. Selective reduction of the exo-configurated double bond with Mg/MeOH[52] yielded the 17β-derivative **14b**, which was reduced to the aldehyde **14a** using DibaH. The latter was converted to the oxime **14** by NH_2OH treatment and deprotection or was transferred by Grignard reaction (Mg, MeI) and subsequent oxidation of the resulting alcohol to the ketone **15a**. The oxidation was performed with PCC adsorbed on Al_2O_3, which simplifies the purification of **15a.** NH_2OH treatment of **15a** gave the corresponding oxime **15**.

Selected compounds (**6, 9**−**11, 14, 15**) were transferred into their progesterone derivatives (**16**−**21**) by a modified Oppenauer oxidation using *N*-methylpiperidone and aluminum isopropoxide as reagents (Scheme 4). The 5-en-3β-ol AB-ring of compounds **6** and **15** was converted to a 4-ene-3,6-dione system (**22**, **23**; Scheme 4) by treatment with PDC in DMF as previously described.[53]

Biological Properties

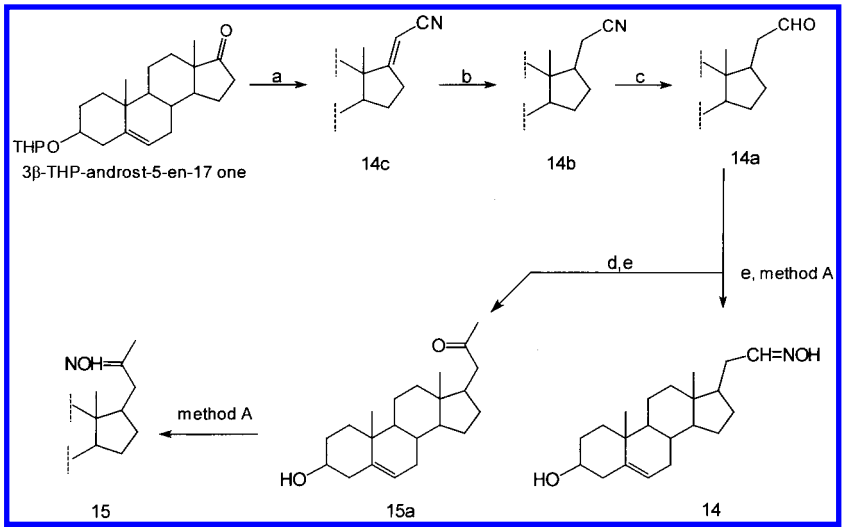
The inhibitory activity toward P450 17 rat and human enzymes was tested using testicular microsomes. Progesterone was used as substrate, and RP-HPLC with UV

Scheme 2[a]



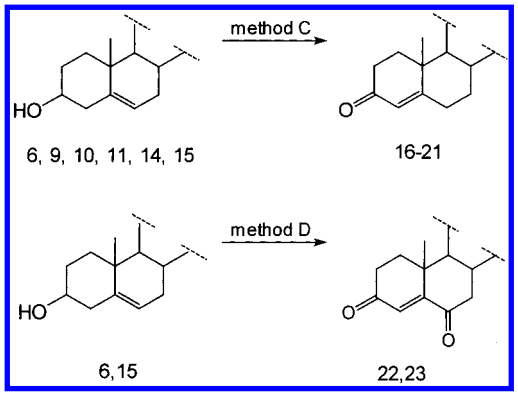
[a] Reagents and conditions: (a) 2,6-di-*tert*-butyl-4-methylpyridine, $(F_3CSO_2)_2O$; (b) $P(Ph_3)_4Pd$, $CH_3CH_2OCH{=}CH_2$; (c) 1. Br_2, CH_3COOH, 2. NBS, AIBN, 3. NaI, acetone; (d) $HSn(n\text{-butyl})_3$, *h*; Method A: NH_2OH, NaOAc; Method B: deacetylation, KOH (10%), MeOH.

Scheme 3[a]



[a] Reagents and conditions: (a) $(EtO)_2POCH_2CN$, NaH; (b) Mg, MeOH; (c) DibaH, −76 °C; (d) 1. CH_3I, Mg, 2. PCC−Al_2O_3; Method A: NH_2OH, NaOAc; (e) deprotection, PPTS.

Scheme 4[a]

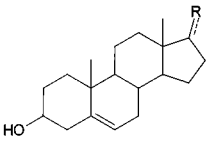
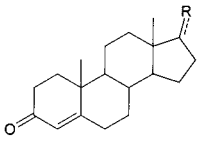
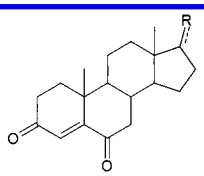


[a] Reagents and conditions: Method C: $Al(OCH(CH_3)_2)_3$, *N*-methylpiperidone; Method D: PDC, DMF.

detection was employed for product determination as recently described by us.[14,24] Compounds **1**−**5** (Scheme 1) bearing one or two oxime groups connected directly to the steroidal D-ring show only poor or no inhibition of the P450 17 rat and human enzymes (data not given). In contrast to these compounds the C20-oxime **6** shows a rather good inhibition of the human enzyme, exhibiting an IC_{50} of 1.65 μM (Table 1). Introduction of a 17α-hydroxy group into compound **6** causes a loss of activity (compound **7**), whereas introduction of a Δ16-double bond increases activity toward the human enzyme dramatically (Table 2, compounds **10** and **11**). The increase of activity is probably due to the conjugation of the oxime group with the D-ring double bond, because compound **12**, bearing an isolated Δ14-double bond, shows only poor activity. Exchanging the side chain of compound **12** from the β-position into the α-position (compound **13**, Scheme 2) does not increase activity (IC_{50} values toward rat and human enzyme: >125 and >2.5 μM, respectively). It is striking that all pregnenolone C20-oximes exhibit almost no inhibition of the rat enzyme.

The C21-oximes **14**, **15**, **8,** and **9** show moderate (**8**) to excellent (**9**) inhibitory activity toward the human enzyme (Tables 1 and 3) and reasonable activity toward

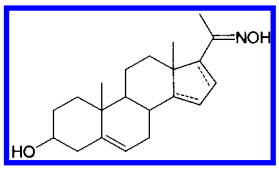
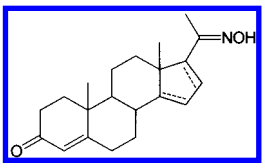
Table 1. Inhibition of P450 17 Rat and Human Enzymes by 17-Substituted Steroidal Oximes

R	Compound	IC_{50}[a] rat[b] [μM]	IC_{50}[a] human[c] [μM]	Compound	IC_{50}[a] rat[b] [μM]	IC_{50}[a] human[c] [μM]	Compound	IC_{50}[a] rat[b] [μM]	IC_{50}[a] human[c] [μM]
	**6**[d]	n.i.	1.65	**16**	>125	>2.5	**22**	n.i.	>2.5
	**7**	n.i.	n.i.						
	**14**	2.76	0.27	**20**	0.30	0.52			
	**15**	9.8	1.18	**21**	3.6	>2.5	**23**	n.i.	n.i.
	**8**	>125	>2.5						
	**9**	0.52	0.077	**17**	0.14	0.18			
	ketoconazole	67	0.74	ketoconazole	67	0.74	ketoconazole	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, deviations within ±5%. [d] See ref 8. ni = no inhibition (at 125 μM for rat and 2.5 μM for human enzymes, respectively).

Table 2. Inhibition of P450 17 Rat and Human Enzymes by Pregnenolone and Progesterone Oximes: Influence of Saturation on the D-Ring

		IC_{50} (μM)[a]			IC_{50} (μM)[a]	
D-ring	compd	rat[b]	human[c]	compd	rat[b]	human[c]
saturated	**6**[d]	ni	1.65	**16**	>125	>2.5
Δ16	**10**[e]	>125	0.17	**18**[e]	>125	0.10
Δ14,16	**11**	125	0.20	**19**	>125	0.20
Δ14	**12**	>125	>2.5			
	ketoconazole	67	0.74	ketoconazole	67	0.74

[a] Concentration of inhibitor required to give 50% inhibition. ni = no 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, deviations within ±5%. [d] See ref 8. [e] See ref 11.

Table 3. Inhibition of P450 17 Rat and Human Enzymes by Steroidal Oximes and Ketoconazole (descending order of activity[a])

compd	type	D-ring	R	rat enzyme IC_{50} (μM)[b]	compd	type	D-ring	R	human enzyme IC_{50} (μM)[b]
17	prog	satd	CHCHNOH	0.14	**9**	preg	sat	CHCHNOH	0.077
20	prog	satd	CH_2CHNOH	0.30	**18**	prog	Δ16	CCH_3NOH	0.10
9	preg	satd	CHCHNOH	0.52	**10**	preg	Δ16	CCH_3NOH	0.17
14	preg	satd	CH_2CHNOH	2.76	**17**	prog	satd	CHCHNOH	0.18
21	prog	satd	CH_2CCH_3NOH	3.6	**11**	preg	Δ14,16	CCH_3NOH	0.20
15	preg	satd	CH_2CCH_3NOH	9.8	**19**	prog	Δ14,16	CCH_3NOH	0.20
ketoconazole				67	**14**	preg	satd	CH_2CHNOH	0.27
11	preg	Δ14,16	CCH_3NOH	125	**20**	prog	satd	CH_2CHNOH	0.30
					ketoconazole				0.74
					15	preg	satd	CH_2CCH_3NOH	1.18
					6	preg	satd	CCH_3NOH	1.65

[a] IC_{50} values of compounds not listed were >125 and >2.5 μM for the rat and human enzymes, respectively. [b] See Tables 1 and 2.

the rat enzyme (compound **14** and **9**). Compound **9** is the most active inhibitor of the human enzyme in this study, being 64 times more potent than its *E*-isomer (compound **8**). The progesterone compounds with a saturated D-ring show reduced inhibition of the human enzyme compared to the corresponding pregnenolone

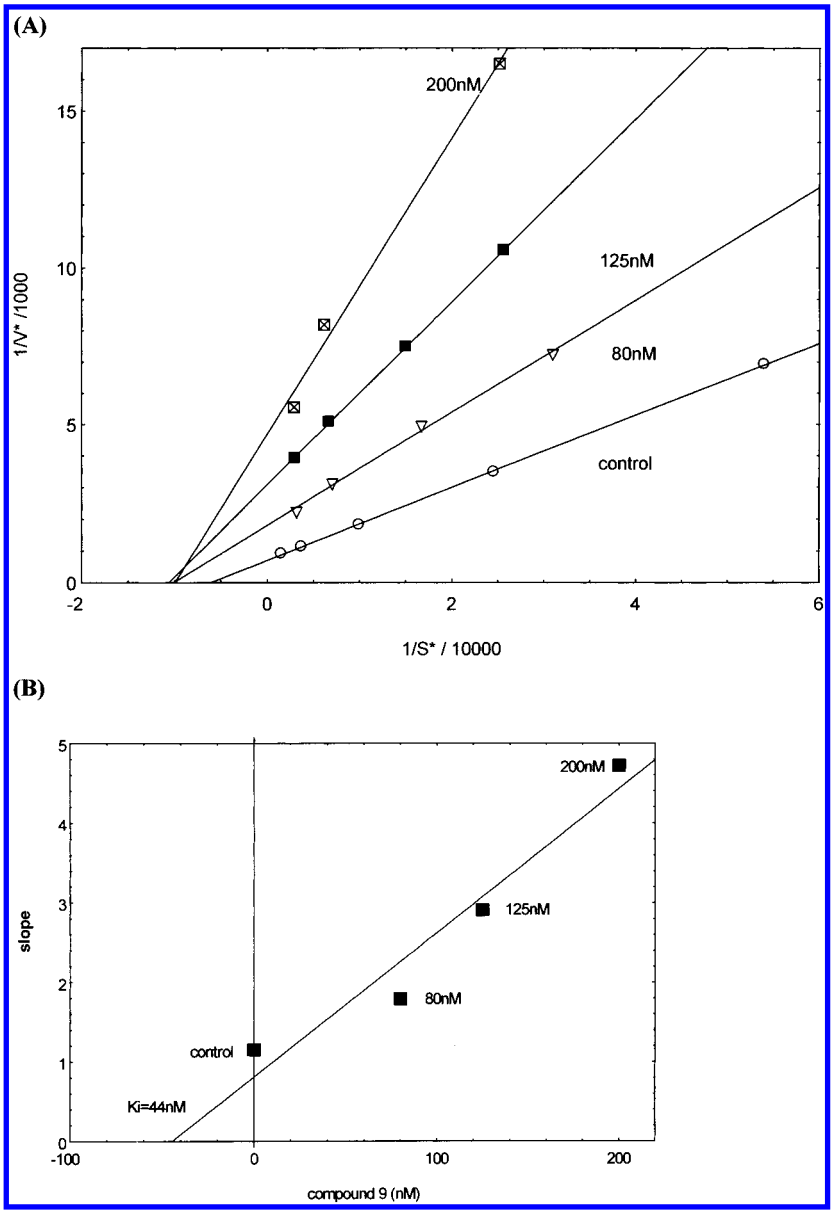


Figure 1. Inhibition of human P450 17 by compound **9**. (A) Lee−Wilson plot of enzyme activities at various substrate and inhibitor concentrations (see Experimental Section). The K_m for progesterone was 4.3 μM. (B) Slope of each reciprocal plot against inhibitor concentration. The given values are mean values of at least two experiments. The deviations were within ±5%.

analogues, whereas inhibitory activity toward the rat enzyme is enhanced (Tables 1 and 3). Thus, compounds **20** and **17** are potent inhibitors of the rat enzyme, exhibiting IC_{50} values of 0.30 and 0.14 μM, respectively. In case of the unsaturated D-ring compounds, however, the change of the pregnenolone to the progesterone skeleton does not influence inhibitory activity toward both enzymes strongly (Table 2). The introduction of a keto group into the 6-position of compounds **16** and **21** is not suitable for increasing inhibition (compounds **22** and **23**, Table 1). It is worth mentioning that several compounds show stronger inhibition of the two P450 17 enzymes than the reference ketoconazole (Table 3).

For compound **9** K_i values of 44 nM (human enzyme: K_m progesterone = 4.3 μM, Figure 1) and 3.4 nM (rat enzyme: K_m progesterone = 1.4 μM) were determined. The chemical nature of the complex formed was studied using UV−vis difference spectroscopy following the procedure previously described.[8] Figure 2 shows the characteristic type II spectrum (trough: 392 nm, peak: 419 nm) indicating the formation of a coordinate bond between the oxime nitrogen and the heme iron of the P450 17 enzyme. This effect is concentration-dependent (data not shown). Interestingly the type II spectrum was not reversed by the addition of high substrate concentrations, suggesting that **9** forms a rather tight complex with the heme iron.

Selected compounds were tested for inhibitory potency versus human 5α-reductase isozymes 1 and 2. Prostate homogenate of BPH patients was used as the source for the type 2 enzyme (pH 5.5) and the DU 145 cell line as the source for isozyme 1. Inhibition assays were performed with radiolabeled substrate, and HPLC was applied for product separation. While the pregnenolone compounds do not show inhibitory activity toward both isozymes (data not given), some of the progesterone compounds exhibit marked inhibition (Table 4). The C20-oxime **16** is a strong inhibitor of both isozymes (IC_{50} values toward types 1 and 2: 1.63 and 0.58 μM, respectively). The introduction of one or two double

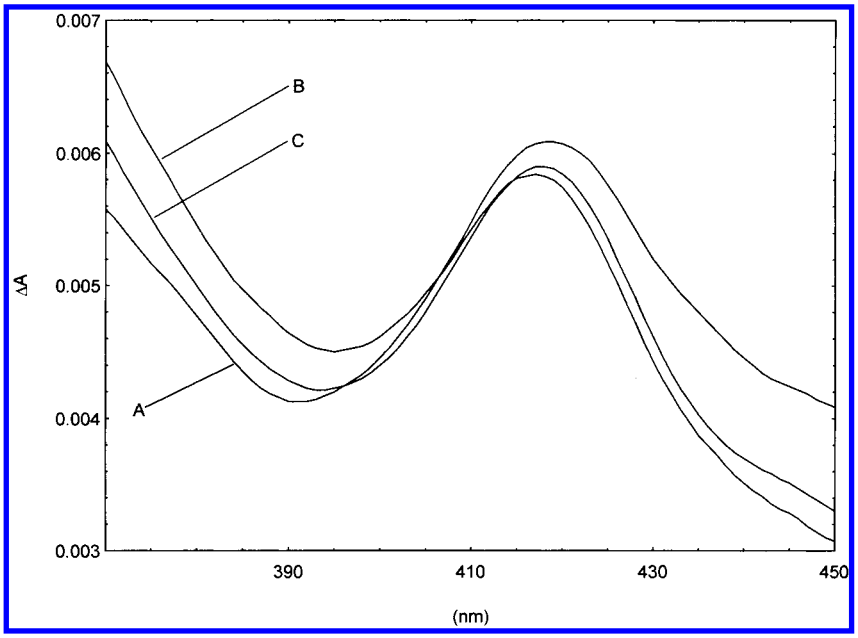
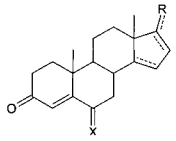


Figure 2. Rat enzyme: type 2 difference spectrum of compound **9** (A: 31 μM). Addition of excess substrate (progesterone) does not reverse the effect (B: 300 μM, 5 min; C: 600 μM, 10 min).

Table 4. Inhibition of Human 5α-Reductase Isozymes 1 and 2 by Progesterone-Derived Oximes



R	D-ring	X	Compound	Type 1[a] IC_{50} [μM]	Type 2[b] IC_{50} [μM]
	saturated	H, H	**16**	1.63	0.58
	Δ16	H, H	**18**	>10	>10
	Δ14, 16	H, H	**19**	>10	n.i.
	saturated	O	**22**	>10	3.2
	saturated	H, H	**20**	1.95	0.30
	saturated	H, H	**21**	0.90	0.47
	saturated	O	**23**	0.90	0.43
	saturated	H, H	**17**	n.d.	0.43
			finasteride	0.04	0.003

[a] Human DU-145 cell assay, concentration of androstenedione 5 nM. [b] Enzyme preparation from human BPH tissue, concentration of testosterone 210 nM, pH = 5.5. The given values are mean values of at least two experiments, deviations within ±5%. ni = no inhibition, nd = not determined.

bonds (Δ16 and Δ14,Δ16) into the D-ring decreases activity dramatically (compounds **18** and **19**). A keto group in position 6 leads to an almost complete loss of activity toward type 1 isozyme and reduces type 2 inhibition (compound **22**). Transferring the oxime group from position 20 to 21 enhances type 2 inhibitory potency. Thus, compound **20** is the most potent inhibitor toward type 2 isozyme. The C22-steroids **21** and **23** show an increased inhibition of type 1 isozyme and are strong inhibitors of type 2 isozyme. The *Z*-configurated Δ17-compound **17** is also a strong inhibitor of isozyme 2. However, none of the compounds reaches the activity of the reference finasteride (IC_{50} values in our tests: 45 and 3 nM for types 1 and 2, respectively). Interestingly the structure modifications performed in this study resulted in selective inhibitors of 5α-reductase (compounds **16**, **21**, and **23**) as well as dual inhibitors of 5α-reductase and P450 17 (compounds **20** and **17**).

The selectivity of the most potent P450 17 inhibitors was tested toward P450 arom, P450 TxA_2, and P450 scc taking into account that 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. High concentrations of compounds **9**, **10**, and **14** were tested using the procedures described recently (P450 scc,[54] P450 arom,[54] and P450 TxA_2[55]). None of the compounds inhibit P450 arom (inhibitor concentration: 25 μM, reference CHAG[56] IC_{50}: 0.15 μM). Only compound **9** shows weak inhibition of P 450 TxA_2 (35% inhibition, 50 μM, reference dazoxiben IC_{50}: 1.1 μM). Tested for inhibition of P450 scc, compound **14** inhibits the enzyme by 74% at an inhibitor concentration of 25 μM, while the other compounds were not active.

A precondition for in vivo activity is the ability of the compounds to permeate cell membranes. Therefore selected inhibitors were tested for inhibition of the target enzymes using the whole-cell assays recently developed by our group[57,58] Employing *E. coli* cells coexpressing P450 17 and NADPH−P450 reductase,[57] compounds **9**−**11**, which were the most potent inhibitors of the microsomal P450 17 enzyme, turned out to be very potent in this assay as well. Exhibiting IC_{50} values from 0.23 to 0.53 μM, the compounds are again more potent than the reference ketoconazole (IC_{50}: 2.8 μM, Table 5). Tested on HEK293 cells expressing isozymes 1 and 2[58] of 5α-reductase, compounds **16**, **20**, and **23** are similarly active, showing IC_{50} values between 0.86 and 2.9 μM (Table 6).

Table 5. Inhibition of Human P450 17 by Selected Compounds Using *E. coli* Cells Coexpressing P450 17 and NADPH−P450 Reductase

compd	IC_{50}[a] (μM)
9	0.23
10	0.52
11	0.42
ketoconazole	2.8

[a] Concentration of inhibitor required to give 50% inhibition. Recombinant *E. coli* cells were used; concentration of progesterone (substrate) 25 μM. The given values are mean values of at least two experiments, deviations within ±10%.

Table 6. Inhibition of Human 5α-Reductase Isozymes 1 and 2 by Selected Compounds Using HEK293 Cells Expressing Isozymes 1 and 2

	IC_{50}[a] (μM)	
compd	HEK293-5α1	HEK293-5α2
16	1.77	1.17
20	2.44	0.89
23	2.90	0.86
finasteride	0.54	0.06

[a] Concentration of inhibitor required to give 50% inhibition; 300 000 cells/well transfected with type 1 5α-reductase expression plasmid pRcCMV-I were used and 300 000 cells/well transfected with type 2 5α-reductase expression plasmid pRcCMV-II were used; concentration of [^{3}H]androstenedione 5 nM; incubation time 30 and 13 min for types 1 and 2, respectively. The given values are mean values of at least three experiments, deviations within ±10%.

A prerequisite for performing in vivo experiments in the rat is the effectiveness of the compounds toward the rat enzyme. It is apparent from Tables 1 and 2 that several highly potent inhibitors of human P450 17 do not show satisfactory inhibition of the rat enzyme to be tested in vivo. Fortunately the most potent inhibitor of human P450 17, compound **9**, inhibits the rat enzyme sufficiently. It was administered intraperitoneally to adult SD rats equimolar to 10 mg/kg ketoconazole (0.019 mmol/kg). In contrast to the reference compound, which is not active at this dose, **9** decreases the plasma testosterone concentration after 2 and 6 h by 57% and 44%, respectively (plasma testosterone concentration ng/mL: control (2 h) 1.51, (6 h) 0.71; ketoconazole (2 h) 1.60, (6 h) 0.64; **9** (2 h) 0.66, (6 h) 0.39; $n = 7-8$).

Discussion and Conclusion

The present study shows that some of the synthesized steroidal oximes are selective inhibitors of either human P450 17 (**16**, **9**, **10**,[11] **11**, **14**, **15**, **18**,[11] **19**) or 5α-reductase (**16**, **21**, **23**). Interestingly, a few compounds are dual inhibitors of both enzymes (**17**, **20**).

It is obvious that the oxime group is appropriate to coordinate with the heme iron of P450 17. It might also be a suitable functional group for the design of inhibitors of other P450 enzymes. A high species difference is apparent from the differing inhibition values for the P 450 17 isozymes shown by many compounds. Although 17α-OH-pregnenolone is a high-affinity substrate of P 450 17, introduction of a OH group into position 17α of compound **6** leads to complete loss of inhibitory activity toward the human enzyme **7**. The present data shows that the inhibitory potency strongly depends on the position of the oxime group: the C21-oximes are more active than the C20-oximes. Inhibition, however, is not related to the basic steroidal structure: the progesterones are similarly active as the corresponding pregnenolones. In rat enzyme, **9** and its progesterone analogue **17** are very potent inhibitors. Compound **17** exceeds the activity of ketoconazole, being 478 times more potent, and in addition is superior to the steroidal inhibitor **II**[10] (IC_{50}: 0.18 μM in our assay). In human enzyme, **9** and the D-ring unsaturated C20-oximes **10**, **11**, **18**, and **19** exhibit strong inhibitory potency. Compound **9**, being 10 times more potent than ketoconazole, shows an activity close to that of **II** (IC_{50}: 0.04 μM in our assay).

Inhibition of related P450 enzymes (P450 arom, P450 TxA_2, P450 scc) was determined as an indication of possible side effects. Especially inhibiton of P450 scc, the key enzyme of steroid biosynthesis, could result in dramatic effects by blockade of aldo- and glucocorticoid formation. The compounds tested turned out to be rather selective: i.e., they show either no effect or only inhibition in high concentrations. In vivo compound **9** is active showing a strong reduction of the testosterone plasma concentration in rats. There is no cause for concern that inhibition of P450 17 could reduce adrenal 17α-hydroxylation and thus glucocorticoid synthesis. In clinical trials with P450 17 inhibitors ketoconazole[59] and liarozole,[60] it has been shown that the androgen plasma concentration was reduced without glucocorticoid plasma levels being affected.

Some of the title compounds show potent inhibition of the second target enzyme 5α-reductase, compounds **16**, **17**, **20**, **21**, and **23**. They have a saturated D-ring in common and are progesterone-based. Compounds **16**, **20**, and **23**, tested for activity using whole cells, are highly active as well.

Potent dual inhibitors of both target enzymes are compounds **17** and **20**. They might be leads for the development of drugs for the treatment of androgen-dependent diseases. Being active in vivo, compound **9** might be a lead for further development of a prostate cancer drug.

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 disks. ^{1}H NMR spectra were recorded on a Bruker AM-400 (400 MHz) or DRX 500 (500 MHz) instrument. ^{13}C NMR spectra were recorded on a Bruker AM-400 (120 MHz) instrument. Chemical shifts are given in parts per million, and TMS was used as internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. 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 in the Inorganic Chemistry Department, University of the Saarland. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography (LCC) was performed using silica gel 60 (50−200 μm), flash-column-chromatography (FCC) using silica gel 60 (40−63 μm), and HPLC using a semi-preparative RP-18-column (16 mm × 250 mm, particle size: 5 μm, Nucleosil-C18), and reaction progress was determined by TLC analysis on ALUGRAM SIL G/UV_{254} (Macherey-Nagel).

Method A. General Procedure for the Synthesis of Oxime Compounds 2, 3, 6, 7, 8a−12a, 14, and 15. 983 mg NaOAc (12 mmol) and 421 mg NH_2OH·HCl (6 mmol) were dissolved in 100 mL MeOH and the solution was refluxed for 10 min. The hot solution was dropped to the steroidal ketone

(5.95 mmol) and stirred for 2 h at 4 °C. The reaction mixture was diluted with 65 mL water and extracted four times with 65 mL EtOAc. The organic phase was washed twice with water, brine and dried over $MgSO_4$. The compounds were purified by FCC and recrystallization from EtOH/H_2O (1:1).

16,17-Dihydroxyiminoandrost-5-en-3β-ol (2): purification FCC (dichloromethane:ethyl acetate 1:1); yield 80%, white crystals, mp 248−50 °C; ^{1}H NMR (DMSO-d_6) δ 0.86 (s, C18-Me, 3H); 0.95 (s, C19-Me, 3H); 5.28 (m, =CH−, 1H); 10.73 (s, =NOH, 1H); 11.11(s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3525, 2910, 1430, 1460, 1060, 960. Anal. ($C_{19}H_{27}N_2O_3$) C, H, N.

17-Hydroxyiminoandrost-5-en-3β-ol (3): purification recrystallization (EtOH:H_2O 1:1); yield 80%, white crystals, mp 200−2 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (s, C18-Me, 3H); 0.96 (s, C19-Me, 3H); 5.28 (m, =CH−, 1H); 10,06 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3400, 2900, 1660, 1450, 1060, 950. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

20-Hydroxyiminopregn-5-ene-3β,17α-diol (7): purification recrystallization (H_2O:EtOH 1:1); yield 94%, white solid, mp 258−9 °C; ^{1}H NMR (DMSO-d_6) δ 0.65 (s, C18-Me, 3H); 1.01 (s, C19-Me, 3H); 1.99 (s, C21-Me, 3H); 3.67 (m, C3αH, 1H); 5.35 (d, C6, =CH−, 1H, ^3J = 4.8 Hz); 10.38 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3200, 2950, 1460, 1370, 1050. Anal. ($C_{21}H_{33}NO_3$) C, H, N.

3β-Acetoxy-21-hydroxyiminopregna-5,17(20)-diene (8a, *E*-isomer): purification FCC (toluene:ether 8:1); yield 38%, white solid, mp 197−9 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.78 (s, C18-Me, 3H); 1.01 (s, C19-Me, 3H); 1.98 (s, CH_3COO−, 3H); 4.43 (m, C3αH, 1H); 5.36 (d, C6, =CH−, 1H, ^3J = 4.0 Hz); 5.70 (d, C20, =CH−, 1H, ^3J = 10.0 Hz); 7.76 (d, C21 = CH−, 1H, ^3J = 10.5 Hz); 10.67 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3520, 2950, 1750, 1650, 1370/60, 1230, 1040, 940. Anal. ($C_{23}H_{33}NO_3$) C, H, N.

3β-Acetoxy-21-hydroxyiminopregna-5,17(20)-diene (9a, *Z*-isomer): purification FCC (toluene:ether 8:1); yield 38%, white solid, mp 152−6 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.79 (s, C18-Me, 3H); 1.01 (s, C19-Me, 3H); 1.98 (s, CH_3COO−, 3H); 4.43 (m, C3αH, 1H); 5.40 (d, C6, =CH−, 1H, ^3J = 4.2 Hz); 6.23 (d, C20, =CH−, 1H, ^3J = 9.6 Hz); 7.14 (d, C21 = CH−, 1H, ^3J = 9.6 Hz); 10.84 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3520, 2950, 1750, 1650, 1370/60, 1230, 1040, 940. Anal. ($C_{23}H_{33}NO_3$) C, H, N.

3β-Acetoxy-20-hydroxyiminopregna-5,16-diene (10a): purification LCC (petrol ether:EtOAc 1:1); yield 55%, white solid, mp 180−5 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (s, C18-Me, 3H); 1.05 (s, C19-Me, 3H); 2.00 (s, C21-Me, 3H); 2.03 (s, CH_3COO−, 3H); 4.62 (m, C3αH, 1H); 5.38 (d, C6, =CH−, 1H, ^3J = 5.1 Hz); 6.06 (m, C16 =CH−, 1H); IR (KBr) cm^{-1} ν_{max} 3480, 2950, 1725, 850. Anal. ($C_{23}H_{33}NO_3$) C, H, N.

3β-Acetoxy-20-hydroxyiminopregna-5,14,16-triene (11a): purification FCC (petrol ether:EtOAc 1:1); yield 26%, white solid, mp 165−8 °C; ^{1}H NMR (DMSO-d_6) δ 1.09(s, C18-Me, 3H); 1.15 (s, C19-Me, 3H); 1.95 (s, C21-Me, 3H); 1.99 (s, CH_3COO−, 3H); 4.47 (m, C3αH, 1H); 5.46 (m, C6, =CH−, 1H); 5.96 (m, C16 =CH−, 1H); 7.62 (d, C15 =CH−, 1H, ^3J = 2.2 Hz); 10.72 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3470, 2950, 1720, 850. Anal. ($C_{23}H_{31}NO_3$) C, H, N.

3β-Acetoxy-20-hydroxyiminopregna-5,14-diene (12a): purification recrystallization (H_2O:EtOH 1:1); yield 99%, white solid, mp 183−6 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, C18-Me, 3H); 1.00 (s, C19-Me, 3H); 1.77 (s, C21-Me, 3H); 1.98 (s, CH_3-COO−, 3H); 4.44 (m, C3αH, 1H); 5.21 (m, C15 =CH−, 1H); 5.41 (s, C6, =CH−, 1H); 10.42 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3300, 2950, 1730, 1440, 1370, 1250, 1050.

21-Hydroxyiminopregn-5-en-3β-ol (14): purification FCC (CH_2Cl_2:EtOH 10:1); yield 62%, white solid, mp 185−8 °C; ^{1}H NMR (DMSO-d_6) δ 0.59 (s, C18-Me, 3H); 0.95 (s, C19-Me, 3H); 3.24 (m, C3αH, 1H); 5.26 (d, C6, =CH−, 1H, ^3J = 4.8 Hz); 6.63 (t, C21 CH=NOH, 0.5H, ^3J = 5.2 Hz); 7.26 (t, C21 CH=NOH, 0.5H, ^3J = 6.4 Hz); 10.30 (s, =NOH, 0.5H); 10.71 (s, =NOH, 0.5H); IR (KBr) cm^{-1} ν_{max} 3500−3200, 2950, 1440/50/70, 1050; GC−MS *m/e* 331 (M^+), 314, 298, 296, 246, 220. Anal. ($C_{21}H_{33}NO_2$) C, H, N.

21-Hydroxyimino-21-methylpregn-5-en-3β-ol (15): purification recrystallization (H_2O:EtOH 1:1); yield 97%, white solid, mp 146−50 °C; ^{1}H NMR (DMSO-d_6) δ 0.59 (s, C18-Me, 3H); 0.94 (s, C19-Me, 3H); 1.70 (s, C22-Me, 3H); 3.25 (m, C3αH, 1H); 5.26 (d, C6, =CH−, 1H, ^3J = 5.26 Hz); 10.16 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3300, 2950, 1730, 1440, 1370, 1250, 1050; GC−MS *m/e* 345 (M^+), 330, 328, 313, 260, 234. Anal. ($C_{22}H_{35}NO_2$) C, H, N.

Method B. General Procedure for the Synthesis of Compounds 8−13. 1 mmol 3β-acetoxy compound was dissolved in 3% KOH (MeOH) and heated to 70 °C for 10 min. The reaction mixture was cooled to room temperature, poured onto ice and extracted two times 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 and recrystallization from EtOH/H_2O (1:1).

21-Hydroxyiminopregna-5,17(20)-dien-3β-ol (8, *E*-isomer): purification recrystallization (H_2O:EtOH 1:1); yield 98%, white solid, mp 229−33 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, C18-Me, 3H); 1.01 (s, C19-Me, 3H); 3.25 (m, C3αH, 1H); 5.27 (d, C6, =CH−, 1H, ^3J = 5.0 Hz); 5.69 (d, C20, =CH−, 1H, ^3J = 10.1 Hz); 5.69 (d, C21 =CH−, 1H, ^3J = 10.0 Hz); 10.71 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3200, 2950, 1650, 1440, 1320, 1050. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

21-Hydroxyiminopregna-5,17(20)-dien-3β-ol (9, *Z*-isomer): purification recrystallization (H_2O:EtOH 1:1); yield 98%, white solid, mp 207−11 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, C18-Me, 3H); 1.08 (s, C19-Me, 3H); 3.25 (m, C3αH, 1H); 5.27 (s, C6, =CH−, 1H); 6.23 (d, C20, =CH−, 1H, ^3J = 10.0 Hz); 7.13 (d, C21 =CH−, 1H, ^3J = 9.6 Hz); 10.83 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3200, 2950, 1650, 1440, 1320, 1050. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

20-Hydroxyiminopregna-5,16-dien-3β-ol (10): purification recrystallization (H_2O:EtOH 1:1); yield 99%, white solid, mp 244−7 °C (lit. mp 217−8 °C[48]); ^{1}H NMR (DMSO-d_6) δ 0.90 (s, C18-Me, 3H); 0.98 (s, C19-Me, 3H), 1.86 (s, C21-Me, 3H); 3.30 (m, C3αH, 1H); 5.28 (d, C6, =CH−, 1H, ^3J = 4.8 Hz); 6.03 (m, C16 =CH−, 1H); 10.70 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3200, 2950, 1440, 1370, 1050. Anal. ($C_{21}H_{31}NO_2$·0.6H_2O) C, H, N.

20-Hydroxyiminopregna-5,14,16-trien-3β-ol (11): purification recrystallization (H_2O:EtOH 1:1); yield 99%, white solid, mp 175−80 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (s, C18-Me, 3H); 1.20 (s, C19-Me, 3H); 2.09 (s, C21-Me, 3H); 3.53 (m, C3αH, 1H); 5.46 (d, C6, =CH−, 1H, ^3J = 5.0 Hz); 6.96 (s, C16 =CH−, 1H); 7.15 (s, C15 =CH−, 1H); 10.70 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3300, 2950, 1530, 1440, 1040, 1060, 980. Anal. ($C_{21}H_{29}NO_2$·0.75H_2O) C, H, N.

20-Hydroxyiminopregna-5,14-dien-3β-ol (12): purification recrystallization (H_2O:EtOH 1:1); yield 99%, white solid, mp 180−5 °C; ^{1}H NMR (DMSO-d_6) δ 0.77 (s, C18-Me, 3H); 0.96 (s, C19-Me, 3H); 1.77 (s, C21-Me, 3H); 2.57 (dd, C17αH, 1H, ^3J = 7.52; 10.16 Hz); 3.26 (m, C3αH, 1H); 5.20 (s; C15 =CH−, 1H); 5.32 (s, C6, =CH−, 1H); 10.42 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3400−3200, 2950, 1450/40, 1380/70, 1050. Anal. ($C_{21}H_{31}NO_2$·0.5H_2O) C, H, N.

20-Hydroxyimino-17α-pregna-5,14-dien-3β-ol (13): purification recrystallization (H_2O:EtOH 1:1); yield 99%, white solid, mp 103−5 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (s, C18-Me, 3H); 0.97 (s, C19-Me, 3H); 1.70 (s, C21-Me, 3H); 2.75 (dd, C17αH, 1H, ^3J = 4.8 Hz, 8.4 Hz); 3.24 (m, C3αH, 1H); 5.17 (s; C15 =CH−, 1H); 5.32 (s, C6, =CH−, 1H); 10.30 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3400−3200, 2950, 1460/40, 1370, 1050; GC−MS *m/e* 329 (M^+), 312, 297, 284, 271. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

21-Methylpregn-5-en-3β-ol-21-one (15a). 1 mmol 3β-THP compound[61] (330.5 mg) was dissolved in 100 mL EtOH and 300 mg (1.2 mmol) pyridinium *p*-toluenesulfonic acid and stirred for 3 h at 55 °C. The reaction mixture was cooled to room temperature and 100 mL EtOAc was added. The organic phase was washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by FCC and recrystallization from EtOH/H_2O (1:1): purification FCC (CH_2Cl_2:EtOAc 3:1); yield 99%, white solid, mp 168−70

°C (lit. mp 177−8 °C[61]); ^{1}H NMR ($CDCl_3$) δ 0.60 (s, C18-Me, 3H); 1.01 (s, C19-Me, 3H), 2.14 (s, C22-Me, 3H), 2.46−2.48 (dd, 17αH, 1H, ^3J = 15.44 Hz, 4.02 Hz); 3.53 (m, C3αH, 1H); 5.35 (t, C6, =CH−, 1H, ^3J = 2.08 Hz); ^{13}C NMR ($CDCl_3$) δ 209 (C21); 140.9 (C5); 121.6 (C6); 71.8 (C17); 55.6; 50.5; 50.3; 46.0; 44.8; 42.4; 42.0; 37.4; 36.7; 36.6; 32.1; 31.9; 30.2; 28.4; 24.7; 20.8; 19.4 (C19); 12.6 (C18); IR (KBr) cm^{-1} ν_{max} 3520, 2950, 1700, 1440, 1380/70/60, 1250, 1060; GC−MS *m/e* 330 (M^+), 312, 297, 245, 239. Anal. ($C_{22}H_{34}O_2$) C, H, N.

Method C. General Procedure for the Synthesis of Oxime Compounds 16−21. 3.38 mL methylpiperidone (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 via 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 ether. The reaction mixture was washed with water and brine, dried over Na_2SO_4 and was evaporated. The crude product was purified by FCC.

20-Hydroxyiminopregn-4-en-3-one (16): purification FCC (CH_2Cl_2:EtOAc 7:1); yield 72%, white solid, mp 208−11 °C; ^{1}H NMR (DMSO-d_6) δ 0.59 (s, C18-Me, 3H); 1.14 (s, C19-Me, 3H); 1.72 (s, C21-Me, 3H); 5.63 (s, C4, =CH−, 1H); 10.36 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500, 2950, 1670, 1620, 1640, 1370, 1230, 970, 920. Anal. ($C_{21}H_{31}NO_2$·0.3toluene) C, H, N.

21-Hydroxyiminopregna-4,17(20)-dien-3-one (17, *Z*-isomer): purification FCC (CH_3Cl:MeOH 40:1); yield 25%, white solid, mp 172−9 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (s, C18-Me, 3H); 1.17 (s, C19-Me, 3H); 5.64 (s, C4, =CH−, 1H); 6.23 (d, C20, =CH−, 1H, ^3J = 9.6 Hz); 7.14 (d, C21 =CH−, 1H, ^3J = 9.6 Hz); 10.85 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3200, 2950, 1660, 1050/30/10; GC−MS *m/e* 328 (M^+), 313, 310, 285, 282. Anal. ($C_{21}H_{29}NO_2$) C, H, N.

20-Hydroxyiminopregna-4,16-dien-3-one (18): purification FCC (CH_2Cl_2:EtOH 10:1); yield 83%, white solid, mp 253−8 °C (lit. mp 254−9 °C[11]); ^{1}H NMR (DMSO-d_6) δ 0.92 (s, C18-Me, 3H); 1.17 (s, C19-Me, 3H); 1.89 (s, C21-Me, 3H); 5.84 (s, C4, =CH−, 1H); 6.02 (s, C16 =CH−, 1H); 10.74 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3340, 2950, 1660/50, 1640, 1610, 1440, 1370/60, 1285, 1245, 1190, 1000. Anal. ($C_{21}H_{29}NO_2$) C, H, N.

20-Hydroxyiminopregna-4,14,16-trien-3-one (19): purification FCC (CH_2Cl_2:EtOAc 15:2); yield 70%, white solid, mp 282−4 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (s, C18-Me, 3H); 1.26 (s, C19-Me, 3H); 1.95 (s, C21-Me, 3H); 5.67 (s, C4, =CH−, 1H); 5.94 (t, C15, =CH−, 1H, ^3J = 2 Hz); 6.61 (d, C16, =CH−, 1H, ^3J = 2 Hz); 10.76 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3320, 2950, 1660/50, 1615, 1450, 1360, 1290/40, 1190, 990/80/30, 900, 850, 725. Anal. ($C_{21}H_{27}NO_2$) C, H, N.

21-Hydroxyiminopregn-4-en-3-one (20): purification FCC (CH_3Cl:MeOH 40:1); yield 54%, white solid, mp 149−51 °C; ^{1}H NMR (DMSO-d_6) δ 0.63 (s, C18-Me, 3H); 1.15 (s, C19-Me, 3H); 5.62 (s, C4, =CH−, 1H); 6.63 (t, C21 CH=NOH, 0.7H, ^3J = 5.2 Hz); 7.25 (t, C21 CH=NOH, 0.3H, ^3J = 5.2 Hz); 10.31 (s, =NOH, 0.7H); 10.71 (s, =NOH, 0.3H); IR (KBr) cm^{-1} ν_{max} 3300, 2950, 1680/60, 1620; GC−MS *m/e* 330 (M^+), 313, 288, 217. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

21-Hydroxyimino-21-methylpregn-4-en-3-one (21): purification FCC (CH_2Cl_2:EtOAc 8:1); yield 53%, white solid, mp 159−60 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (s, C18-Me, 3H); 1.15 (s, C19-Me, 3H); 1.70 (s, C22-Me, 3H); 5.76 (s, C4, =CH−, 1H); 10.13 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3320, 2950, 1650, 1615; MS *m/e* 344 (M^+), 327. Anal. ($C_{22}H_{33}NO_2$) C, H, N.

Method D. General Procedure for the Synthesis of Oxime Compounds 22 and 23. 5.53 mmol pyridinium dichromate (PDC) in 20 mL dry MeOH was added to 250 mg (0.79 mmol) pregnenolone compound in 20 mL dry DMF. The reaction mixture was stirred for 6 h at room temperature. The reaction mixture was poured into 350 mL water and was extracted twice with EtOAc. The organic phase was washed with water and brine, dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by FCC and recrystallization from EtOH/H_2O (1:1).

20-Hydroxyiminopregn-4-ene-3,6-dione (22): purification FCC (CH_2Cl_2:EtOAc 5:1); yield 50%, white solid, mp 124−9 °C; ^{1}H NMR ($CDCl_3$) δ 0.59 (s, C18-Me, 3H); 1.11 (s, C19-Me, 3H); 1.74 (s, C21-Me, 3H); 5.91 (s, C4, =CH−, 1H); 10.40 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3300, 2950, 1740, 1690, 1670, 1450, 1370, 1250/40, 1220. Anal. ($C_{21}H_{29}NO_3$) C, H, N.

21-Hydroxyimino-21-methylpregn-4-ene-3,6-dione (23): purification FCC (CH_2Cl_2:EtOAc 5:1); yield 26%, white solid, mp 70−5 °C; ^{1}H NMR ($CDCl_3$) δ 0.67 (s, C18-Me, 3H); 1.11 (s, C19-Me, 3H); 1.71 (s, C22-Me, 3H); 5.90 (s, C4, =CH−, 1H); 10.19 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3500−3300, 2950, 1740, 1690/80, 1265/20. Anal. ($C_{22}H_{31}NO_3$·EtOH) C, H, N.

20-Carbonitrilopregna-5,17(20)-diene 3β-Tetrahydropyranyl Ether (14c). Under a N_2 atmosphere 3.6 g diethyl cyanomethylphosphonate (20 mmol) in 20 mL DME was dropped to 834 mg NaH (60% suspension in oil) in 25 mL DME. The clear solution was stirred and refluxed for 10 min. To this solution 1.93 g (5.19 mmol) THP-androstenolone was added and refluxing was continued for 5 h. The reaction mixture was cooled to room temperature and diluted with 50 mL ether and 25 mL water. Using additional 25 mL ether the mixture was extracted. The organic phase was washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by FCC toluene/ether (8:1). yield 99%. Compound **14c** was converted to **14b** without further analysis.

20-Carbonitrilopregn-5-ene 3β-Tetrahydropyranyl Ether (14b). 15 g **14c** (38 mmol) was dissolved in 750 mL MeOH. 73 g Mg turnings were added (3 mol). After 30 min the reaction started. The reaction mixture was stirred for 2 h, and the reaction temperature kept below 25 °C. Once again 18.7 g Mg turnings (0.77 mol) and 450 mL MeOH were added and the reaction mixture was stirred at room temperature for 24 h. At 0 °C, ether and 6 N HCl was added and the organic phase was washed with aqueous $NaHCO_3$, water and brine. After drying over $MgSO_4$, the solvent was evaporated in vacuo. The crude product was purified by FCC toluene/EtOAc (12:1). yield 77%. Compound **14b** was converted to **14a** without further analysis.

Pregn-5-en-21-al 3β-Tetrahydropyranyl Ether (14a). 23.5 g **14b** (59 mmol) was diluted in 250 mL dry toluene and cooled to −76 °C. 170 mL DibaH (20% in toluene) was added and the reaction mixture was stirred at −76 °C for 1 h. 94 mL MeOH and 47 mL water were added and the reaction mixture was stirred for 3 h at room temperature. After the extraction with ether, the organic phase was washed with aqueous 5% citric acid, aqueous $NaHCO_3$, water and brine. After drying over $MgSO_4$, the solvent was evaporated in vacuo. The crude product was purified by FCC: purification FCC (toluene:ether 9:1); yield 42%, white solid, mp 142−6 °C; ^{1}H NMR ($CDCl_3$) δ 0.62 (s, C18-Me, 3H); 1.02 (s, C19-Me, 3H), 3.47−3.54 (m, THP, 2H); 3.91 (m, THP, 1H); 4.71 (d, THP, 1H, ^3J = 4.4 Hz); 5.35 (t, C6, =CH−, 1H, ^3J = 5.72 Hz); 9.77 (t, C21, CHO, 1H, ^3J = 2.24 Hz); IR (KBr) cm^{-1} ν_{max} 2950, 1730, 1200, 1120, 1060/40.

3β-Acetoxypregna-5,14-dien-20-one (12b)[51] and 3β-Acetoxy-17α-pregna-5,14-dien-20-one (13b). 200 mg **11b** (0.56 mmol) was diluted in 40 mL xylene. 2 mL (0.75 mmol) tri-*n*-butyl-SnH was added under a N_2 stream to this solution. The reaction mixture was stirred under irradiation (2 × 150 W Osram-Sunbeam lamps) for 1 h at room temperature and 12 h under reflux. After that 20 mL MeOH was added and the mixture was refluxed for an additional hour to terminate the reaction. The reaction mixture was cooled to room temperature, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by FCC (toluene:ether 10:1). A 11:2 mixture of compounds **12b** and **13b** was obtained. Compound **12b**: yield 39%, white solid, mp 156−8 °C (lit. mp 158−61 °C[51]); ^{1}H NMR ($CDCl_3$) δ 0.88 (s, C18-Me, 3H); 1.02 (s, C19-Me, 3H); 2.03 (s, C21-Me, 3H); 2.17 (s, CH_3COO−, 3H); 2.93 (dd, 17αH, 1H, ^3J = 9.76 Hz, 8.4 Hz); 4.61 (m, C3αH, 1H); 5.18 (d, C15 =CH−, 1H, ^3J = 2 Hz); 5.43 (t, C6, =CH−, 1H,

^3J = 2 Hz). Compound **13b**: yield 7%, white solid, mp 148−50 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, C18-Me, 3H); 1.04 (s, C19-Me, 3H); 2.04 (s, C21-Me, 3H); 2.17 (s, CH_3COO−, 3H); 3.08 (dd, 17αH, 1H, ^3J = 8.84 Hz, 5.32 Hz); 4.61 (m, C3αH, 1H); 5.17 (d, C15 =CH−, 1H, ^3J = 2.68 Hz, 4.44 Hz); 5.43 (t, C6, =CH−, 1H, ^3J = 2 Hz).

3β-Acetoxypregna-5,17(20)-dien-21-al (8b) and 3β-Acetoxypregna-5,16-dien-20-one (10b).[50] 11.5 g **8c** (25 mmol) in 125 mL dry DMSO was treated with 6.21 g (8.63 mL, 61 mmol) triethylamine and 520 mg $(PPh_3)_4Pd$. 8.95 g (12.05 mL, 124 mmol) ethyl vinyl ether was dropped to the reaction mixture. After heating to 60 °C, the reaction mixture was stirred for 4 h. 0.5 M HCl was added and the aqueous solution was extracted twice with EtOAc. The combined organic phases were washed with aqueous $NaHCO_3$, water and brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by FCC petrol ether/EtOAc (7:1). A 2:9 mixture of compounds **8b** and **10b** was obtained. Compound **8b**: yield 11%, white solid, 1:1 *E*/*Z*-mixture; ^{1}H NMR ($CDCl_3$) δ 0.88 (s, C18-Me, 3H); 1.04 (s, C19-Me, 3H); 2.03 (s, Acetyl-Me, 3H); 4.60 (m, C3αH, 1H); 5.38 (d, C6, =CH−, 1H, ^3J = 4.96 Hz); 5.75 (t, C20, =CH−, 0.5H, ^3J = 2.64 Hz); 5.77 (t, C20, =CH−, 0.5H, ^3J = 2.64 Hz); 9.86 (s, C21−CHO, 0.5H); 9.88 (s, C21−CHO, 0.5H); IR (KBr) cm^{-1} ν_{max} 2950, 1740, 1680, 1620, 1380/60, 1260, 1140, 1040. Anal. ($C_{23}H_{32}O_3$) C, H, N. Compound **10b**: yield 62%, white solid, mp 170−3 °C (lit. mp 166−8 °C[11]); ^{1}H NMR ($CDCl_3$) δ 0.92 (s, C18-Me, 3H); 1.05 (s, C19-Me, 3H); 2.03 (s, $COOCH_3$, 3H); 2.26 (s, C21-Me, 3H); 4.59 (m, C3αH, 1H); 5.38 (d, C6, =CH−, 1H, ^3J = 5.1 Hz); 6.69 (dd, C16, =CH−, 1H, ^3J = 3.52 Hz, 1.76 Hz); IR (KBr) cm^{-1} ν_{max} 2950, 1730, 1665, 850.

16-Hydroxyiminoandrost-5-en-3β-ol-17-one (1). 0.5 g potassium was dissolved in 20 mL *tert*-butyl alcohol and 2 g androstenolone (6.93 mmol) was added under a N_2 atmosphere. It was kept overnight, then diluted with water, acidified and extracted with chloroform: purification recrystallization (EtOH:H_2O 10:1); yield 80%, white crystals, mp 247−9 °C (lit. mp 248−9 °C[46]); ^{1}H NMR (DMSO-d_6) δ 0.86 (s, C18-Me, 3H); 0.98 (s, C19-Me, 3H); 5.29 (m, C6, =CH−, 1H); 12.3 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3400, 3200, 1745, 1690, 950. Anal. ($C_{19}H_{27}NO_3$) C, H, N.

16-Hydroxyiminoandrost-5-en-3β-ol-17-one Hydrazone (4). 0.5 g **1** (1.57 mmol) in 250 mL ethanol and 5 mL hydrazine hydrate were refluxed for 1 h in the presence of a few drops of glacial acetic acid: purification recrystallization (EtOH:H_2O 10:1); yield 75%, white crystals, mp 279−82 °C (lit. mp 280−3 °C[47]); ^{1}H NMR (DMSO-d_6) δ 0.83 (s, C18-Me, 3H); 0.97 (s, C19-Me, 3H); 5.29 (m, C6, =CH−, 1H); 7.6 (s, =NNH_2, 2H); 11.19 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3385, 3200, 1600, 1450, 1360, 1130, 1055, 950. Anal. ($C_{19}H_{29}N_3O_2$) C, H, N.

16-Hydroxyiminoandrost-5-en-3β-ol (5). 1 g **4** (2.88 mmol), 0.8 g KOH and 40 mL ethylene glycol were refluxed for 2 h, poured into water, and extracted with dichloromethane: purification FCC (dichloromethane: ethyl acetate 1:1); yield 50%, white crystals, mp 200−2 °C (lit. mp 202−5 °C[47]); ^{1}H NMR (DMSO-d_6) δ 0.75 (s, C18-Me,3H); 0.96 (s, C19-Me, 3H); 5.28 (m, =CH−, 1H); 10.19 (s, =NOH, 1H); IR (KBr) cm^{-1} ν_{max} 3375, 2930, 1660, 1450, 1070, 950. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

Biological Methods. 1. Enzyme Preparations. The enzymes were prepared according to described methods: human and rat testicular P450 17,[14] human placental P450 arom,[54] bovine adrenal P450 scc,[54] and 5α-reductase type 2.[36] For the P450 TxA_2 assay citrated human whole blood was used.[55]

2. Enzyme Assays. The following enzyme assays were performed as described: rat P450 17,[14] human P450 17,[24] P450 arom,[54] P450 TxA_2,[55] P450 scc,[54] DU145 cells (5α-reductase type 1),[62] and 5α-reductase type 2.[36]

K_i and K_m values were determined according to Lee and Wilson.[63] Inhibitor concentrations were between the IC_{40} and IC_{70} values 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.[8]

***E. coli* (P450 17/NADPH−P450 Reductase) Assay.**[57] To test the inhibitory activity of compounds on human P450 17 coexpressed with rat NADPH−P450 reductase, 0.1 M sodium phosphate, pH 7.4, was preincubated with 25 μM 1,2-[^{3}H]-progesterone and an appropriate concentration of inhibitor at 37 °C for 10 min. The reaction was started by the addition of a suspension of recombinant *E. coli* XL1 pJL17/OR.[57] A_{578} was 3.0. After 45 min of vigorous shaking of the horizontally positioned cups at 37 °C, the reaction was stopped by heating at 95 °C for 5 min. Steroids were extracted for 5 min with ethyl acetate. The samples were evaporated, dissolved in methanol and analyzed by HPLC as described.[57]

HEK293-5α1 and -5α2 Assays.[58] The 5α-reductase expression plasmids pRcCMV-I and pRcCMV-II were constructed by insertion of the full-length human cDNA encoding the 5α-reductase type 1 or 2, respectively. Human embryonic kidney cells HEK293 were transfected with either pRcCMV-I or pRcCMV-II using the lipid transfection reagent Roti-Fect (Roth, Karlsruhe, Germany). By selection of stable transfected cells (using G418-sulfate), clones with high 5α-reductase activity were identified and named HEK293-5α1 and HEK293-5α2, respectively. For the inhibition assay, 300 000 cells were seeded in each well of a 24-well tissue culture plate and incubated overnight in a humidified 95% O_2 and 5% CO_2 atmosphere at 37 °C to allow attachment of the cells. The medium (DMEM with 10% FCS) was removed and replaced by 0.5 mL of fresh medium containing substrate (5 nM [^{3}H]-androstenedione) and inhibitor. Inhibitors were dissolved in dimethyl sulfoxide (DMSO). DMSO concentration in control and inhibitor incubations was 1%. After incubation, the supernatant was removed and extracted with ether. The organic phase was evaporated and the residue dissolved in methanol and subjected to HPLC analysis as described.[36]

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 equimolar 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 double-antibody testosterone [^{125}I]RIA (ICN Eschwege; detection limits: 0.1 ng/mL under assay conditions) and are given in ng/mL plasma.

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