Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 9, pp 1447–1453, 1996

PII: S0968-0896(96)00138-1
0968-0896/96 $15.00+0.00

20-Amino and 20,21-Aziridinyl Pregnene Steroids: Development of Potent Inhibitors of 17α-Hydroxylase/C17,20-Lyase (P450 17)

Vincent C. O. Njar,† Markus Hector and Rolf W. Hartmann*
Fachrichtung 12.1 Pharmazeutische Chemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany

Abstract—In the search for potent inhibitors of P450 17, the key enzyme of androgen biosynthesis, the 20,21-aziridinyl- and 20-aminopregnene steroids **1**–**11** were synthesized and tested toward rat testicular P450 17. Only the aziridinyl-substituted pregnenolones (**1** and **2**) and progesterones (**3** and **4**), respectively, showed inhibitory activity, which strongly depends on C20 stereochemistry. The most active compound **1** [20(*S*)-20,21-aziridinylpregn-5-en-3β-ol; IC_{50} 0.21 μM, progesterone 25 μM; K_i = 1.7 nM, K_m progesterone = 7.0 μM] is the strongest inhibitor of rat P450 17 described so far. Using UV–vis difference spectroscopy, complexation of the aziridinyl nitrogen to the heme iron, Fe^{3+}, of P450 17 was observed, which could not be reversed by high concentrations of substrate. Preincubation of the enzyme with **1** in the absence and presence of NADPH followed by charcoal treatment results in a strong decrease of enzyme activity within 30 s. However, a recovery of enzyme activity was observed: 90 min after charcoal treatment 75% of the activity was restored. Copyright © 1996 Elsevier Science Ltd

Introduction

Androgens have been implicated in the development and progression of several diseases, most notably prostatic cancer. A promising alternative to the treatment with antihormones and LHRH analogues might be the use of selective inhibitors of androgen biosynthesis.[1] The appropriate target is the 17α-hydroxylase/C17,20-lyase (P450 17, CYP 17), a cytochrome P450 enzyme, which catalyses hydroxylation of progesterone and pregnenolone in the 17α-position as well as conversion of the C21 steroids

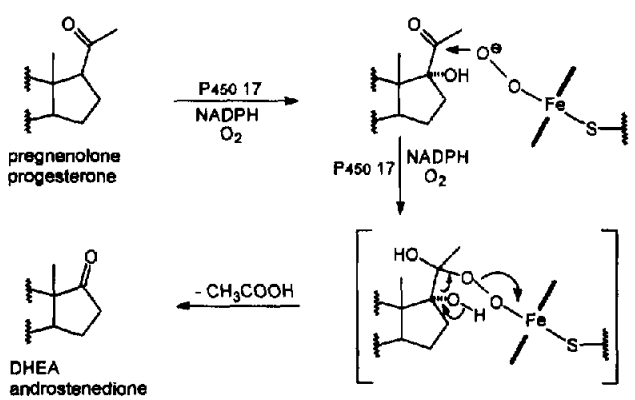


Chart 1. Mechanism of the P450 17 catalysed androgen formation.

to the corresponding C19 androgens by a side-chain cleavage process (Chart 1). As with the other P450 hydroxylases, the heme iron, Fe^{3+}, is responsible for oxygen activation. The hydroxylation step is accomplished by an oxene transfer, the lyase step probably by attack of a ferric peroxy species at carbonyl C20.[2]

Several categories of steroidal[3–8] and non-steroidal[9–16] inhibitors of P450 17 have been designed and the most potent of these to date is 17-(3-pyridyl)androsta-5,16-dien-3β-ol (K_i < 1 nM for the lyase activity of human P450 17).[7,8] The concept of drug design presented in this paper makes use of the P450 17 substrates progesterone and pregnenolone. At the site of enzymatic reaction, *N*-containing functional groups were introduced into a supposed appropriate position to form a coordinative bond with the heme iron and thus prevent oxygen activation (Chart 2). In the case of another steroidogenic P450 enzyme, aromatase, a similar approach with 10β-aziridinylestr-4-ene-3,17-diones recently has resulted in potent inhibitors.[17]

In the following paragraphs we describe the synthesis of compounds **1**–**11** and their in vitro activity toward the target enzyme P450 17. In the case of a very potent inhibitor of P450 17, compound **1**, further studies on the interaction with the enzyme as well as on its selectivity (inhibition of other steroidogenic P450 enzymes) will be presented.

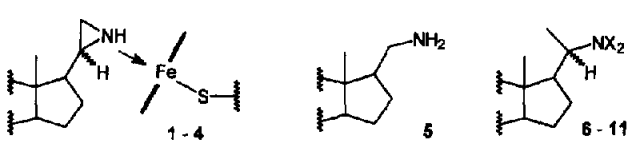


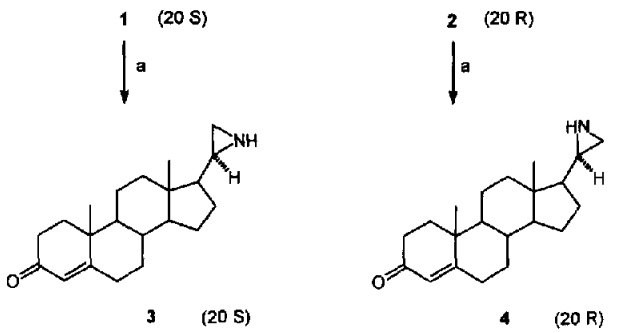
Chart 2. Synthesized potential mechanism-based inhibitors of P450 17; pregnenolone- or progesterone-type, X = H or Ac.

Key words: 17α-hydroxylase/C17,20 lyase (P450 17) inhibitors, 20-aminopregnenes, 20,21-aziridinylpregnenes, androgen-dependent diseases.
†On leave from Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

Results

Synthesis

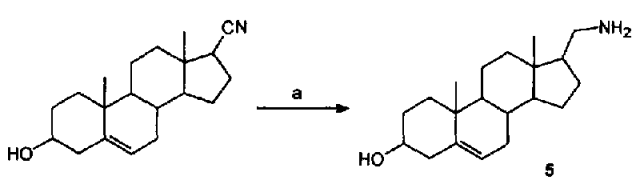
The pregnenolone aziridines, 20(S)-20,21-aziridinyl-pregn-5-en-3β-ol (**1**) and the 20(*R*)-isomer (**2**), were obtained as reported by Tzikas et al.[18] Separation of the diastereomeric mixture was performed by flash column chromatography (SiO_2; CH_2Cl_2:EtOAc 25:1) via their N,O-diacetyl derivatives. After saponification each of them was oxidized separately using a modified Oppenauer oxidation to give the 20(*S*)- and 20(*R*)-20,21-aziridinylpregn-4-en-3-ones **3** and **4** (Scheme 1).



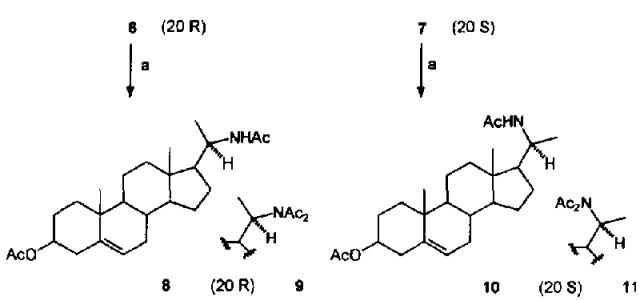
Scheme 1. (a) Modified Oppenauer oxidation.

The 21-nor compound, 21-nor-20-aminopregn-5-en-3β-ol (**5**), was synthesized by reaction of androst-5-en-3β-ol-17β-nitrile[19] with $AlCl_3/LiAlH_4$ (Scheme 2).

The synthesis of the 20-amino compounds 20(R)-20-aminopregn-5-en-3β-ol (**6**) and the 20(S)-isomer (**7**) was accomplished as described.[20] Each were acetylated by Ac_2O/pyridine and the resulting *N,O*-diacetyl compounds **8** and **10** and the *N,N,O*-triacetyl compounds **9** and **11** were obtained by flash chromatography (SiO_2; (1) toluene:ether 6:1, (2) CH_2Cl_2:EtOH 9:1; Scheme 3).



Scheme 2. (a) $AlCl_3/LiAlH_4$, dry THF, N_2.



Scheme 3. (a) Ac_2O/pyridine.

Biological properties

The compounds were assayed using an enzyme preparation from rat testes and the procedure recently described.[14] Table 1 summarizes the results of the test compounds. From the IC_{50} values it becomes apparent that the aziridine compounds **1**–**4** are highly active inhibitors of P450 17. They are at least twice as active as ketoconazole,[21] which was used as a reference. In contrast to the aziridines, the amino compounds **5**–**7** and their N-mono- or N-diacetylated derivatives (**8**–**11**) show only very weak activity: Exact IC_{50} values could not be obtained because of limited inhibitor solubility. The aziridines exhibit a wide variety of inhibitory potencies depending on the C20 stereochemistry. The 20(*S*) isomers **1** and **3** are more powerful inhibitors than the corresponding 20(*R*) isomers **2** and **4**: In the case of the pregnenolone compounds **1** and **2**, the *S* and *R* isomers differ by a factor of 162, whereas in the case of the progesterone aziridines **3** and **4**, the factor is 30. The most active compound is **1**, being 319 times more potent an inhibitor of the enzyme than ketoconazole. Compound **1** is the most potent inhibitor (IC_{50} 0.21 μM, $K_i = 1.7$ nM) of rat P450 17 described so far. Surprisingly the ratio substrate concentration/$K_M = 3.5$ is not seen for compound **1** (0.21/1.7 = 0.12) and compound **3** (1.2/2.1 = 0.57). The reason for this observation is unknown at this time.

The chemical nature of the complex formed between the 20S aziridine **1** and rat testicular P450 17 was studied using UV–vis difference spectroscopy following standard procedure.[22–24] Figure 1 shows that **1** induces a type II spectrum having a peak at 425 nm and a trough at 390 nm and that this effect depends on the concentration of **1**. This indicates coordination of the steroidal nitrogen atom to the heme iron of P450 17 with the formation of low spin iron. The less potent aziridines **2** and **3** also induced type II spectra similar (but with smaller ΔA values) to those induced by **1**. Interestingly, the type II spectrum generated by **1** (5 μM) was not reversed by a high concentration of substrate (Fig. 2), suggesting that **1**, after binding to the steroid site and coordinating with the heme iron, forms a rather tight complex with the enzyme.

Consequently, the effect of compound **1** on enzyme activity was evaluated. After preincubation of **1** with enzyme in the presence of NADPH for various time intervals, unbound inhibitor was removed by charcoal treatment and enzyme activity was determined after 20 min [Fig. 3(a)]. At a concentration of 1.25 μM (which causes ca. 100% inhibition in the usual enzyme inhibition test), compound **1** decreased enzyme activity by 50–60%. This effect was achieved within 30 s. A similar experiment with ketoconazole (50 μM) did not show any 'inactivation' of the enzyme (data not shown). The loss of enzyme activity caused by **1** is not dependent on the presence of NADPH [Fig. 3(b)] and was not prevented by the presence of the nucleophile cysteine (0.5 mM, data not shown). Lower concentrations of **1** in the preincubation mixture resulted in less pronounced effects (dose-dependent), whereas higher

Table 1. Inhibitory activity of 20-substituted pregnene compounds toward rat testicular P450 17[a]

Compd	Pregn-	17 β-substituent	C-20	IC_{50} (μM)[b,c]	RP value[d]
1[e]	-5-en-3-ol		*S*	0.21	319
2	-5-en-3-ol		*R*	34	2.0
3[e]	-4-en-3-one		*S*	1.2	59
4	-4-en-3-one		*R*	36	1.9
5	-5-en-3-ol		–	>125[f]	
6	-5-en-3-ol		*R*	>125[f]	
7	-5-en-3-ol		*S*	>125[f]	
8	-5-en-3-OAc		*R*	>125[f]	
10	-5-en-3-OAc		*S*	>125[f]	
9	-5-en-3-OAc		*R*	>125[f]	
11	-5-en-3-OAc		*S*	>125[f]	
	ketoconazole			67	1

[a]Microsomal fraction.
[b]Substrat: progesterone, 25 μM.
[c]Inhibitor concentration required to inhibit enzyme activity by 50%.
[d]Relative potency, calculated from the IC_{50} values and relative to ketoconazole.
[e]K_i value (**1**) = 1.7 nM, K_i (**3**) = 2.1 nM (K_m for progesterone = 7.0 μM).
[f]At a concentration of 125 μM the inhibition values were between 5 and 30%. At higher concentrations the compounds were not soluble.

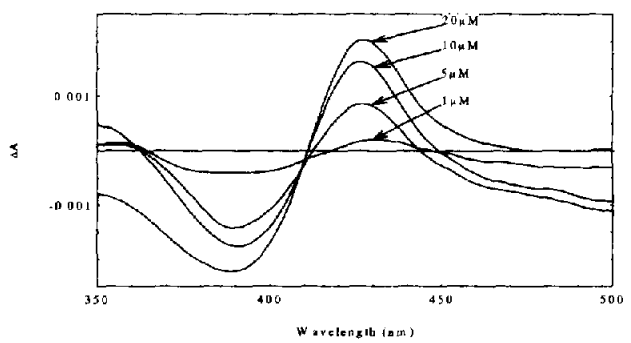


Figure 1. Type II difference spectra. The sample and reference cuvette contained rat testicular P450 17 (protein concn 1 mg/mL) and progesterone (62.5 μM). After addition of **1** the spectra were recorded.

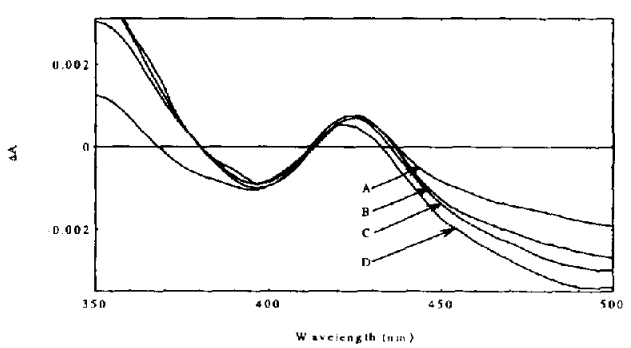


Figure 2. (A) Type II difference spectrum of **1** (5 μM). Addition of excess substrate to reference and sample cuvette did not reverse the effect. (B) −157.5 μM after 1 min. (C) +157.5 μM after 10 min. (D) +315 μM after 10 min.

concentrations did not cause a complete loss of enzyme activity: even at a concentration of 125 μM the 'inactivation' did not exceed 70% (data not shown).

In order to find out more details about the nature of this 'inactivation', especially to elucidate the question as to whether the steroidal inhibitor is bound covalently to the enzyme, a further study on the reversibility of the inhibitor enzyme interaction was performed. After preincubation of **1** with the enzyme for 15 min and removal of the inhibitor with charcoal, enzyme activity was determined after various time intervals (Fig. 4). After 5 min the loss of enzyme activity was greater than 70%. Interestingly, recovery of enzyme activity could be observed with time: After 90 min the loss of enzyme activity caused by **1** was only 25%.

For the evaluation of the selectivity of P450 17 inhibition, compound **1** was tested for inhibition of the related P450 enzymes, P450 arom (aromatase, estrogen synthetase, CYP 19) and P450 scc (cholesterol side chain cleavage enzyme, desmolase, CYP 11A1) using our standard procedure.[25] Inhibition was observed only at high concentrations (human placental P450 arom: 21% at 12.5 μM, bovine adrenal P450 scc: 36% at 25 μM), indicating the specificity of **1** toward P450 17.

Discussion

The structure modifications performed at C20 (and C21) of the pregnenolone or progesterone skeleton resulted in compounds with very strong differences in inhibitory potencies. Only the aziridinyl compounds were powerful inhibitors of P450 17. As expected, their inhibitory activities markedly depend on the stereo-

chemistry at C20. This result reflects the significance of the relative positions of the aziridine nitrogens with respect to the position of the heme iron after the inhibitors are bound to the enzyme. Differences in inhibitory potencies are also observed between the pregnenolone aziridines and the corresponding progesterone derivatives, the former being more potent. This observation is unexpected since progesterone is the preferred substrate for the rat enzyme. The inhibitory potencies of these aziridines versus the human P450 17 (pregnenolone is the preferred substrate) should be of interest. Our finding that the 20(*S*)-aziridinyl steroids are potent inhibitors is in accordance with a recent abstract reporting effective inhibition of rat and human testicular P450 17 by 20(*S*)-oxiranyl steroids without

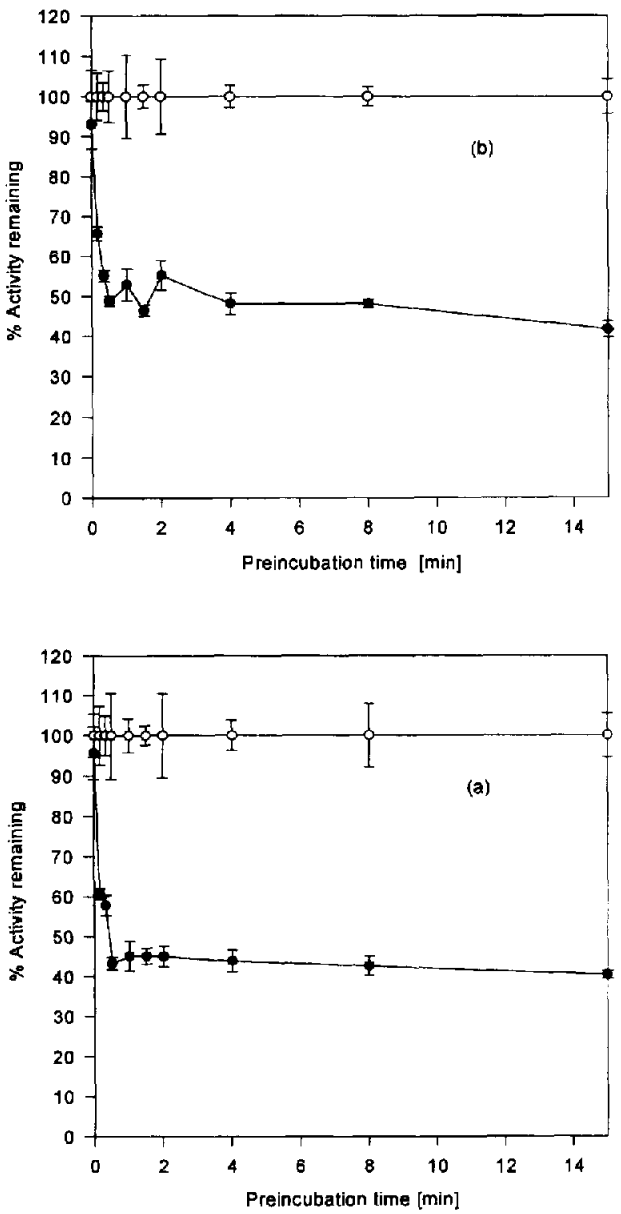


Figure 3. Time-dependent 'inactivation' of rat testicular P450 17 by **1** (1.25 μM [●]) in the presence of NADPH (a), in the absence of NADPH (b); control [○]; the enzyme activity remaining following the preceding preincubation intervals was determined in the usual manner (after 20 min).

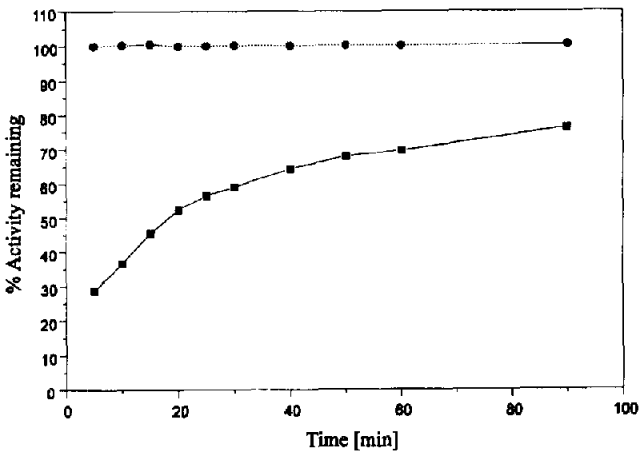


Figure 4. Recovery of enzyme activity (preincubation 15 min, charcoal treatment for removal of inhibitor, determination of enzyme activity after different time intervals).

giving concrete inhibition values.[26] In contrast to the 20,21-amino steroids, the corresponding 20-amino compounds are only very weak inhibitors of P450 17. This finding might be due to the differences in basicity and/or to the different configurations in space due to the divergence from the tetrahedral arrangement at C20 in the case of the aziridines. The finding that *N*-acetylation is not an appropriate means to increase the inhibitory properties of the amino-substituted steroids is not surprising, since the process of *N*-acylation results in a delocalization of the π electrons of the nitrogen and the newly introduced acetyl group(s) might have adverse steric effects hindering the interaction with the heme group. Interestingly, acetylation of the aziridinyl compounds **1** and **2** also led to a dramatic decrease of inhibitory properties (unpublished results).

The results of the mechanistic studies performed with compound **1**, which is the most potent inhibitor of rat testicular P450 17 described so far, are very intriguing. In contrast to the 10β-aziridinylestr-4-ene-3,17-diones, which show slow binding properties,[17] compound **1** binds very fast to the enzyme. There is a coordination of the steroidal nitrogen atom to the heme iron as shown by type II difference spectra. No covalent binding could be observed: the interaction of **1** with the enzyme is reversible. However, **1** forms a tight complex with the active site of the enzyme (i.e., in contrast to other reversible inhibitors of P450 enzymes) it shows a very slow dissociation from the active site. Presently further experiments are being performed with **1**, determining the inhibition of human testicular P450 17 in vitro as well as in vivo activity (reduction of the plasma androgen concentration).

Experimental

General methods

Melting points were determined with a Kofler hot-stage apparatus (Reichert, Wien) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded

with TMS as an internal standard at 400 or 100 MHz, respectively, on a Bruker AM-400 spectrometer; chemical shifts are presented in ppm; the following abbreviations were used: s (singlet); d (doublet); m (multiplet). IR spectra were recorded on a Perkin–Elmer 398 IR spectrophotometer as KBr discs. MS were recorded on a Finnigan MAT 311 or MAT 90 spectrometer. Column chromatography was carried out on columns packed with Macherey–Nagel silica gel 60 (40–60 μM).

20(*S*)-20,21-Aziridinylpregn-4-en-3-one (3). 20(*S*)-20,21-Aziridinylpregn-5-en-3β-ol, **1** (100 mg, 0.31 mmol), was dissolved in 10 mL of dry toluene. After additon of 3.38 mL methylpiperidone, the mixture was heated under reflux until 1–2 mL toluene was collected via a Dean–Stark trap. Al-isopropoxide (112 mg, 0.56 mmol) was added and the mixture was refluxed for 4 h. Al-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 was diluted with 20 mL of ether. The solution was washed with water and brine, dried (Na_2SO_4) and was evaporated. The crude product was purified using flash column chromatography (SiO_2, 40–60 μM; CH_2Cl_2:EtOH 9:1) to obtain 43 mg of **3**. Yield 44%; white powder; mp 136–139 °C; ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, s, C18-CH_3), 1.20 (3H, s, C19-CH_3), 5.72 (1H, s, C4 =CH—); ^{13}C NMR ($CDCl_3$): δ 199.3 (C-3), 171.2 (C-5), 123.8 (C-4), 55.6, 55.1, 54.1, 42.9, 38.7, 38.0, 35.7, 35.4, 33.9, 32.9, 32.0, 30.3 (C-20), 25.7 (C-21), 24.5, 23.3, 20.7, 17.4 (C-19), 13.4 (C-18). IR (KBr): 3400–3500 (br), 3290, 1670, 1615; HRMS: calcd 313.2405 ($C_{21}H_{31}NO$), found 313.2251.

20(*R*)-20,21-Aziridinylpregn-4-en-3-one (4). Yield 36%; white powder; mp 124–127 °C; ^{1}H NMR ($CDCl_3$): δ 0.85 (3H, s, C18-CH_3), 1.20 (3H, s, C19-CH_3), 5.72 (1H, s, C4 =CH—); ^{13}C NMR ($CDCl_3$): δ 199.4 (C-3), 171.2 (C-5), 123.2 (C-4), 56.3, 55.2, 54.2, 43.1, 38.7, 38.4, 35.8, 35.6, 34.0, 32.9, 32.2, 30.4 (C-20), 25.4 (C-21), 24.6, 23.1, 20.8, 17.5 (C-19), 13.2(C-18); IR (KBr): 3400–3500 (br), 3300, 1675, 1615 cm^{-1}; HRMS: calcd 313.2405 ($C_{21}H_{31}NO$), found 313.2324.

21-Nor-20-aminopregn-5-en-3β-ol (5). Androst-5-en-3β-ol-17β-nitrile[19] (250 mg, 0.84 mmol) was dissolved in 5 mL of dry THF. This solution was dropped over 45 min into a solution of $AlCl_3$ (280 mg, 2.1 mmol) and $LiAlH_4$ (80 mg, 2.1 mmol) in 10 mL dry THF (under N_2 atmosphere). After stirring for 2.5 h at room temperature, once again $LiAlH_4$ (80 mg, 2.1 mmol) was added and stirring was continued for additional 2 h. At 0 °C 1 mL of a NaOH soln (20%) was added. The mixture was suspended twice in 20 mL of ether and solids were filtered off. The resulting solution was washed with water, dried (Na_2SO_4) and evaporated. The crude product was purified using flash column chromatography (SiO_2, 40–60 μM; CH_2Cl_2:MeOH 9:2) to give after recrystallization (MeOH) 97.3 mg of **5**. Yield 38%; white crystals; mp 135–40 °C; ^{1}H NMR ($CDCl_3$): δ 0.62 (3H, s, C18-CH_3), 1.01 (3H, s, C19-CH_3), 3.49 (1H, m, C3, αH), 5.34 (1H, s, C6 =CH—); ^{13}C NMR ($CDCl_3$): δ 140.8 (C-5), 121.2 (C-6), 71.3 (C-3), 56.1, 56.0, 53.3 (C-20), 50.3, 42.6, 41.8, 38.1, 37.1, 36.5, 31.7, 31.5, 31.1, 26.6, 24.4, 20.6, 19.1 (C-19), 12.2 (C-18); IR (KBr): 3340–3320, 2920, 1600, 1460, 1450, 1370, 1060 cm^{-1}; Anal. calcd $C_{20}H_{33}NO$ (C,H,N).

20(*S*)-20,21-Aziridinylpregn-5-en-3β-ol (1). Compound **1** was prepared according to ref 18. Additional analytical data: ^{13}C NMR ($CDCl_3$): δ 140.9 (C-5), 121.2 (C-6), 71.6 (C-3), 56, 55.7, 50.5, 42.8, 42.3, 38.2, 37.3, 36.6, 31.9, 31.7, 31.6, 30.6 (C-20), 25.9 (C-21), 24.6, 23.4, 20.7, 19.4 (C-19), 13.3 (C-18); HRMS calcd 315.2562 ($C_{21}H_{33}NO$), found 315.2551.

20(*R*)-20,21-Aziridinylpregn-5-en-3β-ol (2). Compound **2** was prepared according to ref 18. Additional analytical data: ^{13}C NMR ($CDCl_3$): δ 140.9 (C-5), 121.2 (C-6), 71.3 (C-3), 55.9, 55.7, 50.3, 42.8, 42.0, 38.3, 37.2, 36.5, 31.8, 31.7, 31.2, 30.5 (C-20), 25.1 (C-21), 24.5, 22.7, 20.6, 19.2 (C-19), 13.0 (C-18); HRMS calcd 315.2562 ($C_{21}H_{33}NO$), found 315.2546.

3-β-Acetoxy-20(*R*)-acetaminopregn-5-en (8). Compound **8** was prepared according to ref 19. Additional analytical data: ^{1}H NMR ($CDCl_3$): δ 0.71 (3H, s, C18-CH_3), 1.01 (3H, s, C19-CH_3), 1.07 (3H, d, ^2J=6.36 Hz, C21-CH_3), 1.94 (3H, s, $NCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 3.96 (1H, m, C3αH), 4.59 (1H, m, C20-H), 5.19 (1H, d, ^2J=8.5 Hz, —NH—), 5.36 (1H, d, ^2J=4.8 Hz, C6 =CH—); MS *m/e* (CI): (M^-), 341 (M^+ −60), 326 (M^+-60 − 15), 282 (M^+-60–59).

3-β-Acetoxy-20(*R*)-diacetaminopregn-5-en (9). Compound **9** was obtained together with **8**).[19] In contrast to the literature, **9** was isolated (LC) and characterized. Yield 43%; white crystals; mp 152–54 °C; ^{1}H NMR ($CDCl_3$): δ 0.63 (3H, s, C18-CH_3), 0.98 (3H, s, C19-CH_3), 1.36 (3H, d, ^2J=6.84 Hz, C21-CH_3), 2.03 (3H, s, $OCOCH_3$), 1.56 and 2.34 (3H, two s, $NCOCH_3$), 3.73 (3H, m, C3αH), 4.58 (1H, m, C20-H), 5.37 (1H, d, ^2J=5.1 Hz, C6=CH—); IR (KBr) 1730, 1700, 1365, 1250, 1035 cm^{-1}; MS *m/e* (CI): 444 (M^+), 402 (M^+-42), 341 (M^+-42–61); Anal. calcd $C_{27}H_{42}NO_4$ (C,H,N).

3-β-Acetoxy-20(*S*)-acetaminopregn-5-en (10). Compound **10** was prepared according to ref 19. Additional analytical data: ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, s, C18-CH_3), 1.01 (3H, s, C19-CH_3), 1.26 (3H, d, ^2J=6.4 Hz, C21-CH_3), 1.94 (3H, s, $NCOCH_3$), 2.03 (3H, s, $OCOCH_3$), 3.98 (1H, m, C3αH), 4.60 (1H, m, C20-H), 5.27 (1H, d, ^2J=8.56 Hz, —NH—), 5.37 (1H, d, ^2J=4.84 Hz, C6=CH—); MS *m/e* (CI): (M^+), 341 (M^+-60), 326 (M^+-60–15), 282 (M^+-60–59).

3-β-Acetoxy-20(*S*)-diacetaminopregn-5-en (11). Compound **11** was obtained together with **10**.[19] In contrast to the literature, **11** was isolated (LC) and characterized. Yield 28%; white crystals; mp 165–67 °C; ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, s, C18-CH_3), 1.02 (3H, s,

C19-CH_3), 1.43 (3H, d, $^2J = 6.74$ Hz, C21-CH_3), 2.03 (3H, s, $OCOCH_3$), 2.32 and 2.36 (3H, two s, $NCOCH_3$), 3.95 (1H, m, C3αH), 4.60 (1H, m, C20-H), 5.37 (1H, d, $^2J = 5.05$ Hz, C6=CH—); IR (KBr): 1730,1705,1360,1250,1030 cm^{-1}; MS *m/e* (CI): 444 (M^+), 402 (M^+-42), 341 (M^+-42-61); Anal. calcd $C_{27}H_{42}NO_4$ (C,H,N).

Biological methods

The microsomal fraction (containing P450 17) was prepared from rat testes as described recently.[14] The determination of enzyme activity and IC_{50} values, respectively, was performed according to our procedure:[14] the microsomes were incubated with excess progesterone (25 μM), NADPH (500 μM) and inhibitor in phosphate buffer (temperature: 32 °C, termination after 20 min by addition of 1 N HCl.

After extraction of the steroids, fluorocortisol acetate was added as internal standard. The samples were submitted to HPLC (RP-8 column, $CH_3OH:H_2O$ 1:1, v/v and detected by UV. Peak areas (fluorocortisol, progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone) were determined using a data evaluation software.

In the test for 'irreversible' inhibition, a preincubation was performed using the same experimental procedure without substrate. The test compound was separated by charcoal treatment and after 20 min the enzyme activity was determined as described above. In the test for recovery of enzyme activity, a 15 min preincubation was performed. After charcoal treatment the enzyme activity was determined after time intervals from 5 to 90 min.

The UV–vis difference spectra were recorded using a Perkin–Elmer Lambda 2 two-beam spectrophotometer with the corresponding computer software program PECSS. Rat testicular microsomes were suspended in 0.1 M sodium phosphate buffer containing 20% glycerol and 0.5% sodium cholate (pH 7.2) to a final concentration of 1 mg protein/mL. At 22 °C the enzyme suspension was distributed between two 1 cm pathlength cuvettes and a baseline was recorded from 350 to 500 nm. Difference spectra were then recorded at appropriate intervals following the addition of steroids dissolved in ethanol to the sample cuvette and an equivalent amount of ethanol added to the reference cuvette. The final concentration of ethanol in the cuvettes did not exceed 2%.

Statistical limits. The IC_{50} values and the enzyme activity data are mean values of at least three experiments. The deviations were within ±10% and ±5%, respectively.

Acknowledgements

We thank Dr H. L. Holland for the mass spectra and Mr J. Frotscher, Mr M. Mitrenga, and Mr M. Schommer for the ^1H and ^{13}C NMR spectra. Thanks are due to Mrs T. Kany, Mrs R. Würtz and Mr J. Düerkop for performing the P450 17, P450 arom, and P450 scc inhibitory assays, respectively. We are grateful to the Alexander von Humboldt Foundation for award of a Research Fellowship to Dr V.C.O. Njar and the Hermann–Schlosser–Stiftung for award of a Stipendium to Mr M. Hector. This work was supported in part by Verband der Chemischen Industrie, Fonds der Chemischen Industrie.

References

1. Van Wauwe, J. P.; Janssen, P. A. *J. Med. Chem.* **1989**, *32*, 2231.

2. Akhtar, M.; Corina, D. L.; Miller, S. L.; Shyadehi, A. Z.; Wright, J. N. *Biochemistry* **1994**, *33*, 4410; Swinney, D. C.; Mak, A. Y. *Biochemistry* **1994**, *33*, 2185.

3. Arth, G. E.; Patchett, A. A.; Jefopoulus, T.; Bugianesi, R. L.; Peterson, C. H. *J. Med. Chem.* **1971**, *14*, 675.

4. Angelastro, M. R.; Laughlin, M. E.; Schatzman, G. L.; Bey, P.; Blohm, T. R. *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1571.

5. Nakajin, S.; Takahashi, K.; Shinoda, M. *Chem. Pharm. Bull.* **1989**, *37*, 1855.

6. Li, J.; Li, Y.; Son, C.; Banks, P.; Brodie, A. *J. Steroid Biochem. Molec. Biol.* **1992**, *42*, 313.

7. Barrie, S. E.; Potter, G. A.; Goddard, P. M.; Haynes, B. P.; Dowsett, M.; Jarman, M. *J. Steroid Biochem. Molec. Biol.* **1994**, *50*, 267.

8. Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. *J. Med. Chem.* **1995**, *38*, 2463.

9. Hall, F.; Eik-Nes, K. B.; Samuels, L. T. *Endocrinology* **1963**, *73*, 547.

10. Neher, R.; Kahnt, F. W. *Experientia* **1965**, *21*, 310.

11. Ayub, M.; Levell, M. J. *J. Steroid Biochem.* **1987**, *28*, 521.

12. Vanden Bossche, H.; Willemsens, G.; Bellens, D.; Roels, J.; Janssen, P. A. J. *Biochem. Soc. Trans.* **1990**, *18*, 10.

13. McCague, R.; Rowlands, M. G.; Barrie, S. E.; Houghton, J. *J. Med. Chem.* **1990**, *33*, 3050.

14. Sergejew, T.; Hartmann, R. W. *J. Enz. Inhib.* **1994**, *8*, 113.

15. Hartmann, R. W.; Wächter, G. A.; Sergejew, T.; Würtz, R.; Düerkop, J. *Arch. Pharm.* **1995**, *328*, 573.

16. Wächter, G. A.; Hartmann, R. W.; Sergejew, T.; Grün, G. L.; Ledergerber, D. *J. Med. Chem.* **1996**, *39*, 834.

17. Njar, V. C. O.; Safi, E.; Silverton, J. V.; Robinson, C. H. *J. Chem. Soc. Perkin Trans 1* **1993**, 1161.

18. Tzikas, A.; Tamm, C.; Boller, A.; Fürst, A. *Helv. Chim. Acta* **1976**, *59*, 1850.

19. Bull, J. R.; Tuinman, A. *Tetrahedron* **1975**, *31*, 2151.

20. Van de Woude, G.; Van Hove, L. *Bull. Soc. Chim. Belges* **1967**, *76*, 566.

21. The antimycotic ketoconazole is the only commercially available inhibitor of P450 17. It has shown benefit in the treatment of prostate cancer (ref 27).

22. Jefcoate, C. R. *Methods Enzymol.* **1978**, *52*, 258.

23. Higashi, Y.; Omura, M.; Suzuki, K.; Inano, H.; Oshima, H. *Endocrinol. Japan.* **1987**, *34*, 105.

24. Hartmann, R. W.; Grün, G.; Bartz, U.; Palzer, M. *J. Steroid Biochem. Molec. Biol.* **1992**, *43*, 641.

25. Hartmann, R. W.; Batzl, C. *J. Med. Chem.* **1986**, *29*, 1363.

26. Li, J.; Li, Y.; Son, C.; Brodie, A. M. H. *207th Meeting of the ACS, Division of Med. Chem.*, 13–17 March, San Diego, **1994**, Abstract No 155.

(*Received in U.S.A.* 29 *September* 1995)