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Neighboring group participation Part 15. Stereoselective synthesis of some steroidal tetrahydrooxazin-2-ones, as novel presumed inhibitors of human 5α-reductase☆

János Wölfling[a], László Hackler[a], Erzsébet Mernyák[a], Gyula Schneider[a,*], István Tóth[b], Mihály Szécsi[b], János Julesz[b], Pál Sohár[c,d], Antal Csámpai[c]

[a] *Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary*
[b] *Endocrine Unit and Research Laboratory, University of Szeged, Korányi fasor 8, H-6720 Szeged, Hungary*
[c] *Department of General and Inorganic Chemistry, Eötvös Lóránt University, P.O. Box 32, H-1518 Budapest, Hungary*
[d] *Research Group of Structural Chemistry and Spectroscopy, Hung. Acad. Sci., P.O. Box 32, H-1518 Budapest, Hungary*

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Abstract

During the alkaline methanolysis of 3β-acetoxy-21-chloromethyl-pregn-5-ene-20β-*N*-phenylurethane, and its *p*-substituted phenyl derivatives, cyclization occurs, in the course of which 17β-[3-(*N*-phenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol and its *p*-substituted phenyl derivatives are formed. The cyclization takes place with (N^--6) neighboring group participation. Oppenauer oxidation of the 3β-hydroxy-*exo*-heterocyclic steroids yielded the corresponding Δ^4-3-ketosteroids. The structures of the new compounds were proved by IR, ^{1}H and ^{13}C NMR spectroscopy, using up-to-date measuring techniques such as 2D-COSY, HMQC, and HMBC. The inhibitory effects (CI_{50}) of the Δ^4-3-ketosteroids on 5α-reductase were studied.

Keywords: Heterocyclic steroids; Neighboring group participation; 5α-Reductase; In vitro inhibition; Structure determination by IR and NMR

1. Introduction

Prostatic cancer is the second leading cause of cancer-related mortality worldwide. Considerable attention has been devoted to the development of drugs that are active against prostate cancer by intervening in the processes of tumor growth, and especially in the steroid metabolism pathways of testosterone production [2]. Androgen deprivation by the inhibition of human cytochrome $P450_{17\alpha}$ (17α-hydroxylase/$C_{17,20}$-lyase), the enzyme responsible for the conversion of C_{21} steroids to C_{19} androgens, has been proposed as a potential approach for the therapeutic treatment of this disease, which is androgen-dependent in the majority of cases [3]. The syntheses of abiraterone [17-(3-pyridyl)androsta-5,16-dien-3β-ol] and its 4-en-3-one analog were reported recently; these display a high inhibitory activity against $P450_{17\alpha}$. It has been suggested that this activity is related to the presence of the heterocyclic moiety in ring D [4]. Guarna et al. have reported on the synthesis of (+)-17-(3-pyridyl)-(5β)-10-azaestra-1,16-dien-3-one, which may be a fundamental 5α-reductase inhibitor [5]. Since 5α-reductase converts testosterone to the more potent androgen dihydrotestosterone in prostatic tissues, the dual inhibition of 5α-reductase and $P450_{17\alpha}$ might be particularly beneficial in the treatment of prostate cancer [6]. Therefore, it was hypothesized that anchoring a 3′-pyridyl moiety on the D ring of 19-nor-10-azasteroid would produce novel steroid derivatives, as presumed inhibitors of both 5α-reductase and $P450_{17\alpha}$ [7].

We set out to synthesize a novel series of steroidal tetrahydrooxazin-2-ones containing heterocycles involving O and N heteroatoms at position 17β of androst-5-en-3β-ol and position 17β of androst-4-en-3-one, respectively. The present work was stimulated by the importance

☆ For a preceding paper in this series, see reference [1].
* Corresponding author. Tel.: +36-62-544276; fax: +36-62-544200.
E-mail address: schneider@chem.u-szeged.hu (G. Schneider).

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of pentacyclic steroids offering novel binding sites and binding distances to the receptors by introducing heteroatoms and various functionalities into the *exo*-heterocycles.

The discovery of two different genes encoding for two 5α-reductase isoenzymes, types 1 and 2 [8], with different biochemical characteristics and tissue distributions [9], added a further impulse to this research. In human, the type 1 isoenzyme is present mainly in the nongenital skin and in the liver whereas the type 2 is active in the prostate, genital skin, epididymis, seminal vesicle, and also in the liver. Cancerous and hyperplastic prostate are abundant in the type 2 isoenzyme, therefore tissue specimens of these tumors are suitable enzyme sources for the in vitro study of the 5α-reductase type 2 inhibitors. In vitro experiments on the newly synthesized steroid compounds **8a**–**g** were carried out with homogenates of human prostates.

2. Experimental

2.1. General

Melting points (mp) were determined on a Kofler block and are uncorrected. Specific rotations were measured in $CHCl_3$ (*c* 1) at 20 °C with a POLAMAT-A (Zeiss-Jena) polarimeter and are given in units of 10^{-1} degree $cm^2\ g^{-1}$. Elemental analysis was carried out in the analytical laboratory of the University of Szeged with a Perkin-Elmer model 2400 CHN analyzer. The reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent systems (ss) (A): acetone/benzene/light petroleum (30:35:35 v/v), (B): ethyl acetate/chloroform (10:90 v/v), (C): ethyl acetate/chloroform (5:95 v/v), (D): ethyl acetate/chloroform (1:99), and (E): dichloromethane. The spots were detected by spraying with 5% phosphomolybdic acid in 50% aqueous phosphoric acid. The R_f values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm. All solvents were distilled prior to use. The IR spectra were run in KBr disks on a Bruker IFS-113v vacuum optic FT-spectrometer with an Aspect 2000 computer (**3d** and **6**–**8a**, **b**, **d**, **f**, **h**) or on a Bruker IFS-55 FT-spectrometer controlled by Opus 3.0 software (**4d**, **5a**, **b** and **6**–**8c**, **e**, **g**). The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or in $(CD_3)_2SO$ solution in 5 or 10 mm tubes at room temperature, on a Bruker WM-250 and/or WP-80-SY FT-spectrometer controlled by an Aspect 2000 computer at 250.13 MHz (^1H) and 62.89 or 20.14 MHz (^{13}C), and on a Bruker DRX 500 spectrometer at 500.13 (^1H) and 125.76 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as internal standard. DEPT spectra were run in a standard manner, using only the $\Theta = 135^\circ$ pulse to separate CH/CH_3 and CH_2 lines phased "up" and "down," respectively. The HMQC and HMBC spectra were obtained by using the standard Bruker pulse programs.

The characteristic IR band frequencies and the ^1H and ^{13}C NMR data on the compounds investigated (**3d**, **4d**, **5a**, **b**, **6a**–**h**, **7a**–**h**, and **8a**–**h**) are listed in Tables 1–3.

2.2. 21-Hydroxymethylpregn-5-ene-3β,20-diol (3a)

21-Hydroxymethylenepregn-5-en-3β-ol-20-one (**2**) (3.44 g, 10 mmol), prepared by the procedure of Hirsch and Fujimoto [10] (mp 209–211 °C), was suspended in ethanol (50 ml) and cooled in an ice-bath, and KBH_4 (1.62 g, 0.03 mol) was added in small portions. The mixture was allowed to stand for 6 h, and was then neutralized with dilute HCl. The resulting solution was poured onto ice (300 g), and the precipitate that separated out was filtered off, dried over P_2O_5, and crystallized from acetone/water to give **3a** (3.05 g, 87%), mp 184–186 °C, R_f = 0.30 (ss A); $[\alpha]_D$ − 26 (*c* 1 in methanol) ([10] mp 183–185 °C).

2.3. 3β-Acetoxy-21-hydroxymethylpregn-5-en-20β-ol (3d) and 3β-acetoxy-21-hydroxy methylpregn-5-en-20α-ol (4d)

3β-Acetoxypregn-5-ene-21-hydroxymethyl-20-hydroxy cyclic acetonide (**3b**) (4.30 g, 10 mmol), prepared by the procedure of Hirsch and Fujimoto [10] (mp 154–166 °C), was dissolved in methanol (60 ml) and *p*-toluenesulfonic acid (0.5 g) in water (3 ml) was added to the solution. The reaction mixture was allowed to stand for 6 h and was then poured onto water (500 ml). The precipitate that separated out was filtered off, dissolved in chloroform, and subjected to chromatographic separation on silica gel in ethyl acetate/chloroform (5:95), yielding pure **3d** (3.1 g, 79%), mp 165–168 °C, R_f = 0.35 (ss C); $[\alpha]_D^{20}$ − 53 (*c* 1 in chloroform) (found: C, 73.76; H, 9.95. $C_{24}H_{38}O_4$ requires C, 73.81; H, 9.81%); continued elution resulted in a mixture of **3d** and **4d** (500 mg, 13%).

Continued elution furnished **4d** (170 mg, 4%), mp 183–186 °C, R_f = 0.25 (ss B); $[\alpha]_D^{20}$ − 45 (*c* 1 in chloroform) (found: C, 73.58; H, 9.65. $C_{24}H_{38}O_4$ requires C, 73.81; H, 9.81%).

2.4. 3β-Acetoxy-21-p-toluenesulfonyloxymethylpregn-5-ene-20β-ol (5a)

Compound **3d** (3.9 g, 10 mmol) was dissolved in pyridine (30 ml), and a solution of *p*-toluenesulfonyl chloride (2.1 g, 11 mmol) dissolved in pyridine (10 ml) was added during cooling in ice. The reaction mixture was allowed to stand at room temperature for 20 h, and was then saturated with water. The precipitate was filtered off, dissolved in chloroform and chromatographed on silica gel with a mixture of ethyl acetate/chloroform (2.5:75.5) to obtain **5a** (4.8 g, 88%), mp 176–178 °C, R_f = 0.45 (ss C); $[\alpha]_D^{20}$ − 20 (*c* 1 in chloroform) (found C, 68.42; H, 8.02. $C_{31}H_{44}O_6S$ requires C, 68.35; H, 8.14%).

Table 1
^{1}H NMR data[a] and characteristic IR frequencies[b] of compounds **3d**, **4d**, **5a**, **b**, **6a–h**, **7a–h**, and **8a–h**

Compound	CH_3[c] (OAc)	CH_3[d] (Ar)	CH_3[c] Pos. 18	CH_3[c] Pos. 19	H-3[e] *m* (1H)	H-6/4[f] *d* (1H)	OCH[g] *m* (1H)	XCH_2[g,h] *m* (2H)	OH/NH br (1H)	ArH-2′,6′ ∼*d* (2H)[i]	ArH-3′,5′ ∼*d* (2H)[i]	νC=O band[j]	νNCOO band	γC_ArH band
3d	2.03	–	0.79	1.03	4.62	5.40	∼3.85	∼3.85	∼2.65[h]	–	–	1733	–	–
4d	2.03	–	0.70	1.02	4.60	5.38	3.85	3.85	∼2.65	–	–	1732	–	–
5a	2.02	2.44	0.73	1.01	4.59	5.36	3.63	4.14		7.78	7.34	1713	–	903
5b	2.02	–	0.78	1.02	4.59	5.36	3.75	3.70		–	–	1717	–	–
6a	2.03	2.36	0.70	0.98	4.62	5.36	4.95	4.12	6.69	7.74	7.05–7.4[k]	1731	1731	813[l]
6b	2.03	2.30	0.74	0.99	4.60	5.3	5.00	3.56	6.73	7.10	7.30	1740	1723	817
6c	2.04	1.22	0.75	1.00	4.61	5.37	5.02	3.57	6.73	7.33	∼7.14	1721	1721	832
6d	2.03	3.79	0.74	0.99	4.62	5.38	5.00	3.57	6.62	∼7.35	6.85	1740	1722	829
6e	2.03	1.39	0.74	1.02	4.60	5.37	5.10	3.56	∼6.6	∼7.3	6.83	1731	1731	830
6f	2.04		0.74	1.00	4.60	5.36	5.00	3.56	6.95	7.00	∼7.4	1735	1716	833
6g	2.04		0.74	0.99	4.61	5.36	5.02	3.56	6.91	7.26	∼7.38	1734	1714	828
6h	2.04		0.74	0.99	4.65	5.40	5.05	3.56	∼6.8	7.2–7.5	7.2–7.5	1740	1724	822
7a	–	–	0.78	0.97	∼3.25[m]	5.27	4.38	3.55	4.56	7.22[k]	∼7.35[k]	–	1667	759[l]
7b	–	2.29	0.78	0.97	∼3.25[m]	5.27	4.35	3.55	4.50	∼7.20	∼7.20		1676	823
7c	–	1.24	0.85	1.03	3.52	5.35	4.36	3.62		∼7.22	∼7.22		1672	840
7d	–	3.80	0.84	1.03	∼3.6[n]	5.35	4.35	∼3.6[n]		7.25	6.90	–	1680	832
7e	–	1.40	0.76	1.01	3.51	5.35	4.32	3.57		7.19	6.87	–	1686	830
7f	–	–	0.84	1.03	∼3.65[n]	5.40	∼4.4	∼3.65[n]		7.21	7.49	–	1692	827
7g	–	–	0.84	1.03	3.53	5.36	4.36	3.62	∼1.6	7.26	7.35	–	1702	826
7h	–	–	0.84	1.03	∼3.55	5.35	4.36	∼3.75		∼7.05	∼7.35	–	1673	837
8a	–	–	0.88	1.19	–	5.72	4.36	∼3.7	–	∼7.3	∼7.3	1675	1695	754[l]
8b	–	2.33	0.87	1.19	–	5.72	4.35	∼3.65	–	∼7.18	∼7.18	1680	1680	816
8c	–	1.22	0.86	1.19	–	5.71	4.34	3.62	–	∼7.20	∼7.20	1670	1686	840
8d	–	3.80	0.87	1.20	–	5.72	4.35	∼3.72	–	7.21	6.90	1683	1683	850
8e	–	1.40	0.80	1.20	–	5.74	4.34	3.58	–	7.19	6.88	1686	1686	840
8f	–	–	0.87	1.20	–	5.73	∼4.4	∼3.7	–	7.20	7.49	1670	1700	824
8g	–	–	0.86	1.19	–	5.72	4.36	3.62	–	7.25	7.33	1682	1682	845
8h	–	–	0.87	1.20	–	5.72	4.38	∼3.65	–	∼7.1	∼7.3	1670	1692	850

[a] Measuring frequency: 500 (**4d**, **5a**, **b**, and **6–8c**, **e**, **g**) or 250 MHz (**3d** and **6–8a**, **b**, **d**, **f**, **h**). Solvent: $CDCl_3$ or DMSO-d_6 (for **7a**, **b**). Chemical shifts in ppm ($\delta_{TMS} = 0$ ppm), coupling constants in Hz. Assignments were supported by 2D-HMQC (for **4d**, **5a**, **6c**, **7g**, and **8c**) and for **4d** also by 2D-COSY measurements.

[b] In KBr discs (cm^{-1}). Further bands, νOH (doubled for **7e**, **h**): 3380–3500 (**3d**, **4d**, **5a**, **b**, and **7a–h**), νNH: 3315–3430 (**6a–h**, doubled for **6d**, **e**), νSO_2: 1358, 1190, 1178 (**5a** and **6a**).

[c] *s* (3H).

[d] *s* (3H), OMe group (**6–8d**), (Ar)Et group (**6–8c**, **e**): *t* (*J*: 7.0), CH_2 (ArEt): 2.62 (**6–8c**), OCH_2 (ArEt): 4.02 (**7e** and **8e**).

[e] For **5a**, **b** and **6a–h** tt with coalesced lines.

[f] Pos. 6 (**3–7**), Pos. 4 (**8**),∼*d*, *J*: 4.5 ± 0.6 (**5**, **6**), 5.1 ± 0.1 (**7**), ∼1 (**8**).

[g] In the side-chain or oxazinone ring, X: O (**3d**, **4d**, and **5a**, **6a**), Cl (**5b** and **6b–h**), or *N* (**7** and **8**).

[h] Two *m*'s (2 × 1H) with the second signal at ∼2.95 (2H, **3d**), 4.25 (**5a**), 3.72 ± 0.03 (**7a**, **b**, **c**, **g**, and **8c**, **g**), 3.64 ± 0.02 (**7e** and **8e**).

[i] Rudimentaly AA′BB′-type spectrum: *J*: 8.3 (**5a** and **6a–c**), 8.8 (**5d**, **e**, **g**, **7d–g**, and **8d–g**), ∼*s*, broad signals with coalesced fine structure (due to F,H-couplings for **6–8h**): 2 × *m* (2 × 2H, **6–8h**), *m* (4H) for **6h**, **7b**, **c**, and **8b**, **c**, *m* (5H) for **8a**.

[j] Ester (**3d**, **4d**, **5a**, **b**, and **6a–h**) or ketone group (**8a–h**).

[k] Intensity: 7/1/4H.

[l] *p*-Disubstituted benzene ring, 748 (phenyl), $\gamma C_{Ar}C_{Ar}$ (phenyl): 698 (**6a**, **7a**, and **8a**).

[m] Hidden by the water signal of the solvent.

[n] Overlapping signals.

Table 2
^{13}C NMR chemical shifts[a] of compounds **3d**, **4d**, **5a**, **b**, **6a–h**, **7a–h**, and **8a–h**[b,c]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19
3d	38.0[d]	37.1	74.0[d]	39.8	139.8	122.4	31.9	31.8	50.1	36.6	21.0	38.1[d]	42.4	56.2[d]	25.4	27.8	56.5[d]	12.3	19.3
4d	38.4[d]	37.4	74.3	39.3	140.1	122.9	32.2	31.9	50.4	37.0	21.1	38.5[d]	42.1	56.9[d]	25.4	28.2	57.1[d]	13.1	19.7
5a	37.4	36.3	74.3	38.5	140.1	122.8	32.3	32.1	50.4	37.0	21.3	40.3	42.8	56.9	25.6	28.1	56.4	12.8	19.7
5b	38.5	37.4	74.3	40.0[d]	140.1	122.8	32.3	32.1	50.4	37.0	21.3	40.3[d]	42.9	57.0	25.7	28.1	56.5	12.8	19.7
6a	38.2	37.1	74.0	39.1	140.0	122.3	31.9[e]	31.9[e]	50.3	36.7	21.0	33.6	42.4	56.1	25.4	27.9	53.4	12.3	19.2
6b	38.1[d]	37.0	74.0	39.1[d]	139.9	122.3	31.9	31.8	50.2	36.6	21.0	38.2[d]	42.4	56.1	25.5	27.8	53.6	12.4	19.3
6c	38.4[d]	37.4	74.4	38.5[d]	139.9	122.8	32.3	32.1	50.5	37.0	21.4	39.5	42.8	56.4	25.9	28.2	54.0	12.8	19.7
6d	38.1[d]	37.1	74.0[d]	39.1	139.9	122.3	31.8[e]	31.8[e]	50.2	36.6	21.0	38.2[d]	42.5	56.1	25.5	27.8	53.7	12.3	19.3
6e	38.5[d]	37.4	74.3[e]	39.1	140.1	120.8	32.2	31.9	50.4	37.0	21.1	38.7[d]	42.2	56.8	25.1	28.1	54.6	12.9	19.7
6f	38.0[d]	37.1	74.0[d]	39.2	139.9	122.3	31.9	31.8	50.2	37.0	21.1	38.7[d]	42.2	56.8	25.1	28.1	54.6	12.9	19.7
6g	38.5[d]	37.4	74.4	39.5[d]	140.2	120.3	32.2	32.1	50.5	37.0	21.4	38.3[d]	42.8	56.4	25.8	28.2	53.9	12.8	19.7
6h	38.2[d]	37.0	74.0	39.1[d]	139.9	122.3	31.83	31.75	50.2	36.7	210	37.9[d]	42.4	56.1	25.5	27.8	53.5	12.4	19.3
7a	38.7	33.1[e]	71.7	43.9	143.2	121.8	33.1[e]	33.1[e]	51.6	37.8	22.0	40.4	43.7	57.4	25.7[d]	28.1	54.9	13.7	20.8
7b	38.6	33.0[e]	71.7	43.8	143.1	121.6	33.0[e]	33.0[e]	51.5	37.8	21.9	40.3	43.6	57.3	25.5[d]	28.0	54.9	13.6	20.6
7c	37.6	32.1[d]	72.2	42.7	143.1	121.7	32.4[d]	32.2	50.6	37.0	21.2	39.4	43.0	56.7	25.1[d]	27.3	54.0	12.7	19.8
7d	37.4	32.1[d]	71.9	42.4	143.3	121.3	31.9[d]	32.0	50.5	36.7	20.9	39.2	42.7	56.5	24.8[d]	27.1	53.9	12.3	19.5
7e	37.7	32.2[d]	72.0	42.6	141.2	121.8	32.0[d]	31.9	50.5	36.9	21.2	39.3	42.2	56.8	26.0[d]	28.1	54.9	13.0	19.2
7f	38.5	33.0[e]	71.6	43.8	143.1	121.5	33.0[e]	33.0[e]	51.5	37.7	21.9	40.2	43.5	57.2	25.5[d]	27.9	54.8	13.5	20.5
7g	37.6	32.1	72.2	42.7	141.5	121.7	32.4	32.2	50.6	37.0	21.2	39.4	43.0	56.7	24.9[d]	27.2	54.1	12.7	19.8
7h	37.5	32.2[d]	71.8	42.6	141.4	121.3	32.0[d]	32.1[d]	50.6	36.8	21.0	39.3	42.8	56.6	24.9[d]	27.1	54.0	12.3	19.5
8a	35.6[d]	33.9[d]	199.1	123.8	170.9	32.8[d]	32.2[d]	35.7[d]	53.7	38.7	20.8	39.0	42.7	55.5	24.6[d]	26.9[d]	54.0	12.3	17.4
8b	35.7[d]	34.0[d]	199.5	123.8	171.3	32.9[d]	32.1[d]	35.6[d]	53.7	38.7	20.8	38.9	42.7	55.5	24.7[d]	26.9[d]	53.9	12.3	17.4
8c	36.1	34.4	200.0	124.2	171.7	33.3	32.6	35.9	54.3	39.1	21.2	39.3	43.0	55.8	24.8[d]	27.2[d]	54.0	12.7	17.8
8d	35.7[d]	33.9	199.4	123.8	171.3	32.8	32.1	35.5[d]	53.6[d]	38.6	20.8	38.9	42.6	55.4[e]	24.6[d]	26.9[d]	53.9[d]	12.3	17.4
8e	36.1	34.4	199.8	124.3	171.4	33.2	32.3	35.6	54.1[d]	39.0	21.1	39.1	42.3	55.9	24.5[d]	28.2[d]	54.9[d]	13.2	17.8
8f	35.6	33.8	199.1	123.7	171.0	32.7[d]	32.0[d]	35.4	53.6[d]	38.5	20.7	38.8	42.5	55.3	24.4[d]	26.7[d]	53.8[d]	12.2	17.3
8g	36.1	34.4	200.0	124.2	171.7	33.3[d]	32.5[d]	35.9	54.0[d]	39.1	21.2	39.3	43.0	55.8	24.8[d]	27.2[d]	54.3[d]	12.8	17.8
8h	35.8	34.0	199.4	123.9	171.1	32.9[d]	32.2[d]	35.6	53.7[d]	38.7	20.8	39.0	42.7	55.5	24.4[d]	26.9[d]	54.0[d]	12.4	17.5

[a] In ppm ($\delta_{TMS} = 0$ ppm) at 126.7, 62.9 (**3d**, **6b**, **f**, **h**, **7b**, **d**, **f**, **h**, and **8b**, **d**, **f**, **h**) or 20.1 MHz (**6a**, **d**, **7a**, and **8a**). Solvent: $CDCl_3$ or DMSO-d_6 (for **7a**, **b**), respectively.

[b] Assignments were supported by DEPT (except for **3d**, **6a**, **7b**, **f**, **h**, and **8a**, **b**, **d**), and for **4d**, **5a**, **6c**, **7g**, and **8c** also by HMQC and HMBC measurements.

[c] Further lines: see Table 3 and the footnote to it.

[d] Interchangeable assignments.

[e] Overlapping lines.

2.5. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-ol (**5b**)

Compound **3d** (3.9 g, 10 mmol) was suspended in CCl_4 (40 ml) and Ph_3P (2.88 g, 3.1 mmol) was added. After an 6-h reflux, the solvent was evaporated off. The resulting crude product was chromatographed on silica gel with a mixture of ethyl acetate/chloroform (2.5:97.5) to obtain **5b** (2.9 g, 74%), mp 183–184 °C, $R_f = 0.50$ (ss C); $[\alpha]_D^{20} - 31$ (*c* 1 in chloroform) (found C, 70.56; H, 8.98. $C_{24}H_{37}O_3Cl$ requires C, 70.48; H, 9.12%).

2.6. 3β-Acetoxy-21-p-toluenesulfonyloxymethylpregn-5-ene-20β-N-phenylurethane (**6a**) and 3β-acetoxy-21-chloromethylpregn-5-ene-20β-N-phenylurethane (**6b**)

2.6.1. General procedure

Compound **5a** (1.09 g, 2 mmol) or **5b** (818 mg; 2 mmol) was dissolved in dichloromethane (25 ml) and phenyl isocyanate (960 mg, 8 mmol) and triethylamine (0.1 ml) were added. The reaction mixture was refluxed for 6 h, and was then poured into 10% $NaHCO_3$ solution (50 ml). The dichloromethane fraction was washed thoroughly with water, dried and evaporated to dryness. The residue was subjected to chromatographic separation with dichloromethane.

Compound **5b** (818 mg; 2 mmol) was dissolved in benzene (25 ml) and triethylamine (0.3 ml) and a solution of phosgene in benzene (5 ml; 10%) were added. The reaction mixture was allowed to stand at room temperature for 24 h. Diethyl ether (30 ml) was then added, and the triethylamine HCl that separated out was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in benzene (30 ml), and triethylamine (0.3 ml) and the corresponding substituted aniline (2.2 mmol) were added. The mixture was allowed to stand for 6 h, and was then diluted with water. The precipitate that separated out was filtered off and dried over P_2O_5. The resulting crude product was chromatographed on silica gel with dichloromethane.

2.6.2. 3β-Acetoxy-21-p-toluenesulfonyloxymethylpregn-5-ene-20β-N-phenylurethane (**6a**)

6a (1.18 g; 85%), mp 166–167 °C, $R_f = 0.6$ (ss D); $[\alpha]_D^{20} - 51$ (*c* 1 in chloroform) (found C, 68.82; H, 7.55. $C_{38}H_{49}O_7SN$ requires C, 68.75; H, 7.44%).

Table 3
^{13}C NMR chemical shifts[a] of compounds **3d**, **4d**, **5a**, **b**, **6a–h**, **7a–h**, and **8a–h**[b,c]

Compound	CH_3 (OAc)	CH_3 (Ar)	Side chain or oxazinone ring			Aryl group				NCOO	C=O (OAc)
			CH(OH)	CCH_2C	XCH_2	C-1′	C-2′,6′	C-3′,5′	C-4′		
3d	21.3	–	74.3[d]	24.6	61.4	–	–	–	–	–	170.6
4d	21.8	–	74.6	24.5	62.3	–	–	–	–	–	171.0
5a	21.8	22.0	70.8	24.9	68.3	133.6	128.3	130.3	145.2	–	170.9
5b	21.8	–	71.9	24.9	42.3	–	–	–	–	–	170.6
6a	21.3[d]	21.5[d]	73.6	24.3	67.1	133.5[e]	129.0[e]	129.8[e]	144.6[e]	152.9	170.4
6b	21.4	20.7	74.5	24.4	40.7	133.0	119.0	129.5	135.5	153.2	170.5
6c	21.8	16.2	74.8	24.7	41.1	136.0	119.2	128.8	140.2	153.5	170.6
6d	21.3	55.0	74.5	24.3	40.6	131.3	121.0	114.4	156.2	153.5	170.4
6e	21.8	15.3	74.3[d]	24.4	41.3	131.4	122.8	115.3	155.7	154.1	170.6
6f	21.4	–	74.8	24.4	0.6	134.1	∼120.7	115.6[f]	159.1[f]	153.3	170.6
6g	21.9	–	75.2	24.4	41.0	137.1	122.7	129.4	128.8	153.4	171.2
6h	21.4	–	74.8[d]	24.3	40.6	137.4	120.4	131.9	115.9	153.0	170.7
7a	–	–	80.4	25.6[d]	48.7	145.2	127.4	130.3	127.5	153.2	–
7b	–	22.0	80.2	25.6[d]	48.7	142.6	127.1	130.6	136.7	153.6	–
7c	–	15.8	79.6	24.9[d]	48.2	141.1[d]	126.1	129.0	141.6[d]	153.1	–
7d	–	55.6	79.3	24.8[d]	48.2	136.4	127.2	114.6	158.3	152.8	–
7e	–	15.2	80.7	24.6[d]	48.8	136.2	127.6	115.4	157.9	153.8	–
7f	–	–	80.4	25.4[d]	48.3	144.3	129.2	132.9	119.7	152.9	–
7g	–	–	79.9	25.1[d]	48.0	142.0	127.4	129.6	132.4	152.8	–
7h	–	–	79.5	24.6[d]	48.0	139.2	127.6[f]	116.0[f]	161.0[f]	152.6	–
8a	–	–	79.1	26.9	47.6	143.3	125.7	129.0	126.4	152.3	–
8b	–	21.0	79.1	26.9	47.8	140.6	125.7	129.8	136.4	152.6	–
8c	–	15.8	79.5	27.2	48.2	141.1	126.1	129.0	143.1	153.0	–
8d	–	55.4[g]	79.1	26.9	48.1	136.1	127.1	114.4	158.1	152.8	–
8e	–	15.2	80.2	28.2	48.8	136.1	127.6	115.5	158.0	153.7	–
8f	–	–	79.2	26.7	47.3	142.1	127.1	131.9	119.5	152.0	–
8g	–	–	79.8	27.2	48.0	142.0	127.4	129.6	132.5	152.7	–
8h	–	–	79.3	26.9	48.0	139.1[f]	127.6[f]	116.0[f]	161.0[f]	152.5	–

[a] In ppm ($\delta_{TMS} = 0$ ppm) at 125.7 and 62.9 (**3d**, **6b**, **f**, **h**, **7b**, **d**, **f**, **h**, and **8b**, **d**, **f**, **h**) or 20.1 MHz (**6a**, **d**, **7a**, and **8a**). Solvent: $CDCl_3$ or DMSO-d_6 (for **7a**, **b**), respectively.
[b] Assignments were supported by DEPT (except for **3d**, **6a**, **7b**, **f**, **h**, and **8a**, **b**, **d**), for **4d**, **5a**, **6c**, **7g**, and **8c** also by HMQC and HMBC measurements.
[c] Further lines (see also Table 2): phenyl (**6a**), C-1: 138.3, C-2,6: 118.9, C-3,5: 127.9, C-4: 123.4, $ArCH_2$: 28.7 (**6–8c**), OCH_2: 64.1 (**5–7e**).
[d] Interchangeable assignments.
[e] Tosyl group.
[f] Doublet due to F,C-couplings, ^1J: 242.5 (**6f**) and 246.2 (**7h** and **8h**), ^2J: 22.4 (**6f**) and 22.7 (**7h** and **9h**), ^3J: 8.4 (**7h** and **8h**), ^4J: 3.2 (**8h**).
[g] Two overlapping lines.

2.6.3. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-tolylurethane (6b)

6b (960 mg; 88%), mp 162–164 °C, $R_f = 0.6$ (ss E); $[\alpha]_D^{20} + 8$ (*c* 1 in chloroform) (found C, 70.95; H, 8.05. $C_{32}H_{44}O_4NCl$ requires C, 70.89; H, 8.18%);

2.6.4. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-ethylphenylurethane (6c)

6c (910 mg; 81%) mp 129–131 °C, $R_f = 0.55$ (ss E); $[\alpha]_D^{20} + 4$ (*c* 1 in chloroform) (found C, 71.35; H, 8.22. $C_{33}H_{46}O_4NCl$ requires C, 71.26; H, 8.34%).

2.6.5. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-methoxyphenylurethane (6d)

6d (895 mg; 80%), mp 105–107 °C, $R_f = 0.40$ (ss E); $[\alpha]_D^{20} + 4$ (*c* 1 in chloroform) (found C, 68.75; H, 8.02. $C_{32}H_{44}O_5NCl$ requires C, 68.86; H, 7.95%).

2.6.6. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-ethoxyphenylurethane (6e)

6e (980 mg; 85%), mp 157–159 °C, $R_f = 0.50$ (ss E); $[\alpha]_D^{20} - 50$ (*c* 1 in chloroform) (found C, 69.36; H, 8.28. $C_{33}H_{46}O_5NCl$ requires C, 69.27; H, 8.10%).

2.6.7. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-chlorophenylurethane (6f)

6f (900 mg; 80%), mp 116–118 °C, $R_f = 0.70$ (ss E); $[\alpha]_D^{20} + 11$ (*c* 1 in chloroform) (found C, 66.27; H, 7.35. $C_{33}H_{41}O_4NCl_2$ requires C, 66.18; H, 7.35%).

2.6.8. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-bromophenylurethane (6g)

6g (950 mg; 78%), mp 140–142 °C, $R_f = 0.60$ (ss E); $[\alpha]_D^{20} + 13$ (*c* 1 in chloroform) (found C, 61.52; H, 6.72. $C_{31}H_{41}O_4BrClN$ requires C, 61.34; H, 6.81%).

*2.6.9. 3β-Acetoxy-21-chloromethylpregn-5-ene-20β-N-4-fluorophenylurethane (**6h**)*

6h (920 mg; 84%), mp 107–110 °C, $R_f = 0.65$ (ss E); $[\alpha]_D^{20} - 5$ (*c* 1 in chloroform) (found C, 68.23; H, 7.67. $C_{31}H_{41}O_4NFCl$ requires C, 68.18; H, 7.57%).

*2.7. 17β-[3-(N-Aryl)-tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7a–h**)*

2.7.1. General procedure

The individual compounds **6a–h** (2 mmol) were dissolved in methanol (30 ml), $NaOCH_3$ (432 mg, 8 mmol) was added and the solution was kept at the boiling point. The progress of the reaction was monitored by TLC. Refluxing was continued until the starting material had been fully consumed (about 120 min). The reaction mixture was then neutralized with dilute HCl, and diluted with water. The resulting precipitate was filtered off, dissolved in dichloromethane and chromatographed on silica gel with a mixture of ethyl acetate/chloroform (5:95).

*2.7.2. 17β-[3-(N-Phenyl-tetrahydrooxazin-2-on-6-yl)] androst-5-en-3β-ol (**7a**)*

7a (786 mg; 86%), mp 212–214 °C, $R_f = 0.45$ (ss B); $[\alpha]_D^{20} - 79$ (*c* 1 in chloroform) (found C, 77.58; H, 8.57. $C_{29}H_{39}O_3N$ requires C, 77.47; H, 8.74%).

*2.7.3. 17β-[3-(N-4-Tolyl)tetrahydrooxazin-2-on-6-yl] androst-5-en-3β-ol (**7b**)*

7b (810 mg; 87%), mp 240–243 °C, $R_f = 0.45$ (ss B); $[\alpha]_D^{20} - 82$ (*c* 1 in chloroform) (found C, 77.93; H, 9.05. $C_{30}H_{41}O_3N$ requires C, 77.71; H, 8.91%).

*2.7.4. 17β-[3-(N-4-Ethylphenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7c**)*

7c (805 mg; 84%), mp 190–192 °C, $R_f = 0.40$ (ss B); $[\alpha]_D^{20} - 73$ (*c* 1 in chloroform) (found C, 77.82; H, 8.98. $C_{31}H_{43}O_3N$ requires C, 77.95; H, 9.07%).

*2.7.5. 17β-[3-(N-4-Methoxyphenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7d**)*

7d (790 mg; 82%), mp 232–234 °C, $R_f = 0.35$ (ss B); $[\alpha]_D^{20} - 81$ (*c* 1 in chloroform) (found C, 75.25; H, 8.55. $C_{30}H_{41}O_4N$ requires C, 75.12; H, 8.62%).

*2.7.6. 17β-[3-(N-4-Ethoxyphenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7e**)*

7e (832 mg; 84%), mp 207–210 °C, $R_f = 0.45$ (ss B); $[\alpha]_D^{20} + 2$ (*c* 1 in chloroform) (found C, 75.55; H, 8.87. $C_{31}H_{43}O_4N$ requires C, 75.42; H, 8.78%).

*2.7.7. 17β-[3-(N-4-Bromophenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7f**)*

7f (910 mg; 86%), mp 213–214 °C, $R_f = 0.45$ (ss B); $[\alpha]_D^{20} - 72$ (*c* 1 in chloroform) (found C, 66.08; H, 7.32. $C_{29}H_{38}O_3NBr$ requires C, 65.90; H, 7.25%).

*2.7.8. 17β-[3-(N-4-Chlorophenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7g**)*

7g (915 mg; 94%), mp 179–183 °C, $R_f = 0.30$ (ss B); $[\alpha]_D^{20} - 70$ (*c* 1 in chloroform) (found C, 72.06; H, 7.83. $C_{29}H_{38}O_3NCl$ requires C, 71.96; H, 7.91%).

*2.7.9. 17β-[3-(N-4-Fluorophenyl)tetrahydrooxazin-2-on-6-yl]androst-5-en-3β-ol (**7h**)*

7h (876 mg; 93%), mp 235–237 °C, $R_f = 0.35$ (ss B); $[\alpha]_D^{20} - 69$ (*c* 1 in chloroform) (found C, 74.63; H, 8.02. $C_{29}H_{38}O_3NF$ requires C, 74.49; H, 8.19%).

*2.8. 17β-[3-(N-Aryl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8a–h**)*

2.8.1. General procedure

The individual compounds **7a–h** (2 mmol) were dissolved in toluene (60 ml), aluminium isopropoxide (2.04 g, 10 mmol) and cyclohexanone (20 ml) were added, and the mixture was stirred at the boiling point. The disappearance of the starting material was followed by TLC. When the reaction was complete, dilute H_2SO_4 (1%, 200 ml) was added to the reaction mixture. Most of the organic solvent was removed in vacuo and the residual emulsion was extracted with chloroform. The organic phase was evaporated down and the resulting crude product was chromatographed on silica gel with ethyl acetate/chloroform (5:95).

*2.8.2. 17β-[3-(N-Phenyl)tetrahydrooxazin-2-on-6-yl] androst-4-en-3-one (**8a**)*

8a (720 mg; 80%), mp 234–235 °C, $R_f = 0.55$ (ss B); $[\alpha]_D^{20} + 46$ (*c* 1 in chloroform) (found C, 77.64; H, 8.45. $C_{29}H_{37}O_3N$ requires C, 77.82; H, 8.33%).

*2.8.3. 17β-[3-(N-4-tolyl)tetrahydrooxazin-2-on-6-yl] androst-4-en-3-one (**8b**)*

8b (685 mg; 74%), mp 208–209 °C, $R_f = 0.45$ (ss B); $[\alpha]_D^{20} + 45$ (*c* 1 in chloroform) (found C, 77.94; H, 8.67. $C_{30}H_{39}O_3N$ requires C, 78.05; H, 8.52%).

*2.8.4. 17β-[3-(N-4-Ethylphenyl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8c**)*

8c (692 mg; 75%), mp 183–186 °C, $R_f = 0.50$ (ss B); $[\alpha]_D^{20} + 52$ (*c* 1 in chloroform) (found C, 78.37; H, 8.74. $C_{31}H_{41}O_3N$ requires C, 78.28; H, 8.69%);

*2.8.5. 17β-[3-(N-4-Methoxyphenyl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8d**)*

8d (756 mg; 79%), mp 228–231 °C, $R_f = 0.50$ (ss B); $[\alpha]_D^{20} + 29$ (*c* 1 in chloroform) (found C, 75.62; H, 8.25. $C_{30}H_{39}O_4N$ requires C, 75.44; H, 8.23%).

*2.8.6. 17β-[3-(N-4-Ethoxyphenyl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8e**)*

8e (872 mg; 88%), mp 198–201 °C, $R_f = 0.45$ (ss B); $[\alpha]_D^{20} + 126$ (*c* 1 in chloroform) (found C, 75.61; H, 8.65. $C_{31}H_{41}O_4N$ requires C, 75.73; H, 8.41%).

*2.8.7. 17β-[3-(N-4-Bromophenyl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8f**)*

8f (874 mg; 83%), mp 222–225 °C, $R_f = 0.60$ (ss B); $[\alpha]_D^{20} + 47$ (*c* 1 in chloroform) (found C, 65.98; H, 6.67. $C_{29}H_{36}O_3NBr$ requires C, 66.16; H, 6.89%).

*2.8.8. 17β-[3-(N-4-Chlorophenyl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8g**)*

8g (790 mg; 81%), mp 210–213 °C, $R_f = 0.40$ (ss B); $[\alpha]_D^{20} + 46$ (*c* 1 in chloroform) (found C, 72.43; H, 7.41. $C_{29}H_{36}O_3NCl$ requires C, 72.26; H, 7.53%).

*2.8.9. 17β-[3-(N-4-Fluorophenyl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-one (**8h**)*

8h (780 mg; 83%), mp 237–239 °C, $R_f = 0.55$ (ss B); $[\alpha]_D^{20} + 50$ (*c* 1 in chloroform) (found C, 74.65; H, 7.91. $C_{29}H_{36}O_3NF$ requires C, 74.81; H, 7.79%).

2.9. Determination of 5α-reductase activity and its inhibition in the human prostate

Human prostates were obtained surgically from patients with benign prostatic hyperplasia and homogenized [11]. The protein content was determined according to Lowry et al. [12]. The homogenate was incubated with 200 nM [4-^{14}C]testosterone and the test compound (in four different concentrations) in the presence of 1 mM NADPH for 20 min at 37 °C. The control experiments were performed without the test substances in every series. The enzymatic reaction was stopped by the addition of ethyl acetate and freezing. After extraction the radioactive testosterone and dihydrotestosterone were separated by TLC and a Packard Radiochromatogram Scanner was used to trace the separated steroids. Following measurement of the radioactivity of the dihydrotestosterone formed and the substrate testosterone, the 5α-reductase activity was calculated and expressed in terms of pmol dihydrotestosterone per mg of protein for a 20-min incubation. The inhibitory effects of the compounds investigated are given in terms of IC_{50} values, i.e. the concentration of inhibitor at which the 5α-reductase activity was decreased to 50%.

3. Results and discussion

3.1. Synthetic studies

For the synthesis of tetrahydrooxazinone-2 ring at position 17β of the sterane ring, we chose the basic cyclization of the α,γ-haloalkyl-*N*-arylurethanes in alkaline media. Scott et al. examined the ring-closure reactions of α,β- and α,γ-haloalkyl *N*-arylurethanes in alkaline media [13]. They found that the *N*-arylurethane group and its substituted derivatives reacted at different rates, depending on the inductive and conjugative effects of the substituents. The cyclization typically occurred with neighboring group participation, to yield *N*-phenyloxazolidone-2 or *N*-phenyltetrahydrooxazinone-2 derivatives, depending on the starting compounds [14]. In the solvolysis of vicinal halourethanes, oxazolidinone derivatives condensed to the estrane skeleton are formed, which are likewise potential pharmacons [15]. The cyclization of the four possible isomeric 16-*p*-toluene-sulfonyloxymethyl-17-unsubstituted phenylurethanes on the androstane and estrane skeletons has already been investigated [16,17].

To prepare the α,γ-diol system on the side chain of the sterane skeleton, we chose 3β-acetoxypregn-5-en-20-one (**1**) as starting compound. Elongation of the side-chain with a hydroxymethyl group on C-21 in **1** was accomplished by condensation with ethyl formate in the presence of NaOMe, followed by reduction with KBH_4 to the triols [10].

The simultaneous development of the new chiral centers at C-20 permits the formation of 21-hydroxymethylpregn-5-ene-3β,20β-diol (**3a**) and its 20α epimer (**4a**) in a ratio of 95:5. The separation of the two isomeric compounds in this form was not possible. The cyclic acetonides of the mixture of 21-hydroxymethyl-20-hydroxy compounds were acetylated in the 3β position, and the subsequent acidic hydrolysis of the cyclic ketal produced a chromatographically separable mixture of 3β-acetoxy-21-hydroxymethylpregn-5-en-20-ol isomers (**3d** and **4d**). The separation of the two isomeric compounds in this form was possible. All ^{13}C NMR chemical shifts of the epimeric pair **3d** and **4d** are the same within 0.5 ppm. Only the shifts of C-14, C-17, and C-18, carbons near to the chiral center (in position C-20) with different configurations, differ by 0.7, 0.6, and 0.8 ppm, respectively. Similarly, the ^{1}H NMR chemical shifts of the diastereomers are practically identical ($\Delta\delta \leq 0.02$ ppm). The only exception is the 18-CH_3 group, which is similar close to C-20, the shifts of which differ by 0.09 ppm for the isomers.

For the further reactions, we used only the pure 20β isomer (**3d**). In the earlier literature it was supposed that the reduction of the C-20 ketone is stereoselective and that C-20 has the *R* configuration [10].

The selective *p*-tosyl ester formation from **3d** resulted in **5a**, which, in a subsequent reaction with phenyl isocyanate, was converted to 3β-acetoxy-21-*p*-toluene-sulfonyloxymethylpregn-5-ene-20β-phenylurethane (**6a**). For the preparation of substituted phenylurethane derivatives **6b**–**h**, the starting compound was 3β-acetoxy-21-chloromethylpregn-5-en-20β-ol (**5b**), which was prepared by the Appel reaction of **3d** [18]. The OH → Cl change in **5b** as compared with **3d** is confirmed by the very large upfield shift (by 19.1 ppm) of the ^{13}C NMR line of the neighboring carbon. **5b** Reacted with phosgene in the presence of triethylamine to furnish the desired 3β-acetoxy-21-chloromethylpregn-5-ene-20β-chlorocarbonic acid ester. Without isolation, this compound was reacted with the appropriately substituted anilines to yield **6b**–**h**. The presence of the *O*-tosyl group in **5a** and **6a** is demonstrated by the characteristic spectral data on this substituent (e.g.

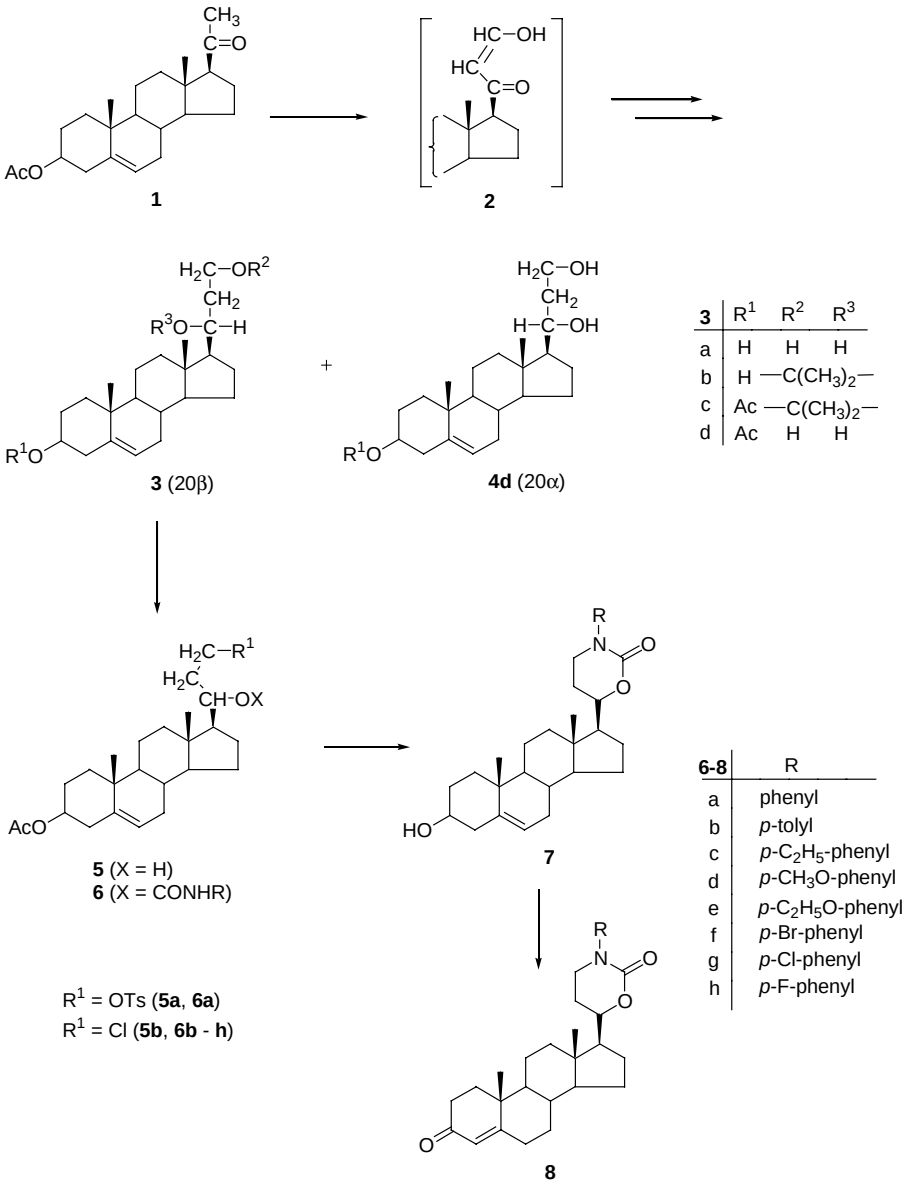
the νSO_2 IR bands, the methyl singlet with 3H intensity and the AA′BB′ multiplet of the *p*-disubstituted aromatic ring in the ^{1}H NMR spectra and the ^{13}C NMR lines of the methyl and aromatic carbons in the ^{13}C NMR spectra) and also by the downfield shifts (by 0.29 and 0.4 ppm) of the 21-methylene signal in the ^{1}H NMR spectra. The urethane carbonyl line appears in the expected interval [19], between 152.3 and 154.1 ppm, for all compounds in the series **6**–**8**. The urethane NH in series **5** gives a broad signal of 1H intensity in all (**a**–**h**) cases (6.6–7.0 ppm).

Compounds **6a**–**h** were subjected to methanolysis in the presence of four equivalents of $NaOCH_3$. Under these experimental conditions, *N*-phenyl-tetrahydrooxazinone-2 rings (**7a**–**h**) were formed in rapid reactions. The cyclization can be explained by the nucleophilic attack of the nitrogen atom of the deprotonated acid amide. This cyclization process is typical neighboring group participation. In the notation proposed by Winstein and Boschan [14], the process can be characterized by the symbol ($N^- - 6$).

The 3-acetoxy group in **3d**, **4d**, **5a**, **b**, and **6a**–**h** gives a high νC=O frequency (1713–1740 cm^{-1}) in the IR spectrum, expected for saturated esters [20], a ^{1}H NMR methyl signal (2.03 ± 0.01 ppm) and a ^{13}C NMR carbonyl line (170.8 ± 0.4 ppm).

Oppenauer oxidation of **7a**–**h** resulted in good yields of the 17β-[3-(*N*-aryl)tetrahydrooxazin-2-on-6-yl]androst-4-en-3-ones **8a**–**h**.

The change OAc → OH causes a moderate upfield shift of the C-3 line, which appears at 74.2 ± 0.2 ppm (series **6** and **3d**, **4d**, and **5**, **a**, **b**) or at 71.9 ± 0.3 ppm (series **7**). This



Scheme 1.

Table 4
In vitro inhibition of 5α-reductase by steroidal tetrahydrooxazin-2-ones (**8a**–**g**)

Compounds	IC_{50} value (nM)	Relative inhibition IC_{50} finasteride IC_{50} compound
Finasteride	55	1
8a	270	0.20
8b	>1000	<0.055
8c	260	0.21
8d	600	0.09
8e	245	0.22
8f	500	0.11
8g	420	0.13

line is dramatically shifted downfield, of course, for series **8**, where it appears at 199.5 ± 0.5 ppm, values characteristic of ketones [21a]. The H-3 signal is also shifted downfield (due to the $-I_S$ effect of the *O*-acetyl group) by ca. 1.2 ppm, as expected [21b] for series **5** as compared with series **7** and **8**. The polarization of the enone moiety leads [21c] to a very great difference (>47 ppm) between the C-4 and C-5 shifts (δC-4: 124.0 ± 0.3 and δC-5: 171.3 ± 0.4) in series **8**.

The ^{1}H and ^{13}C NMR signals of the19-CH_3 group are sensitive to the change of the Δ^5-double bond (series **6** and **7**) to the Δ^4-en-3-one moiety in series **8**: δH(19-CH_3) is 1.00 ± 0.03 for series **6** and **7**, but 1.19–1.20 for series **8**, while δC(19-CH_3) is 20.0 ± 0.8 for series **6** and **7**, but 17.6 ± 0.2 for series **8**. The freely rotating aryl-urethane group in series **6** can lead to a shielding effect of ca. 0.1 ppm on the 18-CH_3 hydrogen relative to series **7** and **8** (where the ring structure forces the aryl group far from 18-CH_3) due to the anisotropic neighboring effect of the benzene ring [21d]. The spectral data characteristic of the substituents (Me, Et, OMe, OEt, halogens) on the aryl group are observed in series **6**–**8** (Tables 1–3; Scheme 1).

It is interesting to note that in the more rigid ring B of the steroidal skeleton in series **6** and **7** (caused by the Δ^5-double bond) the C-9 line is shifted upfield by ca. 3.5 ppm as compared with series **8**, probably because there is no steric hindrance in the latter series (field effect) [21e,22].

3.2. Effects of steroidal tetrahydrooxazin-2-ones on 5α-reductase activity

The inhibitory effects of compounds **8a**–**g** on human prostatic 5α-reductase were investigated with an in vitro incubation technique. Finasteride (17β-(*N-tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one), a potent 5α-reductase inhibitor already applied in medical practice, was used as a reference compound.

IC_{50} values and relative inhibitory efficiencies as compared with the IC_{50} of finasteride are presented in the Table 4. The investigated steroids (**8a**–**g**) exhibit some 5α-reductase inhibitory effect. The IC_{50} values of **8a**–**g** vary between 270 and 600 nM, with the exception of **8b**, which is a very weak inhibitor. The relative inhibitory effect of the unsubsituted *N*-phenyl compound **8a** is 0.20. As concerns the effects of substituents at position 4 of the phenyl ring in **8a**, the introduction of an ethyl (**8c**) or ethoxy (**8e**) group resulted in a weak enhancement of 5α-reductase inhibition. Substitution with halogens (**8f** and **8g**), methyl (**8b**) or methoxy (**8d**) caused a drop-off in inhibition ability.

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