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Synthesis of *N*-Substituted Piperidine-4-(benzylidene-4-carboxylic acids) and Evaluation as Inhibitors of Steroid-5α-Reductase Type 1 and 2

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Abstract—The synthesis of *N*-substituted piperidine-4-(benzylidene-4-carboxylic acids) is described [benzoyl (**1**), benzyl (**2**), adamantanoyl (**3**), cyclohexanoyl (**4**), cyclohexylacetyl (**5**), diphenylacetyl (**6**), dicyclohexylacetyl (**7**), 2-propylpentanoyl (**8**), diphenylcarbamoyl (**9**), trimethylacetyl (**10**), 3,3-dimethylacryloyl (**11**), dicyclohexylacetyl derivative of the benzyl compound (**12**)]. Compounds were tested for inhibitory activity toward 5α-reductase isozymes 1 and 2 in human and rat. The test compounds inhibited 5α-reductase, showing a broad range of inhibitory potencies. In rat, compounds **6** (IC_{50} = 3.44 and 0.37 μM for type 1 and 2, respectively) and **9** (IC_{50} = 0.54 and 0.69 μM for type 1 and 2, respectively) displayed the best inhibition toward both isozymes. Compound **7** showed a strong inhibition toward type 2 human and rat enzyme (IC_{50} = 60 and 80 nM) but only a moderate activity versus type 1 enzyme (IC_{50} approximately 10 μM for rat and human enzyme). In vivo, selected compounds reduced prostate weights in castrated testosterone treated rats.

Introduction

5α-Reductase (EC 1.3.99.5) is an NADPH-dependent enzyme that catalyzes the conversion of testosterone to the more potent androgen dihydrotestosterone (DHT).[1] High concentrations of DHT are associated with the etiology and growth of diseases like benign prostatic hyperplasia (BPH),[2] prostatic carcinoma,[3] male pattern baldness[4] and hirsutism in women.[5] BPH is the most common benign tumor affecting over 40% of men above the age of 70.[6] Surgical therapy (TURP) is currently used in the treatment of BPH,[7] and until recently no non invasive method was available. That is why 5α-reductase has attracted a considerable interest as a new therapeutic target and the search for potent inhibitors of this enzyme has become the goal of several research groups.[8–10] However, the difficulty with developing inhibitors of 5α-reductase is related to the fact that there are two isozymes (type 1 and 2) with a different tissue distribution pattern and with distinct biochemical and pharmalogical properties.[11] The most currently used 5α-reductase inhibitor in BPH treatment is finasteride (Proscar®), a steroidal compound inhibiting mainly isozyme 2. The long term pharmacotherapy with finasteride has generally been well tolerated.[12] Nevertheless, its limited activity and its side effects which are related with sexual function (libido, impotence, ejaculatory disorder)[13] have caused us to look for a new class of 5α-reductase inhibitors. The fact that finasteride decreases circulating DHT levels by only 70%, whereas a combination of selective type 1 (MK 386) and type 2 (finasteride) inhibitors shows a nearly complete suppression of serum DHT,[14] a strategy of dual inhibition, i.e. inhibition of both isozymes by one compound, became attractive. The steroidal compound dutasteride (GG 745) is up to now the most efficient dual inhibitor exhibiting K_i values towards isozymes 1 and 2 of 6 and 7 nM, respectively.[15] In vivo, it shows a nearly complete suppression of serum DHT.[15–18] It is generally accepted that some of the side effects of steroidal compounds are related to their steroidal structures.[19] Therefore, it is our goal to synthesize nonsteroidal dual inhibitors of 5α-reductase isozymes 1 and 2. Potent nonsteroidal inhibitors have already been described so far,[9,10,19] some of which show dual type of inhibition.[10,19] Recently, we found in the class of *N*-substituted 4-(5-indolyl) benzoic acids (Scheme 1) dual inhibitors of the human isozymes exhibiting mediocre potency (best IC_{50} values

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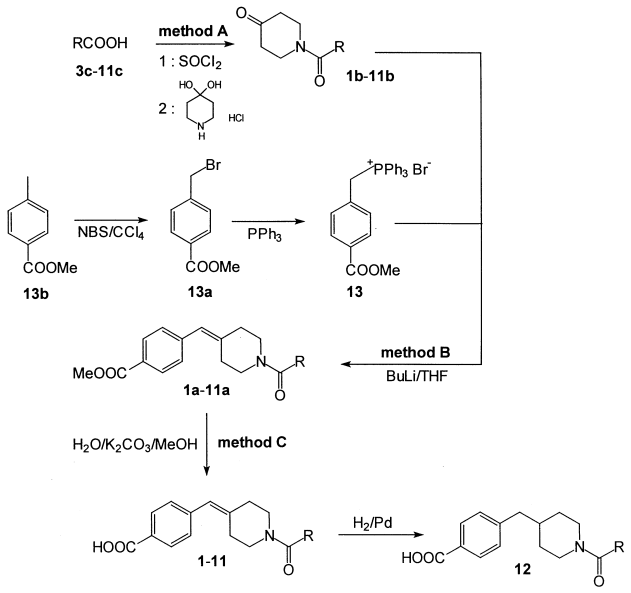
Scheme 1. General structures.

type 1: 2 μM, type 2: 6 μM).[20] These compounds had been designed as mimics of the steroidal substrate. In contrast to steroidal inhibitors which bear a lipophilic substituent in the 17β position, the substituent R of the indole compounds is located in the plane of the molecule. Aiming at the enhancement of inhibitory potency we looked for compounds which are closer to the steroidal structure. *N*-Substituted piperidine-4-(benzylidene-4-carboxylic acids) (Scheme 1) are mimics having the substituent R located outside the plane. In the following we describe the synthesis of a series of these compounds and the evaluation of their inhibitory activity toward rat and human 5α-reductase isozymes 1 and 2 as well as their in vivo activity in the rat.

Chemistry

For the preparation of compounds **1**–**12** (Scheme 2) the corresponding *N*-acyl piperidones **1b**–**11b** were prepared from the corresponding acids **3c**–**11c** as described for *N*-benzoyl piperidone.[21] After reaction with thionyl chloride, the acid chlorides were reacted with piperidone hydrochloride in a solution of triethylamine (method A). The phosphonium bromide **13** necessary for Wittig reaction with **1b**–**11b** was obtained by bromination of methyl toluene-4-carboxylate (**13b**) with NBS using benzoylperoxide[22] and subsequent reaction of **13a** with triphenylphosphine.[23] The *N*-acylpiperidine-4-benzylidene compounds **1a**–**11a** were obtained by Wittig reaction of the acylated piperidones **1b**–**11b** and the phosphonium bromide **13** using BuLi/THF (method B). The crude compounds were purified by chromatography



Scheme 2. Synthetic strategy.

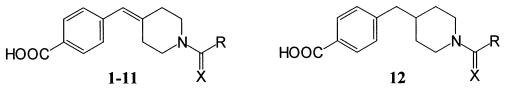
using an appropriate solvent. In a last step (method C), saponification of the methyl esters **1a**–**11a** was performed with potassium carbonate and methanol/H_2O to give the corresponding carboxylic acids **1**–**11**. Compound **12** was prepared by hydrogenation of **7** using palladium on charcoal as a catalyst (Scheme 2).

Biological Results

In vitro

The inhibitory activity of compounds **1**–**12** and finasteride in vitro was determined using rat prostate homogenates (pH 6.6, type 1; pH 5.5, type 2) and human prostate homogenate (BPH tissue for type 2) according to the method of Liang et al.,[24] and the DU145 cell line (for human type 1 enzyme) as described in the literature.[25] As can be seen in Table 1, in rat type 1 isozyme, most of the compounds only displayed a moderate inhibitory activity. The most active compounds in this study were **6** and **9**, showing IC_{50} values of 3.44 and 0.54 μM, respectively. In rat type 2 isozyme, the test compounds showed a broad range of inhibitory potencies from no inhibition for compounds **1** and **2** to strong inhibition for the methyldicyclohexyl compound **7** ($IC_{50} = 80$ nM). In human type 1 isozyme (DU145 cell line), compounds were inactive or rather poor inhibitors. The most active one was the methyldicyclohexyl compound **7**, showing a 46% inhibition at a concentration of 10 μM. Most of the compounds which were potent inhibitors of the rat isozyme 2 were potent inhibitors of the human type 2 isozyme as well. Compounds **3**, **6**, **8** and **12** showed IC_{50} values between 100 and 530 nM. The most active derivative was the methyldicyclohexyl compound **7**, exhibiting an IC_{50} value of 60 nM. Taking a closer look at the structure–activity relationships it becomes apparent that the compounds should be amides. Reduction of the carbonyl group of compound **1** resulting in the amine **2** leads to a complete loss of human type 2 enzyme inhibitory activity. There seems to be a correlation between the number of C-atoms of the substituent R and the activity of the corresponding compounds. The 2-methylpropylidene compound **11** shows almost no inhibition and the *t*-butyl compound **10** is only a mediocre inhibitor. A much better inhibitor is the cyclohexyl compound **4**. The introduction of one more C-atom increases activity further (compounds **5** and **8**). The fact that the 4-heptyl compound **8** is more active than the methylcyclohexyl compound **5** is probably due to the higher flexibility of the open ring substituent. Compound **3** containing an adamantyl (10 C-atoms) substituent is also a highly active inhibitor. Aromatic groups are less appropriate than aliphatic residues. The phenyl compound **1** shows a lower activity than the cyclohexyl compound **4** and the diphenylamino and the methyldiphenyl compounds (**9** and **6**) are less active than the methyldicyclohexyl compound **7**. The high activity of the latter compound is probably caused by the bulkiness and the flexibility of the substituent. A further increase of the flexibility of **7** by reducing the double bond of the benzylidene moiety, however, decreases inhibitory activity (compound **12**).

Table 1. Inhibition of rat and human 5α-reductase type 1 and 2 in vitro by compounds **1**–**12** and **finasteride**



Compound	X	R	RVP[a]: % inhibition (10 μM) [IC_{50} (μM)]		Human: % inhibition (10 μM) [IC_{50} (μM)]	
			type 1[c]	type 2[c]	DU145[b,c,e]	BPH[c,d]
1	O	Phenyl	n.i.	n.i.	17	53 [9.3]
2	H_2	Phenyl	8	n.i.	n.t.	n.i.
3	O	1-Adamantyl	61 [5.6]	66 [2.5]	44	94 [0.26]
4	O	Cyclohexyl	20	[1.50]	23	[1.65]
5	O	Methylcyclohexyl	44	[1.02]	31	[1.02]
6	O	Methyldiphenyl	71 [3.44]	[0.37]	26	[0.53]
7	O	Methyldicyclohexyl	51	[0.08]	46	[0.06]
8	O	4-Heptyl	28	[0.29]	20	[0.26]
9	O	Diphenylamino	[0.54]	[0.69]	22	[0.83]
10	O	*t*-Butyl	25	62	20	75 [3.10]
11	O	2-Methylpropylidene	15	37	12	16
12	O	Methyldicyclohexyl	n.t.	[0.75]	35	[0.10]
Finasteride			[0.01]	[0.011]	[0.039]	[2–3 nM]

[a]Enzyme of rat ventral prostate, 200–250 μg protein, substrate [1β,2β-^{3}H] testosterone, 0.21 μM.
[b]Substrate: [^{3}H] androstenedione, 5 nM.
[c]Mean value; tests have been run in duplicate. The standard deviation for IC_{50} is 20%, for percent inhibition is ±10%.
[d]Enzyme from BPH tissue (type 2), 200–300 μg protein, substrate [1β,2β-^{3}H] testosterone, 0.21 μM.
[e]Prostatic tumor cell line expressing type 1 enzyme. n.i.: no inhibition; n.t.: not tested.

In vivo

As the most active inhibitors of the human enzyme in vitro were highly active towards the rat isozyme 2 as well, they were tested for their in vivo activity using immature castrated SD rats. According to the procedure of Häusler et al., the animals were applied testosterone propionate.[26] This leads to a dramatic stimulation of prostate weights caused by the conversion of testosterone to DHT. 5α-Reductase inhibitors can inhibit this stimulation. Table 2 shows the effects of compounds **3**, **6**, **7**, **8** and **12** administered subcutaneously at doses between 9.2 and 11.4 mg/kg (ten times the dose of finasteride, 1 mg/kg). With the exception of compound **12**, all other compounds showed a significant inhibition (33–47%) of the prostate weight increase. However, they did not reach the effect of finasteride (85% inhibition).

Table 2. In vivo activity of compounds **3**, **6**, **7**, **8**, **12** and **finasteride** on ventral prostate weights in juvenile castrated rats treated with testosterone propionate

Test group	In vivo		
	Effect MV±SD[a]	Doses (mg/kg)	% Inhibition[c]
Vehicle (not castrated)	56.6±8.6		
Vehicle (castrated)	22.9±13.1		
Testosterone propionate (tp)	78.6±25.4	1	
tp + **6**[b]	60±10.5	11.0	33 ($P < 0.025$)
tp + **7**[b]	54.9±15.7	11.3	42 ($P < 0.025$)
tp + **8**[b]	56.7±12	9.2	39 ($P < 0.025$)
tp + **12**[b]	64.6±15.5	11.4	25 (n.s.)
tp + **finasteride**	31.1±9.5	1	85 ($P < 0.01$)
Vehicle (castrated)	17.6±3.7		
Testosterone propionate (tp)	61.4±9.6	1	
tp + **3**[b]	41±6.2	10.1	47 ($P < 0.01$)
tp + **finasteride**	31±9	1	86 ($P < 0.01$)

[a]mg Prostate/100 g body weight (mean values±standard deviation).
[b]All compounds were applied in doses 10 times equimolar to finasteride, 1 mg/kg.
[c]Significance according to U-test (Wilcoxon, Mann and Whitney).

Discussion and Conclusion

The structure–activity study described in this paper demonstrates that it is possible to increase human type 2 isozyme inhibitory activity of the indolyl benzoic acids[20] by a factor of 100 (IC_{50} 6 μM and 60 nM, respectively). However, the structure modifications also decreased isozyme 1 inhibition by a factor of 4–5. The low activity of the present compounds toward human type 1 isozyme might be due to the fact that the assay (DU145 cell line) is based on intact cells. It cannot be excluded that the cell permeability is reduced in this class of carboxylic acids compared to the indolyl benzoic acids. Further experiments regarding this issue are in progress. Comparing enzyme inhibitory activity of the title compounds toward the target isozymes, it is striking that the best correlation exists between the human and rat type 2 isozyme. The correlation between type 1 and 2 isozymes in the human and in the rat is not as good. This observation is in accordance with the finding that the percentage of identical amino acids in human and rat type 2 enzyme is higher than in the other groups.[11,27] The results of this study, showing that very bulky and flexible substituents in a position which corresponds to the 17β position of the steroidal substrate are optimal for high activity, support the hypothesis of the existence of a hydrophobic pocket in the active site proposed by others.[28] The most potent compounds in vitro were active in vivo as well, i. e., the compounds reduced the prostate weights in castrated testosterone treated rats. However, the in vivo activity was not very strong, which might to

some extent be due to the high lipophilicity of the compounds leading to a low concentration of free compound in the plasma. Thus, the fact that compound **7**, which in vitro is more active than **8** (by a factor of 4), is not more potent than the latter compound in vivo might be explained by its stronger lipophilicity.

As a conclusion, we have presented in this paper an interesting new class of nonsteroidal 5α-reductase inhibitors which might lead to the development of highly potent nonsteroidal dual inhibitors of 5α-reductase isozymes 1 and 2.

Experimental

^{1}H NMR spectra were measured on an AM 400 (400 MHz) in DMSO-d_6 or $CDCl_3$. Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane (δ = 0 ppm). IR spectra were performed with KBr film on a Perkin–Elmer PE 398 spectrometer. Melting points were determined on a Kofler melting point apparatus Thermophan (Reichert). Column chromatography was performed on Merck silica gel 60 (40–63 μm). All reactions were followed by thin layer chromatography using Alugram silica gel 60. Chemicals and solvents used were commercially available and were used without further purification.

Method A

***N*-Cyclohexanoyl-4-piperidone (4b).** A mixture of cyclohexane carboxylic acid **4c** (13 g, 0.1 mol), one drop of dimethyl formamide and thionyl chloride (50 mL) was refluxed at 40 °C for 2 h. After this, thionyl chloride was removed by distillation. The crude acid chloride was dissolved in 20 mL of CH_2Cl_2 and was added dropwise to a suspension of 4-piperidone monohydrate hydrochloride (15 g, 9.7 mmol) and dry triethylamine (40 mL, 0.3 mol) in dry CH_2Cl_2 (170 mL). The solution was stirred for 2 h. The organic phase was washed with water (2×20 mL) and dried over magnesium sulfate. After filtration, the solvent was evaporated in vacuo and the crude product was purified by recrystallization in *n*-hexane/ethyl acetate to yield **4b** (10.45 g, 53%): mp 57–58 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18–1.94 (m, 11H, cyclohexane H), 2.49–2.59 (m, 4H, pip. H), 3.80–3.88 (m, 4H, pip. H). $C_{12}H_{19}NO_2$ (209.28). Compounds **1b** and **2b** were obtained commercially.

***N*-(1-Adamantanoyl)-4-piperidone (3b).** Synthesized from 1-adamantane carboxylic acid chloride. ^{1}H NMR (80 MHz, $CDCl_3$): δ = 1.70 (s, 6H, ada. H), 1.90 (s, 3H, ada. H), 2.0 (s, 6H, ada. H), 2.42 (t, ^3J = 6 Hz, 4H, pip. H), 3.90 (t, ^3J = 6 Hz, 4H, pip. H). Oil, yield: 64%, $C_{16}H_{23}NO_2$ (261.36).

***N*-(Cyclohexyl)acetyl-4-piperidone (5b).** Synthesized from cyclohexylacetyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.94–1.79 (m, 11H, cyclohexane H), 2.17 (s, 2H, -COC$\underline{H}_2$-), 2.26 and 2.49 (2s, 4H, pip. H), 3.46–3.70 (t, 4H, broad, ^3J = 6.2 Hz, pip. H); mp: 50–52 °C, yield: 31%, $C_{13}H_{21}NO_2$ (223.31).

***N*-(Diphenyl)acetyl-4-piperidone (6b).** Synthesized from diphenylacetyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.61 (s, 1H, -COC$\underline{H}$-), 2.0 and 2.14 (2t, 4H, broad, ^3J = 6 Hz, pip. H), 3.76 and 3.94 (2t, 4H, broad, ^3J = 6 Hz, pip. H), 7.25 (m, 10H, aromat. H); mp: 135 °C, yield: 46%, $C_{19}H_{19}NO_2$ (293.36).

***N*-(Dicyclohexyl)acetyl-4-piperidone (7b).** Synthesized from dicyclohexylacetyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.94–1.16 (m, 11H, cyclohexane H), 1.56 (s, 1H, -COC$\underline{H}$-), 1.69 (s, 11H, broad, cyclohexane H), 2.46 (t, 4H, ^3J = 6 Hz, pip. H), 3.86 and 3.93 (2t, 4H, broad, ^3J = 6 Hz, pip. H); mp: 168 °C, yield: 68%, $C_{19}H_{31}NO_2$ (305.45).

***N*-(2-Propyl)pentanoyl-4-piperidone (8b).** Synthesized from 2-propylpentanoic acid chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88–1.43 (m, 14H, propylpentane H), 2.76 (s, 1H, -COC$\underline{H}$-), 2.47 (s, 4H, pip. H), 3.85 and 3.93 (2t, 4H, broad, ^3J = 6 Hz, pip. H). Oil, yield: 75%, $C_{13}H_{23}NO_2$ (225.33).

***N*-(Diphenyl)carbamoyl-4-piperidone (9b).** Synthesized from diphenylcarbamoyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.31 (t, ^3J = 6.2 Hz, 4H, pip. H), 3.64 (t, 4H, ^3J = 6.2 Hz, pip. H), 7.08 (d, 4H, broad, aromat. H), 7.16 (t, 2H, ^3J = 7.5 Hz, aromat. H), 7.33 (t, 4H, ^3J = 7.9 Hz, aromat. H); mp: 141–143 °C, yield: 61%, $C_{18}H_{18}N_2O_2$ (294.35).

***N*-(Trimethyl)acetyl-4-piperidone (10b).** Synthesized from trimethylacetyl chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33 (s, 9H, -C(C$\underline{H}_3$)$_3$), 2.46 (t, 4H, ^3J = 6.2 Hz, pip. H), 3.89 (t, 4H, ^3J = 6.2 Hz, pip. H); mp: 87–89 °C, yield: 60%, $C_{10}H_{17}NO_2$ (183.24).

***N*-(3,3-Dimethyl)acryloyl-4-piperidone (11b).** Synthesized from 3,3-dimethyl acrylic acid chloride. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.84–1.90 (m, 6H, -C(C$\underline{H}_3$)$_2$), 2.09 (s, 1H, -COC$\underline{H}$-), 2.41 (t, 4H, pip. H), 3.61 (m, 4H, pip. H). Oil, yield: 65%, $C_{10}H_{15}NO_2$ (181.23).

4-(Carboxymethyl)benzyl bromide (13a). A mixture of methyl *p*-toluate **13b** (10 g, 0.066 mol), NBS (13 g, 0.073 mol) and benzoylperoxide (0.6 g, 2.4 mmol) in dry CCl_4 (100 mL) was refluxed for 3 h at 100 °C. After cooling the suspension was filtered. The organic phase was washed twice with water (50 mL) and dried over magnesium sulfate. The solvent was evaporated in vacuo to yield 11.64 g (77%) **13a**. No further purification was necessary: mp 54 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.89 (s, 3H, -COOC$\underline{H}_3$), 4.49 (s, 2H, Ar-C$\underline{H}_2$-Br), 7.44 and 8.02 (AA′BB′, 4H, ^3J = 8 Hz, aromat. H). $C_9H_9O_2Br$ (229.07).

4-(Carboxymethyl)benzyltriphenylphosphonium bromide (13). A mixture of **13a** (5 g, 22 mmol) and triphenylphosphine (5.7 g, 22 mmol) in dry toluene (165 mL) was refluxed for 5 h at 120 °C. The clear solution became quickly turbid due to precipitation of salt. The solution was filtered to yield 8.1 g (75%) of compound **13**. No further purification was necessary: mp 238 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.86 (s, 3H, -COOC$\underline{H}_3$), 5.67 (d,

2H, ^2J(H,P) = 16 Hz, C$\underline{H}_2$-PPh_3^+ Br^-), 7.59 and 7.76 (AA′BB′, 4H, ^3J = 8 Hz, aromat. H), 7.26–7.76 (m, 15H, PPh_3^+ Br^-). $C_{27}H_{24}O_2P$ Br (491.36).

Method B

***N*-Cyclohexanoyl-4-[4-(carboxymethyl)benzylidene]piperidine (4a).** To a suspension of 4-(carboxymethyl)benzyltriphenylphosphonium bromide (**13**, 4 g, 8.14 mmol) in dry THF (40 mL) under nitrogen was added dropwise a solution of butyllithium (1.6 M in *n*-hexane, 5.6 mL, 8.14 mmol). After 15 min the solution had changed to orange and a solution of *N*-cyclohexanoyl-4-piperidone (**4b**, 1.7 g, 8.14 mmol) in dry THF (30 mL) was added dropwise at room temperature. The solution was stirred overnight under nitrogen. The solvent was evaporated in vacuo, and the residue was dissolved in CH_2Cl_2 (75 mL) and was washed twice with water (20 mL). The solution was dried over magnesium sulfate. After filtration, the solvent was evaporated in vacuo. The crude product was chromatographed using hexane/ethyl acetate 1/1 as eluent to yield 388 mg of **4a** (14%): mp 88–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25–1.80 (m, 11H, cyclohexane H), 2.39–2.51 (d, 4H, ^3J = 5.8 Hz, pip. H), 3.34–3.59 (s, 4H, broad, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.42 (s, 1H, vinyl H), 7.26 and 8.0 (AA′BB′, 4H, ^3J = 8.4 Hz, aromat. H). $C_{21}H_{27}NO_3$ (341.22).

***N*-Benzoyl-4-[4-(carboxymethyl)benzylidene]piperidine (1a).** Synthesized from *N*-benzoyl-4-piperidone (**1b**). Chromatography was performed using benzin A/ethyl acetate 1/1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.50 (m, 4H, pip. H), 3.41–3.80 (m, 4H, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.43 (s, 1H, vinyl H), 7.26 and 7.99 (2s, 4H, 1,4-disubst. aromat.), 7.42 (s, 5H, aromat. H); mp: 105–106 °C, yield: 41%, $C_{21}H_{21}NO_3$ (335.40).

***N*-Benzyl-4-[4-(carboxymethyl)benzylidene]piperidine (2a).** Synthesized from *N*-benzyl-4-piperidone (**2b**). Chromatography was performed using benzin A/ethyl acetate 1/1. ^{1}H NMR (80 MHz, $CDCl_3$): δ = 2.4 (s, 8H, pip. H), 3.5 (s, 2H, aromat-C$\underline{H}_2$-), 3.9 (s, 3H, -COOC$\underline{H}_3$), 6.15 (s, 1H, vinyl H), 7.1 (m, 7H, aromat. H, 2H of AA′BB′ systems and 5H aromat-CH_2), 7.9 (d, 2H, ^3J = 8.2 Hz, of AA′BB′ systems). Oil, yield: 31%, $C_{21}H_{23}NO_2$ (321.41).

***N*-(1-Adamantanoyl)-4-[4-(carboxymethyl)benzylidene]piperidine (3a).** Synthesized from *N*-(1-adamantanoyl)-4-piperidone (**3b**). Chromatography was performed using *n*-hexane/ethyl acetate 1/1 . ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.73 (s, 6H, ada. H), 2.03 (s, 6H, ada. H), 2.05 (s, 3H, ada. H), 2.39 and 2.50 (2t, 4H, ^3J = 5.52 Hz, pip. H), 3.65–3.74 (2t, 4H, broad, ^3J = 5.52 Hz, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.39 (s, 1H, vinyl H), 7.25 and 7.99 (AA′BB′, 4H, ^3J = 8.12 Hz, aromat. H); mp: 147 °C, yield: 60%, $C_{25}H_{31}NO_3$ (393.52).

***N*-(Cyclohexyl)acetyl-4-[4-(carboxymethyl)benzylidene]piperidine (5a).** Synthesized from *N*-(cyclohexyl)acetyl-4-piperidone (**5b**). Chromatography was performed using *n*-hexane/ethyl acetate 1/1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.93–1.78 (m, 11H, cyclohexane H), 2.17 (s, 2H, -COC$\underline{H}_2$-), 2.24–2.49 (m, 4H, pip. H), 3.58 (t, 4H, broad, ^3J = 6.0 Hz, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.41 (s, 1H, vinyl H), 7.24 and 7.98 (AA′BB′, 4H, ^3J = 8.12 Hz, aromat. H); mp: 65 °C, yield: 17%, $C_{22}H_{29}NO_3$ (355.47).

***N*-(Diphenyl)acetyl-4-[4-(carboxymethyl)benzylidene]piperidine (6a).** Synthesized from *N*-(diphenyl)acetyl-4-piperidone (**6b**). Purified by flash chromatography using *n*-hexane/ethyl acetate 7/3. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.58 (s, 1H, -COC$\underline{H}$-), 2.04–2.48 (m, 4H, pip. H), 3.44–3.77 (m 4H, pip. H), 3.93 (s, 3H, -COOC$\underline{H}_3$), 6.37 (s, 1H, vinyl H), 7.26–7.32 (m, 10H, aromat. H), 7.30 and 7.96 (AA′BB′, 4H, ^3J = 7.96 Hz, aromat. H); mp: 135 °C, yield: 13%, $C_{28}H_{27}NO_3$ (425.52).

***N*-(Dicyclohexyl)acetyl-4-[4-(carboxymethyl)benzylidene]piperidine (7a).** Synthesized from *N*-(dicyclohexyl)-acetyl-4-piperidone (**7b**). Purified by flash chromatography using *n*-hexane/ethyl acetate 8/2. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.95–1.93 (m, 22H, cyclohexane H), 2.00 (s, 1H, -COC$\underline{H}$-), 2.50 (m, 4H, pip. H), 3.44–3.75 (m, 4H, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.40 (s, 1H, vinyl H), 7.16–7.96 (m, 4H, 1,4 aromat. H): mp: 131 °C, yield: 38%, $C_{28}H_{39}NO_3$ (437.62).

***N*-(2-Propyl)pentanoyl-4-[4-(carboxymethyl)benzylidene]piperidine (8a).** Synthesized from *N*-(2-propyl)pentanoyl-4-piperidone (**8b**). Purified by flash chromatography using *n*-hexane/ethyl acetate 7/3. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87–1.43 (m, 14H, heptane H), 2.04 (s, 1H, -COC$\underline{H}$-), 2.39–2.50 (m, 4H, pip. H), 3.72 (s, 4H, broad, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.41 (s, 1H, vinyl H), 7.24 and 8.0 (AA′BB′, 4H, ^3J = 8.4 Hz, aromat. H). Oil, yield: 33%, $C_{22}H_{31}NO_3$ (357.49).

***N*-(Diphenyl)carbamoyl-4-[4-(carboxymethyl)benzylidene]piperidine (9a).** Synthesized from *N*-(diphenyl)carbamoyl-4-piperidone (**9b**). Purified by flash chromatography with *n*-hexane/ethyl acetate 7/3. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.26 and 2.35 (2t, 4H, ^3J = 6 Hz, pip. H), 3.36 and 3.47 (2t, 4H, ^3J = 6 Hz, pip. H), 3.89 (s, 3H, -COOC$\underline{H}_3$), 6.32 (s, 1H, vinyl H), 7.05 (d, 4H, ^3J = 6.7 Hz, aromat. H), 7.12 (t, 2H, ^3J = 7.5 Hz, aromat. H), 7.30 (t, 4H, ^3J = 7.5 Hz, aromat. H), 7.18 and 7.94 (AA′BB′, 4H, ^3J = 8.2 Hz, aromat. H). Whitish paste, yield: 38%, $C_{27}H_{26}N_2O_3$ (426.51).

***N*-(Trimethyl)acetyl-4-[4-(carboxymethyl)benzylidene]piperidine (10a).** Synthesized from *N*-(trimethyl)acetyl-4-piperidone (**10b**). Chromatography was performed using *n*-hexane/ethyl acetate 1/1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 (s, 9H, -C(C$\underline{H}_3$)$_3$), 2.39 and 2.50 (2t, 4H, ^3J = 6 Hz, pip. H), 3.60 and 3.71 (2t, 4H, ^3J = 6 Hz, pip. H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.39 (s, 1H, vinyl H), 7.24 and 7.97 (AA′BB′, 4H, ^3J = 8.4 Hz, aromat. H); mp: 129–131 °C, yield: 10%, $C_{19}H_{25}NO_3$ (315.41).

***N*-(3,3-Dimethyl)acryloyl-4-[4-(carboxymethyl)benzylidene]piperidine (11a).** Synthesized from *N*-(3,3-dimethyl)acryloyl-4-piperidone (**11b**). Purified by flash chromatography with *n*-hexane/ethyl acetate 7/3. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.86 (m, 6H, -C(C$\underline{H}_3$)$_2$), 2.09 (s, 1H, -COC$\underline{H}$-), 2.39 (m, 4H, pip. H), 3.59 (m, 4H, pip.

H), 3.91 (s, 3H, -COOC$\underline{H}_3$), 6.42 (s, 1H, vinyl H), 7.24 and 7.98 (AA′ BB′, 4H, $^3J=7.9$ Hz, aromat. H); mp: 110–112 °C, yield: 11%, $C_{19}H_{23}NO_3$ (313.39).

Method C

***N*-Cyclohexanoyl-piperidine-4-(benzylidene-4-carboxylic acid) (4).** A mixture of *N*-cyclohexanoyl-4-[4-(carboxymethyl)benzylidene]piperidine (**4a**, 0.4 g, 1.16 mmol) and potassium carbonate (0.7 g) in methanol/water 9/1 was refluxed for 3 h at 90 °C. The solution was stirred overnight at room temperature. After this, the reaction was acidified with 1 N hydrochloric acid. The compound was extracted with CH_2Cl_2 (3×30 mL), washed with water, and dried over magnesium sulfate. The solvent was evaporated in vacuo to yield 322 mg (85%) of **4**: mp 166 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.16–1.70 (m, 11H, cyclohexane H), 2.29–2.50 (m, 4H, pip. H), 3.32–3.50 (m, 4H, pip. H), 6.44 (s, 1H, vinyl H), 7.34 and 7.91 (AA′BB′, 4H, $^3J=7.96$ Hz, aromat. H), 12.86 (s, 1H, COOH). IR (KBr): 2940, 2860, 1680, 1645, 1610, 1430, 1355, 1315, 1290, 1260, 1205, 1180, 995, 795, 755, 710. Anal. $C_{20}H_{25}NO_3$ (327.42).

***N*-Benzoyl-piperidine-4-(benzylidene-4-carboxylic acid) (1).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.5 (m, 4H, pip. H), 3.31–3.72 (m, 4H, pip. H), 6.47 (s, 1H, vinyl H), 7.36 and 7.90 (2s, 4H, 1,4-disubst. aromat), 7.46 (m, 5H, aromat. H), 12.89 (s, 1H, COOH). IR (KBr): 3000, 2850, 1690, 1640, 1605, 1430, 1365, 1310, 1270, 1240, 1175, 1070, 990, 885, 790, 750, 730, 710; mp: 166 °C, yield: 74%. $C_{20}H_{19}NO_3$ (321.37), MS (CI) 321.

***N*-Benzyl-piperidine-4-(benzylidene-4-carboxylic acid) (2).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.5–3.2 (m, 8H, pip. H), 4.30 (s, 2H, Ar-C$\underline{H}_2$-), 6.51 (s, 1H, vinyl H), 7.35 and 7.91 (AA′BB′, 4H, $^3J=8.4$ Hz), 7.45 (m, 3H, aromat. H), 7.63 (m, 2H, aromat H), 11.36 (s, 1H, COOH). IR (KBr): 3000, 2950, 1680, 1610, 1430, 1325, 1220, 1180, 1105, 950, 890, 755, 705: mp: 285 °C, yield: 68%. $C_{20}H_{21}NO_2$ (307.39); MS (CI) 307.4.

***N*-Adamantanoyl-piperidine-4-(benzylidene-4-carboxylic acid) (3).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.69 (s, 6H, ada. H), 1.93 (s, 6H, ada. H), 1.99 (s, 3H, ada. H), 2.33 and 2.45 (2t, 4H, broad, pip. H), 3.59 and 3.65 (2t, 4H, broad, pip. H), 6.51 (s, 1H, vinyl H), 7.35 and 7.89 (AA′BB′, 4H, $^3J=7.96$ Hz, aromat. H), 12.85 (s, 1H, COOH). IR (KBr): 3000, 2900, 1705, 1590, 1430, 1380, 1260, 1230, 1170, 1100, 1060, 990, 880, 770, 750; mp: 256 °C, yield: 42%. Anal. $C_{24}H_{29}NO_3$ (379.49).

***N*-(Cyclohexyl)acetyl-piperidine-4-(benzylidene-4-carboxylic acid) (5).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.91–1.66 (m, 11H, cyclohexane H), 2.19 (s, 2H, -COC$\underline{H}_2$-), 2.50–3.32 (m, 8H, pip. H), 6.44 (s, 1H, vinyl H), 7.33 and 7.90 (AA′BB′, 4H, $^3J=7.96$ Hz, aromat. H), 12.89 (s, 1H, COOH). IR (KBr): 2950, 2870, 1720, 1610, 1460, 1360, 1310, 1230, 1180, 1080, 995, 885, 775, 765, 750; mp: 173 °C, yield: 44%. Anal. $C_{21}H_{27}NO_3$ (341.45).

***N*-(Diphenyl)acetyl-piperidine-4-(benzylidene-4-carboxylic acid) (6).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.29 (s, 1H, -COC$\underline{H}$-), 2.39–2.50 (m, 4H, pip. H), 3.33–3.60 (m, 4H, pip. H), 6.41 (s, 1H, vinyl H), 7.22–7.31 (m, 10H, aromat. H), 7.21 and 7.89 (AA′BB′, 4H, $^3J=7.96$ Hz, aromat. H), 12.85 (s, 1H, COOH). IR (KBr): 2960, 2860, 1695, 1645, 1610, 1430, 1410, 1300, 1285, 1210, 1185, 1070, 995, 880, 790, 750, 740, 700; mp: 228–229 °C, yield: 62%, Anal. $C_{27}H_{25}NO_3$ (411.49).

***N*-(Dicyclohexyl)acetyl-piperidine-4-(benzylidene-4-carboxylic acid) (7).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.95–1.93 (m, 22H, cyclohexane H), 2.0 (s, 1H, -COC$\underline{H}$-), 2.50 (m, 4H, pip. H), 3.34–3.71 (m, 4H, pip. H), 6.44 (s, 1H, vinyl H), 7.21 and 7.91 (AA′BB′, 4H, $^3J=7.96$ Hz, aromat. H), 12.86 (s, 1H, COOH). IR (KBr): 2920, 2850, 1690, 1630, 1610, 1430, 1360, 1295, 1180, 995, 880, 790, 750, 710; mp: 167–168 °C, yield: 37%, Anal. $C_{27}H_{37}NO_3$ (423.59).

***N*-(2-Propyl)pentanoyl-piperidine-4-(benzylidene-4-carboxylic) acid (8).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.81–1.22 (m, 14H, heptane H), 2.29 (s, 1H, -COC$\underline{H}$-), 2.39–2.50 (m, 4H, pip. H), 3.51–3.63 (m, 4H, pip. H), 6.45 (s, 1H, vinyl H), 7.34 and 7.91 (AA′BB′, 4H, $^3J=7.96$ Hz, aromat. H), 12.89 (s, 1H, COOH). IR (KBr): 2960, 2940, 1680, 1640, 1470, 1420, 1360, 1310, 1290, 1260, 1200, 1185, 1110, 990, 880, 790, 755, 705; mp: 124–125 °C, yield 48%, Anal. $C_{21}H_{29}NO_3$ (343.46).

***N*-(Diphenyl)carbamoyl-piperidine-4-(benzyl-4-carboxylic acid) (9).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.31 (s, 4H, pip. H), 3.37 (s, 4H, pip. H), 6.37 (s, 1H, vinyl H), 7.02 (d, 4H, $^3J=7.5$ Hz, aromat. H), 7.14 (t, 2H, $^3J=7.5$ Hz, aromat. H), 7.34 (t, 4H, $^3J=7.5$ Hz, aromat. H), 7.30 and 7.88 (AA′BB′, 4H, $^3J=8.1$ Hz, aromat.), 12.89 (s, 1H, COOH). IR (KBr): 2980, 2810, 1690, 1650, 1610, 1500, 1410, 1300, 1280, 1240, 1180, 1070, 1020, 990, 900, 760, 700; mp: 189 °C, yield: 66%, Anal. $C_{26}H_{24}N_2O_3$ (412.48).

***N*-(Trimethyl)acetyl-4-piperidine-4-(benzyl-4-carboxylic acid) (10).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.21 (s, 9H, -(C$\underline{H}_3$)$_3$), 2.33 (s, 4H, pip. H), 3.52 (s, 4H, pip. H), 6.43 (s, 1H, vinyl H), 7.33 and 7.88 (AA′BB′, 4H, $^3J=8.4$ Hz, aromat. H), 12.92 (s, 1H, -COOH). IR (KBr): 2980, 2800, 1670, 1600, 1410, 1290, 1160, 980, 880, 750, 700, 650; mp: 220–222 °C, yield: 66%, Anal. $C_{18}H_{23}NO_3$ (301.38).

***N*-(3,3-Dimethyl)acryloyl-4-piperidine-4-(benzyl-4-carboxylic acid) (11).** ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.81 (s, 6H, -(C$\underline{H}_3$)$_2$), 2.33 (s, 1H, -COC$\underline{H}$-), 2.5 (m, 8H, pip. H), 6.45 (s, 1H, vinyl H), 7.34 and 7.89 (AA′BB′, 4H, $^3J=7.9$ Hz, aromat. H), 12.91 (s, 1H, -COOH). IR (KBr): 2980, 2960, 2850, 1680, 1620, 1600, 1420, 1360, 1320, 1280, 1220, 1170, 1060, 990, 880, 750, 700; mp: 155–157 °C, yield: 57%, Anal. $C_{18}H_{21}NO_3$ (299.36).

***N*-(Dicyclohexyl)acetyl-piperidine-4-(benzyl-4-carboxylic acid) (12).** *N*-(Dicyclohexyl)-acetyl-piperidine-4-(benzylidene-4-carboxylic acid) (**7**) (300 mg) dissolved in methanol was subjected to a hydrogenation under

atmospheric pressure for 2 h using Pd/C as a catalyst. The solvent was extracted in vacuo, and the compound was recrystallized from *n*-hexane/ethyl acetate to yield 271 mg (90%) of compound **12**: mp: 190–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 0.89$–1.59 (m, 22H, cyclohexane H), 2.00 (s, 1H, -COCH), 2.34–2.57 (m, 8H, pip. H), 2.58 (s, 2H, -CH_2-arom.), 7.14 and 7.86 (AA′BB′, 4H, $^3J = 7.96$ Hz, aromat. H), 12.74 (s, 1H, COOH). IR (KBr): 2920, 2850, 1690, 1630, 1610, 1450, 1430, 1310, 1290, 1230, 1175, 1110, 1070, 1020, 960, 860, 760, 700. Anal. $C_{27}H_{39}NO_3$ (425.61).

Enzyme inhibition test

Reagents. [1,2-^{3}H]Androstenedione (4-androstene-3,17-dione, AD) and [1,2-^{3}H]testosterone (17β-hydroxy-4-androstene-3-one, T) were purchased from DuPont, Bad Homburg, Germany.

Preparation of tissue. Rat prostatic enzyme was prepared according to the method of Liang et al.[24] with slight modifications.[29] Male rats were killed and prostates were taken within 5 min and put in ice cold 0.9% NaCl solution. All the following operations were performed at 0–4 °C. The prostates were dissected free from fat and connective tissue, cut into pieces and weighed. Per 1 g of tissue, 3 mL of 20 mM phosphate buffer, pH 6.5, containing 0.32 mM sucrose and 1 mM DTT were added. The tissue was homogenized by ten 10-s strokes at 20,500 rpm of an ultraturax (IKA) in 60-s intervals, filtered through cheesecloth and centrifuged for 60 min at 105,000 g. The pellet obtained was resuspended in phosphate buffer. The centrifugation was repeated, the final pellet resuspended in a minimum volume of phosphate buffer and stored in 300 μL portions at −70 °C. The 105,000 g pellet contains nuclei, mitochondria and microsomes and is referred to as the enzyme preparation. The protein content was determined and was in the range of 15–25 mg/mL. Human prostatic tissue from BPH patients was processed in the same way using citrate buffer, pH 5.5.

Incubation procedure. The assay was performed as described[24] with modifications.[29] All values were run in duplicate. The incubation was carried out for 30 min at 37 °C in a total volume of 250 μL. In the case of rat enzyme preparation phosphate buffer (40 mM, pH 6.6 for type 1 and pH 5.5 for type 2) and in the case of human enzyme preparation citrate buffer (40 mM, pH 5.5) was used. The incubation mixture contained approximately 250 μg rat protein (125 μg human protein), 200 μM NADPH (human enzyme: 100 μM NADPH), 0.21 μM T including 45 nCi [1,2-^{3}H]T, and 2% DMSO with or without test compound (10 μM). In case of exceeding 60% inhibition, three concentrations were chosen for measurement of IC_{50} values. The reaction was started by adding the prostatic enzyme preparation and stopped by addition of 50 μL NaOH (10 M). The steroids were extracted using 500 μL of diethylether. The mixture was shaken for 10 min and centrifuged 10 min at 4000 rpm. The water layer was frozen and the ether layer was decanted in fresh tubes and evaporated to dryness.

Human type 1 inhibition: DU145-assay. Intact human prostatic carcinoma DU145 cells were used as the source of type 1 5α-reductase.[25] The inhibitory potency of the compounds was determined by monitoring the conversion of the tritiated substrate androstenedione (5 nM) to androstanedione during an incubation period of 6 h. A day before the experiment DU145 cells were seeded in a 24-multiwell-plate at a density of 200,000 cells/well and allowed to become adherent overnight. Compounds to be tested were dissolved in DMSO and 5 μL of each were added to the cells in a final volume of 0.5 mL complete medium. Inhibitors were screened at a concentration of 10 μM. As control of conversion (typically about 35% under these conditions) served a triplicate of wells without inhibitors and as positive control for inhibition finasteride (80, 60, 40, 20 nM) was used. After the 6 h incubation period in 5% CO_2 at 37 °C the medium samples were extracted twice with 1 mL diethylether and the steroids were separated by HPLC. Results are expressed as amount of formed androstanedione as percentage of control values.

HPLC procedure. The procedure was carried out[29] similar to the method of Cook et al.[30] The steroids were dissolved in 50 μL methanol and 25 μL injected into the computer-controlled HPLC system, which was checked before using labelled reference controls. Radioactivity was measured using a Berthold LB 506C monitor. Using methanol/water (55/45, w/w) for T and DHT, with a flow of 0.4 mL/min and an additive flow of 1.0 mL for scintillator, base-line-separation of T and DHT was achieved within 20 min. For the steroids androstenedione and dihydroandrostenedione we used methanol/water (50/50, w/w).

Calculation procedure. The amount of DHT formed was calculated (% DHT). The zero value was subtracted from the control (*cv*) and inhibition (*iv*) values (cv_{corr} and iv_{corr}). Inhibition (I) was calculated using the following equation: $\%I = (1 - iv_{corr}/cv_{corr})100$.

In vivo assay.[26] One day after their arrival, 21 day old male rats were castrated by scrotal incision under ether anesthesia. All animals were fed commercially available chow and housed in temperature controlled rooms with lights on between 08:00 and 18:00. Rats were divided in 7 groups with 8 rats each. Oil solution of compounds (or vehicle) and testosterone propionate were applied by separate subcutaneous injections to the rats once daily for 4 days at doses of 9.2–11.4 mg/kg for title compounds, 1 mg/kg for testosterone propionate and finasteride. Twenty four h after the last application, the rats were killed by CO_2 inhalation, and the ventral prostates were removed. The prostates were dissected free from fat and connective tissue and weighed. The mean percentage of inhibition of the T-induced hypertrophic response was calculated according to the following equation: % Inhibition $= 100 \times (Ct - D)/(Ct - Cc)$, where Ct, Cc, and D are the mean prostate weights of T-treated control, castrated control and drug treated group, respectively. Mean values and standard deviation were calculated. For determination of significance the U-test according to Wilcoxon, Mann and Whitney was used.

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