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Cyclohex-1-ene Carboxylic Acids: Synthesis and Biological Evaluation of Novel Inhibitors of Human 5α Reductase

In search of novel nonsteroidal mimics of steroidal inhibitors of 5α reductase, 4-(2-phenylethyl)cyclohex-1-ene carboxylic acids **1–5** were synthesized with different substituents in *para* position of the phenyl ring (**1**: *N,N*-diisopropylcarbamoyl, **2**: phenyl, **3**: phenoxy, **4**: benzoyl, and **5**: benzyl). The principal synthetic approach for the desired compounds consisted of a Wittig olefination between 1,4-dioxaspiro[4.5]-decane-8-carbaldehyde (**4 g**) and the appropriate phosphonium salts. The compounds were tested for inhibition of human 5α reductase isozymes 1 and 2 using DU 145 cells and preparations from prostatic tissue, respectively. They turned out to be good inhibitors of the prostatic isozyme 2 with compound **1** being the most potent one (IC_{50} = 760 nM). Isozyme 1 was only slightly inhibited. It is concluded that the novel structures are appropriate for being further optimized, aiming at the development of a novel drug for the treatment of benign prostatic hyperplasia.

Keywords: Steroid 5α reductase; Isozymes 1 and 2; DU 145 cells; Nonsteroidal inhibitors; Steroidomimetics; Benign prostatic hyperplasia; Cyclohex-1-ene carboxylic acids

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Introduction

The enzyme steroid 5α reductase (EC 1.3.99.5) irreversibly catalyzes the reduction of testosterone (T) to the tissue-specific androgen 5α-dihydrotestosterone (DHT). T, the androgen secreted by the testes, is the main circulating androgen in man, responsible for the increase in muscle mass, spermatogenesis, and libido. In some tissues, such as the skin or the prostate, T acts as a prehormone, which is converted to the more potent androgen DHT through local 5α reductase [1]. DHT is believed to be the causative agent in a variety of conditions, including benign prostatic hyperplasia (BPH), acne, hirsutism, and androgenic alopecia [2–5]. Two different 5α reductase isozymes, named type 1 and type 2, were identified, which are characterized by distinct molecular genetics, structural, and biochemical properties and by different tissue localization [6]. Isozyme 2 is mainly located in the prostate, whereas isozyme1 is found in the periphery [6]. Finasteride and Epristeride (Chart 1) are highly potent steroidal inhibitors, the former having been used for the treatment of BPH for some years [7, 8]. Because of the side effects of steroidal drugs which in part are due to their steroidal structure, we and other groups have been trying to develop nonsteroidal compounds [9]. In different classes we found highly active compounds *in vitro.* One class of compounds turned out to be very potent *in vivo* as well [10]. In this paper, we describe a novel approach to the synthesis of conformationally flexible mimics of Epristeride. This investigation was initiated by our finding that the more rigid nonsteroidal mimics MK73 [11] as well as EB2 and 16 [12] (Chart 1) had shown strong inhibition of human 5α reductase type 2 (IC_{50} values of 410 nM, 460 nM, and 200 nM, respectively). In the following paragraphs we describe the synthesis of novel 4(2-phenylethyl)cyclohex-1-ene carboxylic acids **1–5** (Chart 1) and the evaluation of their biological activity toward human 5α reductase isozymes 1 and 2.

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Chemistry

For the preparation of the cyclohex-1-ene carboxylic acid derivatives **1–5** a convergent synthetic approach was chosen, including a Wittig reaction as key step. The phosphonium salt **1 e** (Scheme 1) was synthesized from 4-methylbenzoic acid via aminolysis of the acid chloride with diisopropylamine (**1 g**), bromination using *N*-bromo-

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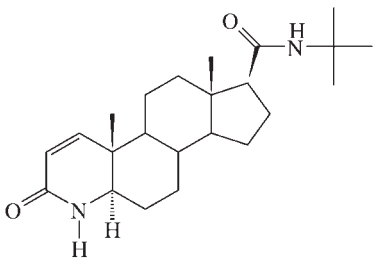
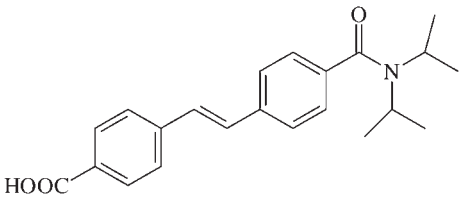
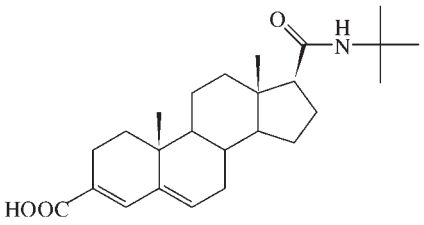


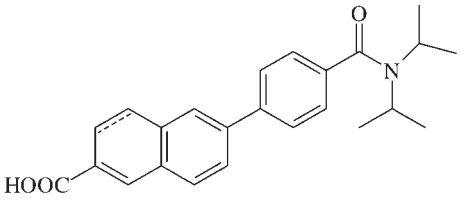
Finasteride



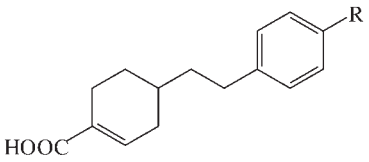
MK 73



Epristeride

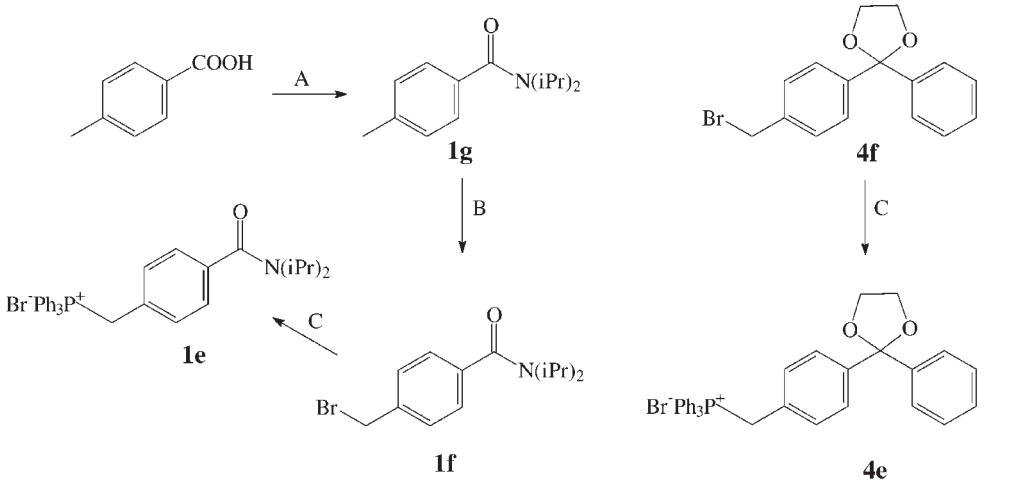


EB 2; saturated
EB 16; unsaturated



1-5

Compd	R
1	CON(*i*-Propyl)$_2$
2	Phenyl
3	Phenoxy
4	Benzoyl
5	Benzyl



[a] Reagents and conditions: A: $SOCl_2$, then HN(*i*-Pr)$_2$, CH_2Cl_2, NEt_3; B: NBS, CCl_4, DBPO, 90°C; C: PPh_3, acetone, reflux.

Scheme 1[a]

succinimide (**1 f**), and finally reaction with triphenylphosphine. In a similar way the phosphonium salt **4 e** was obtained from 2-(4-bromomethylphenyl)-2-phenyl-[1,3]-dioxolane [13] and subsequent treatment with triphenylphosphine. The 4-phenoxy and 4-phenyl substituted benzyltriphenylphosphonium bromides were prepared in very good yields according to published procedures [14, 15].

The preparation of the aldehyde **4 g** started from commercially available 1,4-dioxaspiro[4.5]decan-8-one, which was converted into 8-methylene-1,4-dioxaspiro[4.5]decane using methyltriphenylphosphonium bromide in dimethyl sulfoxide with sodium hydride as a base [16]. The obtained exocyclic olefin was subjected to a hydroboration, directly followed by an oxidation using pyridinium chlorochromate, resulting in **4 g** in an acceptable yield [17, 18].

With compound **4 g** and the corresponding phosphonium salts a Wittig reaction was performed to yield the olefins **1 d–4 d** (Scheme 2). *E-Z* isomeric mixtures were obtained, which were not separated but directly hydrogenated using palladium on charcoal to give quantitatively the saturated analogs **1 c–4 c**. Upon hydrogenation of compound **4 d** for 24 h in ethyl acetate additional deketalization and subsequent reduction of the carbonyl group of the benzophenone moiety was oberved (**5 c**) [19].

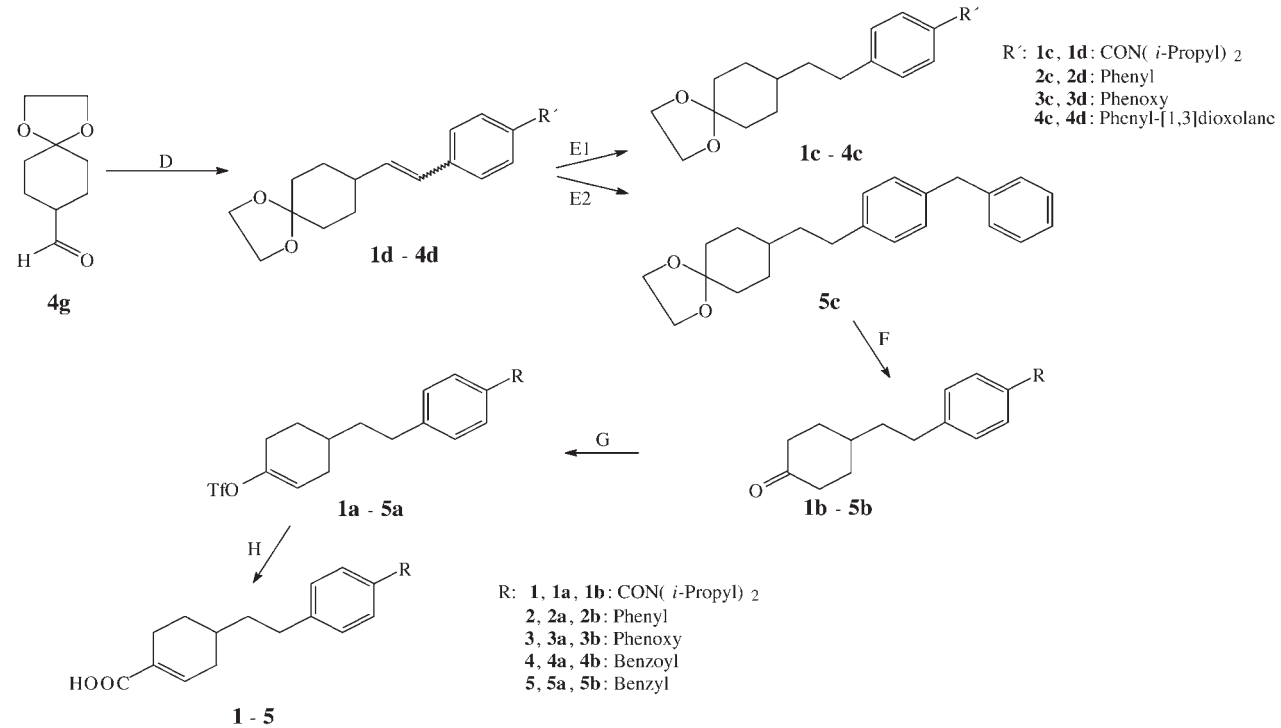
Deprotection of **1 c–5 c** with diluted hydrochloric acid gave the cyclohexanone derivatives **1 b–5 b** in high yields. Conversion into the enol triflates **1 a–5 a** was achieved using 2,6-di-*tert*-butyl-4-methylpyridine as a sterically hindered base and trifluoromethanesulfonic acid [20].

Subsequent Heck-type carboxylation with palladium(II) acetate, triphenylphosphine, and potassium acetate under a carbon monoxide atmosphere according to a recent procedure developed by Cacchi [21] gave direct access to the desired carboxylic acids **1–5** in good yields. This direct procedure is superior to the strategy via the corresponding carboxylic acid ester intermediates [22, 23].

Biological results and discussion

Inhibition of human 5α reductase isozymes

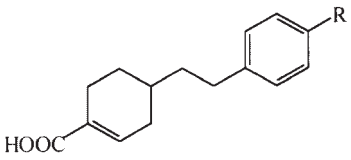
The synthesized compounds were tested for inhibitory potency using prostate homogenates for the human type 2 isozyme and the DU 145 cell line – a prostate cancer metastasis of the brain – for the human type 1 isozyme [24, 25]. As can be seen from Table 1, compound **1** bearing the diisopropylcarboxamide moiety turned out to be the most potent compound toward the prostatic isozyme.



[b] Reagents and conditions: D: phosphoniumsalt (e.g. **1e**), BuLi, THF, O°C, then **4g**, 25°C; E1: H_2/Pd/C, ethyl acetate, 25 °C, 30 min - 2 h; E2: H_2/Pd/C, ethanol, 25 °C, 24 h; F: HCl (3 %)/ethanol (6/1), 25 °C; G: Tf_2O, 2,6-di(*tert*-butyl)-4-methylpyridine, CH_2Cl_2, 25°C; H: KOAc, PPh_3, Pd(II)OAc_2, CO, DMF, 25°C.

Scheme 2[b]

Table 1. Inhibition of human steroid 5α reductase isozymes by 4-substituted cyclohex-1-enecarboxylic acids **1–5**.



1-5

Compd	R	Human Type 2[a,c] % inh. 10 μM [IC_{50}, μM]	Human Type 1[b,d] % inh. 10 μM [IC_{50}, μM]
1	CON(*i*-Propyl)$_2$	[0.76]	16
2	Phenyl	[1.00]	57
3	Phenoxy	[1.50]	n.d.
4	Benzoyl	[2.10]	48
5	Benzyl	[4.10]	n.d.
Finasteride	[0.005]	[0.045]	
Epristeride	[0.003]	[1.10]	

[a] Human prostate homogenates; 200–300 μg protein, pH 5.5.
[b] DU 145 cell culture.
[c] Substrate 1β2β ^{3}H testosterone 210 nM.
[d] Substrate ^{3}H androstene-dione 5 nM. n.d: not determined.

This is not a surprise as it is in closest structural analogy to Epristeride. Although showing an IC_{50} value in the nanomolar range, compound **1** is approximately two orders of magnitude less potent than the parent steroidal inhibitor. The fact that the aromatic substituted compounds **2–5** are only a little less potent than **1** is encouraging, since they might be lead compounds for the development of more potent derivatives. It is striking that the phenyl compound **2** is more active than the phenoxy, benzoyl, and benzyl derivatives (in decreasing order of activity, compounds **3–5**). Obviously the increase in the length of the molecule and/or its conformational flexibility has a negative effect on inhibitory potency. Towards the human isozyme 1, the tested compounds were less active. Compound **1** only showed a marginal inhibitory potency. Compounds **2** and **4** were more active, but showed less inhibition than toward type 2 isozyme. It cannot be excluded that this phenomenon is due to pharmacokinetic effects, as in the assay whole cells are used and the carboxylic acids might not easily permeate the cell wall at the pH value of the assay.

Conclusion

From our results it can be concluded that the phenylethylcyclohex-1-ene carboxylic acid moiety is a good mimic of the basic structure of the potent steroidal inhibitor Epristeride. The phenyl ring introduced into this molecule results in an active inhibitor (compound **2**). As this group can easily be further substituted, the synthesis of more potent inhibitors is feasible. We are currently working on the preparation of such compounds.

Acknowledgements

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Experimental

General

Materials obtained from commercial suppliers were used without further purification. Solvents for reactions under anhydrous conditions were dried according to standard procedures. All reactions, except those involving water as a reagent, were conducted under nitrogen atmosphere. Melting points were measured on a Reichert Thermometer hot stage microscope and are uncorrected. Silica gel TLC and column chromatography were performed on Merck TLC 60F-254 (0.25 mm) and Merck Kieselgel 60. IR spectra were determined with a Perkin Elmer Infrared Spectrometer 398 (KBr). ^{1}H NMR spectra were measured on a Bruker AM 400 at 400 MHz in $CDCl_3$ or d_6-DMSO solution and chemical shifts are reported in δ parts per million downfield from tetramethylsilane. Elemental analyses indicated by the symbols of the elements were within ±0.4 % of the theoretical values and were performed by the Institute of Inorganic Chemistry, Saarland University, Saarbrücken, Germany.

(E/Z) 4-[2-(1,4-Dioxaspiro[4.5]dec-8-yl)-vinyl]-N,N-diisopropylbenzamide (**1 d**)

[4-(*N,N*-Diisopropylcarbamoyl)benzyl]triphenylphosphonium bromide (**1 e**, 10.76 g, 19.2 mmol) was suspended in tetrahydrofuran (100 L) and butyllithium (12.5 mL, 1.6 M in hexane) was injected via a syringe at 0 °C. The red mixture was warmed to 25 °C and stirred for 15 min. 1,4-Dioxaspiro[4.5]decane-8-carbaldehyde (**4 g**) (3.40 g, 20 mmol)) in tetrahydrofuran (15 mL) was added and stirring was continued for 12 h. After evaporation of the solvent, dichloromethane (200 mL) was added and the organic layer was washed with brine (2 × 50 mL), dried (magnesium sulfate), and evaporated *in vacuo*. The residue was chromatographed on silica gel using petroleum ether (40–60 °C)-diethyl ether (1:1) as an eluent. Yield: 59 %, colorless solid, mp 97–98 °C. IR (KBr) 2930, 2880, 1630, 1445, 1340, 1090, 1035, 830 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.33 (m; 12 H); 1.43–1.82 (m; 8 H); 2.19 (m; 1 H, Ar-CH=CH-CH, *E*-Isomer); 2.60 (m; 1 H Ar-CH=CH-CH, *Z*-Isomer); 3.69 (m; 2 H); 3.95 (s; 4 H); 5.54 (dd; 1 H, *J* = 11.54 Hz, Ar-CH=CH-, *Z*-Isomer); 6.35 (d; 1 H, *J* = 11.82 Hz, Ar-CH=CH-, *Z*-Isomer); 6.20 (dd; 1 H, *J* =

15.94 Hz, Ar-CH=CH-, *E*-Isomer); 6.38 (d; 1 H, *J* = 16.04 Hz, Ar-CH=CH-, *E*-Isomer); 7.23 and 7.33 (dd; 4 H, *J* = 8.18 Hz). *E:Z* ratio 73:27.

Compounds **2 d–4 d** were prepared accordingly:

(E/Z) 8-(2-Biphenyl-4-yl-vinyl)-1,4-dioxaspiro[4.5]decane (**2 d**)

Yield: 34 %, colorless solid, mp 65–75 °C. ^{1}H NMR ($CDCl_3$) δ 1.54–1.84 (2 m; 8 H); 2.21 (m; 1 H, Ar-CH=CH-CH, *E*-Isomer); 2.67 (m; 1 H, Ar-CH=CH-CH, *Z*-Isomer); 3.96 (s; 4 H); 5.54 (dd; 1 H, *J* = 11.50 Hz , Ar-CH=CH-, *Z*-Isomer); 6.39 (d; 1 H, *J* = 11.92 Hz, Ar-CH=CH-, *Z*-Isomer); 6.22 (dd; 1 H, *J* = 15.92 Hz, Ar-CH=*CH*-, *E*-Isomer); 6.42 (d; 1 H, *J* = 16.36 Hz, Ar-CH=*CH*-, *E*-Isomer); 7.30–7.33 (m; 9 H). *E:Z* ratio 53:47.

(E/Z) 8-[2-(4-Phenoxy-phenyl)vinyl]-1,4-dioxaspiro[4.5]decane (**3 d)**

Yield: 73 %, colorless solid, mp 97–98 °C. ^{1}H NMR ($CDCl_3$) δ 1.61 and 1.79 (2 m; 8 H); 2.18 (m; 1 H, Ar-CH=CH-*CH*, *E*-Isomer); 2.62 (m; 1 H, Ar-CH=CH-*CH*, *Z*-Isomer); 3.96 (s; 4 H); 5.48 (dd; 1 H, *J* = 11.52 Hz , Ar-CH=*CH*-, *Z*-Isomer); 6.32 (d; 1 H, *J* = 11.52 Hz, Ar-*CH*=CH-, *Z*-Isomer); 6.10 (dd; 1 H, *J* = 15.92 Hz, Ar-CH=*CH*-, *E*-Isomer); 6.36 (d; 1 H, *J* = 15.92 Hz, Ar-*CH*=CH-, *E*-Isomer); 6.92–7.10 (m; 9 H). *E*:*Z* ratio 80:20.

(E/Z) 8-{2-[4-(2-Phenyl-[1.3]dioxolan-2-yl)-phenyl]-vinyl}-1,4-dioxaspiro[4.5]decane (**4 d**)

Yield: 85 %, colorless solid, mp 63–67 °C. ^{1}H NMR ($CDCl_3$) δ 1.51–1.81 (2 m; 8 H); 2.10 (m; 1 H, Ar-CH=CH-*CH*, *E*-Isomer); 2.60 (m; 1 H, Ar-CH=CH-*CH*, *Z*-Isomer); 3.95 (s; 4 H); 4.05 (s; 4 H); 5.49 (dd; 1 H, *J* = 11.48 Hz, Ar-CH=*CH*-, *Z*-Isomer); 6.32 (d; 1 H, *J* = 11.52 Hz, Ar-*CH*=CH-, *Z*-Isomer); 6.16 (dd; 1 H, *J* = 15.92 Hz, Ar-CH=*CH*-, *E*-Isomer); 6.36 (d; 1 H, *J* = 15.92 Hz, Ar-*CH*=CH-, *E*-Isomer); 7.19–7.56 (m; 9 H). *E:Z* ratio 75:25.

4-[2-(1,4-Dioxaspiro[4.5]dec-8-yl)-ethyl]-N,N-diisopropylbenzamide (**1 c**)

Compound **1 d** (4.0 g, 10.8 mmol) was hydrogenated using palladium on charcoal (10 %) in ethanol (100 mL) for 1 h. The catalyst was filtered off and the solvent was evaporated and the pure product was obtained. Yield: 98 %, colorless oil. IR (Film) 2920, 2880, 1630, 1440, 1340, 1005 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.05–1.41 (d; 12 H); 1.47–1.89 (m; 11 H); 3.55–3.83 (m; 4 H); 3.92 (s; 4 H); 7.18 (s; 4 H).

Compounds **2 c–5 c** were prepared accordingly:

8-(2-Biphenyl-4-ylethyl)-1,4-dioxaspiro[4.5]decane (**2 c**)

The reaction mixture was hydrogenated for 30 min using ethyl acetate as a solvent. Yield: 85 %, colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.01–1.80 (m; 11 H); 2.28–2.45 (m; 2 H); 3.89 (s; 4 H); 6.93–7.70 (m; 9 H).

8-[2-(4-Phenoxyphenyl)ethyl]-1,4-dioxaspiro[4.5]decane (**3 c**)

The reaction mixture was hydrogenated for 2 h using ethyl acetate as a solvent. Yield: 85 %, colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.83–1.79 (m; 11 H); 2.34–2.74 (m; 2 H); 3.91 (s; 4 H); 6.62–7.46 (m; 9 H).

8-{2-[4-(2-Phenyl-[1,3]dioxolan-2-yl)phenyl]ethyl}-1,4-dioxaspiro[4.5]decane (**4 c**)

The reaction mixture was hydrogenated for 30min using ethyl acetate as a solvent. Yield: 98 %, colorless solid, mp 93–94 °C. ^{1}H NMR ($CDCl_3$) δ 1.06–1.88 (m; 11 H); 2.59 (t; 2 H, *J* = 6.80 Hz); 3.88 (s; 4 H); 4.01 (s; 4H); 6.93–7.59 (m; 9 H).

8-[2-(4-Benzylphenyl)ethyl]-1,4-dioxaspiro[4.5]decane (**5 c**)

The reaction mixture was hydrogenated for 24 h using ethanol as a solvent. Yield: 80 %, colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.01–2.11 (m; 11 H); 2.41–2.67 (m; 2 H); 3.89 (s; 4 H); 3.94 (s; 2 H); 7.04–7.18 (m; 9 H).

N,N-Diisopropyl-4-[2-(4-oxocyclohexyl)ethyl]benzamide (**1 b**)

Compound **1 c** (3.92 g, 10.5 mmol) was dissolved in ethanol (20 mL) and diluted hydrochloric acid (120 mL, 3 %) was added. The mixture was stirred at 90–95 °C for 1 h and was then extracted with ethyl acetate (3 × 150 mL) after being cooled to 25 °C. The combined organic layers were washed with water (2 × 50 mL), dried (magnesium sulfate) and the solvent was evaporated. An oil was obtained, that crystallized upon standing. Yield: 87 %, colorless solid, mp 75–80 °C. IR (KBr) 2920, 1710, 1620, 1435, 1365, 1335, 1160, 840 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.21–1.46 (d; 12 H); 1.49–2.11 (m; 7 H); 2.18–2.51 (m; 4 H); 2.55–2.87 (m; 2 H); 3.54–3.95 (m; 2 H); 7.20 (s; 4 H).

Compounds **2 b–5 b** were prepared accordingly:

4-(2-Biphenyl-4-ylethyl)cyclohexanone (**2 b**)

Yield: 77 %, colorless solid, mp 72–74 °C. ^{1}H NMR ($CDCl_3$) δ 1.42–2.18 (m; 7 H); 2.34 (m; 4 H); 2.72 (t; 2 H, *J* = 7.94 Hz); 7.25–7.60 (m; 9 H).

4-[2-(4-Phenoxyphenyl)ethyl]cyclohexanone (**3 b**)

Yield: 58 %, colorless solid, mp 59–60 °C. ^{1}H NMR ($CDCl_3$) δ 1.38–2.17 (m; 7 H); 2.38 (m; 4 H); 2.66 (t; 2 H, *J* = 7.94 Hz); 6.93–7.34 (m; 9 H).

4-[2-(4-Benzoylphenyl)ethyl]cyclohexanone (**4 b**)

Yield: 85 %, colorless solid, mp 103–107 °C. ^{1}H NMR ($CDCl_3$) δ 1.43–2.14 (3 m; 7 H); 2.29–2.47 (m; 4 H); 2.77 (t; 2 H, *J* = 7.74 Hz); 7.29–7.80 (m; 9 H).

4-[2-(4-Benzylphenyl)ethyl]cyclohexanone (**5 b**)

Yield: 52 %, colorless solid, mp 38–40 °C. ^{1}H NMR ($CDCl_3$) δ 1.43–2.26 (m; 7 H); 2.28–2.40 (m; 4 H); 2.72 (t; 2 H, *J* = 7.96 Hz); 3.95 (s; 2 H); 7.08–7.19 (m; 9 H).

Trifluoromethanesulfonic acid 4-{2-[4-(diisopropyl-carbamoyl)-phenyl]-ethyl}-cyclohex-1-enyl ester (**1 a**)

Trifluoromethanesulfonic anhydride (0.6 g, 0.35 mL, 2.13 mmol) was injected into a solution of compound **1 b** (0.5 g, 1.5 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (0.33 g, 1.6 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was warmed to 25 °C and stirred for 12 h. Ethyl acetate (100 mL) was added and the organic layer was washed with diluted hydrochloric acid (5 %, 25 mL) and brine (25 mL), dried (magnesium sulfate), filtered over celite and evaporated. Yield: 73 %, colorless oil. IR 2910, 1625, 1440, 1365, 1330, 1210, 1130, 1100, 1030, 925 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.3 (d; 12 H); 1.51–1.88 (m; 9 H); 3.92 (m; 4 H); 6.28 (m; 1 H); 7.24 (s; 4 H).

Compounds **2 a–5 a** were prepared in a similar way:

Trifluoromethanesulfonic acid 4-(2-biphenyl-4-ylethyl)cyclohex-1-enyl ester (**2 a**)

Yield: 79 %, pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.25–2.35 (m; 9 H); 2,69 (t; 2 H, *J* = 7.52 Hz); 5.74 (s; 1 H); 7.23–7.59 (m; 9 H).

Trifluoromethanesulfonic acid 4-[2-(4-phenoxyphenyl)ethyl]-cyclohex-1-enyl ester (**3 a**)

The reaction mixture was stirred for 3 h at 25 °C and the compound was isolated by column chromatography (silica gel)

using hexane-ethyl acetate (4:1) as an eluent. Yield: 63 %, pale yellow solid, mp 68–70 °C. ^{1}H NMR ($CDCl_3$) δ 1.49–2.34 (m; 9 H); 2.63 (t; 2 H, ^3J = 7.74 Hz); 5.73 (s; 1 H); 6.94 and 7.13 (dd; 4 H, *J* = 8.84 Hz); 6.98–7.10 and 7.30–7.34 (2 m, 5 H).

Trifluoromethanesulfonic acid 4-[2-(4-benzoylphenyl)ethyl]-cyclohex-1-enyl ester (**4 a**)

The product was isolated by column chromatography (silica gel) using hexane-ethyl acetate (7:3) as an eluent. Yield: 53 %, pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.51–2.37 (m; 9 H); 2.74 (t; 2 H, *J* = 7.74 Hz); 5.74 (s; 1 H); 7.28 and 7.75 (dd; 4 H, *J* = 8,40 Hz); 7.46–7.81 (m; 5 H).

Trifluoromethanesulfonic acid 4-[2-(4-benzylphenyl)ethyl]cyclohex-1-enyl ester (**5 a**)

The reaction mixture was stirred for 4 h at 25 °C and the product was isolated by column chromatography (silica gel) using hexane-ethyl acetate (4:1) as an eluent. Yield: 36 %, colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.44–2.36 (m; 9 H); 2.61 (t; 2 H, *J* = 7.74 Hz); 3.95 (s; 2 H); 5.72 (s; 1 H); 7.07–7.30 (m; 9 H).

4-{2-[4-(N,N-Diisopropylcarbamoyl)phenyl]ethyl}cyclohex-1-ene carboxylic acid (**1**)

Compound **1 a** (0.50 g, 1 mmol), potassium acetate (0.39 g, 4 mmol), palladium(II) acetate (9 mg) and triphenylphosphine (21 mg) were stirred in *N,N*-dimethylformamide (10 mL) under CO atmosphere at 25 °C for 14 h. Water (30 mL) was added and the solution was acidified with diluted hydrochloric acid (20 mL, 0.5 N). The mixture was extracted with dichloromethane (3 × 25 mL) and the combined organic layers were washed with brine (25 mL), dried (magnesium sulfate) and the solvent was evaporated. The remaining brownish oil was dissolved in diluted sodium hydroxide solution (0.5 N, 50 mL) and extracted with diethyl ether (2 × 25 mL). The aqueous layer was acidified (hydrochloric acid, 2 N, 30 mL) and extracted with diethyl ether (3 × 25 mL), dried (magnesium sulfate) and evaporated. The remaining residue was recrystallized from petroleum ether (40–60 °C)/ethyl acetate of increasing polarity. Yield: 42 %, colorless crystals, mp 175–177 °C. IR (KBr) 3000, 2930, 1685, 1640, 1450, 1345, 1275, 1040 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.23 (s; 12 H); 1.83–2.37 (3 m; 9 H); 2.64 (t; 2 H, **J** = 7.74 Hz); 3.62 (m; 2 H); 6.84 (s; 1 H); 7.17 and 7.24 (dd; 4 H, *J* = 7.96 Hz); 12.07 (s; 1 H). Anal. ($C_{22}H_{31}NO_3$) C, H, N.

Compounds **2–5** were prepared accordingly:

4-(2-Biphenyl-4-ylethyl)cyclohex-1-ene carboxylic acid (**2**)

Yield: 40 %, colorless crystals, mp (petroleum ether (40–60 °C)/acetone): 225–226 °C. IR (KBr) 3000, 2920, 1670, 1645, 1485, 1430, 1280 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.24–2.34 (m; 9 H); 2.67 (t; 2 H, *J* = 7.74 Hz); 6.85 (s; 1 H); 7.30 and 7.57 (dd; 4 H, *J* = 8.18 Hz); 7.33–7.46 and 7.63 (2 m; 5 H); 12.06 (s; 1 H). Anal. ($C_{21}H_{22}O_2$) C, H, N.

4-[2-(4-Phenoxyphenyl)ethyl]cyclohex-1-ene carboxylic acid (**3**)

Yield: 24 %, colorless crystals, mp (petroleum ether (40–60 °C)/acetone): 206–207 °C. IR (KBr) 3000, 1680, 1640, 1590, 1505, 1490, 1430, 1280, 1235, 1165, 870, 850 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.22–2.31 (m; 9 H); 2.61 (t; 2 H, *J* = 7.74 Hz); 6.84 (s; 1 H); 6.92 and 7.22 (dd; 4 H, *J* = 8.62 Hz); 6.95–7.12 and 7.35–7.39 (2 m; 5 H); 12.05 (s; 1 H). Anal. ($C_{21}H_{22}O_3$) C, H, N.

4-[2-(4-Benzoylphenyl)ethyl]cyclohex-1-ene carboxylic acid (**4**)

Yield: 18 %, white powder, mp (hexane/ethyl acetate): 197–198 °C. IR (KBr) 3000, 2930, 1680, 1660, 1610, 1320, 1280, 940, 930, 700 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.25–2.32 (m; 9 H); 2.73 (s; 2 H); 6.83 (s; 1 H); 7.38 (d; 2 H, *J* = 7.52 Hz); 7.54–7.71 (m; 7 H); 11.91 (s; 1 H). Anal. ($C_{22}H_{22}O_3$) C, H, N.

4-[2-(4-Benzylphenyl)ethyl]cyclohex-1-ene carboxylic acid (**5**)

Yield: 32 %, colorless crystals, mp (petroleum ether (40–60 °C)/ethyl acetate): 177–178 °C. IR (KBr) 3000, 2940, 1680, 1640, 1515, 1490, 1450, 1430, 1420, 1280 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.15–1.73 (m; 9 H); 2.57 (t; 2 H, *J* = 7.74 Hz); 3.88 (s; 2 H); 6.83 (s; 1 H); 7.10–7.33 (m; 9 H); 12.10 (s; 1 H).

Inhibition of 5α reductase in vitro

Enzyme inhibition test

Preparation of tissue

Prostatic enzyme was prepared according to the method of Liang et al. [24] with slight modifications [26]. All the following operations were performed at 0–4 °C. The human prostates were dissected free from fat and connective tissue, cut into pieces and weighed. Per 1 g of tissue, 3 mL of 20 mM citrate buffer, pH 5.5, containing 0.32 mM sucrose and 1 mM dithiothreitol (DTT) were added. The tissue was homogenized by 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 citrate buffer. The centrifugation was repeated, the final pellet resuspended in a minimum volume of citrate 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.

Incubation procedure

The assay was performed as described [24] with modifications [26]. All values were determined in duplicate. The incubation was carried out for 30 min at 37 °C in a total volume of 250 µL using citrate buffer (40 mM, pH 5.5). The incubation mixture contained approximately 200–300 µg human protein, 100 µM NADPH, 0.21 µM T including 100 nCi [1β,2β-^{3}H]T and 2 % DMSO with or without test compound (10 µM). In case of exceeding 60 % inhibition, three concentrations were chosen for the determination of IC_{50} values. The reaction was started by adding the prostatic enzyme preparation and terminated by addition of 50 µL aqueous solution of NaOH (10 M). The steroids were extracted with diethyl ether (500 µL) by shaking for 10 min. Subsequent centrifugation was performed for 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: DU 145-assay [27]

Intact human prostatic carcinoma DU145 cells were used as the source of type1 5α reductase [28]. The inhibitory potencies of the compounds were 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 180,000 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 first screened at concentrations of 10 µM in an initial test and in cases exceeding 80 % inhibition, three concentrations were chosen for measurement of IC_{50} values. As control of conversion (typically about 35 % under these conditions) a triplicate of wells without inhibitors was used and as a positive control for inhibition Finasteride (80, 60, 40, 20 nM). After the 6h incubation period in 5 % CO_2 at 37 °C the medium samples were extracted twice

with 1 mL of diethyl ether and the steroids were separated by HPLC. Results are expressed as amount of formed androstanedione as percentage of control values.

HPLC procedure

Steroid separation was performed [26] similarly to the method of Cook et al. [29]. The steroids were dissolved in 50 µL methanol and 25 µL was 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, baseline separation of T, and DHT was achieved within 20 min. For the steroids androstenedione and dihydroandrostenedione methanol/water (50/50, *w/w*) was used.

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: $\% I = (1 - iv_{corr}/cv_{corr})100$.

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