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N-substituted 4-(4-carboxyphenoxy)benzamides. Synthesis and Evaluation as Inhibitors of Steroid-5α-reductase Type 1 and 2

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In search of non-steroidal inhibitors of human prostatic 5α-reductase, we recently described *N*-substituted 4′-biphenyl-4-carboxylic acids. Here, we report the optimisation of this series of compounds by increasing the conformational flexibility using an ether linker between the steroidal A–C ring mimetics. Ten new compounds were synthesised and tested against human and rat isozymes 1 and 2. The substances showed a broad range of activity from 36% inhibition at a concentration of 10 μM to an IC_{50} value of 60 nM for compounds 22 and 29 respectively. The most potent compound 26 showed an IC_{50} value improved by a factor of 5 from 1.9 μM to 0.38 μM in comparison with the parent biphenyl compound 15.

Keywords: 5α-reductase; Non-steroidal inhibitors; Isozymes 1 and 2; *N*-substituted 4-(4-carboxyphenoxy)benzamides

INTRODUCTION

We recently reported the discovery of new lead compounds for the design of non-steroidal 5α-reductase (5α-R) inhibitors: 4′-carboxamide substituted biphenyl-4-carboxylic acids[1] (Figure 1, **A**), mimetics of the steroidal A–C ring of the substrate testosterone (T). 5α-R, which converts T to the most potent androgen dihydrotestosterone (DHT), is believed to play a stimulating role in benign prostatic hyperplasia (BPH), the most common non-malignant tumour in elderly men.[2–4] Among all the potential targets to treat BPH 5α-R plays a prominent role. To date, the only inhibitor of this enzyme on the market is finasteride,[5] a steroidal inhibitor whose side effects are believed to be due to its steroidal backbone. Therefore, we[6–10] and others[11–14] have focused our interest on the development of non-steroidal inhibitors. Recently one very potent carboxylic compound (Figure 1, **B**) has been described bearing an ether linker between the steroidal A–C ring mimetics.[15] As this structural modification increases conformational flexibility and thus might enable an inhibitor to better accommodate into the active site of the enzyme, we report in this paper the introduction of an ether group into type **A** compounds. We describe the synthesis of new carboxamide type compounds **21**–**28** as well as the preparation of **29**, a homologue of **B**. **29** contains a phenyl acetic acid moiety as an A-ring mimetic. This moiety has also been successfully used in our group.[16] The compounds are evaluated for 5α-R inhibition toward rat and human isozymes 1 and prostatic isozymes 2.

MATERIALS AND METHODS

Chemistry

^{1}H NMR spectra were recorded on a Bruker AM-400 (400 MHz) in $CDCl_3$ or DMSO-d_6 for compounds **21**–**30** and **B**. Chemical shifts are reported as δ values (ppm) relative to internal tetramethylsilane (δ 0 ppm). Elemental analyses were performed in the Department of Inorganic Chemistry, University of the Saarland. IR spectra were performed with KBr disks or films as indicated on a Perkin–Elmer 398 infrared spectrometer. Melting points were determined on a Kofler melting point apparatus Thermopan (Reichert) and are uncorrected. Column

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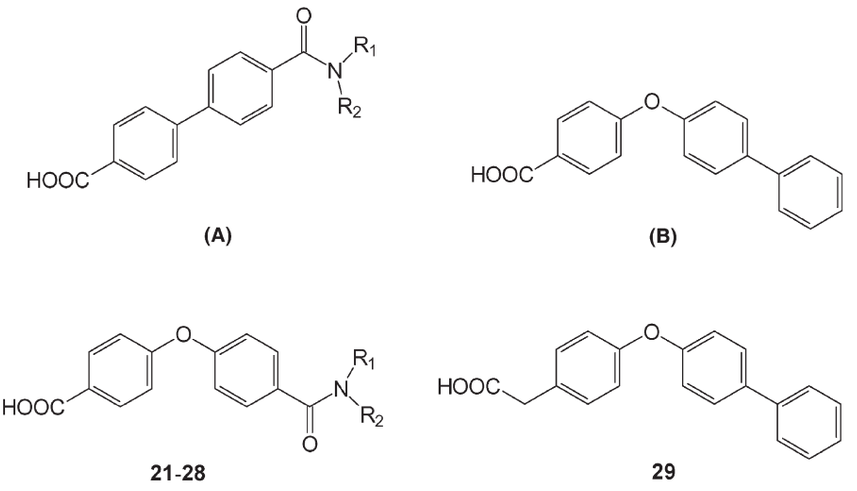


FIGURE 1 General structures.

chromatography was performed on Merck silicagel 60 (40–63 μm) or (50–200 μm). All reactions were followed by thin layer chromatography using Alugram® Silica gel 60. Chemicals and solvents used were commercially available (Lancaster, Fluka, Acros) and were used without further purification.

[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.

4-(4′-[1,3]Dioxolan-2-yl-phenoxy)benzonitrile (30a)

A mixture of 4-(4′-formylphenoxy)benzonitrile (**30b**)[17] (20.0 g, 89.6 mmol), ethylene glycol (8.34 g, 134 mmol), (±)-10-camphor sulfonic acid (25.0 g, 107 mmol) in toluene (250 mL) was refluxed overnight in a Dean-Stark trap. After cooling the solvent was evaporated under reduced pressure. The mixture was diluted with diethyl ether (300 mL), washed with brine (3 × 150 mL) and dried over $MgSO_4$. After evaporation of the solvent the resulting solid was recrystallised (hexane/ethyl acetate) to afford **30a**. Yield 97%, white crystals, mp 68–69°C. ^{1}H NMR δ 4.03–4.07 (m, 2H, $-OCH_2CH_2O-$), 4.14–4.17 (m, 2H, $-OCH_2CH_2O-$), 5.80 (s, 1H, –CH–), 7.00 and 7.53 (d, 4H, $J = 8.8$ Hz, Ar H), 7.07 and 7.59 (d, 4H, $J = 8.8$ Hz, Ar H). IR (KBr): $\nu = 2220, 1690, 1600, 1500, 1250\ cm^{-1}$.

4-(4′-Formylphenoxy)benzoic Acid (30)

4-(4′-[1,3]Dioxolan-2-yl-phenoxy)benzonitrile (**30a**) (10.0 g, 37.4 mmol) and KOH (19.0 g, 336 mmol) were dissolved in water (89 mL) and ethylene glycol (89 mL) and refluxed for 18 h. The light orange solution was acidified with aqueous HCl (10N) and heated for 3 h. The mixture was extracted with diethyl ether (4 × 100 mL) and washed with brine (150 mL). Drying over $MgSO_4$ and evaporation of the solvent under reduced pressure left a slightly brown solid which was recrystallised (hexane/ethyl acetate) to give pure compound **30**. Yield 88%, white powder, mp 171°C (lit. 170–173°C).[18] ^{1}H NMR δ 7.20 and 7.97 (d, 4H, $J = 8.4$ Hz, Ar H), 7.25 and 8.01 (d, 4H, $J = 8.4$ Hz, Ar H), 9.97 (s, 1H, –CHO), 12.91 (s, 1H, –COOH). IR (KBr): $\nu = 3000$ (OH), 1690, 1600, 1500, 1440, 1250 cm^{-1}. Found: C, 69.35; H, 4.18. $C_{14}H_{10}O_4$ requires: C, 69.42; H, 4.16%.

General Procedure for the Synthesis of 21a–25a, 27a–28a

N-Cyclohexyl-4-(4′-formylphenoxy)benzamide (**27a**)

A solution of 4-(4′-formylphenoxy)benzoic acid (**30**) (500 mg, 2.06 mmol), oxalyl chloride (180 μL, 2.06 mmol) and 2 drops of DMF in anhydrous CH_2Cl_2 (25 mL) under nitrogen was stirred overnight. A mixture of cyclohexylamine (408 mg, 4.12 mmol) and triethylamine (347 μL, 2.47 mmol) was added dropwise and the solution stirred for a further 2 h, washed with brine (3 × 15 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting oil was purified by flash column chromatography (FCC) (hexane/ethyl acetate 17:4 v/v) to afford **27a**. Yield 90%, white crystals, mp 166–167°C. ^{1}H NMR δ 1.20–1.78 (m, 10H, cyclohexyl-H), 3.97 (m, broad, 1H, –NHC*H*–), 5.92 (s, broad, 1H, –N*H*CH–), 7.09 and 7.80 (d, 4H, $J = 8.4$ Hz, Ar H), 7.10 and 7.87 (d, 4H, $J = 8.4$ Hz, Ar H), 9.95 (s, 1H, –CHO). IR (KBr): $\nu = 3300$ (NH), 2840, 1700, 1630, 1600, 1500, 1240 cm^{-1}.

N-tert-Butyl-4-(4′-formylphenoxy)benzamide (**21a**)

Synthesised from 4-(4′-formylphenoxy)benzoic acid (**30**) and *tert*-butylamine. Purified by FCC (hexane/ethyl acetate 7:3 v/v). Yield 75%, colourless oil. ^{1}H NMR δ 1.48 (s, 9H, −NH(CH_3)$_3$), 5.95 (s, broad, 1H, −NH(CH$_3$)$_3$), 7.09 and 7.78 (d, 4H, J = 8.8 Hz, Ar H), 7.10 and 7.88 (d, 4H, J = 8.8 Hz, Ar H), 9.94 (s, 1H, −CHO). IR (film): ν = 3400 (NH), 2750, 1690, 1650, 1600, 1500, 1240 cm^{-1}.

N,N-Diisopropyl-4-(4′-formylphenoxy)benzamide (**22a**)

Synthesised from 4-(4′-formylphenoxy)benzoic acid (**30**) and diisopropylamine. Purified by FCC (hexane/ethyl acetate 7:3 v/v). Yield 60%, colourless oil. ^{1}H NMR δ 1.26 (s, broad, 12H, −CH(CH_3)$_2$), 3.68 (s, broad, 2H, −CH(CH$_3$)$_2$), 7.09 and 7.38 (d, 4H, J = 8.4 Hz, Ar H), 7.10 and 7.87 (d, 4H, J = 8.8 Hz, Ar H), 9.94 (s, 1H, −CHO). IR (film): ν = 2960, 1720, 1650, 1600, 1500, 1230 cm^{-1}.

N,N-Diisobutyl-4-(4′-formylphenoxy)benzamide (**23a**)

Synthesised from 4-(4′-formylphenoxy)benzoic acid (**30**) and diisobutylamine. Purified by FCC (hexane/ethyl acetate 6:4 v/v). Yield 86%, colourless oil. ^{1}H NMR δ 0.77 (s, 6H, −CH(CH_3)$_2$), 0.98 (s, 6H, −CH(CH_3)$_2$), 1.87 (s, broad, 1H, −CH(CH$_3$)$_2$), 2.13 (s, broad, 1H, −CH(CH$_3$)$_2$), 3.14 (s, 2H, −NCH_2−), 3.36 (s, 2H, −NCH_2−), 7.09 and 7.42 (d, 4H, J = 8.4 Hz, Ar H), 7.10 and 7.84 (d, 4H, J = 8.8 Hz, Ar H), 9.94 (s, 1H, −CHO). IR (film): ν = 2960, 1740, 1700, 1650, 1600, 1500−1400, 1230 cm^{-1}.

N-Phenyl-4-(4′-formylphenoxy)benzamide (**24a**)

Synthesised from 4-(4′-formylphenoxy)benzoic acid (**30**) and aniline. Purified by FCC (hexane/ethyl acetate 7:3 v/v). Yield 40%, white solid, mp 199–200°C. ^{1}H NMR δ 7.10 and 7.65 (d, 4H, J = 8.4 Hz, Ar H), 7.12 and 7.91 (d, 4H, J = 8.8 Hz, Ar H), 7.16–7.33 (m, 5H, Ar H), 8.46 (s, 1H, −NH−), 9.96 (s, 1H, −CHO). IR (KBr): ν = 3400, 2820, 1650, 1600, 1500, 1450, 1250 cm^{-1}.

N,N-Diphenyl-4-(4′-formylphenoxy)benzamide (**25a**)

Synthesised from 4-(4′-formylphenoxy)benzoic acid (**30**) and diphenylamine. Purified by FCC (hexane/ethyl acetate 7:3 v/v). Yield 35%, white solid, mp 173–174°C. ^{1}H NMR δ 6.90 and 7.50 (d, 4H, J = 8.4 Hz, Ar H), 7.02 and 7.85 (d, 4H, J = 8.8 Hz, Ar H), 7.15–7.33 (m, 10H, Ar H), 9.93 (s, 1H, −CHO). IR (KBr): ν = 2830, 1700, 1660, 1600, 1500, 1450, 1350, 1250 cm^{-1}.

N,N-Dicyclohexyl-4-(4′-formylphenoxy)benzamide (**28a**)

Synthesised from 4-(4′-formylphenoxy)benzoic acid (**30**) and dicyclohexylamine. Purified by FCC (hexane/ethyl acetate 8:2 v/v). Yield 47%, slightly yellow oil. ^{1}H NMR δ 1.16–1.79 (m, 20H, cyclohexyl-H), 3.32 (s, broad, 2H, −N(CH)$_2$−), 7.02 (d, 4H, J = 8.4 Hz, Ar H), 7.31 and 7.55 (d, 4H, J = 8.8 Hz, Ar H), 9.96 (s, 1H, −CHO). IR (KBr): ν = 1700, 1660, 1600, 1500, 1300, 1250 cm^{-1}.

N-*Adamantyl-4-(4′-formylphenoxy)benzamide (26a)*

Under nitrogen, 4-(4′-formylphenoxy)benzoic acid (**30**) (73.0 mg, 0.30 mmol), 2-chloro-1-methylpyridinium iodide (115 mg, 0.45 mmol), triethylamine (45.7 mg, 0.45 mmol) and adamantylamine (68.3 mg, 0.45 mmol) were dissolved in anhydrous CH_2Cl_2 (25 mL). The reaction mixture was stirred for 2 h at room temperature. After evaporation of the solvent, the residue obtained was purified by FCC (hexane/ethyl acetate 8:2 v/v) to give **26a**. Yield 72%, white powder, mp 160–161°C. ^{1}H NMR δ 1.73 (s, 6H, ada. H), 2.13 (s, 9H, ada. H), 5.75 (s, 1H, −NH), 7.09 (d, 4H, J = 8.8 Hz, Ar H), 7.76 and 7.87 (d, 4H, J = 8.8 Hz, Ar H), 9.94 (s, 1H, −CHO). IR (KBr): ν = 3400 (NH), 2900–2840, 1700, 1660, 1600, 1500, 1300, 1250 cm^{-1}.

4-(Biphenyl-4′-yloxy)phenylacetaldehyde (29a)

To a stirred suspension of methoxymethyltriphenylphosphonium chloride (5.00 g, 14.6 mmol) in anhydrous THF (30 mL) at −78°C under nitrogen, was added dropwise *n*-BuLi (1.6 M in hexane, 9.12 mL, 14.6 mmol). After 30 min a solution of **29c**[15] (4.66 g, 17.0 mmol) in anhydrous THF (35 mL) was added and the resulting mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was chromatographed directly (petroleum/ethyl acetate 0–20%). Yield 43%, white paste. To a cooled solution of the enol ether **29b** (1.90 g, 6.28 mmol) in THF (30 mL) and water (5.60 mL) was added concentrated H_2SO_4 (0.6 mL). The reaction mixture was refluxed for 10 h during which time all the starting material was consumed as evidenced by TLC. The organic solvent was evaporated under reduced pressure and the remaining aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was washed repeatedly with water until the washings were free of acid. After drying ($MgSO_4$) the solvent was evaporated and the residue purified by FCC (hexane/ethyl acetate 9:1 v/v) to give **29a**. Yield 73%, white powder, mp 108–110°C. ^{1}H NMR δ 3.69 (d, 2H, J = 2.2 Hz, −CH_2CHO), 7.06 (d, 4H, J = 8.4 Hz, Ar H), 7.20 (d, 2H, J = 8.4 Hz, Ar H), 7.33 (t, 1H, J = 7.4 Hz, Ar H), 7.43 (t, 2H, J = 7.7 Hz, Ar H), 7.55 (d, 4H, J = 8.4 Hz, Ar H), 9.77 (d, 1H, J = 2.2 Hz, −CH$_2$CHO). IR (KBr): ν = 3400 broad, 1730, 1600, 1500, 1300, 1270 cm^{-1}.

General Procedure for the Synthesis of 21–29, B

N-Cyclohexyl-4-(4′-carboxyphenoxy)benzamide (**27**)

To a solution of *N*-cyclohexyl-4-(4′-formylphenoxy)benzamide (**27a**) (300 mg, 0.93 mmol) in THF (5 mL) was added aqueous NaH_2PO_4 (29.6 mg, 0.24 mmol) in H_2O (370 μL) and H_2O_2 (35%) (93 μL). After stirring for 5 min $NaClO_2$ (80%, 148 mg, 1.63 mmol) in H_2O (1.30 mL) was added over a period of 30 min. The mixture was stirred overnight at room temperature. After evaporation of THF the residue was diluted with water (15 mL) and extracted with CH_2Cl_2 (3 × 25 mL). Drying over $MgSO_4$ and evaporation of the solvent gave a solid which was purified by recrystallisation (hexane/ethyl acetate) to yield **27**. Yield 53%, white powder, mp >300°C. ^{1}H NMR δ 1.13–1.80 (m, 10H, cyclohexyl-H), 3.75 (s, broad, 1H, –NHC*H*–), 7.04 and 7.90 (d, 4H, $J = 8.4$ Hz, Ar H), 7.11 and 7.95 (d, 4H, $J = 8.4$ Hz, Ar H), 8.19 (s, broad, 1H, –N*H*CH–), 12.86 (s, 1H, –COOH). IR (KBr): $\nu = 3340$ (NH), 2840, 2760, 1700, 1630, 1600, 1500, 1240 cm^{-1}. Found: C, 70.73; H, 6.11; N, 4.18. $C_{20}H_{21}NO_4$ requires: C, 70.78; H, 6.24; N, 4.13%.

N-*tert*-Butyl-4-(4′-carboxyphenoxy)benzamide (**21**)

Synthesised from *N*-*tert*-butyl-4-(4′-formylphenoxy)benzamide (**21a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 46%, white powder, mp 215–216°C. ^{1}H NMR δ 1.38 (s, 9H, –N*H*(CH_3)$_3$), 7.07 and 7.88 (d, 4H, $J = 8.2$ Hz, Ar H), 7.12 and 7.97 (d, 4H, $J = 8.2$ Hz, Ar H), 7.74 (s, 1H, –N*H*(CH_3)$_3$), 12.86 (s, 1H, –COOH). IR (film): $\nu = 3380$ (NH), 2800 broad, 1690, 1650, 1600, 1540, 1500, 1300, 1240, 1170 cm^{-1}. Found: C, 59.95; H, 6.10; N, 4.51. $C_{18}H_{19}NO_4$ requires: C, 69.00; H, 6.11; N, 4.47%.

N,N-Diisopropyl-4-(4′-carboxyphenoxy)benzamide (**22**)

Synthesised from *N,N*-diisopropyl-4-(4′-formylphenoxy)benzamide (**22a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 52%, white powder, mp 215–217°C. ^{1}H NMR δ 1.27 (s, broad, 12H, –CH(CH_3)$_2$), 3.66 (s, broad, 2H, –C*H*(CH_3)$_2$), 7.10 and 7.35 (d, 4H, $J = 8.6$ Hz, Ar H), 7.12 and 7.97 (d, 4H, $J = 8.6$ Hz, Ar H), 12.87 (s, 1H, –COOH). IR (KBr): $\nu = 2960$ broad, 1720, 1600, 1460, 1230, 1160 cm^{-1}. Found: C, 70.31; H, 6.80; N, 4.10. $C_{20}H_{23}NO_4$ requires: C, 70.36; H, 6.79; N, 4.10%.

N,N-Diisobutyl-4-(4′-carboxyphenoxy)benzamide (**23**)

Synthesised from *N,N*-diisobutyl-4-(4′-formylphenoxy)benzamide (**23a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 57%, white powder, mp 157–159°C. ^{1}H NMR δ 0.69 (s, 6H, –CH(CH_3)$_2$), 0.93 (s, 6H, –CH(CH_3)$_2$), 1.81 (s, broad, 1H, –C*H*(CH_3)$_2$), 2.04 (s, broad, 1H, –C*H*(CH_3)$_2$), 3.11 (s, 2H, –NCH_2–), 3.26 (s, 2H, –NCH_2–), 7.09 and 7.39 (d, 4H, $J = 8.4$ Hz, Ar H), 7.14 and 7.97 (d, 4H, $J = 8.4$ Hz, Ar H), 12.87 (s, 1H, –COOH). IR (KBr): $\nu = 2960$ broad, 1680, 1640, 1500, 1420, 1300, 1230, 1170 cm^{-1}. Found: C, 71.12; H, 7.35; N, 3.84. $C_{22}H_{27}NO_4$ requires: C, 71.52; H, 7.37; N, 3.79%.

N-Phenyl-4-(4′carboxyphenoxy)benzamide (**24**)

Synthesised from *N*-phenyl-4-(4′-formylphenoxy)-benzamide (**24a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 39%, white solid, mp >300°C. ^{1}H NMR δ 7.13 and 7.60 (d, 4H, $J = 8.4$ Hz, Ar H), 7.15 and 7.95 (d, 4H, $J = 8.8$ Hz, Ar H), 7.19–7.35 (m, 5H, Ar H), 8.46 (s, 1H, –NH–), 12.86 (s, 1H, –COOH). IR (KBr): $\nu = 3360$ broad, 1650, 1600, 1500, 1450, 1260 cm^{-1}. Found: C, 72.12; H, 4.47; N, 4.19. $C_{20}H_{15}NO_4$ requires: C, 72.06; H, 4.54; N, 4.20%.

N,N-Diphenyl-4-(4′-carboxyphenoxy)benzamide (**25**)

Synthesised from *N,N*-diphenyl-4-(4′-formylphenoxy)benzamide (**25a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 47%, slightly yellow paste. ^{1}H NMR δ 6.96 and 7.48 (d, 4H, $J = 8.2$ Hz, Ar H), 7.00 and 7.95 (d, 4H, $J = 8.2$ Hz, Ar H), 7.19–7.36 (m, 10H, Ar H), 12.85 (s, 1H, –COOH). IR (KBr): $\nu = 2920$ broad, 1700, 1660, 1600, 1500, 1450, 1350, 1260 cm^{-1}. Found: C, 76.04; H, 4.68; N, 3.44. $C_{26}H_{19}NO_4$ requires: C, 76.27; H, 4.68; N, 3.42%.

N-Adamantyl-4-(4′-carboxyphenoxy)benzamide (**26**)

Synthesised from *N*-adamantyl-4-(4′-formylphenoxy)benzamide (**26a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 64%, white powder, mp 260–261°C. ^{1}H NMR δ 1.65 (s, 6H, ada. H), 2.07 (s, 9H, ada. H), 7.07 and 7.86 (d, 4H, $J = 8.8$ Hz, Ar H), 7.12 and 7.96 (d, 4H, $J = 8.8$ Hz, Ar H), 7.59 (s, 1H, –NH), 12.83 (s, 1H, –COOH). IR (KBr): $\nu = 3400$, 2900, 1720, 1630, 1600, 1500, 1240 cm^{-1}. Found: C, 73.60; H, 6.38; N, 3.64. $C_{24}H_{25}NO_4$ requires: C, 73.64; H, 6.44; N, 3.58%.

N,N-Dicyclohexyl-4-(4′-carboxyphenoxy)benzamide (**28**)

Synthesised from *N,N*-dicyclohexyl-4-(4′-formylphenoxy)benzamide (**28a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 41%, white powder, mp 211–213°C. ^{1}H NMR δ 1.09–1.69 (m, 20H, cyclohexyl-H), 3.17 (s, broad, 2H, –N(CH_2)$_2$–), 7.09 and 7.33 (d, 4H, $J = 8.4$ Hz, Ar H), 7.12 and 7.97 (d, 4H, $J = 8.4$ Hz, Ar H), 12.84 (s, 1H, –COOH). IR (KBr): $\nu = 3400$ broad, 2940, 1710, 1600, 1240 cm^{-1}. Found: C, 73.98; H, 7.36; N, 3.41. $C_{26}H_{31}NO_4$ requires: C, 74.08; H, 7.41; N, 3.32%.

4-(Biphenyl-4′-yloxy)phenylacetic Acid (**29**)

Synthesised from 4-(biphenyl-4′-yloxy)phenylacetaldehyde (**29a**). Purified by recrystallisation (hexane/ethyl acetate). Yield 51%, white powder, mp

161–163°C. ^{1}H NMR δ 3.65 (s, 2H, –CH_2COOH), 7.02 and 7.55 (d, 4H, $J = 8.4$ Hz, Ar H), 7.08 and 7.57 (d, 4H, $J = 8.4$ Hz, Ar H), 7.27 (d, 2H, $J = 7.4$ Hz, Ar H), 7.33 (t, 1H, $J = 7.4$ Hz, Ar H) 7.43 (d, 2H, $J = 7.4$ Hz, Ar H), 12.88 (s, 1H, –COOH). IR (KBr): $\nu = 3000$ broad, 1700, 1600, 1500, 1300, 1270, 1170 cm^{-1}. Found: C, 78.89; H, 5.33. $C_{20}H_{16}O_3$ requires: C, 78.93; H, 5.30%.

Enzyme Inhibition Test

Preparation of Tissue

Rat prostatic enzyme was prepared according to the method of Liang *et al.*[19] with slight modifications.[1] Male rats were sacrificed and prostates were taken within 5 min and put in ice cold 0.9% aqueous 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 dithiothreitol (DTT) were added. For the preparation of type 2 enzyme citrate buffer pH 5.5 was used. The tissue was homogenised by ten 10-s strokes at 20,500 rpm of an ultraturax (IKA) with 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[19] with modifications.[1] 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, type 1) or citrate buffer (40 mM, pH 5.5, type 2) were used. In the case of human enzyme preparation citrate buffer (40 mM, pH 5.5) was used. The incubation mixture contained approximately 250 μg of rat protein (125 μg of 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 (100 μM). The reaction was started by adding the prostatic enzyme preparation and stopped by addition of 50 μL aqueous solution of NaOH (10 M). The steroids were extracted using 500 μL of diethylether. The mixture was shaken for 10 min and centrifuged for 10 min at 4000 rpm. The water layer was frozen and the ether layer was decanted into fresh tubes and evaporated to dryness.

Human Type 1 Inhibition: DU145-assay[20,21]

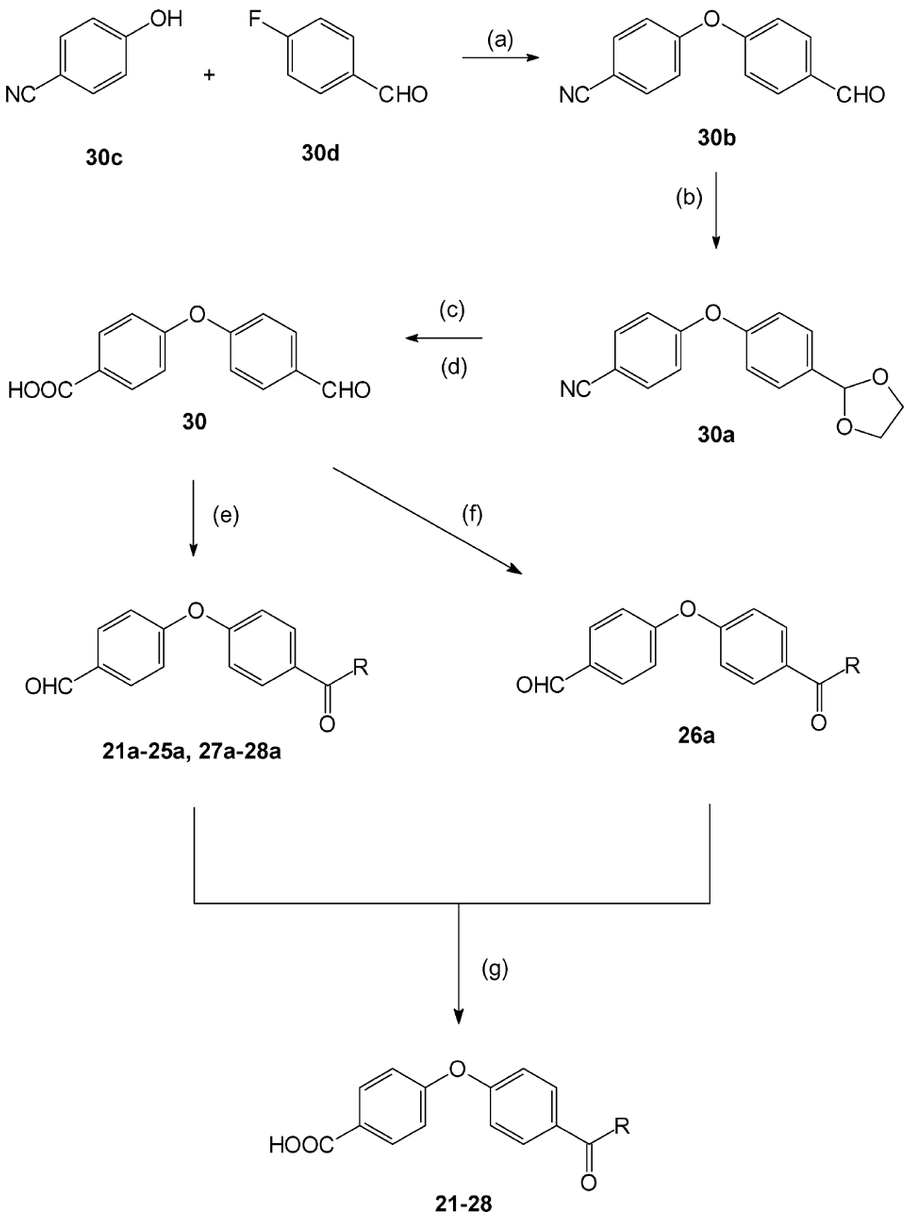
Intact human prostatic carcinoma DU145 cells were used as the source of type 1 5α-reductase.[22] The inhibitory potencies of the compounds were determined by monitoring the conversion of the tritiated substrate androstenedione (5 nM) to androstanedione during an incubation period of 6 h. A day before 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 choosen 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 6 h incubation period in 5% CO_2 at 37°C the medium samples were extracted twice with 1 mL of 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 carried out[1] was similar to the method of Cook *et al.*[23] 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 scintillant (quickszint flow 302) base-line-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) value (cv_{corr} and iv_{corr}). Inhibition (I) was calculated using the following equation: $\% I = (1 - iv_{corr}/cv_{corr})100$.

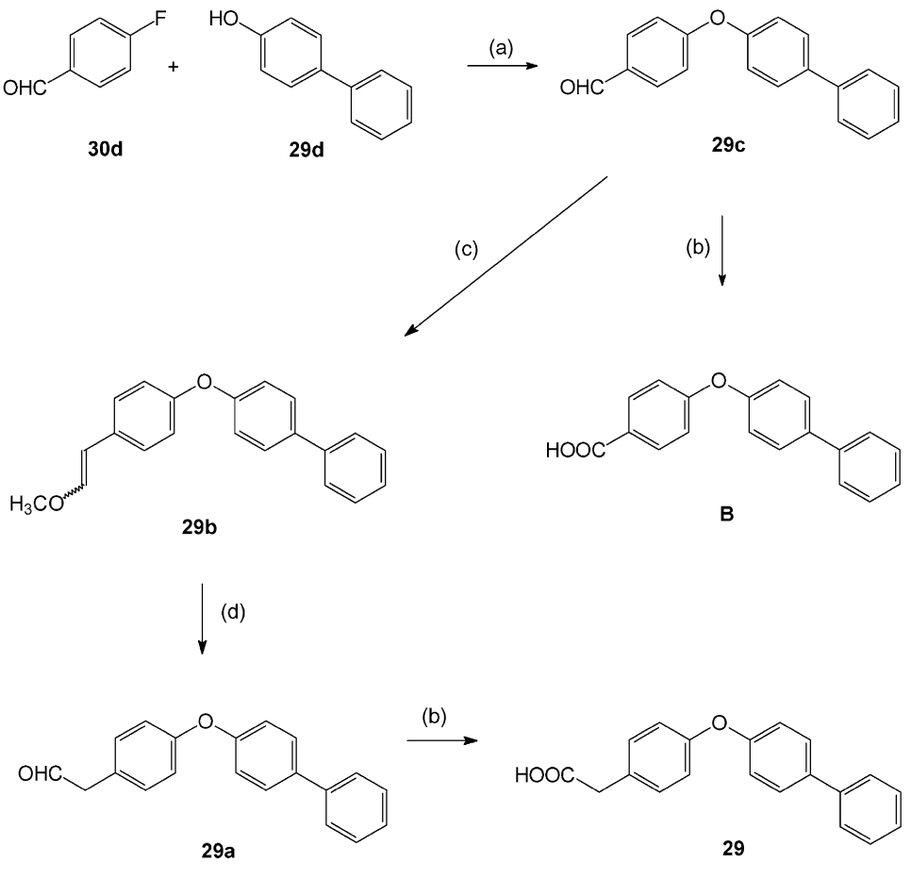


SCHEME 1 Synthesis of compounds **21**–**28**, **30**. Reagents and conditions: (a) DMF, K_2CO_3, 160°C, 6 h; (b) ethylene glycol, CSA, toluene, reflux, 10 h; (c) KOH, H_2O/ethylene glycol, reflux, 18 h; (d) HCl (10N), reflux, 3 h; (e) oxalyl chloride, CH_2Cl_2, rt, 12 h, then HNR_1R_2, Et_3N, 2 h; (f) Mukaiyama's reagent, HNR_1R_2, Et_3N, 2 h; (g) $NaClO_2$, NaH_2PO_4, H_2O_2 (35%), THF, rt, 12 h.

RESULTS

Chemistry

The first step in the synthesis of compounds **21**–**28** was the preparation of 4-(4′-formylphenoxy)benzonitrile **30b** from commercially available 4-hydroxybenzonitrile **30c** and 4-fluorobenzaldehyde **30d** as described[17] (Scheme 1). The formyl moiety was protected to yield acetal **30a** before hydrolysing the nitrile to a carboxylic acid. Cleavage of the acetal under acidic conditions led to 4-(4′-formylphenoxy)benzoic acid **30**. Two methods were employed to activate the carboxylic acid for the preparation of the different substituted amides **21a**–**28a**. The amides **21a**–**25a** and **27a**–**28a** could be prepared from the acid chloride, which in turn was made from the acid and oxalyl chloride, and the corresponding amines. Amide **26a** was synthesised from the acid directly using the Mukaiyama reagent[24] (2-chloro-1-methylpyridinium iodide). The resulting aldehydes **21a**–**28a** were subjected to a Lindgren oxidation[25] to obtain the title compounds **21**–**28**. Compound **29** was synthesised in a similar way using 4-hydroxybiphenyl **29d** and 4-fluorobenzaldehyde **30d**



SCHEME 2 Synthesis of compounds **B**, **29**. Reagents and conditions: (a) DMSO, K_2CO_3, 160°C, 6 h; (b) $NaClO_2$, NaH_2PO_4, H_2O_2 (35%), THF, rt, 12 h; (c) *n*-BuLi (1.6 M), $Ph_3PCH_2OCH_3^+$ Cl^-, THF, −78°C, then rt overnight; (d) THF, H_2O/H_2SO_4, reflux, 10 h.

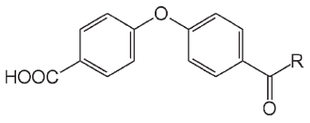
(Scheme 2). The resulting 4-(biphenyl-4′-yloxy) benzaldehyde **29c** was reacted with methoxymethyl-triphenyl-phosphonium chloride to yield the homologue **29a** via the enol ether **29b** (mixture of *E/Z* isomers). The aldehyde **29a** was oxidised under Lindgren conditions[25] to carboxylic acid **29**. Direct oxidation of **29c** gave Igarashi's compound (**B**,[15] Figure 1).

Inhibitory Activity

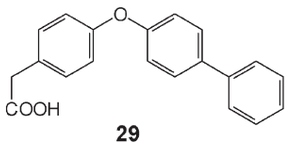
The inhibitory activities of compounds **21**–**30**, **B** and **finasteride** as a reference were determined using rat prostate homogenates (pH 6.6, type 1; pH 5.5, type 2) and human prostate homogenate (BPH tissue for type 2) according to the method of Liang *et al.*,[19] and the DU145 cell line (for human type 1 enzyme) as described in the literature.[20–22] The percent inhibition values at a concentration of 10 μM or, in the case of more potent compounds, the IC_{50} values are presented in Table I.

As expected the nature and the bulkiness of the substituents at the amide nitrogen have a clear impact on the inhibitory activities. In human type 2 isozyme the *tert*-butyl substituted compound **21** displayed a moderate inhibitory activity with an IC_{50} value of 2.8 μM. It is striking that more bulky and flexible substituents such as diisopropyl (**22**) or diisobutyl (**23**) significantly decreased inhibitory activity to 36% and 46% inhibition respectively (at a concentration of 10 μM). The cyclohexyl compound **27** showed a stronger potency than the former compounds with an IC_{50} value of 2.3 μM. However, the difference between **21** and **27** is not significant. On the other hand, compound **28**, bearing two cyclohexyl substituents, displayed a weaker inhibitory activity ($IC_{50} = 6.7$ μM) and was as active as the "unsubstituted" compound 4-(4′-formylphenoxy)benzoic acid **30** ($IC_{50} = 7$ μM). However, the inhibitor bearing an adamantyl substituent (**26**), the bulkiness of which is between the cyclohexyl and the dicyclohexyl group, showed the strongest inhibitory activity in this class of compounds ($IC_{50} = 380$ nM). Also, the phenyl and diphenyl substituted derivatives **24** and **25** were rather good inhibitors with IC_{50} values of 1 and 0.85 μM, respectively. All non-steroidal compounds **21**–**30** and **B** were not active as inhibitors of the human type 1 isozyme. Using the rat enzyme none of the compounds was highly

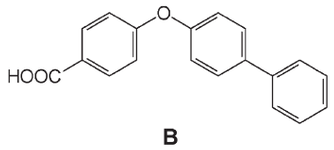
TABLE I Inhibition of rat and human 5α-reductase type 1 and 2 *in vitro* by compounds **21**–**30**, **B** and **finasteride**



21-28, 30



29



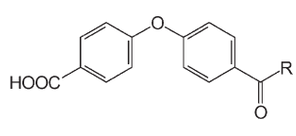
B

R	Compound	RVP*: % inhibition (10 μM); [IC_{50}, (μM)] Type 1†	Type 2†	Human: % inhibition (10 μM); [IC_{50}, (μM)] DU 145†,‡,¶	BPH†,§
*N*HC$(CH_3)_3$	**21**	5	26	n. i.	[2.8]
N(iPr)$_2$	**22**	4	n. i.	4	36
N(iBu)$_2$	**23**	18	22	n. i.	46
*N*H(Phenyl)	**24**	22	39	n. i.	[1.0]
N(Phenyl)$_2$	**25**	[4.5]	[4.6]	n. i.	[0.85]
*N*H(Adamantyl)	**26**	[8.2]	[8.0]	n. d.	[0.38]
*N*H(Cyclohexyl)	**27**	17	35	n. i.	[2.3]
N(Cyclohexyl)$_2$	**28**	[4.1]	[10]	4	[6.7]
-	**29**	29	[9.6]	n. i.	[0.06]
H	**30**	7	16	n. d.	[7.0]
-	**B**	21	15	n. d.	[0.006]
Finasteride		[0.010]	[0.010]	[0.41]	[3–4 nM]

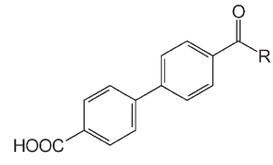
* Enzyme of rat ventral prostate, 250 μg protein, substrate [1β, 2β-^{3}H] testosterone 0.21 μM. † Mean value; tests have been run in duplicate. The standard deviation for IC_{50} is 20%, for percent inhibition it is ± 10%. ‡ Substrate: [^{3}H] androstenedione 5 nM. ¶ Prostatic tumour cell line expressing type 1 enzyme. § Enzyme from BPH tissue (type 2), 125 μg protein, substrate [1β, 2β-^{3}H] testosterone, 0.21 μM. n. i.: no inhibition, n. d.: not determined.

active, either for type 1 or type 2 isozyme. The most active inhibitor was the diphenyl compound **25** which displayed a profile as a weak dual inhibitor with IC_{50} values of 4.5 and 4.6 μM towards rat type 1 and type 2 isozyme respectively. The reference compound **B** showed a strong inhibitory activity toward the human type 2 enzyme (IC_{50} = 6 nM). Structural modification by insertion of a methylene spacer decreased activity (compound **29**).

TABLE II Inhibition of rat and human 5α-reductase type 1 and 2 *in vitro* by compounds **22**, **23**, **25**, **26**, **28** and corresponding type **A** compounds (**11**–**15**)



22-23, **25**, **26**, **28**



11-15

R	Compound	RVP*: % inhibition (10 μM); [IC_{50}, (μM)] Type 1†	Type 2†	Human: % inhibition (10 μM); [IC_{50}, (μM)] DU 145†,‡,¶	BPH†,§
N(iPr)$_2$	**22**	4	n.i.	4	36
	11	4	10	9	15
N(iBu)$_2$	**23**	18	22	n. i.	46
	12	24	37	16	[4.0]
N(Phenyl)$_2$	**25**	[4.5]	[4.6]	n. i.	[0.85]
	13	[0.46]	[3]	28	[2.33]
*N*H(Adamantyl)	**26**	[8.2]	[8]	n. d.	[0.38]
	15	[5.6]	[10]	16	[1.9]
N(Cyclohexyl)$_2$	**28**	[4.1]	[10]	n. i.	[6.7]
	14	[1.4]	30	44	[4.7]

* Enzyme of rat ventral prostate, 250 μg protein, substrate [1β, 2β-^{3}H] testosterone 0.21 μM. † Mean value; tests have been run in duplicate. The standard deviation for IC_{50} is 20%, for percent inhibition it is ± 10%. ‡ Substrate: [^{3}H] androstenedione 5 nM. ¶ Prostatic tumour cell line expressing type 1 enzyme. § Enzyme from BPH tissue (type 2), 125 μg protein, substrate [1β, 2β-^{3}H] testosterone, 0.21 μM. n. i.: no inhibition; n. d.: not determined.

DISCUSSION

Looking for a structure-activity relationship it becomes apparent that the aromatic derivatives **24** and **25** are more active than their aliphatic counterparts **27** and **28** (IC_{50} **24/27**: 1/2.3 μM; **25/28**: 0.85/6.7 μM). This finding suggests that aromatic substituents at the amide nitrogen are advantageous. The finding that the adamantyl inhibitor (**26**) is more active than both the cyclohexyl and the dicyclohexyl compound supports the hypothesis that a bulky substituent shows a greater inhibitory potency but, at the same time, implies that there is an upper limit for the bulkiness. Furthermore it becomes clear that secondary amides (**21**, **24**, **26**, **27**), show a better inhibitory activity than tertiary amides (**22**, **23**, **28**). The only exception to this rule is compound **25**. The use of a secondary amide bearing a bulky aromatic substituent seems to be the most promising modification to obtain potent amides. This finding is in accordance with steroidal and non-steroidal amide type inhibitors described by others.[26–33]

However, it should be noticed that exchange of the substituted amide moieties by a simple phenyl group (compound **B**) leads to an increase in inhibitory potency which is again diminished by insertion of a methylene spacer (compound **29**).

Comparing the amides synthesised in this paper with the corresponding biphenyl (type **A**) compounds, it becomes apparent that in most cases the inhibitory activities towards the human type 2 enzyme could be enhanced. In the case of compound **26** this was by a factor of 5 (Table II: IC_{50} values **26**: 0.38 μM, **15**: 1.9 μM).

Concerning the human and rat type 1 isozyme the structural modifications led to a decrease in inhibitory potency (**22**, **23**, **25**, **26**, **28** vs. **11**, **12**, **13**, **15**, **14** respectively). For rat type 2 isozyme however the results were somewhat different. In fact, the inhibitory activities of compounds bearing bulky substituents (**26**, **28**) were higher than those of the corresponding biphenyls (**15**, **14**).

In conclusion, we have further optimised the type **A** compounds for human 5α-reductase type 2 inhibition. This series of compounds might contribute to a better understanding of the structural features required for strong inhibition of the main isozyme involved in BPH.

Acknowledgements

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