Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 2441–2448

BIOORGANIC & MEDICINAL CHEMISTRY

Indole and Benzimidazole Derivatives as Steroid 5α-Reductase Inhibitors in the Rat Prostate

Hitoshi Takami,† Nobuyuki Kishibayashi, Akio Ishii‡ and Toshiaki Kumazawa*

Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd, Nagaizumi, Shizuoka 411-8731, Japan

Received 30 June 1998; accepted 25 August 1998

Abstract—A novel series of indole and benzimidazole derivatives were synthesized and evaluated for their inhibitory activity of rat prostatic 5α-reductase. Among these compounds, 4-{2-[1-(4,4′-dipropylbenzhydryl)indole-5-carboxamido]phenoxy}butyric acid (**15**) and its benzimidazole analogue **25** showed potent inhibitory activities for rat prostatic 5α-reductase (IC_{50} values of 9.6 ± 1.0 and 13 ± 1.5 nM, respectively), with the potency very close to that of finasteride. Compound **30**, in which the moiety between the benzene ring and amide bond was replaced by quinolin-4-one ring, showed almost equipotent activity ($IC_{50} = 19 \pm 6.2$ nM) with the correspondent amide derivative **13**. This result was consistent with the previous observation that the coplanarity of this moiety might contribute to the potent inhibitory activity.

Introduction

Steroid 5α-reductase is an NADPH-dependent enzyme which catalyzes the reduction of testosterone (T) to dihydrotestosterone (DHT). Inhibition of 5α-reductase is attractive for therapeutic intervention in disorders associated with elevated levels of DHT such as benign prostatic hyperplasia (BPH),[1] acne,[2] male pattern baldness,[3] and hirsutism.[4] Among numerous steroidal 5α-reductase inhibitors reported[5] during the last decade, MK-906 (**1**, finasteride)[5b] has been studied extensively. Although this azasteroid demonstrated clinical efficacy in BPH patients, it also was associated with sexual dysfunctions.[6] Among the steroidal inhibitors, several azasteroids were reported to express the antiandrogenic acitivity.[5b,e] We considered this adverse effect by azasteroids as due to the nature of steroidal skeleton. To develop specific 5α-reductase inhibitors, we designed a novel series of compounds, in which the steroidal skeleton was excluded. Several nonsteroidal compounds have recently been identified as inhibitors of 5α-reductase.[7] However, when we started our research program, **2**, (ONO-3805)[7a] was the only compound reported as a nonsteroidal 5α-reductase inhibitor. Consequentially, we began our research by designing nonsteroidal inhibitors analogous to **2** and successfully discovered a novel series of indole derivatives **3**, exemplified KF18678 (Fig. 1).[8a] During the course of structure–activity relationship (SAR) studies of the indole derivatives, we concluded the coplanarity between the benzene ring and amide moiety was a critical structural requirement for inhibitory activity. We also revealed that the spatial arrangement of the substituent at the position 1 of indole and the link unit between indole skeleton and amide bond were important.[8b] In this paper, considering the above results of parent series, we describe further investigations on SARs of a novel series. Molecular superposition suggested that indole-5-carboxanilide overlaps quite well with the substructure of **2** such that the phenolic oxygen atom corresponds to the nitrogen atom of indole. From this point of view, we considered it was valuable to synthesize indole (**4**) and benzimidazole (**5**) carboxanilides deleting the link unit between indole skeleton and amide moiety of parent indole derivatives (**3**).

Key words: Enzyme inhibitors; substituent effects; 5α-reductase; indole.

*Corresponding author.

†Present address, Kyowa Hakko Kogyo Co., Ltd, 6-1 Ohtemachi, 1-chome Chiyoda-ku, Tokyo 100-8185, Japan.

‡Present address, Toxicological Research Laboratories, Kyowa Hakko Kogyo Co., Ltd, 2548 Fujimagari, Ube, Yamaguchi 755-8501, Japan.

0968-0896/98/$ - see front matter
PII: S0968-0896(98)00176-X

Chemistry

The general synthetic route is shown in Scheme 1. Indole-5-carboxylic acid **6** was condensed with aniline **7**[7a] using Mukaiyama reagent[9] to afford compound **8**. Alkylation on indole nitrogen of **8** was achieved by treatment with alkyl bromides using *t*-BuOK to afford 1-alkylindoles, which were hydrolyzed to give compounds **10–17**. An alternative method was employed when branched alkyl groups were introduced as R^1. Compounds **18** and **19** were obtained by the reaction of **8** with alkyl tosylates using KOH in DMSO,[10] with the simultaneous hydrolysis of ethyl ester. In the case of benzoyl group, compound **8** was hydrolyzed to **9**, which was treated with 4-isobutylbenzoyl chloride to afford **20**.

Benzimidazole derivatives were obtained by the route depicted in Scheme 2. Alkylation of benzimidazole-5-carboxylic acid **21** afforded a 1:1 mixture of benzimidazole 5- and 6-carboxylic acids **22** and **23**, which were separable by silica gel column chromatography. The resulting benzimidazole 5- and 6-carboxylic acids were condensed with aniline **7** to give anilides, which were hydrolyzed to afford **25** and **26**, respectively. Compound **22** was selectively prepared from 4-chloro-3-nitro benzoic acid methyl ester **24** in four steps: condensation with 4,4′-dipropylbenzhydryl amine, hydrogenation, benzimidazole formation, and hydrolysis.

Quinolin-4-one derivative, in which the moiety between benzene ring and amide bond was replaced by planar

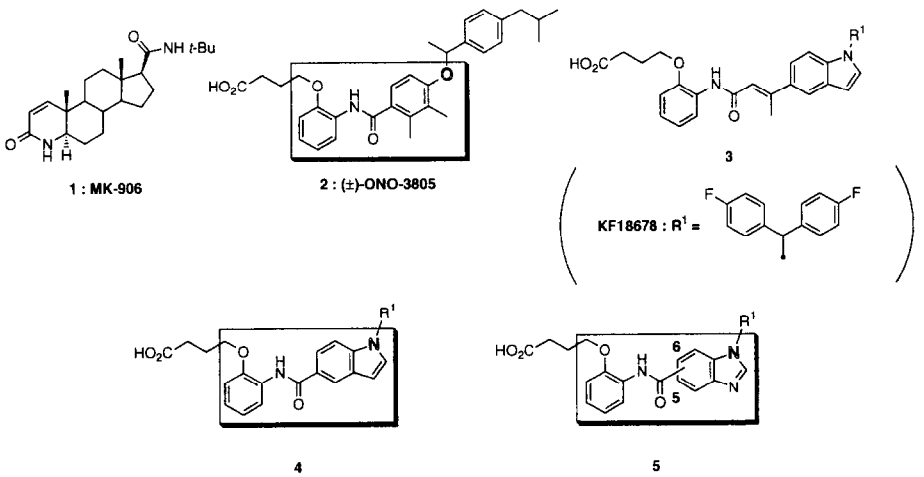
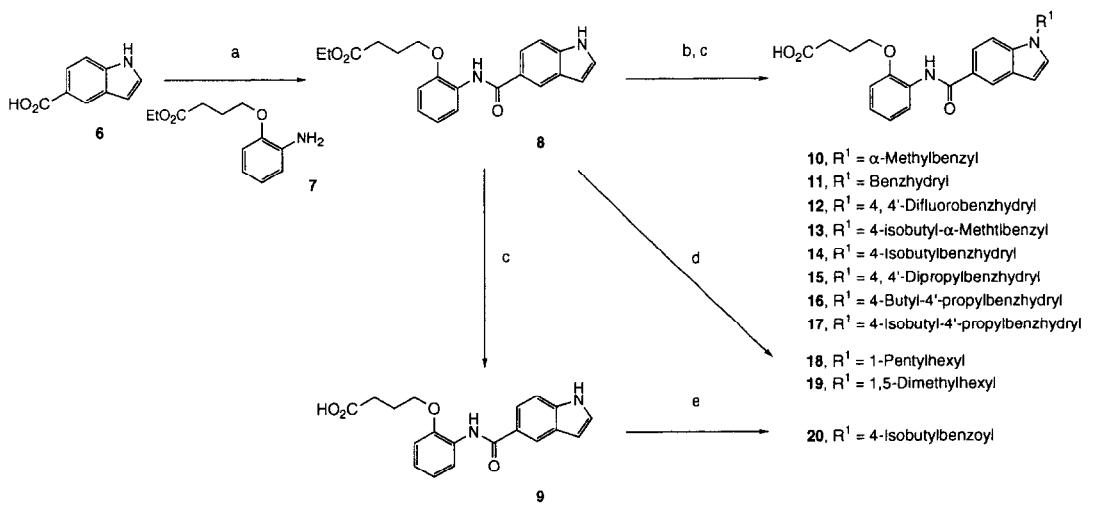


Figure 1.



Scheme 1. (a) 2-Chloro-1-methylpyridinium iodide, NBu_3/CH_2Cl_2; (b) R^1-Br, *t*-BuOK/DMF; (c) NaOH/aq. EtOH; (d) R^1-OTs, KOH/DMSO; (e) *t*-BuOK, 4-isobutylbenzoyl chloride/DMF.

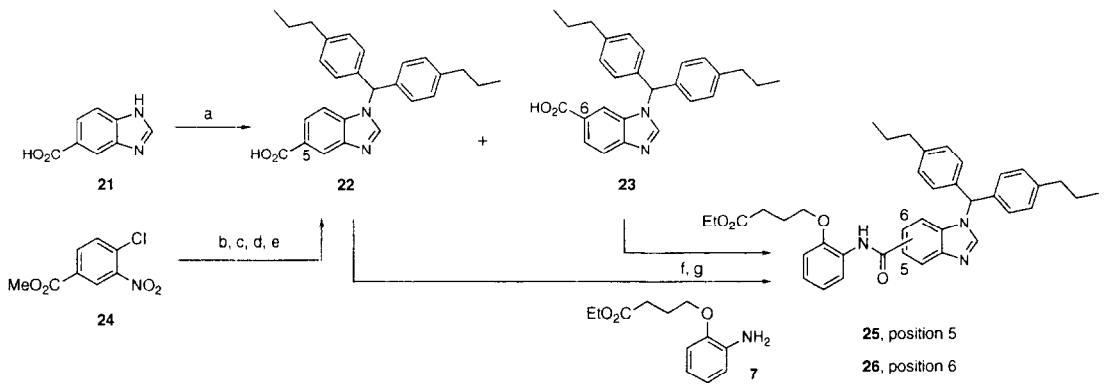
six-membered ring, was synthesized by the method shown in Scheme 3. Compound **28**, obtained by condensation of indole-5-carboxylic acid **6** with aniline **27**,[11] was cyclized through intramolecular aldol condensation using NaOMe in EtOH with hydrolysis of ethyl ester to give **29**. Nitrogen of indole was alkylated by the method described above to afford compound **30**.

Results and Discussion

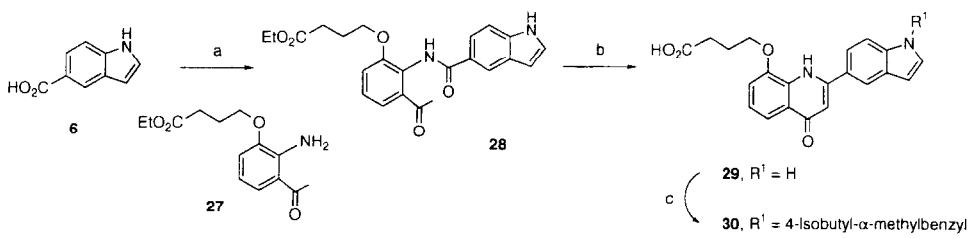
The compounds were evaluated for their ability to inhibit rat prostatic 5α-reductase. The inhibitory activity was expressed as percent inhibition at 100 nM or as an IC_{50} value. The results of 5α-reductase assays are presented in Table 1, with **1**, **2** and **3** included for comparison.

Initially, the effect of the several types of substituents at nitrogen of indole were investigated. Compound **11** possessing benzhydryl as substituent R^1, expressed moderate inhibitory activity (55% inhibition at 100 nM). On the other hand, non-substituted (**9**) or α-methylbenzyl substituted (**10**) compounds remarkably reduced inhibitory activity. Compound **13** possessing the same substructure as **2**, exhibited a moderate inhibitory activity ($IC_{50} = 31 \pm 5.0$ nM), but was less potent in comparison with **2** ($IC_{50} = 2.5 \pm 0.033$ nM). Though reasons of the dropped potency of **13** are not clear, subtle changes in structural arrangement or electronic nature of connecting heteroatoms may be affected. Branched alkyl substituents at the position 1 of indole decreased potency (**18** and **19**). Introduction of 4-isobutylbenzoyl (**20**), whose size was considered as large as 4-isobutyl-α-methylbenzyl, resulted in vanishing activity. We supposed that the benzoyl substituent is insufficient in regard to a certain spatial arrangement of R^1 required for potent activity possibly due to the planar structure. Compared with the derivatives possessing the same substituent at position 1 of indole, amide derivatives **10**, **11** and **18** were apparently less potent compared with the parent isocrotonoylamide series.[8a]

Next, we focused on introducing substituents on the benzhydryl group. Fluorine substitution at 4 and/or 4′ positions of the benzhydryl unexpectedly reduced potency (**12** versus **11**), whereas introduction of a lower alkyl group at this position resulted in enhanced activity (**14–17**). Among these compounds, isobutyl and propyl substituted derivative **17** showed the most potent inhibitory activity, with IC_{50} value of 6.3 ± 0.82 nM, which



Scheme 2. (a) 4,4′-Dipropylbenzhydryl bromide, *t*-BuOK/DMF; (b) 4,4′-dipropylbenzhydryl amine/DMSO; (c) PtO_2, H_2/EtOH; (d) $HC(OEt)_3/HCO_2H$; (e) NaOH/aq. EtOH; (f) 2-chloro-1-methylpyridinium iodide, NBu_3/CH_2Cl_2; (g) NaOH/aq. EtOH.



Scheme 3. (a) 2-Chloro-1-methylpyridinium iodide, NBu_3/CH_2Cl_2; (b) NaOMe/EtOH; (c) 4-isobutyl-α-methylbenzyl bromide, *t*-BuOK/DMF.

Table 1. Rat prostatic 5α-reductase inhibitory activity

Compd	Formula[a]	mp, °C	Solvent[b]	% Inhibn. at 100 nM[c]	IC_{50} (nM)[d]
9	$C_{19}H_{18}N_2O_4 \cdot 0.25H_2O$	148–150	HX-EA[e]	3.7%	—
10	$C_{27}H_{25}N_2NaO_4 \cdot 0.5C_2H_6O$[f]	Amorphous	—	16%	—
11	$C_{32}H_{28}N_2NaO_4$	82–87	IPE	55%	—
12	$C_{32}H_{26}F_2N_2O_4 \cdot 0.5C_2H_2O$	168–170	IPE	32%	—
13	$C_{31}H_{34}N_2O_4$	62–67	IPE	73%	31 ± 5.0
14	$C_{36}H_{36}N_2O_4 \cdot 0.25H_2O$	86–88	IPE	93%	24 ± 2.9
15	$C_{38}H_{40}N_2O_4$	61–64	IPE	87%	9.6 ± 1.0
16	$C_{39}H_{42}N_2O_4 \cdot H_2O$	87–89	IPE	88%	32 ± 8.1
17	$C_{39}H_{42}N_2O_4 \cdot 0.5H_2O$	95–100	IPE	93%	6.3 ± 0.82
18	$C_{30}H_{39}N_2NaO_4 \cdot 1.5H_2O$	Amorphous	—	40%	—
19	$C_{27}H_{33}N_2NaO_4 \cdot C_2H_4O_2$[g] $\cdot 0.5H_2O$	Amorphous	—	35%	—
20	$C_{30}H_{30}N_2O_5$	124–125	IPE	24%	—
25	$C_{37}H_{39}N_3O_4 \cdot 0.5H_2O$	82–85	IPE	66%	13 ± 1.5
26	$C_{37}H_{39}N_3O_4 \cdot 0.5H_2O$	72–74	IPE	11%	—
30	$C_{33}H_{34}N_2O_4 \cdot 0.5H_2O$	Amorphous	—	72%	19 ± 6.2
1					10 ± 1.8
2					2.5 ± 0.003
3					3.3 ± 0.23

[a]All new compounds had C, H, N microanalyses within 0.4% of theoretical values.
[b]Solvent of trituration: HX, hexane; EA, ethyl acetate; IPE, diisopropyl ether.
[c]Prostate from male rats (type 1).
[d]IC_{50} values are means ± SE three separate experiments.
[e]Solvent for recrystallization.
[f]C_2H_6O; ethanol.
[g]$C_2H_4O_2$; acetic acid.

is comparable with that of finasteride (**1**; $IC_{50} = 10 \pm 1.8$ nM). Butyl and propyl derivative **16** showed somewhat reduced activity. This result indicates a bulk limitation of substituents on benzhydryl. In the parent isocrotonoyl series, introduction of lower alkyl substituents on benzhydryl resulted in decreased potency.[8a] From these results, introducing a bulky substituent at position 1 of the indole appears to be required for enhancing activity, instead of deleting the link unit of the parent indole derivatives.

Benzimidazole derivatives were also synthesized and evaluated for their inhibitory activities. 5-Amide derivative **25** exhibited almost the same potency ($IC_{50} = 13 \pm 1.5$ nM) as the corresponding indole derivative **15**, whereas 6-amide **26** significantly reduced the potency. This result indicates 1,5-arrangement between the bulky substituents and amide moiety of benzimidazole is essential for the inhibitory activity.

The 5α-reductase inhibitory activities of several analogues of **2** were reported.[7a] Among them, 4,4′-dipropylbenzhydryl dimethylphenoxy derivative also showed potent inhibitory activity ($IC_{50} = 0.011$ μM), although the replacement of the connecting heteroatom, O by S or NH drops the potency to 0.42 μM and 2.8 μM, respectively.[7a] On the other hand, the corresponding indole and benzimidazole derivatives (**15** and **25**) exhibited retained potency in comparison with dimethylphenoxy derivative. These observations added positive characteristics of novel indole and benzimidazole derivatives.

Quinolin-4-one derivative **30** inhibited rat prostatic 5α-reductase with IC_{50} value of 19 ± 6.2 nM that was almost the same inhibitory activity as the corresponding amide derivative **13**. This result is consistent with the previous observation that the planar structure between benzene ring and amide moiety is necessary for exhibiting a potent 5α-reductase inhibitory activity.

Several potent compounds were evaluated for inhibitory effect on DHT production in vivo.[12] Benzimidazole derivative **25** significantly inhibited DHT production at 30 mg/kg, po administration in rat (48% conversion from T versus 65% in control), whereas corresponding indole derivative **15** did not.

In conclusion, we synthesized indole and benzimidazole derivatives deleting the link unit between indole skeleton and amide moiety of parent indole derivatives **3** and evaluated them for 5α-reductase inhibitory activity. Several indole and benzimidazole derivatives showed the potent inhibitory activity with IC_{50} of 10^{-8}–10^{-9} M order by introducing the bulky substituents at the posi-

tion 1. Quinolin-4-one derivative **30** also possessed moderate activity. On the basis of the SARs presented above, further investigations of the benzofused-heteroaromatics in place of dimethylphenoxy moiety of **2** appears promising for more potent 5α-reductase inhibitors.

Experimental Section

Melting points were determined with a Büchi-510 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Jasco IR-810 spectrometer. Proton nuclear resonance spectra (^{1}H NMR) were recorded on a Hitachi R-90H (90 MHz) or a JEOL JNM GX-270 or EX-270 (270 MHz) spectrometer with Me_4Si as internal standard. Elemental analyses were performed by the analytical department of our laboratories.

General procedure for preparation of anilide and hydrolysis. Ethyl 4-[2-(indole-5-carboxamido)phenoxy]-butyrate (8) and -butyric acid (9). To a mixture of ethyl 4-(2-aminophenoxy)butyrate **7** (2.8 g, 13 mmol), 2-chloro-1-methylpyridinium iodide (1.9 g, 7.7 mmol), and tributylamine (3.6 mL, 15 mmol) in 20 mL of CH_2Cl_2 was added under reflux a suspension of indole-5-carboxylic acid **6** (1.0 g, 6.2 mmol) in 10 mL of CH_2Cl_2 and the mixture was stirred at reflux for 1 h. After addition of water, the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with 1 N HCl, water, and brine, dried, and evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt:triethylamine (10:10:1) to afford **8** (1.8 g, 80%) as a yellow powder: mp 125–126 °C; IR (KBr) 3414, 1730, 1651, 1599, 1451 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, J = 7.2 Hz), 2.03–2.32 (m, 2H), 2.46–2.62 (m, 2H), 4.06 (q, 2H, J = 7.2 Hz), 4.12 (t, 2H, J = 5.9 Hz), 6.60–6.64 (m, 1H), 6.83–7.08 (m, 3H), 7.22–7.27 (m, 1H), 7.43 (d, 1H, J = 8.6 Hz), 7.76 (dd, 1H, J = 1.7 Hz and 8.6 Hz), 8.23 (s, 1H), 8.51–8.61 (m, 1H), 8.67 (br, 1H). A mixture of the obtained ethyl ester **8** (1.8 g, 5.0 mmol), 1.5 mL of 10 N NaOH, and 50 mL of EtOH was stirred at room temperature for 3 h. The mixture was evaporated in vacuo and the residue was dissolved in 50 mL of water. The mixture was acidified with 4 N HCl to pH 4 and extracted with AcOEt. The organic layer was washed with brine, dried, and evaporated in vacuo to afford crude **9**. This was recrystallized from EtOH to give pure **9** (1.55 g, 92%) as brown crystals: mp 148–150 °C; IR (KBr) 3276, 2930, 1702, 1649, 1600, 1521, 1449, 1252, 1054, 739 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.10–2.35 (m, 2H), 2.54 (t, 2H, J = 7.1 Hz), 4.13 (t, 2H, J = 6.2 Hz), 6.64 (d, 1H, J = 3.2 Hz), 6.90 (dd, 1H, J = 2.2 Hz and 7.4 Hz), 7.00 (dd, 1H, J = 2.2 and 7.6 Hz), 7.00–7.10 (m, 1H), 7.25 (d, 1H, J = 3.2 Hz), 7.42 (d, 1H, J = 8.6 Hz), 7.71 (dd, 1H, J = 1.8 Hz and 8.6 Hz), 8.22 (d, 1H, 1.8 Hz), 8.48–8.52 (m, 1H); Anal. ($C_{19}H_{18}N_2O_4 \cdot 0.25H_2O$) C, H, N.

4-{2-[1-(α-Methylbenzyl)indole-5-carboxamido]phenoxy}-butyric acid (10). To a solution of **8** (1.3 g, 3.8 mmol) in 25 mL of DMF was added portionwise *t*-BuOK (0.47 g, 4.2 mmol) at 0 °C and the mixture was stirred for 30 min. A solution of 2-phenethylbromide (0.62 mL, 4.5 mmol) in 5 mL of DMF was added dropwise to the reaction mixture at 0 °C. The mixture was stirred at 0 °C for 1 h. After addition of water, the resulting mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried and evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt (2:1) to afford ethyl 4-{2-[1-(α-methylbenzyl)indole-5-carboxamido]phenoxy}butyrate (1.4 g, 85%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.0 Hz), 1.87 (d, 3H, J = 7.0 Hz), 2.08–2.32 (m, 2H), 2.41–2.58 (m, 2H), 4.02 (q, 2H, J = 7.0 Hz), 4.06 (t, 2H, J = 7.0 Hz), 5.64 (d, 1H, J = 7.0 Hz), 6.66 (d, 1H, J = 3.1 Hz), 6.78–7.34 (m, 10H), 7.70 (dd, 1H, J = 1.6 Hz and 8.6 Hz), 8.24 (d, 1H, J = 1.6 Hz), 8.48–8.64 (m, 1H), 8.63 (br, 1H). By the same procedure described above, ethyl ester was converted into carboxylic acid **10** (1.3 g, 92%) as an amorphous solid: IR (KBr) 3428, 2960, 2252, 1706, 1663, 1604, 1456, 1355, 1180, 908 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.86 (d, 3H, J = 7.0 Hz), 2.04–2.27 (m, 2H), 2.53 (t, 2H, J = 7.3 Hz), 4.08 (t, 2H, J = 5.8 Hz), 5.62 (q, 2H, J = 7.0 Hz), 6.65 (d, 1H, J = 3.3 Hz), 6.78–7.31 (m, 10H), 7.67 (dd, 1H, J = 1.5 Hz and 8.6 Hz), 8.20 (d, 1H, J = 1.3 Hz), 8.45–8.72 (m, 1H), 8.61 (br, 1H), 8.87 (br, 1H). To a solution of the obtained carboxylic acid **10** (1.3 g, 2.6 mmol) in 20 mL of MeOH was added NaOMe (28% in MeOH; 0.5 mL, 2.6 mmol) and the resulting mixture was evaporated in vacuo. The residue was triturated with diisopropylether and corrected by filtration to afford sodium salt of **10** (1.2 g, 95%) as an amorphous solid: Anal. ($C_{27}H_{25}N_2NaO_4 \cdot 0.5EtOH$) C, H, N.

4-{2-[1-(1-Pentylhexyl)indole-5-carboxamido]phenoxy}-butyric acid (18). To a suspension of **8** (0.5 g, 1.4 mmol) and KOH (80%; 0.57 g, 8.1 mmol) in 5 mL of DMSO, a solution of 1-pentylhexyl *p*-toluenesulfonate (1.3 g, 4.2 mmol) in 2 mL of DMSO was added dropwise over the period of 15 min. After being stirred at room temperature for 2 h, water was added. The reaction mixture was neutralized (pH 7) with 4 N HCl and extracted with AcOEt. The organic layer was washed with water and brine, dried and evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt:AcOH (10:10:1) to afford **18** (0.51 g, 76%) as a colorless oil: IR (KBr) 3424, 2928, 2856, 1657, 1454, 1118 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.74–0.86 (m, 6H), 1.00–1.35 (m, 12H), 1.80–1.87 (m, 4H), 2.06–2.34 (m, 2H), 2.61 (t, 2H, J = 7.3 Hz), 4.10–4.40 (m, 1H), 4.14 (t,

2H, J=6.1 Hz), 6.65 (d, 1H, J=3.1 Hz), 6.93–7.23 (m, 4H), 7.41 (d, 1H, J=9.0 Hz), 7.76 (dd, 1H, J=1.5 Hz and 9.0 Hz), 8.19 (d, 1H, J=1.5 Hz), 8.45–8.64 (m, 3H). By the same manner described above, this oil was converted into the sodium salt (0.52 g, 98%) as an amorphous solid: Anal. ($C_{30}H_{39}N_2NaO_4 \cdot 1.5H_2O$) C, H, N.

4-{2-[1-(4-Isobutylbenzoyl)indole-5-carboxamido]phenoxy}butyric acid (20). To a solution of **9** (1.0 g, 3.0 mmol) in 20 mL of DMF was added portionwise *t*-BuOK (1.0 g, 8.9 mmol) at 0 °C and the mixture was stirred for 30 min. A solution of 4-isobutylbenzoyl chloride in 10 mL of DMF, prepared from 4-isobutylbenzoic acid (0.63 g, 3.6 mmol) in the usual manner with $SOCl_2$, was added dropwise to the reaction mixture at 0 °C over the period of 1 h. After addition of water, the resulting mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried and evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt:AcOH (10:10:1) to afford **20** (0.82 g, 73%) as a yellow powder: mp 124–125 °C; IR (KBr) 3425, 2926, 1687, 1595, 1463, 1118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (d, 6H, J=6.7 Hz), 1.84–2.04 (m, 1H), 2.07–2.17 (m, 2H), 2.53–2.64 (m, 4H), 4.14 (t, 2H, J=6.2 Hz), 6.69 (d, 1H, J=4.0 Hz), 6.90 (dd, 1H, J=1.7 Hz and 7.7 Hz), 6.98–7.09 (m, 2H), 7.29 (d, 2H, J=7.9 Hz), 7.65 (d, 2H, J=7.9 Hz), 7.90 (dd, 1H, J=1.7 Hz and 8.6 Hz), 8.19 (d, 1H, J=1.7 Hz), 8.47 (d, 1H, J=8.6 Hz), 8.55 (dd, 1H, J=1.7 Hz and 7.2 Hz), 8.73 (s, 1H); Anal. ($C_{30}H_{30}N_2O_5$) C, H, N.

1-(4,4′-Dipropylbenzhydryl)benzimidazole-5-carboxylic acid (22). A solution of methyl 4-chloro-3-nitrobenzoate **24** (0.11 g, 0.52 mmol), 4,4′-dipropylbenzhydryl amine (0.14 g, 0.52 mmol), in 5 mL of DMSO was stirred at 100 °C for 3 h. Upon cooling, the water was added to the reaction mixture, which was extracted with AcOEt. The organic layer was washed with water and brine, dried and evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt (5:1) to afford methyl 4-(4,4′-dipropylbenzhydryl)-amino-3-nitrobenzoate (0.15 g, 66%) as a yellow oil; ^1H NMR ($CDCl_3$) δ 0.93 (t, 6H, J=7.3 Hz), 1.43–1.75 (m, 4H), 2.57 (t, 4H, J=7.5 Hz), 3.87 (s, 3H), 5.73 (d, 1H, J=5.7 Hz), 6.75 (d, 1H, J=8.9 Hz), 7.08–7.29 (m, 8H), 7.91 (dd, 1H, J=1.9 Hz and 8.9 Hz), 8.83–8.90 (m, 1H), 8.89 (d, 1H, J=1.9 Hz). To a solution of methyl 4-(4,4′-dipropylbenzhydryl)amino-3-nitrobenzoate (100 mg, 0.22 mmol) in 2.0 mL of EtOH and 0.1 mL of AcOH, 5.0 mg of platinum(IV) oxide was added and the reaction mixture was stirred under atmospheric hydrogen for 12 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt (3:1) to afford methyl 3-amino-4-(4,4′-dipropylbenzhydryl)aminobenzoate (40 mg, 43%) as a yellow oil; ^1H NMR ($CDCl_3$) δ 0.92 (t, 6H, J=7.3 Hz), 1.42–1.74 (m, 4H), 2.47–2.63 (m, 2H), 2.56 (t, 4H, J=7.1 Hz), 3.80 (s, 3H), 5.53 (s, 1H), 6.41 (d, 1H, J=8.8 Hz), 7.12 and 7.22 (m, AB system, 8H, J=8.4 Hz), 7.40–7.48 (m, 2H). A solution of methyl 3-amino-4-(4,4′-dipropylbenzhydryl)aminobenzoate (40 mg, 0.10 mmol) in 1.0 mL of triethylorthoformate and catalytic amount of formic acid was stirred at 120 °C for 1 h. Upon cooling, the reaction mixture was chromatographed on silica gel eluting with AcOEt:AcOH (10:1) to afford methyl 1-(4,4′-dipropylbenzhydryl)benzimidazole-5-carboxylate (30 mg). The residue was dissolved in 1.0 mL of EtOH and 1.0 mL of aqueous 1 N NaOH was added. After being stirred at room temperature for 30 min, water was added. The resulting mixture was acidified to pH 3, and then extracted with AcOEt. The organic layer was washed with brine, dried and evaporated in vacuo. The residue was chromatographed on silica gel eluting with hexane:AcOEt:AcOH (10:10:1) to afford **22** (27 mg, 68%) as a yellow oil; ^1H NMR ($CDCl_3$) δ 0.92 (t, 6H, J=7.1 Hz), 1.42–1.83 (m, 4H), 2.58 (t, 4H, J=7.5 Hz), 6.00 (br, 1H), 6.69 (s, 1H), 6.97–7.32 (m, 9H), 7.78 (s, 1H), 7.95 (d, 1H, J=8.6 Hz), 8.68 (s, 1H).

1-(4,4′-Dipropylbenzhydryl)benzimidazole-6-carboxylic acid (23). A solution of benzimidazole-5-carboxylic acid **21** (0.4 g, 2.5 mmol), 4,4′-dipropylbenzhydryl bromide (0.25 g, 0.76 mmol), in 8.0 mL of toluene and 1.0 mL of DMF was stirred at reflux for 3 h. Upon cooling, the reaction mixture was evaporated in vacuo. The residue was chromatographed on silica gel eluting with chloroform:MeOH (10:1) to afford **23** (0.4 g, 39%) as an amorphous solid; ^1H NMR ($CDCl_3$) δ 0.93 (t, 6H, J=7.1 Hz), 1.42–1.83 (m, 4H), 2.55–2.73 (m, 4H), 6.80 (s, 1H), 7.80–8.15 (m, 2H), 7.89 (s, 1), 8.03 (s, 1H), 8.93 (br, 1H).

2-[1-(4-Isobutyl-α-methylbenzyl)indol-5-yl]-4-oxo-quinolin-8-oxybutyric acid (30). Ethyl 4-[3-acetyl-2-(indole-5-yl)carboxamido]phenoxybutyrate **28** (0.6 g, 1.5 mmol), derived from the condensation of indole-5-carboxylic acid **6** and ethyl 4-(3-acetyl-2-amino)phenoxybutyrate **27**, was dissolved in 30 mL of EtOH. After NaOMe (28% in MeOH; 1.3 mL, 5.9 mmol) was added, the resulting mixture was stirred under reflux for 2 h. The reaction mixture was acidified with AcOH to pH 5 and evaporated in vacuo. The residue was chromatographed on silica gel eluting with AcOEt:AcOH (10:1) to afford **29** (0.4 g, 76%) as a yellow oil; ^1H NMR ($CDCl_3$) δ 1.94–2.15 (m, 2H), 2.30–2.43 (m, 2H), 4.20–4.33 (m, 2H), 6.61 (d, 1H, J=1.8 Hz), 6.69 (s, 1H), 7.26–7.58 (m, 5H), 7.76 (d, 1H, J=8.4 Hz), 8.30 (s, 1H), 8.73 (s, 1H), 9.32 (br, 1H). By the same procedure described above, **29** (0.39 g, 1.1 mmol) and 4-isobutyl-α-methylbenzyl bromide (0.34 g, 1.40 mmol) was condensed to give **30** (0.1 g, 18%, 51% recovery of **29**) as an amorphous

solid; IR (KBr) 2864, 1714, 1564, 1511, 1174, 1064, 799 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (d, 6H, J=6.6 Hz), 1.55–1.95 (m, 1H), 1.92 (d, 3H, J=7.1 Hz), 2.23–2.54 (m, 2H), 2.44 (d, 2H, J=7.0 Hz), 2.60–2.73 (m, 2H), 4.16–4.30 (m, 2H), 5.66 (q, 1H, J=7.1 Hz), 6.21 (s, 1H), 6.66 (br, 2H), 7.05 (s, 4H), 7.20–7.55 (m, 5H), 7.73 (dd, 1H, J=1.3 Hz and 8.4 Hz), 8.30 (br, 1H), 8.63 (br, 1H); Anal. ($C_{33}H_{34}N_2O_4 \cdot 0.5H_2O$) C, H, N.

Biological methods

Rat prostatic 5α-reductase assay. The preparation of rat prostate particulates and the assay of 5α-reductase were carried out according to the reported procedure. The ventral prostate from male Wistar rats (200–300 g, Japan Cler), sacrificed by cervical dislocation, were minced and homogenized in 3 tissue volumes of ice-cold medium A (0.3 nM sucrose, 1 mM dithiothreitol and 20 mM sodium phosphate, pH 6.5) using a Polytron homogenizer. The homogenate was centrifuged at 140,000 *g* for 1 h at 2 °C. The resulting pellet was washed once with medium A and resuspended in the same medium (30–50 mg protein/mL). The enzyme preparation was stored at −80 °C. The reaction solution contains 1 mM dithiothreitol, 40 mM sodium phosphate, pH 6.5, 150 mM NADPH, [^{14}C]-testosterone (T) (3 mM) and the enzyme preparation (1 mg of protein) in a total volume of 0.5 mL. The test compounds in 10 mL of ethanol were added to the test tubes, whereas control and blank tubes received the same volume of ethanol. The blank tubes also received 2 mL of ethyl acetate. The reaction was started with the addition of the enzyme preparation. After incubation at 37 °C for 20 min, the control and test tubes received 2 mL of ethyl acetate, and the reaction solution was centrifuged at 1,000 *g* for 5 min. The ethyl acetate phase was transferred to another tube and evaporated to dryness. The steroids were taken up in 50 mL of ethyl acetate and chromatographed on a Whatman Silica plate LK6DF, using ethyl acetate:cyclohexane (1:1) as the developing solvent system. The radioactivity of [^{14}C]-T and [^{14}C]-5α-dihydrotestosterone (DHT) on the plate was measured by a thin layer chromatography scanner (Aloka, JTC-601). The rate of the conversion by the enzyme was calculated according to the following formula: Rate of the conversion (%) = [(Radioactivity of [^{14}C]-DHT)/{(Radioactivity of [^{14}C]-T) + (Radioactivity of [^{14}C]-DHT)}]×100.

The rate of the inhibition by the test compound was calculated according to the following formula: Rate of the inhibition (%) = [1−{(Rate of the conversion in the test tube)−(Rate of the conversion in the blank tube)}/{(Rate of the conversion of the control tube)−(Rate of the conversion of the blank tube)}]×100.

The IC_{50} values were calculated as the concentration that inhibited the enzyme activity by 50%.

Acknowledgements

The authors thank E. Sugimoto and K. Namiki for their excellent technical assistance. The authors are also grateful to Dr. T. Hirata for his continuous support.

References and Notes

1. (a) Metcalf, B. W.; Levy, M. A.; Holt, D. A. *Trends Pharmacol. Sci.* **1989**, *10*, 491. (b) Geller, J. *Prostate* **1989**, Suppl. 2, 95. (c) Isaacs, J. T.; Brendler, C. B.; Walsh, P. C. *J. Clin. Endocrinol. Metab.* **1983**, *56*, 139.

2. Sansone, G. L.; Reisner, R. M. *J. Invest. Dermatol.* **1971**, *56*, 366.

3. Diani, A. R.; Mulholland, M. J.; Shull, K. L.; Kubicek, M. F.; Johnson, G. A.; Schostarez, H. J.; Brunden, M. N.; Buhl, A. E. *J. Clin. Endocrinol. Metab.* **1992**, *74*, 345.

4. Brooks, J. R. *J. Clin. Endocrinol. Metab.* **1986**, *15*, 391.

5. For recent studies of steroidal inhibitors of 5α-reductase, see: (a) Rasmusson, G. H.; Reynolds, G. F.; Utne, T.; Jobson, R. B.; Primka, R. L.; Berman, C.; Brooks, J. R. *J. Med. Chem.* **1984**, *27*, 1690. (b) Rasmusson, G. H.; Reynolds, G. F.; Steinberg, N. G.; Walton, E.; Patel, G. F.; Liang, T.; Cascieri, M. A.; Cheung, A. H.; Brooks, J. R.; Berman, C. *J. Med. Chem.* **1986**, *29*, 2298. (c) Holt, D. A.; Levy, M. A.; Ladd, D. L.; Oh, H.-J.; Erb, J. M.; Heaslip, J. I.; Brandt, M.; Metcalf, B. W. *J. Med. Chem.* **1990**, *33*, 937. (d) Holt, D. A.; Levy, M. A.; Oh, H.-J.; Erb, J. M.; Heaslip, J. I.; Brandt, M.; Lan-Hargest, H. Y.; Metcalf, B. W. *J. Med. Chem.* **1990**, *33*, 943. (e) Stoner, E. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 375. (f) Levy, M. A.; Metcalf, B. W.; Brandt, M.; Erb, J. M.; Oh, H.-J.; Heaslip, J. I.; Yen, H.-K.; Rozamus, L. W.; Holt, D. A. *Bioorg. Chem.* **1991**, *19*, 245. (g) Haase-Held, M.; Hatzis, M.; Mann, J. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2999. (h) Frye, S. V.; Haffner, C. D.; Maloney, P. R.; Mook, Jr. R. A.; Dorsey, Jr. G. F.; Hiner, R. N.; Cribbs, C. M.; Wheeler, T. N.; Ray, J. A.; Andrews, R. C.; Batchelor, K. W.; Bramson, H. N.; Stuart, J. D.; Schweiker, S. L.; Arnold, J. V.; Croom, S.; Bickett, D. M.; Moss, M. L.; Tian, G.; Unwalla, R. J.; Lee, F. W.; Tippin, T. K.; James, M. K.; Grizzle, M. K.; Long, J. E.; Schuster, S. V. *J. Med. Chem.* **1994**, *37*, 2352. (i) Haffner, C. *Tetrahedron Lett.* **1994**, *35*, 1349. (j) Li, X.; Singh, S. M.; Luu-The, V.; Côté, J.; Laplante, S.; Labrie, F. *Bioorg. Med. Chem.* **1996**, *4*, 55. (k) Guarna, A.; Belle, C.; Machetti, F.; Occhiato, E. G.; Payne, A. H.; Cassiani, C.; Comerci, A.; Danza, G.; De Bellis, A.; Dini, S. *J. Med. Chem.* **1997**, *40*, 1112.

6. (a) Gormley, G. J.; Stoner, E.; Bruskewitz, R. C.; Imperato-McGinley, J.; Walsh, P. C.; McConnell, J. D.; Andriole, G. L.; Geller, J.; Bracken, B. R.; Tenover, J. S.; Vaughan, E. D.; Pappas, F.; Taylor, A.; Binkowitz, B.; Ng, J. *N. Engl. J. Med.* **1992**, *327*, 1185. (b) Rittmaster, R. S. *N. Engl. J. Med.* **1994**, *330*, 120.

7. For recent studies of nonsteroidal inhibitors of 5α-reductase, see: (a) Nakai, H.; Terashima, H; Arai, Y. Ono Pharmaceutical Co., Ltd. EP 0 291 245, 1988; *Chem. Abstr.* **1989**, *110*, 212384t, 708. (b) Jones, C. D.; Audia, J. E.; Lawhorn, D. E.; McQuaid, L. A.; Nerbauer, B. L.; Pike, A. J.; Pennington, P. A.; Stamm, N. B.; Toomey, R. E.; Hirsch, K. S. *J. Med. Chem.* **1993**, *36*, 421. (c) Audia, J. E.; Lawhorn, D. E.; Deeter, J. B. *Tetrahedron Lett.* **1993**, *34*, 7001. (d) Abell, A. D. *Bioorg. Med. Chem. Lett.*

1994, *4*, 2327. (e) Holt, D. A.; Yamashita, D. S.; Konialian-Beck, A. L.; Luengo, J. I.; Abell, A. D.; Bergsma, D. J.; Brandt, M.; Levy, M. *J. Med. Chem.* **1995**, *38*, 13. (f) Kenny, B.; Ballard, S.; Blagg, J.; Fox, D. *J. Med. Chem.* **1997**, *40*, 1293. (g) Kato, M.; Komoda, K.; Namera, A.; Sakai, Y.; Okada, S.; Yamada, A.; Yokoyama, K.; Migita, E.; Minobe, Y.; Tani, T. *Chem. Pharm. Bull.* **1997**, *45*, 1767. (h) Ishibashi, K.; Nakajima, K.; Sugioka, Y.; Sugiyama, M.; Hamada, T.; Horikoshi, H.; Nishi, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 561.

8. (a) Kumazawa, T.; Takami, H.; Kishibayashi, N.; Ishii, A.; Nagahara, Y.; Hirayama, N.; Obase, H. *J. Med. Chem.* **1995**, *38*, 2887. (b) Takami, H.; Koshimura, H.; Kishibayashi, N.; Ishii, A.; Nonaka, H.; Aoyama, S.; Kase, H.; Kumazawa, T. *J. Med. Chem.* **1996**, *39*, 5047.

9. Bald, E.; Saigo, K.; Mukaiyama, T. *Chem. Lett.* **1975**, *26*, 61.

10. Marzoni, G.; Garbrecht, W. L. *Synthesis* **1987**, 651.

11. Aniline **27** was derived from 3-hydroxyacetophenone in three steps: introduction of butyric acid moiety, nitration, and reduction to amine.

12. Brooks, J. R.; Berman, C.; Primka, R. L.; Reynolds, G. F.; Rasmusson, G. H. *Steroids* **1986**, *47*, 1.