(*E*)-4-{2-[[3-(Indol-5-yl)-1-oxo-2-butenyl]amino]phenoxy}butyric Acid Derivatives: A New Class of Steroid 5α-Reductase Inhibitors in the Rat Prostate. 1

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A series of (*E*)-4-{2-[[3-(indol-5-yl)-1-oxo-2-butenyl]amino]phenoxy}butyric acid derivatives was prepared, and the derivatives were demonstrated to be potent inhibitors of steroid 5α-reductase in the rat prostate. The structure−activity relationships were as follows. An α-branched alkyl or benzyl substituent of proper size at position 1 of the indole is crucial for optimal enzyme inhibitory activity. *N*-Methylation of the amide NH resulted in complete loss of activity. Thus, coplanarity of the benzene ring and amide moiety is essential for such activity. Among the compounds prepared, (*E*)-4-{2-[[3-[1-[bis(4-fluorophenyl)methyl]indol-5-yl]-1-oxo-2-butenyl]amino]phenoxy}butyric acid (**57**, KF18678) was one of the most potent compounds (rat prostate 5α-reductase IC_{50} = 3.3 nM).

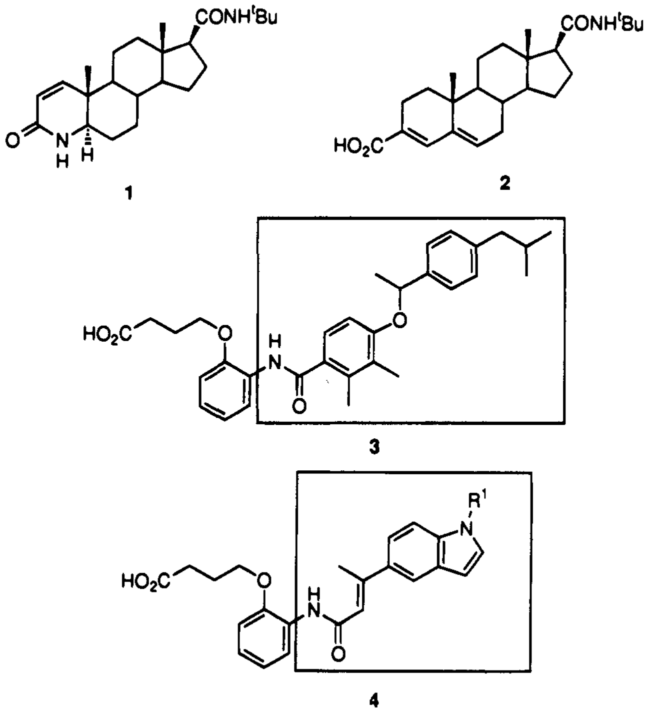

Figure 1.

Benign prostatic hyperplasia (BPH) is a common age-related disease of the urinary tract in men. Testosterone is secreted mainly by the testes and is converted into the more potent androgen dihydrotestosterone (DHT) by steroid 5α-reductase. Elevated DHT has been implicated as a causative factor of BPH. It has also been reported that 5α-reductase activity is increased in BPH patients.[2] Consequently, compounds inhibiting 5α-reductase activity might be useful in preventing the formation of BPH.[3] A number of 5α-reductase inhibitors have been reported,[4−7] including both steroidal inhibitors, MK-906 (finasteride)[4] (**1**) and SKF 105687[5] (**2**), and a nonsteroidal inhibitor, ONO-3805[6] (**3**) (Figure 1).

Recently, different genes encoding for two 5α-reductase isozymes (designated types 1 and 2) have been described in rats[8] and humans,[9] respectively. Finasteride has been reported to strongly inhibit the type 2 enzyme, whereas it is a relatively poor inhibitor of the type 1 in human 5α-reductase. However, the physiological role of these isozymes remains to be elucidated.

Starting our research program to find a new 5α-reductase inhibitor, we focused our attention on the nonsteroidal compound **3**. However, no SAR on this compound has been reported so far. 5α-Reductase is an NADPH-dependent enzyme. We considered that the right lipophilic part of the structure of **3** corresponds to a steroidal skeleton and the carboxylic acid should interact with NADPH or NADP$^+$. This hypothesis led us to design 3-(indol-5-yl)isocrotonoyl skeleton **4**.

In this paper, we describe the synthesis and 5α-reductase inhibitory activity of the (*E*)-4-{2-[[3-(indol-5-yl)-1-oxo-2-butenyl]amino]phenoxy}butyric acid derivatives.

Chemistry

The general synthetic method of compound **4** is shown in Scheme 1. The key intermediate carboxylic acids **9** were prepared from 5-acetylindole **5**[10] using two different routes. Compound **5** was alkylated to **6**, which was reacted with ethyl (diethylphosphono)acetate to afford ester **7** (method A). In the case where R^1 is an α-branched alkyl group, the reaction of **5** with an alkyl tosylate using t-BuOK gave **6** in poor yield. Thus, **5** was reacted with alkyl tosylate using KOH in dimethyl sulfoxide.[11] Otherwise, compound **5** was first reacted with ethyl (diethylphosphono)acetate to afford ester **8**, which was then alkylated to **7** (method B). Ester **7** was hydrolyzed with LiOH to (*E*)-3-(indol-5-yl)-2-butenoic acid **9**. The obtained carboxylic acid **9** was reacted with aniline **10**[6] using Mukaiyama reagent[12] to afford amide **11**, which was hydrolyzed to the carboxylic acids **12**−**72**.

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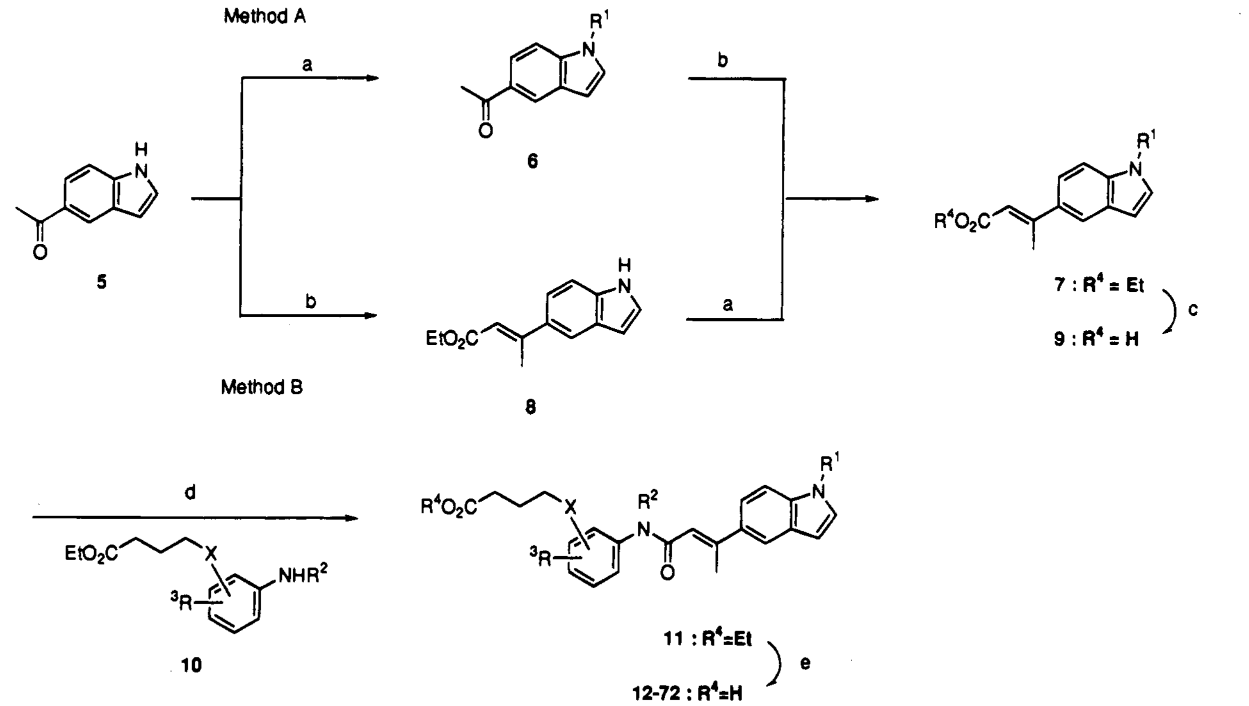
Scheme 1^a



^{a} (a) R^1X, t-BuOK/DMF or R^1OTs, KOH/DMSO; (b) $(EtO)_2POCH_2CO_2Et$, NaH/THF; (c) LiOH/aqueous dioxane; (d) 2-chloro-1-methylpyridinium iodide, NBu_3/CH_2Cl_2; (e) NaOH/aqueous EtOH.

Single-Crystal X-ray Analysis

The structure of **28** was confirmed unequivocally by X-ray crystallographic analysis. The X-ray data were obtained on nonresolved material. A summary of the crystal data and data collection parameters for **28** is listed in Table 2. Colorless needle crystals are obtained from a methanol solution. Unit cell parameters were determined from angular settings of 25 carefully centered reflections. The intensities of three standard reflections were monitored periodically for stability control during data collection. Intensities were corrected for Lorentz and polarization and secondary extinction effects but not for absorption. A total of 3574 reflections with $I > 3.0\sigma(I)$ were used in the structure determination. The structure was solved by direct methods using SAPI91[13] and Fourier synthesis. The structure was refined by full-matrix least-squares methods. The temperature factors of carbon atoms of the group are so large that relatively high residual peaks were observed around the relevant atoms on the final different Fouler map. The positions of all H atoms were calculated geometrically and included in the structure factor calculations, but the atomic parameters were not refined.[14] The final R value with a unit weight is 0.093. The structure of **28** is shown in Figure 2.

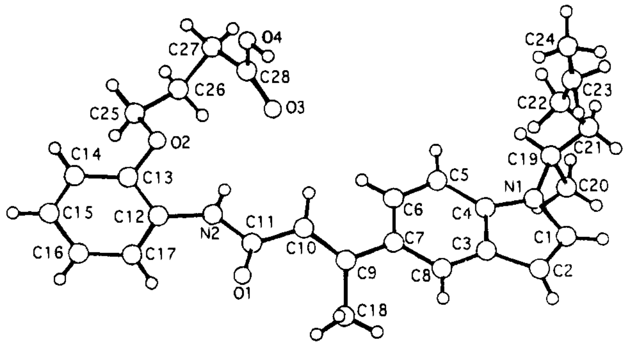
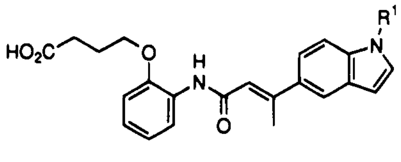


Figure 2.



4'

Figure 3.

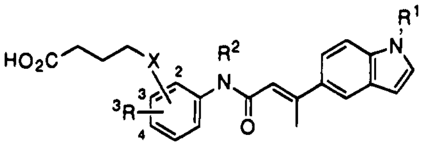
Results and Discussion

From the results of the single-crystal X-ray analysis of **28**, the conformation of the 4-{2-[[3-(indol-5-yl)-1-oxo-2-butenyl]amino]phenoxy}butyric acid derivative is not **4** but **4'**. However, the conformation **4'** can still be regarded as a steroid template (Figure 3).

The prepared compounds were evaluated for their ability to inhibit the rat prostatic 5α-reductase. The inhibitory activity was expressed as the IC_{50} value. The results of 5α-reductase inhibitory activity are shown in Table 1. In our biological assays, 1 and (±)-**3** showed inhibitory activity with IC_{50} values of 10 ± 1.8 and 2.5 ± 0.03 nM, respectively.

The substituents (R^1) at position 1 of the indole influenced the inhibitory activity. At first, an alkyl group was introduced. In the case of a normal alkyl group (**12**–**17**), the inhibitory activity increased with lengthening the chain of the substituents, with maximum potency being obtained with a pentyl group (**15**) while further extension reduced the activity. Branching at the α-position of the alkyl group significantly increased potency (**13**–**16** vs **21**–**33**). Among them, the 1-propylbutyl substituted one (**24**) showed the most potent inhibitory activity with an IC_{50} value of 2.3 ± 0.18 nM. The effect of branching at the α-position can be explained as follows. A normal alkyl group can rotate freely, while branching at the α-position regulates the

Table 1. Indole Derivatives



compd	R¹	R²	R³	X	formula[a]	mp (°C)	5α-reductase inhibitory activity[b] IC_{50} (nM)
12	methyl	H	H	2-O	$C_{23}H_{24}N_2O_4$	132–133.5	780 ± 76
13	*n*-propyl	H	H	2-O	$C_{25}H_{28}N_2O_4$	153–154	190 ± 48
14	*n*-butyl	H	H	2-O	$C_{26}H_{30}N_2O_4{\cdot}0.5H_2O$	154–155.5	43 ± 4.0
15	*n*-pentyl	H	H	2-O	$C_{27}H_{32}N_2O_4$	129–132	23 ± 4.1
16	*n*-hexyl	H	H	2-O	$C_{28}H_{34}N_2O_4$	107–108	77 ± 20
17	*n*-heptyl	H	H	2-O	$C_{29}H_{36}N_2O_4$	95.5–96.5	120 ± 13
18	isobutyl	H	H	2-O	$C_{26}H_{30}N_2O_4$	153–155.5	160 ± 41
19	2,2-dimethylpropyl	H	H	2-O	$C_{27}H_{32}N_2O_4{\cdot}0.33C_7H_8$[d]	141–143	84 ± 13
20	3-methyl-2-butenyl	H	H	2-O	$C_{27}H_{30}N_2O_4$	137–140	120 ± 2.7
21	1-methylpropyl	H	H	2-O	$C_{26}H_{30}N_2O_4$	137–140	67 ± 26
22	1-methylbutyl	H	H	2-O	$C_{27}H_{32}N_2O_4$	167–168	7.8 ± 0.68
23	1-ethylbutyl	H	H	2-O	$C_{28}H_{34}N_2O_4{\cdot}0.3C_6H_{14}O$[e]	137–140	9.3 ± 1.1
24	1-propylbutyl	H	H	2-O	$C_{29}H_{35}N_2NaO_4{\cdot}H_2O$	amorphous	2.3 ± 0.18
25	1-propylbutyl	H	H	3-O	$C_{29}H_{36}N_2O_4{\cdot}0.1C_2H_6O$[f]	87–89	19%[c]
26	1-propylbutyl	H	H	4-O	$C_{29}H_{36}N_2O_4{\cdot}0.2H_2O$	124–125	28%[c]
27	1-isopropylbutyl	H	H	2-O	$C_{29}H_{36}N_2O_4{\cdot}0.2H_2O$	119–123	7.2 ± 0.84
28	1-methylpentyl	H	H	2-O	$C_{28}H_{34}N_2O_4$	157–159	6.0 ± 0.58
29	1-ethylpentyl	H	H	2-O	$C_{29}H_{36}N_2O_4$	144–149	7.1 ± 0.58
30	1-propylpentyl	H	H	2-O	$C_{30}H_{37}N_2NaO_4{\cdot}H_2O$	amorphous	4.1 ± 0.94
31	1-butylpentyl	H	H	2-O	$C_{31}H_{39}N_2NaO_4$	amorphous	21 ± 2.5
32	1-pentylhexyl	H	H	2-O	$C_{33}H_{43}N_2NaO_4{\cdot}H_2O$	amorphous	25 ± 1.5
33	1-isopropylisobutyl	H	H	2-O	$C_{29}H_{36}N_2O_4{\cdot}0.2H_2O$	150–152	14 ± 2.3
34	cyclohexyl	H	H	2-O	$C_{28}H_{32}N_2O_4{\cdot}0.3H_2O$	68–75	48%[c]
35	1-isobutyl-3-methylbutyl	H	H	2-O	$C_{31}H_{39}N_2NaO_4{\cdot}2H_2O$	amorphous	8.0 ± 2.4
36	benzyl	H	H	2-O	$C_{29}H_{28}N_2O_4$	162–170	50 ± 15
37	2-methylbenzyl	H	H	2-O	$C_{30}H_{30}N_2O_4$	187–190	69 ± 11
38	3-methylbenzyl	H	H	2-O	$C_{30}H_{30}N_2O_4$	145–150	250 ± 71
39	4-methylbenzyl	H	H	2-O	$C_{30}H_{30}N_2O_4$	150.5–154	88 ± 14
40	4-(trifluoromethyl)benzyl	H	H	2-O	$C_{30}H_{27}FN_2O_4$	134–137	300 ± 64
41	4-fluorobenzyl	H	H	2-O	$C_{29}H_{27}FN_2O_4$	161–163	97 ± 26
42	4-*n*-butylbenzyl	H	H	2-O	$C_{33}H_{36}N_2O_4{\cdot}0.5C_6H_{14}O$[e]	84–87.5	100 ± 19
43	4-*tert*-butylbenzyl	H	H	2-O	$C_{33}H_{36}N_2O_4{\cdot}0.75CCl_4H_2O$	103–105	170 ± 23
44	4-methoxybenzyl	H	H	2-O	$C_{30}H_{30}N_2O_5$	123–130	480 ± 62
45	α-methylbenzyl	H	H	2-O	$C_{30}H_{30}N_2O_4$	50	19 ± 3.2
46	α-ethylbenzyl	H	H	2-O	$C_{31}H_{32}N_2O_4{\cdot}0.1C_6H_{14}O$[e]$\cdot H_2O$	153–160	19 ± 6.5
47	α-*n*-propylbenzyl	H	H	2-O	$C_{32}H_{34}N_2O_4$	162–165.5	9.1 ± 2.4
48	α-*n*-butylbenzyl	H	H	2-O	$C_{33}H_{36}N_2O_4$	129–136	9.8 ± 0.82
49	α-*n*-pentylbenzyl	H	H	2-O	$C_{34}H_{38}N_2O_4$	68–72	25 ± 1.9
50	α-isopropylbenzyl	H	H	2-O	$C_{32}H_{34}N_2O_4$	158–161	25 ± 2.4
51	α-isobutylbenzyl	H	H	2-O	$C_{32}H_{36}N_2O_4{\cdot}0.2C_2H_6O$[f]$\cdot 0.5H_2O$	68–72	6.3 ± 1.0
52	1-(2-naphthyl)ethyl	H	H	2-O	$C_{34}H_{32}N_2O_4{\cdot}0.5H_2O$	amorphous	26%[c]
53	benzhydryl	H	H	2-O	$C_{35}H_{32}N_2O_4{\cdot}0.2C_3H_8O$[g]	158–162	5.6 ± 1.2
54	benzhydryl	H	H	2-S	$C_{35}H_{32}N_2O_3S$	amorphous	39 ± 5.9
55	2.2′-dimethylbenzhydryl	H	H	2-O	$C_{37}H_{36}N_2O_4{\cdot}0.5H_2O$	amorphous	19 ± 6.7
56	4,4′-dimethylbenzhydryl	H	H	2-O	$C_{37}H_{36}N_2O_4{\cdot}0.25H_2O$	139–141	8.8 ± 1.4
57	4,4′-difluorobenzhydryl	H	H	2-O	$C_{35}H_{30}F_2N_2O_4$	148.5–149	3.3 ± 0.23
58	4-methoxybenzhydryl	H	H	2-O	$C_{36}H_{33}N_2NaO_5N{\cdot}1.5H_2O$	amorphous	8.8 ± 1.7
59	4,4′-dimethoxybenzhydryl	H	H	2-O	$C_{37}H_{36}N_2O_6$	147–148	9.8 ± 2.1
60	4-(trifluuromethyl)benzhydryl	H	H	2-O	$C_{36}H_{31}F_3N_2O_4$	166–170	8.1 ± 0.82
61	4,4′-dichlorobenzhydryl	H	H	2-O	$C_{35}H_{30}Cl_2N_2O_4$	181–182	25 ± 3.9
62	α-2-pyridylbenzyl	H	H	2-O	$C_{34}H_{31}N_3O_4$	186.5–188	37 ± 7.1
63	α-3-pyridylbenzyl	H	H	2-O	$C_{34}H_{31}N_3O_4{\cdot}0.25H_2O$	171–172	47 ± 2.1
64	α-4-pyridylbenzyl	H	H	2-O	$C_{34}H_{31}N_3O_4$	amorphous	26 ± 1.5
65	dibenzosuberyl	H	H	2-O	$C_{37}H_{34}N_2O_4$	227–230	31%[c]
66	benzhydryl	H	3-F	2-O	$C_{35}H_{31}FN_2O_4$	179–180	15 ± 1.3
67	benzhydryl	H	4-F	2-O	$C_{35}H_{31}FN_2O_4$	174–176	3.6 ± 0.67
68	benzhydryl	H	4-Me	2-O	$C_{36}H_{34}N_2O_4$	112–115	14 ± 3.7
69	benzhydryl	H	5-F	2-O	$C_{35}H_{31}FN_2O_4$	193.5–195	52%[c]
70	benzhydryl	H	5-Me	2-O	$C_{36}H_{34}N_2O_4$	176–178	45%[c]
71	benzhydryl	H	5-Cl	2-O	$C_{35}H_{31}ClN_2O_4$	138–140	38%[c]
72	α-methylbenzyl	Me	H	2-O	$C_{31}H_{31}N_2NaO_4{\cdot}0.4H_2O$	amorphous	8%[c]

[a] All new compounds had C, H, N microanalyses within 0.4% of theoretical values unless otherwise noted. [b] Prostates from male rats. IC_{50} values are means ± SE of three separate experiments. [c] Inhibition percent at 100 nM. [d] Toluene. [e] Isopropyl ether. [f] EtOH. [g] 2-Propanol.

direction of a substituent at position 1 of the indole. This regulation is supposed to result in more potent activity. Actually, the X-ray crystallographic analysis of compound **28** shows that the plane of the 1-pentyl moiety is almost orthogonal to the indole ring. Although there is little difference in the activity of the methyl, ethyl, propyl, and isopropyl groups as substituents of the α-branched group, the butyl and pentyl groups reduced

Table 2. Crystal Data and Data Collection Parameters for **28**

Crystal Data at 20 °C	
mol formula	$C_{28}H_{34}N_2O_4$
a, Å	17.180(5)
b, Å	10.629(3)
c, Å	14.159(4)
β, deg	102.46(5)
Z	4
space group	$P2_1/a$
crystal size	0.2 × 0.2 × 0.5 mm
Data Measurement Parameters	
radiation	graphite monochromated Cu Kα λ = 1.541 84 Å
diffractometer	Enraf-Nonius CAD-4
θ range, deg	2–75
unique reflections	4950
unique reflections with $I > 3.0\sigma(I)$	3574

the activity (**21**–**24**, **28**–**30** vs **31** and **32**). This indicates a bulk limitation for the substituent R^1. The cyclohexyl derivative **34** showed weak inhibitory activity, which suggests that not only lipophilicity but a certain spatial arrangement of the substituent is required for potent activity. Next, the benzyl group was examined. The unsubstituted benzyl derivative **36** showed moderate activity (IC_{50} = 50 ± 15 nM). Substitution on the benzene ring of **36** reduced the potency irrespective of the nature of the substituents (**37**–**44**). Branching with an alkyl group at the α-position of the benzyl also resulted in enhanced activity (**45**–**51**), with maximum potency being obtained with a butyl group. The 1-(2-naphthyl)ethyl derivative **52**, however, almost lost all activity, which also suggests a bulk limitation. Compound **53**, which introduced a benzhydryl group, that can be regarded as a phenyl substitution at the α-position of the benzyl moiety, showed potent inhibitory activity (IC_{50} = 5.6 ± 1.2 nM). Substitution at the 4 and 4′ positions of the benzhydryl almost retained the same activity except for the dichloro compound **61** (**55**–**60**). The 4,4′-difluorobenzhydryl derivative **57** was one of the most potent compounds (IC_{50} = 3.3 ± 0.23 nM). Substitution of the phenyl group of the benzhydryl with pyridine reduced the activity (**62**–**64**). The conversion of benzhydryl to dibenzosuberyl led to a loss in activity (**65**).

As for the substituents R^3 on the benzene ring of the phenoxybutyric acid part, substitution at the 5 position brought about reduced activity irrespective of the nature of the substituents (**69**–**71**). Replacement of the ether bond of the phenoxy part by thioether also decreased the activity (**54**). The substitution position of the oxybutyric acid group notably influenced the potency, and position 2 was crucial for such activity (**24**–**26**). *N*-Methylation of amide NH resulted in a complete loss of inhibitory effects (**72**). The ^{1}H NMR chemical shift of the hydrogen adjacent to the isocrotonoylamino moiety on the benzene ring of compound **45** was lower than 8 ppm due to the shielding effect of the carbonyl, while the hydrogen of compound **72** was observed at a field higher than 8 ppm. This result means that *N*-methylation of the amide produced a deviation in the plane of the amide moiety from that of the benzene ring and these two moieties must be coplanar for potent inhibitory activity. Indeed, this coplanarity of the two moieties was confirmed by the X-ray crystallographic analysis.

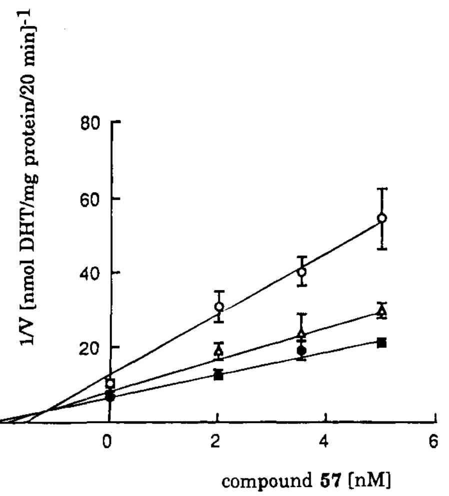


Figure 4. Double inhibition analysis of compound **57** and $NADP^+$. The activity of rat 5α-reductase was evaluated at variable concentrations of compound **57** and $NADP^+$ in 40 mM sodium phosphonate buffer, pH 6.5, containing 3 μM [^{14}C]-testosterone, 150 μM NADPH and 1 mM dithiothreitol. Reactions were initiated by addition of enzyme solution and incubated at 37 °C for 20 min. Each point represents the mean ± SEM. of three experiments. Concentration of $NADP^+$ represented in the figure are 0 (○), 50 (△), and 150 (●) μM. The calculated K_i (apparent dissociation constant of the enzyme and compound **57**) and K_{ii} (apparent dissociation constant of the enzyme–$NADP^+$ complex and compound **57**) values were 2.3 ± 0.23 and 1.2 ± 0.33 nM, respectively.

The inhibitory nature of this series of compounds for rat 5α-reductase was further evaluated. Compound **57**, one of the most potent compounds, exhibited uncompetitive dead-end inhibition kinetics versus T (testosterone) and noncompetitive kinetics versus NADPH, respectively. Furthermore, compound **57** demonstrated reversible binding preferentially to the enzyme–$NADP^+$ complex (Figure 4),[17] in analogy to the steroidal carboxylic acids[5].

In conclusion, we have identified a newly designed series of (*E*)-4-{2-[[3-(indol-5-yl)-1-oxo-2-butenyl]amino]-phenoxy}butyric acid derivatives as potent rat 5α-reductase inhibitors. For this series of compounds a certain spatial arrangement of the functional groups is necessary for optimal enzyme inhibitory activity. Thus, an α-branched alkyl or benzyl substituent of proper size at position 1 of the indole is essential for high potency. Coplanarity of the benzene ring and amide moiety is also crucial. Compound **57** (KF18678) is now being further evaluated for inhibitory activity both for 5α-reductase from other species and for isozymes of 5α-reductase purified subtypes.

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 magnetic resonance spectra (^{1}H NMR) were recorded on a Hitachi R-90H (90 MHz) or a JEOL JNM GX-270 (270 MHz) spectrometer with Me_4Si as internal standard. Elemental analyses were performed by the analytical department of our laboratories.

Method A. 5-Acetyl-1-(diphenylmethyl)indole (6, R^1 = Diphenylmethyl). To a solution of 5-acetylindole **5** (8.0 g, 50 mmol) in 120 mL of DMF was added portionwise t-BuOK (6.76 g, 60 mmol) at 0 °C, and the mixture was stirred for 30 min. A solution of diphenylmethyl bromide (18.6 g, 75 mmol) in 50 mL of DMF was added dropwise to the reaction mixture at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for 3 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 (5:1) to afford **6** (13.71 g, 87.5%) as colorless crystals: IR (KBr) 1669, 1607, 1452, 1361 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.62 (s, 3H), 6.60 (d, 1H, J = 3 Hz), 6.84 (s, 1H), 6.90 (d, 1H, J = 3 Hz), 7.03–7.85 (m, 12H), 8.30 (d, 1H, J = 1 Hz).

5-Acetyl-1-(2-propylbutyl)indole (6, R^1 = 2-Propylbutyl). To a suspension of 5-acetylindole **5** (0.12 g, 0.75 mmol) and powdered KOH (0.29 g, 4.5 mmol) in 1.2 mL of DMSO was added dropwise a solution of 2-propylbutyl *p*-toluenesulfonate (0.3 g, 1.1 mmol) in 0.6 mL of DMSO at room temperature. After being stirred for 1 h, the reaction mixture was diluted with water and 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 (6:1) to afford **6** (0.17 g, 88%) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 0.75 (d, 6H, J = 6 Hz), 0.85–1.24 (m, 4H), 1.66–1.87 (m, 4H), 2.54 (s, 3H), 4.24 (q, 1H, J = 7 Hz), 6.56 (d, 1H, J = 3 Hz), 7.10 (d, 1H, J = 3 Hz), 7.25 (d, 1H, J = 9.5 Hz), 7.79 (dd, 1H, J = 1.5 and 9.5 Hz), 8.27 (d, 1H, J = 1.5 Hz).

(*E*)-3-[1-(Diphenylmethyl)indol-5-yl]-2-butenoic Acid (9, R^1 = Diphenylmethyl). To a suspension of NaH (60% in oil; 8.42 g, 210 mmol) in 110 mL of THF were added 2 drops of EtOH and then dropwise ethyl (diethylphosphono)acetate (47.1 g, 210 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, and then a solution of **6** (13.70 g, 43.7 mmol) in 50 mL of THF was added dropwise. After being stirred at room temperature for 30 min, the reaction mixture was heated under reflux for 7 h. After addition of water, the 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 (3:1) to afford ethyl 3-[1-(diphenylmethyl)indol-5-yl]isocrotonate (**7**) (14.31 g, 85%) as an oil: IR (liquid film) 1708, 1620, 1608, 1451, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, 3H, J = 7 Hz), 2.64 (d, 3H, J = 1 Hz), 4.20 (q, 2H, J = 7 Hz), 6.17 (d, 1H, J = 1 Hz), 6.50 (d, 1H, J = 3 Hz), 6.81 (s, 1H), 6.85 (d, 1H, J = 3 Hz), 7.03–7.36 (m 12H), 7.79 (s, 1H).

A mixture of obtained **7** (14.3 g, 37 mmol), 80 mL of 1 N LiOH, and 130 mL of 1,4-dioxane was stirred at 60–70 °C for 10 h. Upon cooling, the reaction mixture was evaporated in vacuo. The residue was dissolved in 200 mL of water and acidified with 4 N HCl to pH 2. The precipitated crystals were collected by filtration, washed, and dried to afford crude **9** (12.69 g, 96%). This was recrystallized from isopropyl ether to give pure **9** (6.0 g, 45%) as colorless crystals: mp 173–175 °C; IR (KBr) 3500, 1680, 1602, 1447 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.66 (d, 3H, J = 1 Hz), 6.21 (d, 1H, J = 1 Hz), 6.52 (d, 1H, J = 3 Hz), 6.81 (s, 1H), 6.86 (d, 1H, J = 3 Hz), 7.04–7.36 (m, 12H), 7.81 (s, 1H).

Method B. Ethyl (*E*)-3-(Indol-5-yl)-2-butenoate (8). To a suspension of NaH (60% in oil; 12.5 g, 310 mmol) in 180 mL of THF were added 2 drops of EtOH and then dropwise ethyl (diethylphosphono)acetate (70.4 g, 310 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, and then a solution of **5** (10.0 g, 63 mmol) in 70 mL of THF was added dropwise. After being stirred at room temperature for 30 min, the reaction mixture was heated under reflux for 8 h. After addition of water, the 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 (5:1) to afford **8** (9.6 g, 67%) as an oil: IR (liquid film) 1680, 1603, 1195, 1101 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, 3H, J = 7 Hz), 2.67 (d, 3H, J = 1 Hz), 4.22 (q, 2H J = 7 Hz), 6.21 (d, 1H, J = 1 Hz), 6.56 (dd, 1 H, J = 2 and 3 Hz), 7.33 (s, 2H), 7.79 (s, 1H), 8.30 (br s, 1H).

(*E*)-3-(1-Pentylindol-5-yl)-2-butenoic Acid (9, R^1 = Pentyl). To a solution of **8** (2.29 g, 10 mmol) in 30 mL of DMF was added portionwise t-BuOK (1.39 g, 12 mmol) at 0 °C, and the mixture was stirred for 30 min. A solution of 1-iodopentane (2.58 g, 13 mmol) in 10 mL of DMF was added dropwise at 0 °C, and 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 (5:1) to afford **7** (R^1 = pentyl) (2.28 g, 76%) as an oil: IR (liquid film) 1709, 1611, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6 Hz), 1.24–1.39 (m, 7H), 1.65–2.05 (m, 2H), 2.67 (s, 3H), 4.0–4.32 (m, 4H), 6.19 (s, 1H), 6.49 (d, 1H, J = 3 Hz) 7.08 (d, 1H, J = 3 Hz), 7.15–7.62 (m, 2H), 7.77 (s, 1H).

A mixture of obtained **7** (R^1 = pentyl) (2.20 g, 7.4 mmol), 22 mL of 1 N LiOH, and 40 mL of 1,4-dioxane was stirred at 70–80 °C for 4 h. Upon cooling, the reaction mixture was evaporated in vacuo. The residue was dissolved in 50 mL of water and acidified with 4 N HCl to pH 2. The precipitated crystals were collected by filtration, washed, and dried to afford crude **9** (1.92 g, 97%). This was recrystallized from isopropyl ether to give pure **9** (0.91 g, 46%) as colorless crystals: mp 69–75 °C; IR (KBr) 3500, 1692, 1590, 1216 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6 Hz), 1.2–1.6 (m, 4H), 1.65–2.05 (m, 2H), 2.69 (s, 3H), 4.11 (t, 2H, J = 7 Hz), 6.24 (d, 1H, J = 1 Hz), 6.55 (d, 1H, J = 3 Hz), 7.11 (d, 1H, J = 3 Hz), 7.34–7.37 (m, 2H), 7.81 (s, 1H).

General Procedure for Preparation of 4. (*E*)-4-{2-[[3-[1-(Diphenylmethyl)indol-5-yl]-1-oxo-2-butenyl]amino]phenoxy}butyric Acid (53). To a mixture of ethyl 4-(2-aminophenoxy)butyrate (1.76 g, 3.9 mmol), 2-(chloromethyl)pyridinium iodide (1.2 g, 4.7 mmol), and tributylamine (2.25 mL, 9.5 mmol) in 10 mL of CH_2Cl_2 was added at reflux a solution of 3-[1-(diphenylmethyl)indol-5-yl]crotonic acid (1.45 g, 7.8 mmol) in 6 mL of CH_2Cl_2, and the mixture was stirred at reflux for 3 h. Upon cooling, the reaction mixture was diluted with ether, washed with water, 1 N HCl, and brine, dried, and then evaporated in vacuo. The residue was chromatographed on silica gel eluting with toluene–AcOEt (98:2) to afford 11 (1.0 g, 44%) as an oil: IR (liquid film) 3370, 1726, 1672, 1601, 1520, 1449 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11 (t, 3H, J = 7 Hz), 2.05–2.60 (m, 4H), 2.72 (d, 1H, J = 1 Hz), 3.92–4.17 (m, 4H), 6.41 (d, 1H, J = 1 Hz), 6.52 (d, 1H, J = 3 Hz), 6.81–7.45 (m, 16H), 7.83 (d, 1H, J = 1 Hz), 8.03 (br s, 1H), 8.46–8.56 (m, 1H).

A mixture of 11 (0.99 g, 1.7 mmol) in 2 mL of 1 N NaOH, 3 mL of EtOH, and 3.5 mL of 1,4-dioxane was stirred at room temperature overnight. The mixture was evaporated in vacuo, and the residue was dissolved in 10 mL of water. The mixture was acidified with 4 N HCl to pH 2 and stirred at room temperature for 1 h. The resultant crystalline product was collected by filtration, dried, and recrystallized from 2-propanol to give **53** (an adduct of 0.2 i-PrOH) (0.66 g, 70%) as colorless crystals: mp 158–162 °C; IR (KBr) 3450, 3340, 1717, 1638, 1603, 1596, 1539, 1452, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0–2.6 (m, 4H), 2.69 (d, 3H, J = 1 Hz), 4.08 (t, 2H, J = 6 Hz), 6.30 (d, 1H, J = 1 Hz), 6.51 (d, 1H, J = 3 Hz), 6.8–7.4 (m, 17H), 7.79 (s, 1H), 7.90 (s, 1H), 8.3–8.5 (m, 1H). Anal. ($C_{35}H_{32}N_2O_4\cdot 0.2C_3H_8O$) C, H, N.

Biological Methods. 5α-Reductase Assay. The preparation of rat prostate particulates and the assay of 5α-reductase were carried out according to the reported procedure.[16] The ventral prostates 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 (0.32 M sucrose, 1 mM dithiothreitol, and 20 mM sodium phosphate pH 6.5) using a Polytron homogenizer. The homogenate was centrifuged at 140000*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 μM NADPH, [^{14}C]testosterone (T) (3 μM), and the enzyme preparation (1 mg of protein) in a total volume of 0.5 mL. The test compounds in 10 μL 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 1000*g* for 5 min. The ethyl acetate phase was transferred to another tube and evaporated to dryness. The steroids were taken up in 50 μL 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 in the control tube) − (rate of the conversion in the blank tube)}] × 100.

The IC$_{50}$ values were calculated as the concentrations that inhibited the enzyme activity by 50%.

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Supporting Information Available: Listings of atomic parameters and standard deviations (5 pages); observed and calculated structure factors (25 pages). Ordering information is given on any current masthead page.

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