# Indole Derivatives as a New Class of Steroid 5α-Reductase Inhibitors

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A series of indole derivatives with varied substituents on the α,β-unsaturated double bond were synthesized and evaluated for their ability to inhibit rat prostatic 5α−reductase. Compounds possessing an ethyl substituent at the β-position of the double bond showed potent inhibitory activity. Among them, (*Z*)-4-{2-[[3-[1-(4,4′-difluorobenzhydryl)indol-5-yl]-2-pentenoyl]-amino]phenoxy}butyric acid (**16**, KF20405) showed the maximum potency with an IC$_{50}$ value of 0.48 ± 0.086 nM, which was 20-fold higher potency than **1** (MK-906). Compound **16** effectively inhibited DHT production 4 h after a 3 mg/kg oral administration. Several potent indole derivatives, **1** and **2** ((±)-ONO-3805), were tested versus rat and human isozymes. Nonsteroidal inhibitors such as indole derivatives and **2** were 2−3 orders of magnitude less potent for human type 2 isozyme than steroidal inhibitor **1** and expressed a significant species deference for these isozymes.

5α-Reductase inhibitors may provide a novel therapeutic treatment for androgen-related disorders associated with elevated levels of dihydrotestosterone (DHT) including the benign prostatic hyperplasia (BPH),[1] skin disorders such as acne,[2] male pattern baldness,[3] and hirsutism.[4] Several 5α-reductase inhibitors have been reported,[5,6] including the steroidal inhibitor MK-906 (**1**, finasteride)[5b] and the nonsteroidal inhibitor ONO-3805[6a] (**2**) (Figure 1). At the starting point of our research to develop a novel 5α-reductase inhibitor, finasteride had undergone extensive clinical trials in patients with BPH. Although the effectiveness of the steroidal 5α-reductase inhibitor in the treatment of BPH was clinically confirmed, several symptoms of sexual dysfunction were reported.[7] These adverse effects are possibly due to the reduction in DHT; however, the roles of DHT for normal sexual function remains to be elucidated. In general, azasteroidal inhibitors, except for 4-N-H derivative as finasteride, express the antiandrogenic activity.[5b,i] To develop specific 5α-reductase inhibitors, we designed a novel series of compounds, in which the steroidal skeleton was excluded. In contrast to the therapeutical use of the steroidal inhibitor (finasteride), nonsteroidal inhibitors have not been studied clinically. Compound **2** was a promising nonsteroidal inhibitor; however, the structure−activity relationship (SAR) on this compound has not been reported. Thus, we started our research by designing compounds analogous to **2**, in which steroidal skeleton was excluded. We synthesized indole derivatives to mimic the putative testosterone-NADPH-enzyme complex and identified **3** (KF18678), possessing potent inhibitory activity for rat prostatic 5α-reductase as previously reported.[8] An investigation of the SAR of this novel series revealed the key elements as follows: (1) a defined spacial arrangement of the functional groups at position 1 of the indole was important for the enhancement of activity and (2) coplanarity of the benzene ring and amide moiety was crucial for inhibitory activity. On the basis of these findings, further structural modifications of the indole derivatives were performed to enhance the 5α-reductase inhibitory activity of the series. In this paper, further investigations are described which were focused on the linking unit between the anilide moiety and the indole skeleton.

Recently, two 5α-reductase isozymes were identified in rats[9] and humans,[10] although the physiological and pharmacological roles of these isozymes in BPH are yet to be fully elucidated. This paper also investigated the inhibitory activities of some potent indole derivatives for these isozymes.

## Chemistry

The starting material 5-acyl or 5-formylindoles[6] were prepared either by the general procedure described in the preceding paper[8] or by alkylation of indoline 5-aldehydes **5** followed by oxidation of the intermediate alcohols as depicted in Scheme 1. The carboxylic acids **7** were prepared from 5-acetyl or formylindoles **6** by the Horner−Emmons reaction (method A), followed by hydrolysis with aqueous LiOH. When R$^2$ was a larger alkyl group than a methyl, for example, propionyl derivatives, the Horner−Emmons procedure gave the product in low yield under harsh reaction conditions and the Peterson olefination reaction was employed (method B) instead. During the course of the C−C bond formation reaction, a minor fraction of *Z* isomer was produced, which was separable by silica gel chromatography, and that material was converted into carboxylic acids **7Z**. The obtained α,β-unsaturated carboxylic acids **7** were reacted with aniline **8**[6a] using Mukaiyama's reagent (method C)[11] or *N,N*-bis(2-oxo-3-oxazolizinyl)phosphinic chloride (BOP-Cl) (method D)[12] to afford anilides **9**, which were hydrolyzed to carboxylic acids **10−27**.

## Results and Discussion

The prepared compounds were evaluated for their ability to inhibit rat prostatic 5α-reductase. Inhibitory acitivity was expressed as the IC$_{50}$ value or percent inhibition at 100 nM (Table 1). In our 5α-reductase assays, **1** and **2** showed inhibitory activities with IC$_{50}$ values of 10 ± 1.8 and 2.5 ± 0.03 nM, respectively.

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**Scheme 1**[a]
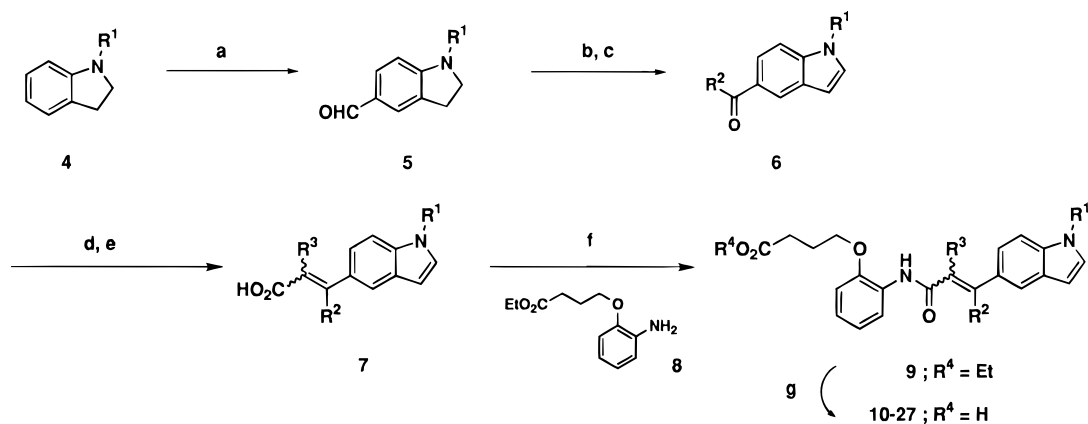


[a] (a) POCl$_3$, DMF/ClCH$_2$CH$_2$Cl; (b) R$^2$Li or R$^2$MgBr/THF; (c) MnO$_2$/toluene; (d) method A, (EtO)$_2$P(O)CHR$^3$CO$_2$Et, NaH/THF, or method B, Me$_3$SiCH$_2$CO$_2$Et, LDA/THF; (e) LiOH/aqueous EtOH; (f) method C, 2-chloro-1-methylpyridinium iodide, NBu$_3$/CH$_2$Cl$_2$, or method D, N,N-bis(2-oxo-3-oxazolidinyl)phosphinic chloride, TEA/CH$_2$Cl$_2$; (g) NaOH/aqueous EtOH.
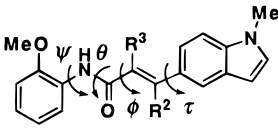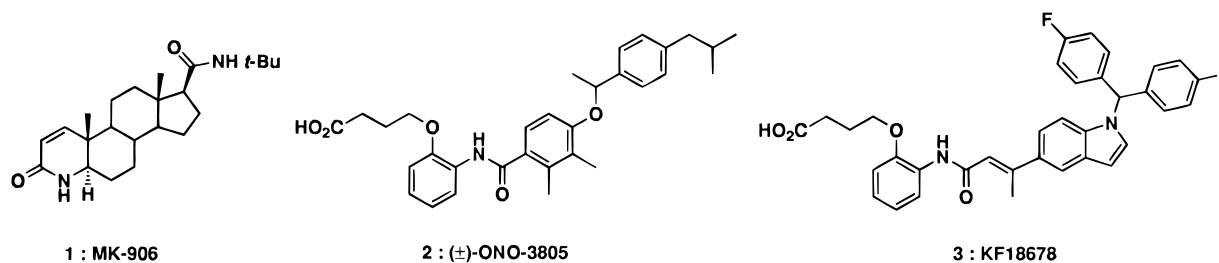


**Figure 1.**

**Table 1.** Indole Derivatives



| compd | R$^1$ | R$^2$ | R$^3$ | geometry | formula[a] | mp (°C) | solvent[b] | 5α-reductase inhibitory activity[c] IC$_{50}$ (nM) |
|---|---|---|---|---|---|---|---|---|
| **10** | benzyl | H | H | E | C$_{28}$H$_{26}$N$_2$O$_4$ | 123−132 | IPA | 1700 ± 250 |
| **11**[d] | benzyl | Me | H | E | C$_{29}$H$_{28}$N$_2$O$_4$ | 135−137 | IPA | 50 ± 15 |
| **12** | benzyl | H | Me | E | C$_{29}$H$_{28}$N$_2$O$_4$ | 162−170 | IPA | 120 ± 30 |
| **13** | benzyl | Me | Me | E | C$_{30}$H$_{30}$N$_2$O$_4$ | 132.5−134 | TL | 23%[e] |
| **14**[d] | benzhydryl | Me | H | E | C$_{35}$H$_{32}$N$_2$O$_4$·0.2C$_3$H$_8$O[f] | 158−162 | IPA | 5.6 ± 1.2 |
| **15** | benzhydryl | Et | H | E | C$_{36}$H$_{34}$N$_2$O$_4$·0.3H$_2$O | 76−78 | IPA | 2.5 ± 1.2 |
| **3**[d] | 4,4′-difluorobenzhydryl | Me | H | E | C$_{35}$H$_{30}$F$_2$N$_2$O$_4$ | 158−162 | IPE[g] | 3.3 ± 0.23 |
| **16** | 4,4′-difluorobenzhydryl | Et | H | E | C$_{36}$H$_{32}$F$_2$N$_2$O$_4$·0.8H$_2$O | 150−152 | IPE[g] | 0.48 ± 0.086 |
| **17** | 4,4′-difluorobenzhydryl | Et | H | Z | C$_{36}$H$_{32}$F$_2$N$_2$O$_4$·0.3H$_2$O | 78−80 | IPE[g] | 100[h] |
| **18** | 4,4′-difluorobenzhydryl | n-Pr | H | E | C$_{37}$H$_{34}$F$_2$N$_2$O$_4$·0.3H$_2$O | amorphous | | 4.9[h] |
| **19** | 4,4′-difluorobenzhydryl | i-Pr | H | E | C$_{37}$H$_{34}$F$_2$N$_2$O$_4$·0.2C$_3$H$_8$O[f], 0.5H$_2$O | amorphous | | 75%[e] |
| **20** | 4,4′-difluorobenzhydryl | c-Pr[i] | H | E | C$_{37}$H$_{32}$F$_2$N$_2$O$_4$ | 81−89 | IPE[g] | 3.4[h] |
| **21**[d] | 1-propylbutyl[j] | Me | H | E | C$_{29}$H$_{35}$N$_2$NaO4·H$_2$O | amorphous | | 2.3 ± 0.18 |
| **22** | 1-propylbutyl | Et | H | E | C$_{30}$H$_{38}$N$_2$O$_4$·0.5H$_2$O | 165−166 | IPA | 2.8 ± 0.18 |
| **23** | 1-propylbutyl[j] | n-Pr | H | E | C$_{31}$H$_{39}$N$_2$NaO4·2H$_2$O | amorphous | | 25%[e] |
| **24**[d] | 1-isobutyl-3-methylbutyl[j] | Me | H | E | C$_{31}$H$_{39}$N$_2$NaO4·2H$_2$O | amorphous | | 8.0 ± 2.4 |
| **25** | 1-isobutyl-3-methylbutyl[j] | Et | H | E | C$_{32}$H$_{41}$N$_2$NaO4·2.5H$_2$O | amorphous | | 4.8[h] |
| **26**[d] | 1-propylpentyl[j] | Me | H | E | C$_{30}$H$_{37}$N$_2$NaO4·H$_2$O | amorphous | | 4.1 ± 0.94 |
| **27** | 1-propylpentyl[j] | Et | H | E | C$_{31}$H$_{39}$N$_2$NaO4·0.2H$_2$O | amorphous | | 3.6[h] |
| **1** (MK-906) | | | | | | | | 10 ± 1.8 |
| **2** [(±)-ONO-3805] | | | | | | | | 2.5 ± 0.03 |

[a] All new compounds had C, H, N microanalyses within 0.4% of theoretical values unless otherwise noted. [b] Solvent of recrystallization: IPA, isopropyl alcohol; TL, toluene; IPE, isopropyl ether. [c] Prostates from male rats. IC$_{50}$ values are means ± SE of three separate experiments. [d] See the preceding paper.[3] [e] Percent inhibition at 100 nM of test compounds. [f] C$_3$H$_8$O, isopropyl alcohol. [g] Trituration solvent. [h] IC$_{50}$ values represents the means of triplicate determinations in a single experiment. [i] Cyclopropyl. [j] Na salt.

Initially, the effect of methyl substituents on the α,β-unsaturated double bond was investigated (**10**−**13**). Isocrotonoylamide **11** possessing a β-methyl on the double bond was confirmed to exhibit the most potent

inhibitory activity among these compounds. Methacrylamide **12** possessing an α-methyl on the double bond showed more than twice the potency of **11**, while the replacement of this portion with an α,β-dimethyl (**13**)

**Table 2.** Torsion Angle Values of Indole Derivatives



| compd | R$^2$ | R$^3$ | torsion angle,[a] deg | | | |
|-------|-------|-------|-----|-----|-----|-----|
| | | | $\tau$ | $\phi$ | $\theta$ | $\psi$ |
| **a** | H | H | −178 (20) | 180 (34) | 180 (1) | 180 (10) |
| **b** | Me | H | 140 (−22) | −179 (33) | 180 (1) | 180 (10) |
| **c** | H | Me | 140 (−22) | −173 (27) | 180 (1) | −148 (42) |
| **d** | Me | Me | 122 (−40) | −168 (22) | −179 (0) | −147 (43) |
| **e**[b] | Me | H | 162 | −146 | −179 | 170 |

[a] Torsion angles were calculated to convergent values by Nemesis [V2.1]. Values in each parentheses indicated the degree of deference from the angle observed from X-ray crystallographic analysis. [b] Corresponding torsion angles observed from X-ray crystallographic analysis of isocrotonoyl compound.[8]

and nonsubstituted (**10**) acrylamide remarkably reduced activity. To investigate whether the 5α-reductase inhibitory activity might be related to conformational changes as the result of differences in the substituents on the α,β-unsaturated double bond, energy minimization was performed on the substructure of the indole derivatives **10**−**13**. Compounds **a**−**d** were considered as candidates for each analog **10**−**13**. The central bond of each torsion angle was defined by one of the Greek letters $\tau$, $\phi$, $\theta$, and $\psi$. The relative low-energy conformers were calculated using the COSMIC force field[13] by Nemesis,[14] and the defined angles are summarized in Table 2. In the case of compound **b**, these angles were different from the angles observed from the X-ray crystallographic analysis of a structurally similar compound (**e**).[8] This result suggested that the crystal structure was not the most stable conformer. In comparison with **b**, compound **a** was most stable almost in a coplanar structure, whereas compound **d** had a twisted form. The decreased potency of **10** and **13**, which correspond to **a** and **d**, indicated that these conformations were not suitable for potent activity. On the other hand, compound **c** can overlap quite well with **b** except for the $\psi$ angle. This result was consistent with the somewhat less potent activity observed in compound **12**. From these results, the conformations were shown to be dependent on the substituents on the α,β-unsaturated double bond, and isocrotonoylamide was indicated as the optimized substructure among them.

Next, the investigation focused on the β-substituents (R$^2$) of the unsaturated double bond. To ascertain the effects of the substituents R$^2$, benzhydryl, difluorobenzhydryl, 1-propylbutyl, 1-(2-methylpropyl)-3-methylbutyl, and 1-propylpentyl were selected as the R$^1$ substituents because these exhibited a potent inhibitory activity in the parent isocrotonoylamide derivatives (**14**, **3**, **21**, **24**, and **26**) as previously reported.[8] Within the derivatives possessing a benzhydryl type substituents as R$^1$, introduction of an ethyl substituent at the β-position resulted in enhanced inhibitory activity, as represented by compounds **15** and **16**. Compounds **18**−**20** were equipotent with the parent compound **3** irrespective of the substructure of the propyl substituent at the β-position. The *Z* geometry of the double bond (**17**) apparently reduced activity. On the other hand, among the compounds possessing branched alkyl substituents at R$^1$, the effect of ethyl substitution at the β-position was

**Table 3.** Effects of 5a-Reductase Inhibitors on DHT Production

| compd | dose (mg/kg, po) | % conversion of T to DHT[a] | compd | dose (mg/kg, po) | % convervsion of T to DHT[a] |
|-------|------|------|------|------|------|
| **16** | 0 | 94 ± 3 | **2** | 0 | 91 ± 4 |
| | 3 | 76 ± 3* | | 1 | 82 ± 3 |
| | 10 | 70 ± 3** | | 3 | 88 ± 3 |
| | 30 | 50 ± 7*** | | 10 | 56 ± 8*** |
| | | | | 30 | 54 ± 2*** |
| **22** | 0 | 96 ± 2 | | | |
| | 3 | 87 ± 5 | **1** | 0 | 84 ± 5 |
| | 10 | 84 ± 4 | | 1 | 64 ± 6 |
| | 30 | 73 ± 6** | | 3 | 49 ± 3* |
| | 10 | 50 ± 10* | | 10 | 50 ± 10* |
| **3** | 0 | 98 ± 2 | | 30 | 39 ± 11* |
| | 10 | 85 ± 6 | | | |
| | 0 | 93 ± 4 | | | |
| | 30 | 62 ± 4‡‡‡ | | | |

[a] Rat prostate was removed and homogenized 4 h after oral administration of test compounds. The concentrations of testosterone (T) and DHT in the homogenate were determined by radio immunoassay. Values are presented as the mean ±SEM of four rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the control (0 mg/kg) group (Dunnet multiple comparison test). ‡‡‡$p < 0.001$, compared with the control (0 mg/kg) group (Student's *t*-test).

neutral. Compounds **22**, **25**, and **27**, possessing an ethyl substituent at R$^2$, were equipotent as the methylated compounds **21**, **24**, and **26**, while propylated compound **23** markedly reduced the potency. These results indicated that a limitation in the bulk of the R$^2$ substituent existed for potent rat prostatic 5α-reductase inhibitory activity. Among the compounds possessing a β-ethyl substituent, **16** exhibited maximum potency for rat prostatic 5α-reductase with an IC$_{50}$ value of 0.48 ± 0.086 nM, which was 20-fold more potent than **1** and 5-fold more potent than **2**.

In addition to inhibitory activity for rat prostatic 5α-reductase in vitro, we examined the inhibitory effect of the several potent indole derivatives, **1** and **2** on DHT production in vivo (Table 3). Four hours after oral dosing with test compounds at 1−30 mg/kg, the rats were sacrificed and prostates were removed. The effect on DHT production was determined by the previously reported method[15] and expressed as the percent conversion of testosterone to DHT. In our experiments, compounds **1** and **2** significantly inhibited the percent conversion at 3 and 10 mg/kg, respectively. Indole derivatives that exhibited potent activity in vitro also inhibited DHT production in vivo. Compound **16**, which had a 20-fold more potent inhibitory activity on rat prostatic 5α-reductase than **1**, significantly inhibited DHT production at 3 mg/kg.

Several potent indole derivatives, **1** and **2**, were tested for inhibitory effects on rat and human 5α-reductase isozymes (Table 4). The rat type 2 isozyme was obtained from epididymis that exhibited an optimal pH of 5.5[16] and human type 2 isozyme expressed in transfected Namalwa cells with an optimal pH of 5.5.[17] Indole derivatives **3**, **16**, and **22** expressed an extremely potent inhibitory acitivity on rat type 2 as the same as **2** with IC$_{50}$ values on the order of $10^{-10}$ M. Compound **1** strongly inhibited the human type 2 isozyme with an IC$_{50}$ value of 0.58 ± 0.17 nM in our assay, whereas indole derivatives and **2** showed less potent activity with IC$_{50}$ values of $10^{-7}$ M and 78.7 ± 16.3 nM, respectively. From the result of assays on rat and human isozymes, it appeared that the indole derivatives and **2** expressed an oppsite selectivity for rats and human isozymes and

**Table 4.** Effects of Indole Derivatives on Rat and Human 5α-Reductase Isozymes

| | $IC_{50}$ (nM ± SE)[a] | |
| --- | --- | --- |
| compd | rat[b] epididymis | transfected[c] Namalwa cell |
| **16** | 0.28[d] | 357[d] |
| **22** | 0.75[d] | 341[d] |
| **3** | 0.21[d] | 215 ± 54.6 |
| **2** | 0.18[d] | 78.7 ± 16.3 |
| **1** | 2.1[d] | 0.58 ± 0.17 |

[a] $IC_{50}$ values are means ± SEM of three separate experiments. [b] Rat epididymises were homogenated and assayed in vitro for steroid 5α-reductase activity. A 10 mg sample of cellular protein was assayed in 40 mM Tris-citrate buffer (pH4.5) in the presence of 150 nM [$^{14}$C]testosterone, 2 mM NADPH, and the indicated concentrations of drugs. [c] Transfected Namalwa cells were homogenated and assayed in vitro for steroid 5α-reductase activity. Then 40 μg of cellular protein were assayed in 0.1 M $Na_2HPO_4$–citrate buffer (pH5.5) in the presence of 0.3 μM [$^{14}$C]testosterone, 2 mM NADPH and the indicated concentrations of drugs.[18] [d] The values represent the means of triplicate determinations in a single experiment.

significant species difference compared with a steroidal inhibitor **1**.

Indole derivatives, such as **3** and **16** were potent inhibitors of rat 5α-reductase with selectivity versus other enzymes taking part in the steroidal biosynthesis. These compounds did not show any significant activities for 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α- and -β-hydroxysteroid dehydrogenase (17-HSD), and aromatase even up to a concentration of 100 mM. These indole derivatives did not show any affinity for the androgen receptor at the same concentration.

In conclusion, the structure of the linking unit between the anilide moiety and indole skeleton was important in determining potent rat 5α-reductase inhibitory activity. An ethyl instead of a methyl substituent in the β-position of **3** resulted in enhancement of inhibitory activity for rat prostatic 5α-reductase. Compound **16**, which showed the maximum potency with an $IC_{50}$ value of 0.48 ± 0.086 nM, also effectively inhibited DHT production in vivo (3 mg/kg, po, in Table 3). 5α-Reductase assays on rat and human isozymes showed that nonsteroidal inhibitors, such as indole derivatives and **2**, exhibited significant species differences, whereas a selective human type 2 isozyme inhibitor **1** was effective both for rat and human type 2 isozymes. To provide a new agent for BPH therapy, further efforts to discover nonsteroidal 5α-reductase inhibitors possessing potent inhibitory activity for the human type 2 isozyme are ongoing.

## 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 or EX-270 (270 MHz) spectrometer with Me$_4$Si as internal standard. Elemental analyses were performed by the analytical department of our laboratories.

**1-(4,4′-Difluorobenzhydryl)indoline-5-carbaldehyde (5).** To a stirred solution of POCl$_3$ (4.30 mL, 45.9 mmol) in 10 mL of dichloroethane was added dropwise DMF (3.5 mL, 46 mmol), and the solution was stirred at room temperature for 1 h. After a solution of 1-(4,4′-difluorobenzhydryl)indoline (**4**) (9.8 g, 31 mmol) in 20 mL of dichoroethane was added, the reaction mixture was stirred at 50 °C for 2 h and then poured into sodium acetate (50 g, 0.61 mol) in 300 mL of water. The mixture was extracted with dichloromethane washed with

aqueous sodium hydrogen carbonate and brine, dried with MgSO$_4$, filtered, and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−AcOEt (2:1) to afford **5** (8.3 g, 78%) as a pale yellow oil: $^1$H NMR (CDCl$_3$) δ 3.02 (t, 2H, $J = 9.6$ Hz), 3.37 (t, 2H, $J = 9.6$ Hz), 5.81 (s, 1H), 6.21 (d, 1H, $J = 11.4$ Hz), 6.83−7.53 (m, 9H), 7.51 (s, 1H), 9.63 (s, 1H).

**1-(4,4′-Difluorobenzhydryl)-5-propionylindole (6).** To a solution of **5** (8.8 g, 25 mmol) in 200 mL of THF was added at −78 °C ethylmagnesium bromide (0.93 M in THF; 38 mL, 35 mmol), and the solution was stirred for 1 h. After addition of water, the mixture was extracted with EtOAc. The organic layer was washed with 1 N HCl, saturated sodium hydrogen carbonate and brine successively, dried, and evaporated in vacuo. To a solution of this residue in 200 mL of toluene was added MnO$_2$ (44 g, 0.50 mol), and the mixture was heated under reflux for 1 h. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was evaporated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−AcOEt (3:1) to afford **6** (6.0 g, 64%) as a colorless oil: $^1$H NMR (CDCl$_3$) δ 1.21 (t, 3H, $J = 6.6$ Hz), 3.01 (q, 2H, $J = 6.6$ Hz), 6.62 (d, 1H, $J = 3.3$ Hz), 6.83 (s, 1H), 6.85 (d, 1H, $J = 3.3$ Hz), 7.00−7.40 (m, 9H), 7.70−7.90 (m, 1H), 8.34 (br, 1H).

**Method A. (E)-3-(1-Benzylindol-5-yl)-2-methyl-2-propenoic Acid (7).** To a suspension of NaH (60% in oil; 0.51 g, 13 mmol) in 30 mL of THF was added triethyl 2-phosphonopropionate (2.7 mL, 13 mmol) dropwise at 0 °C. After the solution was stirred at 0 °C for 15 min, a solution of 1-benzylindole-5-carbaldehyde (1.0 g, 4.3 mmol) in 5 mL of THF was added dropwise. After being stirred at room temperature for 1 h, the reaction mixture was added to water and then 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 (4:1) to afford ethyl (E)-3-(1-benzylindol-5-yl)-2-methyl-2-propenoate (1.25 g, 92%) as a colorless oil: $^1$H NMR (CDCl$_3$) δ 1.35 (t, 3H, $J = 7.1$ Hz), 2.19 (s, 3H), 4.27 (q, 2H, $J = 7.1$ Hz), 5.30 (s, 2H), 6.57 (d, 1H, $J = 3.0$ Hz), 7.08−7.14 (m, 2H), 7.14 (d, 1H, $J = 3.0$ Hz), 7.23−7.33 (m, 5H), 7.74 (s, 1H), 7.84 (s, 1H). A mixture of obtained ethyl ester (1.25 g, 3.91 mmol), 12 mL of 1 N LiOH, and 25 mL of 1,4-dioxane was stirred at 70−80 °C for 2 h. The mixture was evaporated in vacuo. The residue was dissolved in 25 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 **7**. This was recrystallized from 2-propanol to give pure **7** (1.11 g, 97%) as colorless crystals: mp 191−192 °C; IR (KBr) 3450, 1668, 1600, 1447, 1274 cm$^{-1}$; $^1$H NMR (CDCl$_3$) δ 2.09 (d, 3H, $J = 1.0$ Hz), 5.43 (s, 2H), 6.54 (d, 1H, $J = 3.0$ Hz), 7.10−7.50 (m, 8H), 7.71 (s, 2H).

**Method B. (E)-3-[1-(1-Propylbutyl)indol-5-yl]-2-pentenoic Acid (7).** To a solution of diisopropylamine (4.4 mL, 31 mmol) was added dropwise at 0 °C in 50 mL of THF, n-butyllithium (1.65 M in hexane; 19 mL, 31 mmol), and the solution was stirred for 20 min at the same temperature. After this solution was cooled at −78 °C, ethyl (trimethylsilyl)acetate (4.8 mL, 26 mmol) was added, and the mixture was stirred at same temperature for 20 min. A solution of 1-(1-propylbutyl)-5-propionylindole (2.8 g, 11 mmol) in 14 mL of THF was added, and the mixture was stirred at −70 to 0 °C for 2 h. After addition of saturated aqueous NH$_4$Cl, the mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was chromatgraphed on silica gel, eluting with hexane−AcOEt (20:1) to afford ethyl (E)-3-[1-(1-propylbutyl)indol-5-yl]-2-pentenoate (3.2 g, 90%) as a colorless oil: $^1$H NMR (CDCl$_3$) δ 0.83 (t, 6H, $J = 6.9$ Hz), 1.00−1.35 (m, 4H), 1.14 (t, 3H, $J = 7.4$ Hz), 1.30 (t, 3H, $J = 7.2$ Hz), 1.70−2.00 (m, 4H), 3.18 (q, 2H, $J = 7.4$ Hz), 4.10−4.40 (m, 1H), 4.19 (q, 2H, $J = 7.2$ Hz), 6.07 (s, 1H), 6.52 (d, 1H, $J = 3.3$ Hz), 7.12 (d, 1H, $J = 3.3$ Hz), 7.32 (s, 2H), 7.74 (s, 1H). The obtained ethyl ester (3.2 g, 9.4 mmol) was hydrolyzed by the same procedure described above to afford crude **7**. This was recrystallized from diisopropyl ether to give pure **7** (2.4 g, 82%) as pale brown crystals: mp 127−129 °C; IR (KBr) 2954, 1679, 1598, 1413, 1297, 1218, 723 cm$^{-1}$;

$^1$H NMR (CDCl$_3$) δ 0.84 (t, 6H, $J$ = 6.3 Hz), 1.00−1.30 (m, 4H), 1.16 (t, 3H, $J$ = 7.4 Hz), 1.70−2.00 (m, 4H), 3.21 (q, 2H, $J$ = 7.4 Hz), 4.30 (dq, 1H, $J$ = 7.4 Hz), 6.13 (s, 1H), 6.54 (d, 1H, $J$ = 3.3 Hz), 7.15 (d, 1H, $J$ = 3.3 Hz), 7.36 (br, 2H), 7.79 (br, 1H).

**Method C**. **Ethyl (*E*)-4-{2-[[3-[1-(1-Propylbutyl)indol-5-yl]-1-oxo-2-pentenyl]amino]phenoxy}butyrate (9)**. To a mixture of ethyl 4-(2-aminophenoxy)butyrate (2.4 g, 11 mmol), 2-chloro-1-methylpyridinium iodide (2.4 g, 9.6 mmol), and tributylamine (4.4 mL, 19 mmol) in 48 mL of CH$_2$Cl$_2$ was added at reflux a solution of (*E*)-3-[1-(1-propylbutyl)indol-5-yl]-2-pentenoic acid (2.4 g, 7.7 mmol) in 12 mL of CH$_2$Cl$_2$, and the mixture was stirred at reflux for 1 h. Upon cooling, the 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 hexane−AcOEt (5:1) to afford **9** (3.2 g, 81%) as an oil: $^1$H NMR (CDCl$_3$) δ 0.86 (d, 6H, $J$ = 7.2 Hz), 1.09−1.29 (m, 4H), 1.13 (t, 3H, $J$ = 7.1 Hz), 1.19 (t, 3H, $J$ = 7.5 Hz), 1.73−1.91 (m, 4H), 2.14−2.23 (m, 2H), 2.51 (t, 2H, $J$ = 6.7 Hz), 3.29 (q, 2H, $J$ = 7.5 Hz), 4.07 (q, 2H, $J$ = 7.1 Hz), 4.08 (t, 2H, $J$ = 5.7 Hz), 4.25−4.36 (m, 1H), 6.31 (s, 1H), 6.56 (d, 1H, $J$ = 3.0 Hz), 6.83−6.86 (m, 1H), 6.94−7.01 (m, 2H), 7.15 (d, 1H, $J$ = 3.0 Hz), 7.33−7.41 (m, 2H), 7.79 (s, 1H), 8.05 (br, 1H), 8.50−8.60 (m, 1H).

**Method D**. **Ethyl (*E*)-4-{2-[[3-[1-(1-Propylbutyl)indol-5-yl]-1-oxo-2-hexenyl]amino]phenoxy}butyrate (9)**. To a mixture of ethyl 4-(2-aminophenoxy)butyrate (0.34 g, 1.5 mmol), (*E*)-3-[1-(1-propylbutyl)indol-5-yl]-2-hexenoic acid (0.25 g, 0.76 mmol), and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.23 g, 0.92 mmol) in 25 mL of CH$_2$Cl$_2$ was added triethylamine (0.23 mL, 1.7 mmol) dropwise at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was added to water and extracted with ether. The organic layer was washed with 1 N HCl, water, and brine successively, dried, and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−AcOEt (4:1) to afford **9** (0.22 g, 54%) as a yellow oil: $^1$H NMR (CDCl$_3$) δ 0.85 (t, 6H, $J$ = 7.4 Hz), 0.99−1.35 (m, 10H), 1.38−1.80 (m, 2H), 1.72−2.06 (m, 4H), 2.11−2.33 (m, 2H), 2.51 (t, 2H, $J$ = 6.4 Hz), 3.27 (t, 2H, $J$ = 7.6 Hz), 4.07 (q, 2H, $J$ = 7.1 Hz), 4.08 (t, 2H, $J$ = 5.7 Hz), 4.25−4.37 (m, 1H), 6.31 (s, 1H), 6.56 (d, 1H, $J$ = 3.3 Hz), 6.77−7.10 (m, 3H), 7.15 (d, 1H, $J$ = 3.3 Hz), 7.35 (s, 2H), 7.77 (s, 1H), 8.02 (br, 1H), 8.49−8.59 (m, 1H).

**(*E*)-4-{2-[[3-[1-(1-Propylbutyl)indol-5-yl]-1-oxo-2-pentenyl]amino]phenoxy}butyric Acid (22)**. A mixture of ethyl (*E*)-4-{2-[[3-[1-(1-propylbutyl)indol-5-yl]-1-oxo-2-pentenoyl]-amino]phenoxy}butyrate (3.2 g, 6.2 mmol), 1.9 mL of 10 N NaOH, and 30 mL of EtOH was stirred at 50 °C for 1 h. The mixture was evaporated in vacuo, and the residue was dissolved in 30 mL of water. The mixture was acidified with 4 N HCl to pH 3 and extracted with AcOEt. The organic layer was washed with brine, dried, and evaporated in vacuo. The residue was triturated with diisopropyl ether to afford **22** (2.5 g, 83%) as a white powder: mp 165−166 °C; IR (KBr) 3222, 2822, 1647, 1568, 1476, 1253, 1206, 1155 cm$^{-1}$; $^1$H NMR (CDCl$_3$) δ 0.83 (t, 6H, $J$ = 6.6 Hz), 1.03−1.37 (m, 10H), 1.16 (t, 3H, $J$ = 7.5 Hz), 1.70−2.04 (m, 4H), 2.05−2.28 (m, 2H), 2.42−2.61 (m, 2H), 3.24 (q, 2H, $J$ = 7.5 Hz), 4.07 (t, 2H, $J$ = 5.9 Hz), 4.22−4.34 (m, 1H), 5.70−5.90 (m, 1H), 6.16 (s, 1H), 6.54 (d, 1H, $J$ = 3.2 Hz), 6.75−7.14 (m, 3H), 7.28 (d, 1H, $J$ = 3.2 Hz), 7.39 (s, 2H), 7.74 (s, 1H), 7.91 (s, 1H), 8.25−8.55 (m, 1H). Anal. (C$_{30}$H$_{38}$N$_2$O$_4$·0.5H$_2$O) C, H, N.

**Biological Methods**. **Rat Type 1 5α-Reductase Assay.** The preparation of rat prostate particulates and the assay of 5α-reductase were carried out according to the reported procedure.[19] 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 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 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 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%.

**Rat Type 2 5α-Reductase Assay.** The preparation of rat epididymis particulates and the assay of 5α-reductase were carried out according to the reported procedure.[9] The epididymis from male Wistar rats (200−300 g, Japan Cler), sacrificed by cervical dislocation, were minced and homogenized in 10 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 100000$g$ for 0.5 h at 2 °C. The resulting pellet was washed once with medium A and resuspended in the same medium (10−20 mg of protein/mL). The enzyme preparation was stored at −80 °C. The reaction solution contained 1 mM dithiothreitol, 40 mM Tris-citrate, pH 4.5, 2 mM NADPH, [$^{14}$C]-testosterone (T) (150 nM), and the enzyme preparation (10 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 10 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 25 mL of ethyl acetate and chromatographed on a Whatman Silica plate LK6DF, using dichloromethane−diethyl ether (11:1) 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 (Fuji Film, BAS2000). The rate of the conversion by the enzyme and the rate of the inhibition by test compounds were calculated according to the same formula described above.

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