A Novel Class of Inhibitors for Human and Rat Steroid 5α-Reductases: Synthesis and Biological Evaluation of Indoline and Aniline Derivatives. III[1,2)]

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While searching for novel nonsteroidal inhibitors of human and rat prostatic 5α-reductases, we found a new series of indoline and aniline derivatives that showed potent inhibitory activities for both enzymes. Among them, 3-chloro-4-{[1-(4-phenoxybenzyl)indolin-5-yl]oxy}benzoic acid (2e, YM-36117) showed a more potent inhibitory activity for the human enzyme than ONO-3805 with an IC_{50} value of 5.3 nM and a reduced rat prostatic dihydrotestosterone (DHT) concentration by oral administration. The synthesis and the structure–activity relationships of these indoline and aniline derivatives are presented.

Key words 5α-reductase inhibitor; benign prostatic hypertrophy; YM-36117

5α-Reductase is an NADPH-dependent enzyme responsible for the conversion of testosterone to (DHT) which is an active agonist for androgen receptor. Benign prostatic hyperplasia (BPH), skin disorders such as acne, male pattern baldness and hirsutism are androgen-related disorders, associated with elevated levels of DHT.[6)] As a consequence, a 5α-reductase inhibitor is expected to provide a potential treatment for androgen-related disorders. Several 5α-reductase inhibitors have been reported,[7,8)] including both steroidal inhibitors, finasteride[7a)] and epristeride,[7b)] and a nonsteroidal inhibitor, ONO-3805[8a)] (Chart 1). Following the discovery of these inhibitors, the existence of two different 5α-reductase isozymes, called type 1 and type 2 5α-reductases, have been reported in humans and rats.[9—11)] The type 1 enzyme is normal in men with congenital 5α-reductase deficiency and is expressed in skin tissue throughout the body that has an optimal pH of between 6 and 9. The type 2 enzyme is defective in men with congenital 5α-reductase deficiency and is the dominant form of the enzyme in genital tissue, including prostate that has an optimal pH of about 5.5. However, the physiological role of these isozymes has yet to be fully elucidated. Steroidal inhibitors, for example, finasteride, have been shown to be clinically effective for the treatment of BPH, however, the possibility of adverse effects due to their steroidal structures remains.[12)] On the other hand, nonsteroidal inhibitors have not shown clinical efficacy for the treatment of BPH. Finasteride showed strong inhibitory activity (IC_{50}=4.1 nM)[13)] for human prostatic 5α-reductase, whereas ONO-3805 showed moderate activity (IC_{50}= 538 nM)[13)] which might not be sufficient to show clinical efficacy. We considered that a more potent nonsteroidal inhibitor of human prostatic 5α-reductase than ONO-3805 could show clinical efficacy for the treatment of BPH and reduce the potential of side effects compared to steroidal inhibitors.

In our previous studies on novel human prostatic 5α-reductase inhibitors, we found the 4-(1*H*-indol-5-yl)oxy benzoic acid derivative, YM-32906 (**1**), which showed potent inhibitory activity with an IC_{50} value of 0.44 nM.[2)] However,

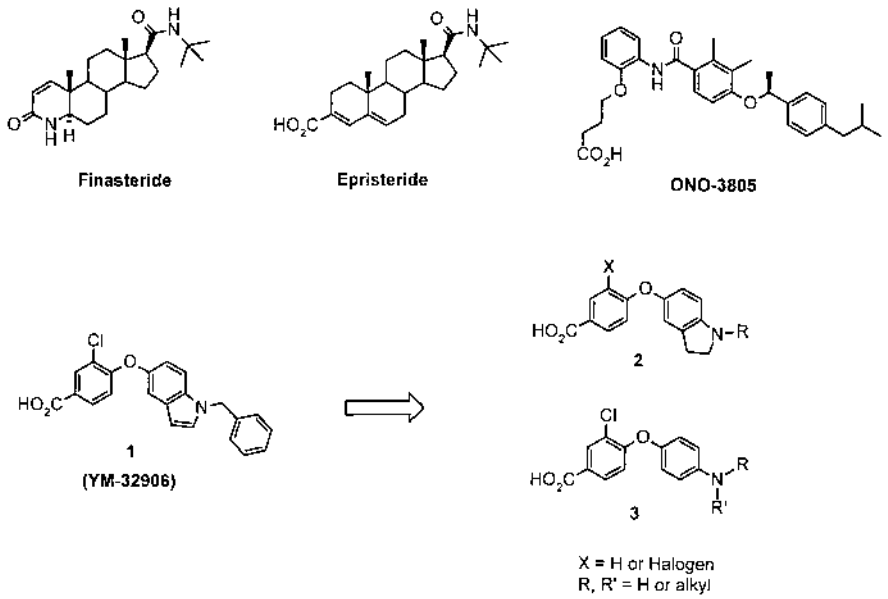

Chart 1

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these indole derivatives showed reduced inhibitory activity for rat prostatic 5α-reductase and were not sufficient to show inhibitory activity in a rat *in vivo* model.[14] In this study, our attention focused on discovering novel benzoic acid derivatives having potent inhibitory activities for both human and rat prostatic 5α-reductases by the modification of the 1*H*-indol-5-yloxy group of **1**. The indolin-5-yloxy group and 4-aminophenoxy group as the substituent at the 4-position of the benzoic acid moiety were newly designed as described in Chart 1. We now report the synthesis and the structure–activity relationships of these benzoic acid derivatives **2** and **3**.

Chemistry The indoline derivatives (**2a**—**2j**) were prepared as described in Charts 2 and 3. Compound **4** was treated with diethyl pyrocarbonate and 4-dimethylaminopyridine (DMAP) to give compound **5**. The reduction of the indole ring and the debenzylation were carried out by catalytic hydrogenation in the presence of Pd–C to afford compound **6**, which was alkylated with 3-chloro-4-fluorobenzonitrile in the presence of K_2CO_3 in *N*,*N*-dimethylformamide (DMF) and hydrolyzed with KOH to give the benzoic acid derivative **8**. The esterification of **8** was performed using H_2SO_4 in EtOH to afford compound **9**, which was alkylated with benzyl bromide or (1-bromoethyl)benzene and subsequent hydrolysis with NaOH to give compounds **2a** and **2b**. Compound **2c** was obtained by reductive alkylation of **8** in the presence of $NaBH(OAc)_3$ with 4-isopropylbenzaldehyde. Compound **11**, prepared using the same procedure for the preparation of **6** with di-*tert*-butyl pyrocarbonate, was alkylated with 4-fluorobenzonitrile derivatives followed by the deprotection with CF_3CO_2H to give compound **12**. Compounds **2d**—**2j** were obtained by the *N*-alkylation of **12** with 2-(bromomethyl)naphthalene or aldehydes and subsequent hydrolysis with KOH.

The aniline derivatives (**3a**—**3h**) were prepared as described in Charts 4 and 5. The *O*-alkylation of the aminophenol derivatives **13** with 3-chloro-4-fluorobenzonitrile afforded **14**, which was converted to the benzoic acids **3a**, **3b** and **3h** in one or two steps as described in Chart 4. Compounds **3c**—**3g** were prepared as described in Chart 5. Compound **15** was alkylated with 1-fluoro-4-nitrobenzene followed by a catalytic hydrogenation in the presence of Raney-Ni to give **16**. Compound **3e** was obtained by the acylation with benzoyl chloride of **16** and subsequent hydrolysis with KOH. Compound **16** was alkylated with 2 eq of benzyl bromide followed by hydrolysis with NaOH to afford **3f**. The reductive alkylation of **16** was performed using $NaBH(OAc)_3$ to give **17**, which was methylated with formaldehyde in the presence of formic acid to afford **18**. Compounds **17** and **18** were converted to the benzoic acids **3c**, **3d** and **3g** by hydrolysis with NaOH.

Results and Discussion

The inhibitory activities of the indoline and aniline derivatives were evaluated on the basis of their ability to inhibit 5α-reductases in human prostate at pH 5.5 and rat prostate at pH 6.5, which are optimal for the activities of the type 2 enzymes. These results are described in Tables 1 and 2.

In our previous paper, we found that the indole derivative,

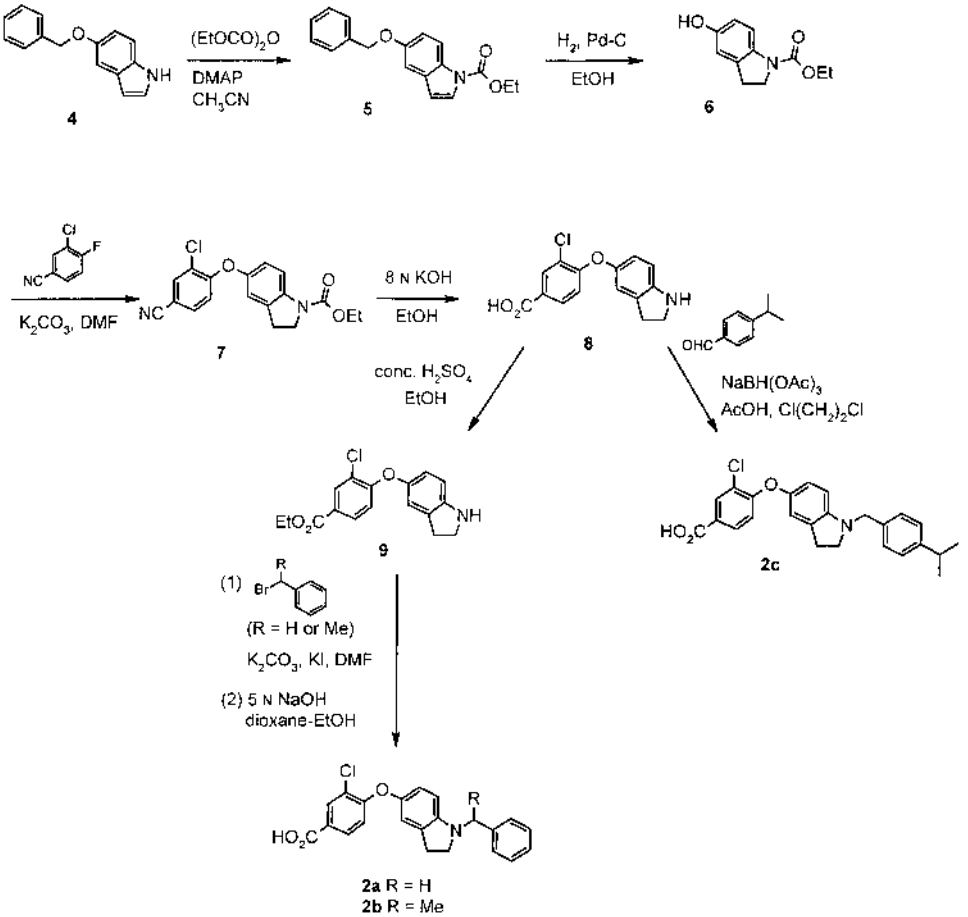


Chart 2

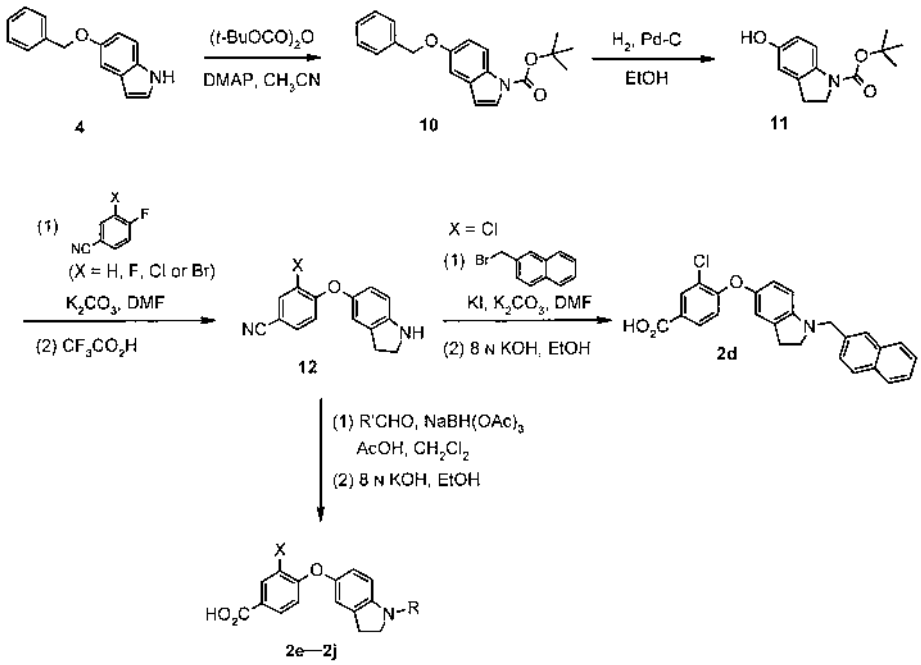


Chart 3

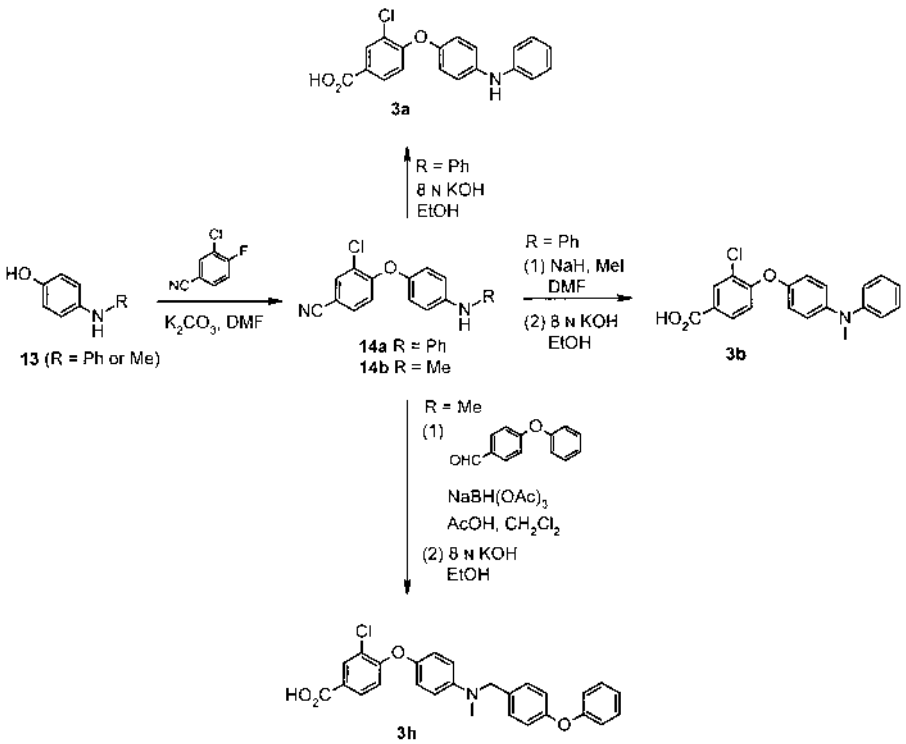


Chart 4

having a hydrophobic substituent such as the 4-phenoxybenzyl group at the 1-position of the indole ring, showed moderate inhibitory activity against the rat enzyme with an IC_{50} value of 73 nM.[2] However, this indole derivative was not sufficient to show inhibitory activity in a rat *in vivo* model. Initially, the effects of substituents at the 1-position of the indoline ring were investigated as shown in Table 1. The benzyl derivative **2a**, the 1-phenylethyl derivative **2b**, the 4-isopropylbenzyl derivative **2c** and the naphthalen-2-ylmethyl derivative **2d** showed potent inhibitory activities for the human enzyme with IC_{50} values below 10 nM but no inhibitory activities for the rat enzyme at 300 nM. On the other hand, the 4-phenoxybenzyl derivative **2e** retained its high potency for the human enzyme and showed moderate inhibitory activity for the rat enzyme with an IC_{50} value of 46 nM. Furthermore, the 4-benzyloxybenzyl derivative **2f** and the 4-(4-isopropylphenoxy)benzyl derivative **2g** resulted in a loss in activity for the rat enzyme, suggesting that the 4-phenoxy-

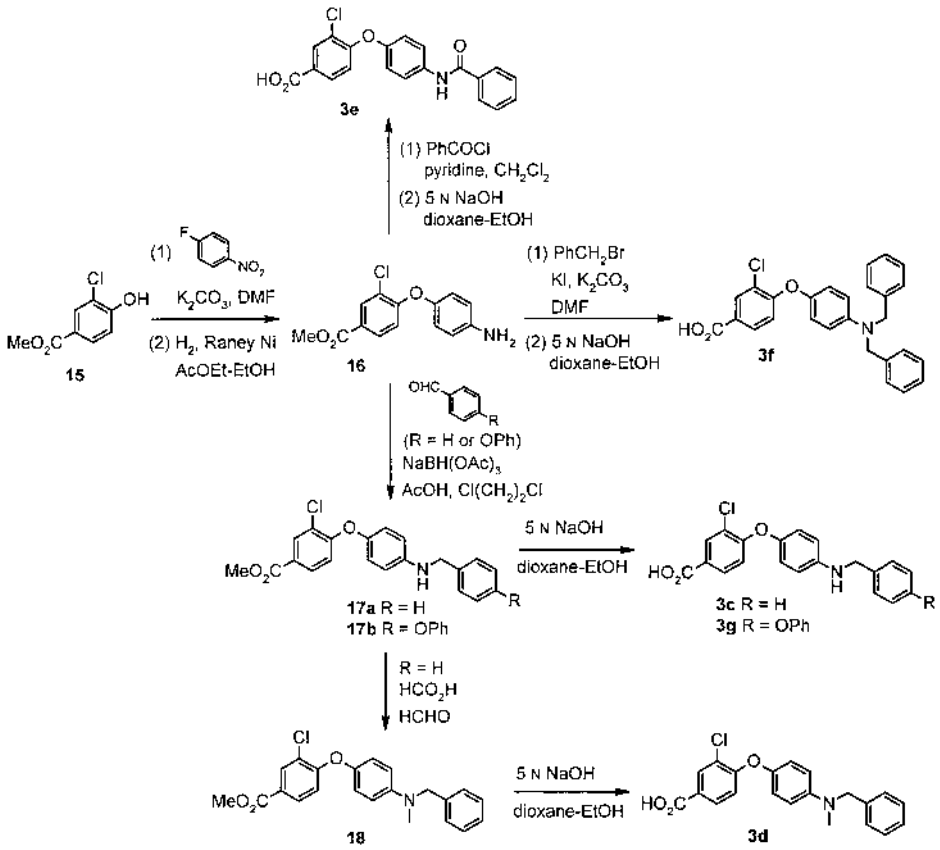


Chart 5

benzyl group plays an important role for showing potent inhibitory activity against the rat enzyme. Next, modification of the chloro group of compound **2e** was investigated. Compound **2h**, which has no substituent at the position, showed reduced activity, while the fluoro derivative **2i** and the bromo derivative **2j** retained potency for the rat enzyme. These findings suggested that a hydrophobic substituent at the 3-position of the benzoic acid moiety such as a halogen is favorable for the inhibitory activity against the rat enzyme.

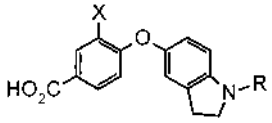
Concerning the inhibitory activities of the aniline derivatives, the 4-anilinophenoxy derivatives (**3a**, **3b**) and 4-benzylaminophenoxy derivatives (**3c**, **3d**, **3f**) were selective human prostatic 5α-reductase inhibitors as shown in Table 2. The *N*-methylated compounds **3b** and **3d** showed more potent inhibitory activity compared to the parent compounds **3a** and **3c**, while the 4-benzoylaminophenoxy derivative **3e** showed reduced potency and the 4-dibenzylaminophenoxy derivative **3f** resulted in a slight loss of potency compared to compound **3c**. These results suggested that a proton of the amino group is unfavorable and a hydrophobic and bulky substituent such as the dibenzylamino group is tolerant for the display of potent inhibitory activity against the human enzyme. On the other hand, the 4-[*N*-(4-phenoxybenzyl)amino]phenoxy derivatives (**3g**, **3h**) resulted in potent inhibitory activities for both the human and rat enzymes. Compound **3g** showed the most potent inhibitory activity against the rat enzyme in these aniline derivatives with an IC_{50} value of 5.5 nM. These results suggested that the 4-phenoxybenzyl group of these aniline derivatives is also effective for providing potent inhibitory activity against the rat enzyme as shown by the indoline derivatives.

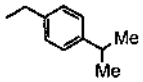
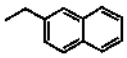
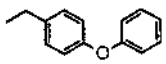
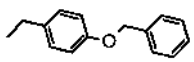
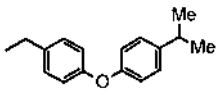
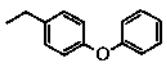
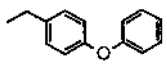
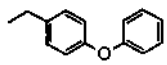
In order to investigate *in vivo* inhibitory activity of these indoline and aniline derivatives, compounds **2e**, **2j**, **3g**, and **3h**, which showed potent inhibitory activities for the rat enzyme, were evaluated for their inhibitory activity of the DHT concentration in rat prostate by oral administration. These results are described in Table 3. In this model, (±)-ONO-3805 showed significant *in vivo* inhibitory activities at 10 mg/kg. The aniline derivative **3g**, having a potent *in vitro* inhibitory activity for the rat enzyme, did not improve the *in vivo* inhibitory activity compared to (±)-ONO-3805. On the other hand, the indoline derivative **2e**, having 18 times less potent *in vitro* inhibitory activity for the rat enzyme than (±)-ONO-3805, reduced prostatic DHT by 44% at a dose of 100 mg/kg. Since compound **2e** showed 100 times more potent *in vitro* inhibitory activity for the human enzyme compared to (±)-ONO-3805, there is a possibility to show clinical efficacy for the treatment of BPH.

Conclusions

In order to prepare a novel potent inhibitor against both human and rat prostatic 5α-reductases, we modified the 1*H*-indol-5-yloxy group of YM-32906 to indolin-5-yloxy group or 4-aminophenoxy group. Consequently, we have identified a new series of indoline and aniline derivatives having a 4-phenoxybenzyl group as potent inhibitors for both human and rat prostatic 5α-reductases. Particularly, compound **2e** (YM-36117) showed potent *in vivo* inhibitory activity on rat prostatic DHT. These derivatives have the possibility to display clinical efficacy for the treatment of BPH.

Table 1. Physicochemical Data and Inhibitory Activities of 5α-Reductases in Human and Rat Prostate Homogenates for Indoline Derivatives



Compound No.	R	X	mp (°C)	Formula	Analysis (%) Calcd (Found)				IC_{50} (nM)	
					C	H	N	Halogen	Human	Rat
2a		Cl	142—143	$C_{22}H_{18}ClNO_3$	69.57 (69.63	4.78 4.87	3.69 3.75	9.33 9.41)	1.8	>300
2b		Cl	123—125	$C_{23}H_{20}ClNO_3$	70.14 (69.95	5.12 5.12	3.56 3.65	9.00 9.03)	8.8	>300
2c		Cl	109—111	$C_{25}H_{24}ClNO_3$	71.17 (71.24	5.73 5.93	3.32 3.22	8.40 8.34)	2.2	>300
2d		Cl	182—183	$C_{26}H_{20}ClNO_3$	72.64 (72.66	4.69 4.63	3.26 3.27	8.25 8.17)	3.9	>300
2e (YM-36117)		Cl	164—165	$C_{28}H_{22}ClNO_4$	71.26 (71.26	4.70 4.69	2.97 2.99	7.51 7.40)	5.3	46
2f		Cl	116—118	$C_{29}H_{24}ClNO_4$	71.68 (71.58	4.98 4.96	2.88 2.73	7.30 7.29)	3.4	>300
2g		Cl	134—136	$C_{31}H_{28}ClNO_4$	72.44 (72.41	5.49 5.50	2.72 2.78	6.90 6.88)	11	>300
2h		H	142—144	$C_{28}H_{23}NO_4$	76.87 (76.70	5.30 5.37	3.20 3.17)		11	48% inhibition at 100 nM
2i		F	116—119	$C_{28}H_{22}FNO_4$	73.84 (73.90	4.87 4.83	3.08 3.07	4.17 4.17)	4.3	54
2j		Br	193—198	$C_{28}H_{22}BrNO_4$	65.13 (64.99	4.29 4.21	2.71 2.75	15.47 15.27)	6.4	19
1 (YM-32906)									0.44	>300
Finasteride									4.1	13
(±)-ONO-3805									5.38	2.6

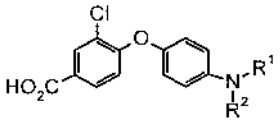
Experimental

Melting points were taken on a Yanaco MP-3 melting point apparatus and are uncorrected. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-90, JNM-LA 300, JNM-LA 400, JNM-GX 400 or JNM-GX 500 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Hitachi M-80 (electron impact (EI)) or JEOL JMS-DX300 (FAB) mass spectrometer. Elemental analyses were performed with a Yanaco MT-5. Column chromatography was carried out on silica gel (Wakogel C-200). Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification. In general, the organic extract was dried over anhydrous Na_2SO_4 or $MgSO_4$, and the organic solvent was evaporated under reduced pressure. All nonaqueous reactions were performed in dry glassware under an atmosphere of dry Ar.

Ethyl 5-Benzyloxy-1*H*-indole-1-carboxylate (5) A mixture of **4** (6.70 g, 30.0 mmol), diethyl pyrocarbonate (5.84 g, 36.0 mmol) and DMAP (370 mg, 3.03 mmol) in 30 ml of CH_3CN was stirred at room temperature for 15 h. After the reaction mixture was concentrated, the residue was extracted with AcOEt. The extract was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=95 : 5) to give **5** (5.38 g, 61%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.45 (t, 3H, J=7 Hz), 4.46 (q, 2H, J=7 Hz), 5.10 (s, 2H), 6.50 (d, 1H, J=3 Hz), 7.02 (dd, 1H, J=9, 2 Hz), 7.10 (d, 1H, J=2 Hz), 7.3—7.4 (m, 3H), 7.45 (d, 2H, J=7 Hz), 7.58 (d, 1H, J=3 Hz), 8.05 (d, 1H, J=8 Hz); EI-MS m/z 295 (M^+).

Ethyl 5-Hydroxyindoline-1-carboxylate (6) To a solution of **5** (5.38 g,

Table 2. Physicochemical Data and Inhibitory Activities of 5α-Reductases in Human and Rat Homogenates for Aniline Derivatives



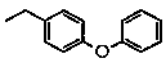
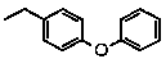
Compound No.	R^1	R^2	mp (°C)	Formula	Analysis (%) Calcd (Found)				IC_{50} (nM)	
					C	H	N	Cl	Human	Rat
3a		H	196—197	$C_{19}H_{14}ClNO_3$	67.16 (67.04	4.15 4.16	4.12 4.14	10.43 10.50)	14	>300
3b		Me	133—134	$C_{20}H_{16}ClNO_3$	67.90 (67.86	4.56 4.57	3.96 3.99	10.02 10.14)	3.3	>300
3c		H	167—168	$C_{20}H_{16}ClNO_3$	67.90 (67.86	4.56 4.60	3.96 3.94	10.02 10.12)	7.5	>300
3d		Me	154—155	$C_{21}H_{18}ClNO_3$	68.57 (68.50	4.93 4.85	3.81 3.80	9.64 9.74)	2.1	>300
3e		H	245—246	$C_{20}H_{14}ClNO_4$	65.31 (65.09	3.84 3.82	3.81 3.84	9.64 9.82)	100	>300
3f			183—184	$C_{27}H_{22}ClNO_3$	73.05 (72.91	5.00 5.11	3.16 3.12	7.99 7.99)	12	>300
3g		H	205—206	$C_{26}H_{20}ClNO_4$	70.03 (69.87	4.52 4.58	3.14 3.08	7.95 7.82)	10	5.5
3h		Me	177—178	$C_{27}H_{22}ClNO_4 \cdot 1/4H_2O$	69.83 (69.90	4.88 5.01	3.02 3.10	7.63 7.87)	11	12

Table 3. Effects of Selected Indoline and Aniline Derivatives on Prostatic Content of DHT in Adult Male Rats

Compd. No.	*In vitro* IC_{50} (nM)		*In vivo*[a)]	
	Human	Rat	Dose (mg/kg, *p.o.*)	% Reduction of prostatic DHT
2e (YM-36117)	5.3	46	100	44± 6**
2j	6.4	19	100	20± 9*
3g	10	5.5	100	25±10*
3h	11	12	100	36± 4**
(±)-ONO-3805	538	2.6	10	25±10*
			30	65± 5**

a) *In vivo* effects of test compounds on reduction of DHT in rat prostate. Rat prostate was removed and homogenized 8 h after oral administration of test compounds. The concentrations of DHT in the homogenate were determined by radioimmunoassay. Results are the mean±SE of five to seven separate assays. Statistically significant at $*p<0.05$ and $**p<0.01$ compared with control value by Student's *t*-test.

18.2 mmol) in 80 ml of EtOH, 10% Pd–C (540 mg) was added. The flask was then placed in a hydrogen atmosphere and stirred at room temperature for 6 h. The catalyst was removed by filtration and the filtrate was concentrated to give **6** (3.72 g, 98%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.33 (br s, 3H), 3.04 (t, 2H, J=8 Hz), 3.99 (br s, 2H), 4.2—4.4 (m, 2H), 6.04 (br s, 1H), 6.66 (d, 1H, J=9 Hz), 6.70 (s, 1H), 7.33 (br s, 0.3 H), 7.69 (br s, 0.7 H); EI-MS *m/z* 207 (M^+).

Ethyl 5-(2-Chloro-4-cyanophenoxy)indoline-1-carboxylate (7) A mixture of **6** (3.69 g, 17.8 mmol), 3-chloro-4-fluorobenzonitrile (2.91 g, 18.7 mmol) and K_2CO_3 (3.69 g, 26.7 mmol) in 60 ml of DMF was stirred at 80 °C for 5 h. After dilution with AcOEt, the reaction mixture was washed with water and brine, dried and concentrated. The residue was washed with EtOH to give **7** (5.72 g, 94%) as a colorless solid: ^{1}H-NMR ($CDCl_3$) δ: 1.36 (br s, 3H), 3.14 (d, 2H, J=9 Hz), 4.0—4.2 (m, 2H), 4.29 (br s, 2H), 6.81 (d, 1H, J=8 Hz), 6.8—6.9 (m, 2H), 7.42 (dd, 1H, J=9, 2 Hz), 7.73 (d, 1H, J=2 Hz), 7.90 (br s, 1H); EI-MS *m/z* 342 (M^+).

3-Chloro-4-(indolin-5-yloxy)benzoic Acid (8) To a solution of **7** (5.71 g, 15.08 mmol) in 50 ml of EtOH, 50 ml of 8 N KOH aqueous solution was added, then the mixture was refluxed for 5 h. The reaction mixture was neutralized with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated. The residue was washed with EtOH to give **8** (3.56 g, 81%) as a gray solid: ^{1}H-NMR (DMSO-d_6) δ: 2.93 (t, 2H, J=8 Hz), 3.4—3.5 (m, 2H), 6.54 (d, 1H, J=8 Hz), 6.71 (dd, 1H, J=8, 2 Hz), 6.81 (d, 1H, J=9 Hz), 6.86 (d, 1H, J=2 Hz), 7.8—7.9 (m, 1H), 7.99 (d, 1H, J=2 Hz); EI-MS *m/z* 289 (M^+).

Ethyl 3-Chloro-4-(indolin-5-yloxy)benzoate (9) To a solution of **8** (870 mg, 3.00 mmol) in 20 ml of EtOH, 2.0 ml of 36 N H_2SO_4 was added, and the mixture was refluxed for 12 h. The reaction mixture was quenched with ice-water, neutralized with 5 N NaOH aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=3 : 1) to give **9** (940 mg, 99%) as a colorless oil: ^{1}H-

NMR ($CDCl_3$) δ: 1.38 (t, 3H, J=7 Hz), 3.0—3.1 (m, 2H), 3.61 (t, 2H, J=8 Hz), 4.35 (q, 2H, J=7 Hz), 6.62 (d, 1H, J=8 Hz), 6.74 (dd, 1H, J=8, 2 Hz), 6.78 (d, 1H, J=9 Hz), 6.85 (s, 1H), 7.80 (dd, 1H, J=9, 2 Hz), 8.10 (d, 1H, J=2 Hz); EI-MS m/z 317 (M^+).

4-[(1-Benzylindolin-5-yl)oxy]-3-chlorobenzoic Acid (2a) A mixture of **9** (620 mg, 1.95 mmol), benzyl bromide (370 mg, 2.16 mmol), KI (10 mg, 0.06 mmol) and K_2CO_3 (400 mg, 2.89 mmol) in 10 ml of DMF was stirred at room temperature for 2 h. After dilution with AcOEt, the reaction mixture was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=95 : 5) to give ethyl 4-[(1-benzylindolin-5-yl)oxy]-3-chlorobenzoate (590 mg, 74%) as a colorless oil. This intermediate (560 mg, 1.37 mmol) was dissolved in 9 ml of EtOH–dioxane (7 : 2), then 5 N NaOH aqueous solution (8 ml) was added, and the mixture was stirred at room temperature for 8 h. The reaction mixture was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was recrystallized from EtOH to give **2a** (270 mg, 52%) as gray crystals: mp 142—143 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.8—3.0 (m, 2H), 3.30 (t, 2H, J=9 Hz), 4.27 (s, 2H), 6.61 (d, 1H, J=8 Hz), 6.79 (dd, 1H, J=9, 2 Hz), 6.83 (d, 1H, J=9 Hz), 6.89 (s, 1H), 7.2—7.3 (m, 1H), 7.3—7.4 (m, 4H), 7.82 (dd, 1H, J=9, 2 Hz), 7.99 (d, 1H, J=2 Hz), 13.07 (br s, 1H); EI-MS m/z 379 (M^+).

(±)-3-Chloro-4-{[1-(1-phenylethyl)indolin-5-yl]oxy}benzoic Acid (2b) Essentially the same procedure as described above for the preparation of **2a** afforded **2b** (50%) as gray crystals: mp 123—125 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.55 (d, 3H, J=7 Hz), 2.95 (t, 2H, J=8 Hz), 3.3—3.5 (m, 2H), 4.67 (q, 2H, J=7 Hz), 6.30 (d, 1H, 8 Hz), 6.71 (dd, 1H, J=8, 2 Hz), 6.8—6.9 (m, 1H), 7.2—7.3 (m, 1H), 7.3—7.4 (m, 2H), 7.41 (d, 2H, J=7 Hz), 7.84 (dd, 1H, J=9, 2 Hz), 8.15 (d, 1H, J=2 Hz); EI-MS m/z 393 (M^+).

3-Chloro-4-{[1-(4-isopropylbenzyl)indolin-5-yl]oxy}benzoic Acid (2c) A mixture of **8** (580 mg, 2.00 mmol), 4-isopropylbenzaldehyde (330 mg, 2.20 mmol), AcOH (120 mg, 2.00 mmol) and $NaBH(OAc)_3$ (640 mg, 3.00 mmol) in 20 ml of dichloroethane (20 ml) was stirred at room temperature for 4 h. The reaction mixture was quenched with water, neutralized with 1 N NaOH aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH=95 : 5) to give **2c** (760 mg, 90%) as colorless crystals: mp 109—111 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.25 (d, 6H, J=7 Hz), 2.8—3.0 (m, 3H), 3.35 (t, 2H, J=8 Hz), 4.22 (s, 2H), 6.49 (d, 1H, J=9 Hz), 6.7—6.9 (m, 3H), 7.21 (d, 2H, J=8 Hz), 7.29 (d, 2H, J=8 Hz), 7.8—7.9 (m, 1H), 8.17 (d, 1H, J=2 Hz); EI-MS m/z 421 (M^+).

***tert*-Butyl 5-Benzyloxy-1*H*-indole-1-carboxylate (10)** A mixture of **4** (2.23 g, 10.0 mmol), di-*tert*-butyl dicarbonate (2.62 g, 12.0 mmol) and DMAP (120 mg, 0.98 mmol) in 20 ml of CH_3CN was stirred at room temperature for 2 h. After dilution with AcOEt, the reaction mixture was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=97 : 3) to give **10** (2.86 g, 89%) as a colorless oil; ^{1}H-NMR ($CDCl_3$) δ: 1.65 (s, 9H), 5.10 (s, 2H), 6.48 (d, 1H, J=4 Hz), 6.9—7.1 (m, 1H), 7.09 (d, 1H, J=2 Hz), 7.31 (t, 1H, J=7 Hz), 7.3—7.4 (m, 2H), 7.45 (d, 2H, J=7 Hz), 7.56 (d, 1H, J=3 Hz), 8.03 (br s, 1H); EI-MS m/z 323 (M^+).

***tert*-Butyl 5-Hydroxyindoline-1-carboxylate (11)** Essentially the same procedure as described above for the preparation of **6** afforded **11** (96%) as a colorless solid; ^{1}H-NMR ($CDCl_3$) δ: 1.55 (s, 9H), 3.01 (t, 2H, J=8 Hz), 3.95 (br s, 2H), 5.77 (br s, 1H), 6.6—6.7 (m, 1H), 6.68 (s, 1H), 7.26 (br s, 0.5 H), 7.67 (br s, 0.5 H); EI-MS m/z 235 (M^+).

3-Chloro-4-(indolin-5-yloxy)benzonitrile (12a) A mixture of **11** (5.51 g, 23.5 mmol), 3-chloro-4-fluorobenzonitrile (3.83 g, 24.6 mmol) and K_2CO_3 (4.86 g, 35.2 mmol) in 80 ml of DMF was stirred at 80 °C for 2 h. After dilution with AcOEt, the reaction mixture was washed with water and brine, dried and concentrated. This intermediate (9.24 g) was dissolved in 100 ml of trifluoroacetic acid, and the mixture was stirred at room temperature for 1 h and concentrated. The residue was neutralized with 1 N NaOH aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was recrystallized from EtOH to give **12a** (5.14 g, 81%) as a colorless solid; ^{1}H-NMR ($CDCl_3$) δ: 3.04 (t, 2H, J=9 Hz), 3.62 (t, 2H, J=9 Hz), 3.80 (br s, 1H), 6.62 (d, 1H, J=8 Hz), 6.73 (dd, 1H, J=9, 2 Hz), 6.80 (d, 1H, J=9 Hz), 6.84 (t, 1H, J=1 Hz), 7.40 (dd, 1H, J=9, 2 Hz), 7.70 (d, 1H, J=2 Hz); EI-MS m/z 270 (M^+).

3-Chloro-4-{[1-(naphthalen-2-ylmethyl)indolin-5-yl]oxy}benzoic Acid (2d) A mixture of **12a** (540 mg, 2.00 mmol), 2-(bromomethyl)naphthalene (490 mg, 2.20 mmol), KI (10 mg, 0.06 mmol) and K_2CO_3 (410 mg, 3.00 mmol) in 10 ml of DMF was stirred at room temperature for 7 h. After dilution with AcOEt, the reaction mixture was washed with water and brine, dried and concentrated. The residue was washed with EtOH to give 3-chloro-4-{[1-(naphthalen-2-ylmethyl)indolin-5-yl]oxy}benzonitrile (680 mg, 84%) as a brown solid. This intermediate (670 mg, 1.63 mmol) was dissolved in 5 ml of EtOH, then 5 ml of 8 N KOH aqueous solution was added, and the mixture was refluxed for 6 h. The reaction mixture was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was washed from EtOH to give **2d** (530 mg, 76%) as pale yellow crystals: mp 182—183 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.00 (t, 2H, J=8 Hz), 3.40 (t, 2H, J=8 Hz), 4.41 (s, 2H), 6.52 (d, 1H, J=8 Hz), 6.79 (dd, 1H, J=8, 2 Hz), 6.82 (d, 1H, J=9 Hz), 6.86 (d, 1H, J=2 Hz), 7.4-7.6 (m, 3H), 7.8—7.9 (m, 5H), 8.17 (d, 1H, J=2 Hz); EI-MS m/z 429 (M^+).

3-Chloro-4-{[1-(4-phenoxybenzyl)indolin-5-yl]oxy}benzoic Acid (2e) A mixture of **12a** (540 mg, 2.00 mmol), 4-phenoxybenzaldehyde (440 mg, 2.20 mmol), AcOH (1.20 g, 20.0 mmol) and $NaBH(OAc)_3$ (640 mg, 3.00 mmol) in 20 ml of dichloromethane was stirred at room temperature for 1 h. The reaction mixture was quenched with water, neutralized with K_2CO_3 and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was recrystallized from EtOH to give 3-chloro-4-{[1-(4-phenoxybenzyl)indolin-5-yl]oxy}benzonitrile (740 mg, 82%) as a colorless solid. This intermediate (720 mg, 1.59 mmol) was dissolved in 5 ml of EtOH, then 5 ml of 8 N KOH aqueous solution was added, and the resulting mixture was refluxed for 7 h. The reaction mixture was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was washed from EtOH to give **2e** (570 mg, 76%) as colorless crystals: mp 164—165 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.97 (t, 2H, J=8 Hz), 3.36 (t, 2H, J=8 Hz), 4.22 (s, 2H), 6.49 (d, 1H, J=9 Hz), 6.78 (d, 1H, J=2 Hz), 6.80 (d, 1H, J=2 Hz), 6.83 (d, 1H, J=9 Hz), 6.99 (d, 2H, J=8 Hz), 7.01 (d, 2H, J=9 Hz), 7.10 (t, 1H, J=7 Hz), 7.3-7.4 (m, 4H), 7.85 (dd, 1H, J=9, 2 Hz), 8.17 (d, 1H, J=2 Hz); EI-MS m/z 471 (M^+).

Essentially the same procedure as described above for the preparation of **2e** afforded **2f** (brown crystals, 58%), **2g** (colorless crystals, 63%), **2h** (pale brown crystals, 59%), **2i** (colorless crystals, 35%), and **2j** (colorless crystals, 44%).

4-(4-Anilinophenoxy)-3-chlorobenzonitrile (14a) Essentially the same procedure as described above for the preparation of **7** afforded **14a** (90%) as a pale yellow solid; ^{1}H-NMR ($CDCl_3$) δ: 6.85 (d, 1H, J=9 Hz), 6.9—7.0 (m, 3H), 7.0—7.2 (m, 4H), 7.2—7.3 (m, 2H), 7.43 (dd, 1H, J=9, 2 Hz), 7.72 (d, 1H, J=2 Hz); EI-MS m/z 320 (M^+).

3-Chloro-4-[4-(*N*-methylamino)phenoxy]benzonitrile (14b) Essentially the same procedure as described above for the preparation of **7** afforded **14b** (58%) as a pale yellow solid; ^{1}H-NMR (DMSO-d_6) δ: 2.70 (d, 3H, J=5 Hz), 5.79 (q, 1H, J=5 Hz), 6.61 (d, 2H, J=9 Hz), 6.80 (d, 1H, J=9 Hz), 6.9—7.0 (m, 2H), 7.70 (dd, 1H, J=9, 2 Hz), 8.11 (d, 1H, J=2 Hz); EI-MS m/z 258 (M^+).

4-(4-Anilinophenoxy)-3-chlorobenzoic Acid (3a) Essentially the same procedure as described above for the preparation of **8** afforded **3a** (25%) as pale brown crystals: mp 196—197 °C; ^{1}H-NMR ($CDCl_3$) δ: 6.87 (d, 1H, J=9 Hz), 6.95 (t, 1H, J=7 Hz), 7.00 (dd, 2H, J=7, 2 Hz), 7.06 (d, 2H, J=8 Hz), 7.1—7.2 (m, 2H), 7.28 (dd, 2H, J=8, 7 Hz), 7.89 (dd, 1H, J=9, 2 Hz), 8.20 (d, 1H, J=2 Hz); EI-MS m/z 339 (M^+).

3-Chloro-4-[4-(*N*-methylanilino)phenoxy]benzoic Acid (3b) To a suspension of 60% NaH (130 mg, 3.30 mmol) in 5 ml of DMF, **14a** (960 mg, 3.00 mmol) was added, and stirred at room temperature for 1 h. Methyl iodide (510 mg, 3.60 mmol) was added and the mixture was stirred at room temperature for 22 h. After the addition of water, the mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=95 : 5) to give 3-chloro-4-[4-(*N*-methylanilino)phenoxy]benzonitrile (860 mg, 87%) as a colorless solid. This intermediate (820 mg, 2.45 mmol) was dissolved in 15 ml of EtOH, then 15 ml of 8 N KOH aqueous solution was added, and the mixture was refluxed for 6 h. The reaction mixture was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was washed from EtOH to give **3b** (440 mg, 51%) as colorless crystals: mp 133—134 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.32 (s, 3H), 6.88 (d, 1H, J=9 Hz), 6.9—7.1 (m, 7H), 7.29 (t, 2H, J=8 Hz), 7.90 (dd, 1H, J=9, 2 Hz), 8.20 (d, 1H, J=2 Hz); EI-MS m/z 353 (M^+).

3-Chloro-4-{4-[*N*-methyl-*N*-(4-phenoxybenzyl)amino]phenoxy}benzoic Acid (3h) Essentially the same procedure as described above for the preparation of **2e** afforded **3h** (42%) as pale orange crystals: mp 177—178 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.02 (s, 3H), 4.50 (s, 2H), 6.8—6.9 (m, 3H), 6.9—7.1 (m, 6H), 7.10 (t, 1H, J=7 Hz), 7.20 (d, 2H, J=9 Hz), 7.3—7.4 (m,

2H), 7.85 (dd, 1H, J=9, 2 Hz), 8.17 (d, 1H, J=2 Hz); EI-MS m/z 459 (M^+).

Methyl 4-(4-Aminophenoxy)-3-chlorobenzoate (16) A mixture of **15** (3.73 g, 20.0 mmol), 1-fluoro-4-nitrobenzene (2.82 g, 20.0 mmol) and K_2CO_3 (4.15 g, 30.0 mmol) in 50 ml of DMF was stirred at 70 °C for 1 d. After dilution with AcOEt, the organic layer was washed with water and brine, dried and concentrated. The residue was recrystallized from EtOH to give methyl 3-chloro-4-(4-nitrophenoxy)benzoate (4.87 g, 79%) as a pale yellow solid. This intermediate (4.84 g, 15.7 mmol) was dissolved in 40 ml of AcOEt, then a suspension of Raney-Ni (400 mg) in 2 ml of EtOH was added. The flask was then placed in a hydrogen atmosphere and stirred at room temperature for 5 h. The catalyst was removed by filtration and the filtrate was concentrated. The residue was recrystallized from EtOH to give **16** (3.19 g, 73%) as colorless crystals: ^{1}H-NMR ($CDCl_3$) δ: 3.68 (br s, 2H), 3.89 (s, 3H), 6.6—6.8 (m, 2H), 6.76 (d, 1H, J=9 Hz), 6.8—6.9 (m, 2H), 7.79 (dd, 1H, J=9, 2 Hz), 8.11 (d, 1H, J=2 Hz); EI-MS m/z 277 (M^+).

Methyl 4-[4-(Benzylamino)phenoxy]-3-chlorobenzoate (17a) Essentially the same procedure as described above for the preparation of **2c** afforded **17a** (77%) as colorless crystals; ^{1}H-NMR (DMSO-d_6) δ: 3.83 (s, 3H), 4.27 (d, 2H, J=4 Hz), 6.35 (br s, 1H), 6.65 (d, 2H, J=9 Hz), 6.79 (d, 1H, J=9 Hz), 6.89 (d, 2H, J=9 Hz), 7.24 (t, 1H, J=7 Hz), 7.34 (dd, 2H, J=8, 7 Hz), 7.38 (d, 2H, J=7 Hz), 7.81 (dd, 1H, J=9, 2 Hz), 8.00 (d, 1H, J=2 Hz); EI-MS m/z 367 (M^+).

Methyl 3-Chloro-4-{4-[*N*-(4-phenoxybenzyl)amino]phenoxy}benzoate (17b) Essentially the same procedure as described above for the preparation of **2c** afforded **17b** (100%) as a colorless oil; ^{1}H-NMR ($CDCl_3$) δ: 3.89 (s, 3H), 4.03 (br s, 1H), 4.30 (s, 2H), 6.6—6.7 (m, 2H), 6.76 (dd, 1H, J=9, 4 Hz), 6.9—7.1 (m, 6H), 7.10 (t, 1H, J=8Hz), 7.3—7.4 (m, 4H), 7.78 (dd, 1H, J=9, 2Hz), 8.10 (d, 1H, J=2Hz); EI-MS m/z 459 (M^+).

Methyl 4-[4-(*N*-Benzyl-*N*-methylamino)phenoxy]-3-chlorobenzoate (18) A mixture of **17a** (350 mg, 0.95 mmol), formic acid (3 ml) and 35% formaldehyde aqueous solution (3 ml) was stirred at 100 °C for 1.5 h. After the addition of water, the reaction mixture was neutralized with K_2CO_3 and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=9 : 1) to give **18** (310 mg, 85%) as colorless crystals; ^{1}H-NMR (DMSO-d_6) δ: 3.03 (s, 3H), 3.83 (s, 3H), 4.57 (s, 2H), 6.78 (d, 2H, J=9 Hz), 6.81 (d, 1H, J=9 Hz), 6.99 (d, 2H, J=9 Hz), 7.23 (d, 3H, J=8 Hz), 7.33 (dd, 2H, J=8, 7 Hz), 7.83 (d, 1H, J=11 Hz), 8.01 (d, 1H, J=1 Hz); EI-MS m/z 381 (M^+).

4-[4-(Benzoylamino)phenoxy]-3-chlorobenzoic Acid (3e) To a solution of **16** (830 mg, 3.00 mmol) in 8 ml of pyridine–CH_2Cl_2 (1 : 1), a solution of benzoyl chloride (440 mg, 3.13 mmol) in 4 ml of CH_2Cl_2 was added dropwise at 5 °C, then the mixture was stirred at room temperature for 5 h. After the addition of water, the mixture was extracted with AcOEt. The organic layer was washed with 1 N HCl aqueous solution, water and brine, dried and concentrated. The residue was washed with EtOH to give methyl 4-[4-(benzoylamino)phenoxy]-3-chlorobenzoate (900 mg, 79%) as a colorless solid. This intermediate (440 mg, 1.15 mmol) was dissolved in 7 ml of dioxane–EtOH (2 : 5), then 7 ml of 5 N NaOH aqueous solution was added, and the mixture was stirred at 50 °C for 2 h. The reaction mixture was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with water and brine, dried and concentrated, and the residue was washed with EtOH to give **3e** (240 mg, 57%) as colorless crystals: mp 245—246 °C; ^{1}H-NMR ($CDCl_3$) δ: 6.88 (d, 1H, J=9 Hz), 7.0—7.1 (m, 2H), 7.4—7.6 (m, 3H), 7.8—7.9 (m, 3H), 7.9—8.0 (m, 2H), 8.11 (d, 1H, J=2 Hz), 9.94 (s, 1H); FAB-MS m/z 368 (M^++H).

3-Chloro-4-(4-dibenzylaminophenoxy)benzoic Acid (3f) A mixture of **16** (520 mg, 1.87 mmol), benzyl bromide (700 mg, 4.09 mmol), potassium iodide (30 mg, 0.18 mmol) and K_2CO_3 (780 mg, 5.64 mmol) in 10 ml of DMF was stirred at 80 °C for 8 h. After the addition of water, the reaction mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried and concentrated, and the residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=95 : 5) to give methyl 3-chloro-4-(4-dibenzylaminophenoxy)benzoate (710 mg, 83%) as a colorless oil. This intermediate (690 mg, 1.51 mmol) was hydrolyzed in the same manner as described for compound **3e** to give **3f** (530 mg, 79%) as colorless crystals: mp 183—184 °C; ^{1}H-NMR ($CDCl_3$) δ: 4.66 (s, 4H), 6.7—6.8 (m, 2H), 6.80 (d, 1H, J=9 Hz), 6.8—7.0 (m, 2H), 7.2—7.3 (m, 6H), 7.3—7.4 (m, 4H), 7.8—7.9 (m, 1H), 8.16 (d, 1H, J=2 Hz); EI-MS m/z 443 (M^+).

4-[4-(Benzylamino)phenoxy]-3-chlorobenzoic Acid (3c) Compound **17a** (350 mg, 0.95 mmol) was hydrolyzed in the same manner as described for compound **3e** to give **3c** (120 mg, 36%) as colorless crystals: mp 167—168 °C; ^{1}H-NMR (DMSO-d_6) δ: 4.27 (s, 2H), 6.36 (br s, 1H), 6.6—6.7 (m, 2H), 6.77 (d, 1H, J=9 Hz), 6.8—7.0 (m, 2H), 7.24 (t, 1H, J=7 Hz), 7.3—7.4 (m, 4H), 7.7—7.9 (m, 1H), 7.98 (d, 1H, J=2 Hz), 13.05 (br s, 1H); EI-MS m/z 353 (M^+).

3-Chloro-4-{4-[*N*-(4-phenoxybenzyl)amino]phenoxy}benzoic Acid (3g) Compound **17b** (470 mg, 1.02 mmol) was hydrolyzed in the same manner as described for compound **3e** to give **3g** (260 mg, 57%) as colorless crystals: mp 205—206 °C; ^{1}H-NMR ($CDCl_3$) δ: 4.31 (s, 2H), 6.67 (d, 2H, J=9 Hz), 6.79 (d, 1H, J=9 Hz), 6.93 (d, 2H, J=9 Hz), 7.0—7.1 (m, 4H), 7.11 (t, 1H, J=7 Hz), 7.3—7.4 (m, 4H), 7.84 (dd, 1H, J=9, 2 Hz), 8.16 (d, 1H, J=2 Hz); EI-MS m/z 445 (M^+).

4-[4-(*N*-Benzyl-*N*-methylamino)phenoxy]-3-chlorobenzoic Acid (3d) Compound **18** (280 mg, 0.73 mmol) was hydrolyzed in the same manner as described for compound **3e** to give **3d** (180 mg, 67%) as colorless crystals: mp 154—155 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.02 (s, 3H), 4.57 (s, 2H), 6.7—6.9 (m, 3H), 6.9—7.0 (m, 2H), 7.24 (t, 3H, J=4 Hz), 7.33 (t, 2H, J=7 Hz), 7.8—7.9 (m, 1H), 7.99 (d, 1H, J=2 Hz), 13.08 (br s, 1H); EI-MS m/z 367 (M^+).

Authentic Materials The 5α-reductase inhibitors, finasteride[7a] and (±)-ONO-3805,[8a] were synthesized in our company according to the methods described in the literature.

Biological Methods. Preparation of 5α-Reductases from Rat Prostate and Human Prostate Male Wistar rats 8—12 weeks of age (Charles River Japan Inc., Atsugi, Japan) were sacrificed and the ventral prostates were removed. The prostatic tissues were rinsed with ice-cold saline and minced with scissors. Unless specified, all the following procedures were carried out at 4 °C. The minced tissues were homogenized with a Polytron homogenizer (Kinematica GMBH, Lucerne, Switzerland) in 3 tissue volumes of 20 mM sodium phosphate buffer, pH 7.0, containing 0.25 M sucrose and 0.1 mM dithiothreitol (DTT). The homogenate was centrifuged at 10000×g for 10 min, and the resulting supernatant was centrifuged again at 140000×g for 60 min. The pellets were resuspended in 40 mM sodium phosphate buffer, pH 6.5. The suspension was stored at −80 °C until use.

Human prostatic tissues from BPH patients who received a transurethral prostatectomy were kindly provided by Dr. T. Tahara at Yamato Hospital, Tokyo, Japan, and stored at −80 °C until preparation of the enzyme fractions. The frozen prostatic tissues were minced with scissors and homogenized with a Polytron homogenizer in 3 tissue volumes of 10 mM Tris–HCl buffer, pH 7.0, containing 0.33 M sucrose, 1 mM DTT and 1 μM NADPH. The homogenate was centrifuged at 1000×g for 5 min. The supernatant was centrifuged at 11000×g for 20 min, and the resulting supernatant was centrifuged again at 140000×g for 60 min. The resulting pellet was resuspended in 10 mM Tris–HCl buffer, pH 7.0, and stored at −80 °C until use.

5α-Reductase Assay *in Vitro* 5α-Reductase activities were assayed according to the method described by Liang *et al.*[15] with a minor modification. Briefly, the reaction solutions contained in a final volume of 0.5 ml: 1 μM [4-^{14}C] testosterone, 1 mM DTT, 50 mM NADPH, 50 mM buffer (Tris–HCl, pH 6.5, for the rat prostate enzyme; Tris–citrate, pH 5.0, for the human prostate enzyme) and the enzyme fractions. To identify the inhibitory effect of the drugs, the various concentrations of the test compounds were also added in 5 μl DMSO (final conc. 1%). The reaction solutions in duplicate were incubated at 37 °C for 60 min, and the reaction was terminated by the addition of 2.0 ml cold AcOEt containing 10 μg testosterone, 5α-DHT, 4-androstene-3,17-dione,5α-androstan-3α,17β-diol and 5α-androstan-3,17-dione as the standards. The organic phase was separated by centrifugation, evaporated under N_2 gas and resuspended with 40 μl AcOEt. 20 μl of AcOEt was spotted on the TLC plate and separated twice using AcOEt/cyclohexane (1 : 1) as the developing solvent. The steroid standards were located by UV (254 nm) and by spraying with a 1% $CeSO_4$–10% H_2SO_4 solution followed by heating. The regions containing 5α-reduced metabolites (5α-DHT, 5α-androstan-3α,17β-diol and 5α-androstan-3,17-dione) were cut from the TLC plate, soaked in 5 ml of Aquasol-2 and the radioactivity was counted by a scintillation counter. The IC_{50} values for 5α-reductase activity were obtained from the linear line drawn using the least-squares fitting method.

***In Vivo* Effects in Rats** Male SD rats at 9 weeks of age (Charles River Japan Inc., Atsugi, Japan) were used to evaluate the effects of test compounds on the prostatic concentration of DHT. The test compounds or a vehicle were orally administered as an aqueous suspension with 0.5% methylcellulose. Eight hours after the dosing, the rat were sacrificed by chloroform anesthesia and the ventral prostate in the treated rats was removed and weighed. The ventral prostates were immediately placed in liquid nitrogen then stored at −80 °C until use.

The frozen prostatic tissues (wet weight 200—500 mg) were minced with scissors and homogenized using a Polytron homogenizer in 3 ml of ice-cold water. One milliliter of the homogenate was twice extracted using 3 ml of

ice-cold diethyl ether. The collected ether layer was concentrated under nitrogen gas and resuspended with 1.5 ml of assay buffer (50 mM Tris–HCl buffer, pH 8.0, containing 0.1% (w/v) gelatin). The DHT contents in the extract samples were determined by radioimmunoassay using testosterone/DHT [^{3}H] assay system (Amersham Pharmacia Biotech, Uppsala, Sweden). The DHT contents were calculated as pg/100 mg of prostatic wet weight.

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