A Novel Class of Inhibitors for Human Steroid 5 α -Reductase: Synthesis and Biological Evaluation of Indole Derivatives. II¹⁾

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In a search for novel nonsteroidal inhibitors of human prostatic 5 α -reductase, we found a new series of indole derivatives that showed potent inhibitory activities for the human enzyme. Among them, 4-[(1-benzyl-1*H*-indol-5-yl)oxy]-3-chlorobenzoic acid (2d, YM-32906) showed more potent inhibitory activity than finasteride with an IC₅₀ value of 0.44 nM. 3-Chloro-4-[[1-(4-phenoxybenzyl)-1*H*-indol-5-yl]oxy]benzoic acid (2m) showed inhibitory activities for both human and rat prostatic 5 α -reductase with IC₅₀ values of 2.1 and 73 nM, respectively. The synthesis and structure–activity relationships of these indole derivatives are presented.

Key words 5 α -reductase inhibitor; benign prostatic hypertrophy; indole derivative; YM-32906

5 α -Reductase is an NADPH-dependent enzyme responsible for the conversion of testosterone to dihydrotestosterone (DHT), an active agonist for androgen receptors. Benign prostatic hyperplasia (BPH), skin disorders such as acne, male pattern baldness and hirsutism are androgen-related disorders, associated with elevated levels of DHT.⁵⁾ As a consequence, a 5 α -reductase inhibitor is expected to provide a potential treatment for various androgen-related disorders. Several 5 α -reductase inhibitors have been reported,^{6,7)} including both steroidal inhibitors such as finasteride^{6a)} and epristeride,^{6b)} and nonsteroidal inhibitors such as ONO-3805^{7a)} (Fig. 1). Following the discovery of these inhibitors, the existence of two different 5 α -reductase isozymes, type 1 and type 2 5 α -reductase, has been reported in humans and rats.^{8–10)} The type 1 enzyme is normal in men with congenital 5 α -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 5 α -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 exact physiological roles of these isozymes has yet to be fully elucidated. Steroidal inhibitors, for example, finasteride, have been shown to be

clinically effective for treatment of BPH, however the possibility of adverse effects due to their steroidal structures remains.¹¹⁾ On the other hand, nonsteroidal inhibitors have not shown clinical efficacy for the treatment of BPH. Finasteride showed strong inhibitory activity (IC₅₀=4.1 nM)¹²⁾ for human prostatic 5 α -reductase, whereas ONO-3805 showed moderate activity (IC₅₀=538 nM)¹²⁾ which might not be sufficient to show clinical efficacy. We considered that a more potent nonsteroidal inhibitor of human prostatic 5 α -reductase than ONO-3805 could show clinical efficacy for the treatment of BPH and reduce the potential for side effects compared to steroidal inhibitors.

In our previous studies on novel human prostatic 5 α -reductase inhibitors, we found the biphenyl-4-yloxy benzoic acid derivative, YM-31758 (1), which showed potent inhibitory activity with an IC₅₀ value of 0.87 nM.¹⁾ During the course of this study, we found that it is favorable for high potency to have a hydrophobic substituent such as a phenyl group at a position from the carboxyl group in the range of 9–11 Å through the benzene ring juncture. These results suggested that it may be possible to find a more potent inhibitor against human prostatic 5 α -reductase through modification of the biphenyl-4-yloxy group in YM-31758. In this

Fig. 1

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study, we focused on modification of the biphenyl-4-yloxy group. A 1*H*-indole-5-yloxy group as a substituent at the 4-position of the benzoic acid moiety was newly designed as described in Fig. 1. We report here the synthesis and the structure–activity relationships of the indole derivatives **2**.

(a) BBr_3 , CH_2Cl_2 ; (b) 3-chloro-4-fluorobenzonitrile, K_2CO_3 , DMSO; (c) MeI, KOH, DMSO; (d) 8 N KOH, EtOH

Chart 1

Chemistry

2-Phenyl-1*H*-indole derivatives (**2a**, **2b**) were prepared as shown in Chart 1. The demethylation of compound **3** was performed using BBr_3 to give compound **4**, which was converted to compound **5** by alkylation with 3-chloro-4-fluorobenzonitrile. Compound **2a** was obtained by hydrolysis with KOH. Compound **2b** was prepared by the *N*-methylation of compound **5** in the presence of KOH in dimethyl sulfoxide (DMSO) with methyl iodide¹³ and subsequent hydrolysis with KOH. The 3-benzyl-1-methyl-1*H*-indole derivative **2c** was prepared as shown in Chart 2. After methylation of compound **7**, benzoylation was carried out by the Vilsmeier method to give compound **8**. Debenzylation and reduction of the carbonyl group were performed using catalytic hydrogenation in the presence of Pd-C to afford compound **9**, which was converted to the benzoic acid derivative **2c** in two steps. The 1-alkyl-1*H*-indole derivatives (**2d**–**2u**) were prepared from compound **7** by three synthetic methods (Methods A, B and C) as described in Chart 3. Compound **11** was obtained by debenzoylation of **7** in the presence of Pd-C and subsequent alkylation with 3-chloro-4-fluorobenzonitrile. *N*-Alkylation of **11** was performed using KOH in DMSO to give compound **13**, which was then hydrolyzed with KOH to

(a) i) MeI, KOH, DMSO, ii) *N*-benzoylpyrrolidine, POCl_3 , 1,2-dichloroethane; (b) $\text{H}_2/\text{Pd-C}$, AcOEt – EtOH – AcOH ; (c) i) 3-chloro-4-fluorobenzonitrile, K_2CO_3 , DMSO, ii) 8 N KOH, EtOH

Chart 2

(a) Pd-C , HCO_2NH_4 , EtOH; (b) 3-chloro-4-fluorobenzonitrile, K_2CO_3 , DMF; (c) R-X (X = Cl, Br or I), KOH, DMSO; (d) 8 N KOH, EtOH; (e) NaH, ClCO_2Et , DMF

Chart 3

Table 1. Physicochemical Data and Inhibitory Activities of 5 α -Reductase in Human Prostate for Indole Derivatives

Compd. No.	R ¹	R ²	R ³	mp (°C)	Formula	Analysis (%) Calcd (Found)				IC ₅₀ (nM)
						C	H	N	Cl	
2a	H		H	268—270	C ₂₁ H ₁₄ NO ₃ Cl	69.33 (69.44)	3.88 3.91	3.85 3.87	9.75 (9.86)	0% inhibition at 100 nM
2b	Me		H	260—263	C ₂₂ H ₁₆ NO ₃ Cl	69.94 (69.71)	4.27 4.35	3.71 3.67	9.38 (9.23)	3.4
2c	Me	H		220—222	C ₂₃ H ₁₈ NO ₃ Cl	70.50 (70.28)	4.63 4.62	3.57 3.43	9.05 (9.06)	12
2d^{a)}		H	H	158—159	C ₂₂ H ₁₆ NO ₃ Cl	69.94 (69.86)	4.27 4.29	3.71 3.68	9.38 (9.44)	0.44

a) The compound was prepared by using Method B as shown in Chart 3.

give the benzoic acid derivatives **2f—2i** and **2l** (Method A). Compound **12**, prepared by the *N*-alkylation of **7** with an alkyl halide and subsequent debenzoylation in the presence of Pd-C, was alkylated with 3-chloro-4-fluorobenzonitrile to afford compound **13**, which was hydrolyzed with KOH to give the benzoic acid derivatives **2d**, **2e** and **2q** (Method B). Compound **7** was acylated with chloroethylformate followed by debenzoylation to afford **14**. Alkylation of **14** with 3-chloro-4-fluorobenzonitrile afforded **15**, which was hydrolyzed with KOH to give the benzoic acid derivative **16**. *N*-Alkylation of **16** was also carried out in the presence of KOH in DMSO to afford the benzoic acid derivatives **2j**, **2k**, **2m—2p** and **2r—2u** (Method C).

Results and Discussion

The inhibitory activities of the indole derivatives were evaluated on the basis of the ability to inhibit 5 α -reductase in human prostate at pH 5.5 which is optimal for the activity of the type 2 enzyme. The results are described in Tables 1—4.

In our previous paper, we found that for high potency it is favorable to have a phenyl group at a position from the carboxyl group in the range of 9—11 Å for phenoxy benzoic acid derivatives. Initially, the favorable position for the phenyl group on the indole ring was investigated as shown in Table 1. Compound **2a**, having an acidic proton at the 1-position on the indole ring, showed in a loss of activity, while the 1-methyl-2-phenyl-1*H*-indole derivative **2b** showed potent inhibitory activity with an IC₅₀ of 3.4 nM. An acidic proton in this position was found to be incompatible with potent inhibitory activity. The 3-benzyl-1-methyl-1*H*-indole derivative **2c** resulted in decreased activity, while the 1-benzyl-1*H*-indole derivative **2d** showed strong potency with an IC₅₀ of 0.44 nM. These findings suggested that the position of the phenyl group is important for high potency. In order to investigate the correlation between distance and inhibitory activity, optimized structures for the indole derivatives **2b—2d** were calculated by a systematic search and minimization using the molecular modeling program Sybyl, version 6.3, and the distances between the carbon of the carboxyl group and centroid of the phenyl group (benzene ring A) were calculated from the optimized structures. The calculated data and the optimized structures of **2b—2d** are shown in Table 2 and Fig. 2, respectively. The distances for the potent com-

Table 2. Distances between the Carbon of the Carboxyl Group and the Centroid of the Benzene Ring A for Compounds **2b—2d**

Compd. No.	Indole junction	C-Centroid distance (Å)	IC ₅₀ (nM) ^{a)}
2b		11.367	3.4
2c		4.657	12
2d		8.705	0.44

a) Inhibitory activities of 5 α -reductase in human prostate homogenates.

pounds **2b** and **2d** are in the range of 9—11 Å, with values of 11.367 Å and 8.705 Å, respectively.

On the other hand, the reduced potent compound **2c** is a non-linear compound, and the position of the benzene ring A is probably too close to the carboxyl group, with a value of 4.657 Å, to show potent inhibitory activity. The 1-benzyl derivative **2d** showed more potent inhibitory activity compared to **2b** or YM-31758, suggesting that the 1-benzyl-1*H*-indole group of **2d** is more flexible compared to the 1-methyl-2-phenyl-1*H*-indole group of **2b** or the biphenyl group of YM-31758 and the position of the phenyl group in **2d** may be more favorable for hydrophobic interaction with the enzyme compared to the position of the phenyl group in **2b** or YM-31758.

Next, we focused our efforts on modification of the benzyl group of compound **2d**. These results are summarized in Table 3. Concerning the distance between the indole ring and the phenyl group, the 3-phenylpropyl derivative **2e** retained high potency, while the 5-phenylpentyl derivative **2f** showed reduced inhibitory activity. These results suggested that the inhibitory activity is affected by the bulkiness of the substituent, *i.e.*, the 3-phenylpropyl group is tolerant but the 5-phenylpentyl group is too bulky. Concerning the substituent on the benzyl group, the 4-methylbenzyl derivative **2i** re-

Table 3. Physicochemical Data and Inhibitory Activities of 5 α -Reductase in Human Prostate Homogenates for Indole Derivatives

Compd. No.	R	Method ^{a)}	mp (°C)	Formula	Analysis (%) Calcd (Found)					IC ₅₀ (nM)
					C	H	N	S	Cl	
2e		B	Oil	C ₂₄ H ₂₀ NO ₃ Cl	71.02 (70.75)	4.97 4.95	3.45 3.45		8.73 8.63)	0.61
2f		A	144—145	C ₂₆ H ₂₄ NO ₃ Cl	71.97 (71.99)	5.57 5.54	3.23 3.22		8.17 7.96)	5.0
2g		A	195—197	C ₂₃ H ₁₈ NO ₃ Cl	70.50 (70.51)	4.63 4.69	3.57 3.54		9.05 9.28)	1.1
2h		A	182—184	C ₂₃ H ₁₈ NO ₃ Cl	70.50 (70.49)	4.63 4.68	3.57 3.56		9.05 9.11)	1.3
2i		A	160—162	C ₂₁ H ₁₆ NO ₃ Cl	70.50 (70.48)	4.63 4.70	3.57 3.54		9.05 9.08)	0.60
2j		C	168—171	C ₂₅ H ₂₂ NO ₃ Cl	71.51 (71.51)	5.28 5.33	3.34 3.29		8.44 8.32)	2.3
2k		C	213—217	C ₂₃ H ₁₅ N ₂ O ₃ Cl	68.58 (68.42)	3.75 3.84	6.95 6.93		8.80 8.79)	0.93
2l		A	173—176	C ₂₃ H ₁₈ NO ₄ Cl	67.73 (68.03)	4.45 4.43	3.43 3.45		8.69 8.87)	0.47
2m		C	213—216	C ₂₈ H ₂₀ NO ₄ Cl	71.57 (71.65)	4.29 4.28	2.98 2.93		7.54 7.50)	2.1
2n		C	185—188	C ₂₀ H ₁₄ NO ₄ Cl	65.31 (65.06)	3.84 3.88	3.81 3.79		9.64 9.55)	0.35
2o		C	191—194	C ₂₀ H ₁₄ NO ₃ SCl	62.58 (62.25)	3.68 3.66	3.65 3.62	8.35 8.48	9.24 9.54)	0.29
2p		C	168—171	C ₂₀ H ₁₄ NO ₃ SCl	62.58 (62.37)	3.68 3.63	3.65 3.54	8.35 8.35	9.24 9.17)	0.31
2q	Me	B	199—201	C ₁₆ H ₁₂ NO ₃ Cl	63.69 (63.62)	4.01 4.09	4.64 4.63		11.75 11.65)	11
2r		C	123—126	C ₁₉ H ₁₈ NO ₃ Cl	66.38 (66.47)	5.28 5.21	4.07 4.13		10.31 10.15)	0.57
2s		C	oil	C ₂₁ H ₂₆ NO ₃ Cl	69.08 (68.97)	6.55 6.60	3.50 3.46		8.87 8.83)	5.1
2t		C	185—188	C ₁₉ H ₁₆ NO ₃ Cl	66.77 (66.93)	4.72 4.78	4.10 4.10		10.37 10.29)	0.64
2u		C	154—157	C ₂₀ H ₁₈ NO ₃ Cl	67.51 (67.53)	5.10 5.18	3.94 3.92		9.96 9.87)	0.56

a) Synthetic methods as shown in Chart 3.

sulted in a slight increase in activity compared to the 2-methylbenzyl derivative **2g** or 3-methylbenzyl derivative **2h**, suggesting that the 4-position is the favorable position for modification of the benzyl group. Replacement of the methyl group in compound **2i** with a cyano group (**2k**) or methoxy group (**2l**) retained high potency, while replacement with a propyl group (**2j**) or phenoxy group (**2m**) resulted in a slight loss of inhibitory activity, suggesting that the propyl and phenoxy groups are too bulky for high potency. Furthermore, the effect of another aromatic substituent such as a furan ring or thiophene ring instead of the benzene ring in compound **2d**

was investigated. The furfuryl derivative **2n**, the 2-thienylmethyl derivative **2o** and the 3-thienylmethyl derivative **2p** showed strong inhibitory activities with IC₅₀ values below 0.5 nM, suggesting that these heteroaromatic rings may enhance the inhibitory activity by an interaction with a hydrophobic pocket in the enzyme. Finally, the inhibitory activities of compounds having no aromatic ring at the 1-position of the indole ring was investigated. The methyl derivative **2q** and octyl derivative **2s** showed reduced inhibitory activities, while the butyl derivative **2r**, cyclopropylmethyl derivative **2t** and prenyl derivative **2u** retained high potency with IC₅₀ val-

ues below 1 nM. These results demonstrated that the inhibitory activity is affected by the bulkiness of the substituent and an aromatic ring at the 1-position of the indole ring is not essential for high potency.

This new series of indole derivatives **2b**–**2u** was further evaluated for rat prostatic 5 α -reductase inhibitory activities in order to investigate *in vivo* inhibitory activity. Compound **1** did not inhibit the rat enzyme, whereas finasteride and ($\pm$)-ONO-3805 showed inhibitory activities with IC₅₀ values of 13 nM and 2.6 nM, respectively, as shown in Table 4. Except for compound **2m**, none of these indole derivatives showed potent inhibitory activity toward the rat enzyme with an IC₅₀ below 100 nM. Compound **2m** showed moderate inhibitory activity against the rat enzyme with an IC₅₀ value of 73 nM (Table 4) which may not be sufficient to show inhibitory activity in an *in vivo* model such as the castrated young rat model.¹⁴ These findings suggest that these indole derivatives **2b**–**2u** are selective human prostatic 5 α -reductase inhibitors.

Conclusions

In order to prepare a novel potent inhibitor against human prostatic enzymes, we modified the biphenyl-4-yl group of YM-31758 to the 1*H*-indol-5-yl group. Consequently, we have identified a new series of indole derivatives as potent human prostatic 5 α -reductase inhibitors. Particularly, compounds **2d** (YM-32906), **2l** and **2n**–**2p** showed potent in-

hibitory activities with IC₅₀ values below 0.5 nM. Replacement of the benzyl group in compound **2d** with a 4-phenoxybenzyl group (**2m**) resulted in an increase in the inhibitory activity against the rat enzyme. Further efforts to discover novel nonsteroidal inhibitors possessing high potency for both human and rat prostatic 5 α -reductases are on going.

Experimental

Melting points were recorded on a Yanaco MP-3 melting point apparatus and are uncorrected. ¹H-nuclear magnetic resonance (¹H-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 analysis was 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 Na₂SO₄ or MgSO₄, and the organic solvent was evaporated under reduced pressure. All non-aqueous reactions were performed in dry glassware under an atmosphere of dry Ar. The three-dimensional structures of the molecules were constructed using the molecular modeling program Sybyl (version 6.3) on an Indigo Elan workstation (Silicon Graphics Inc., Mountain View, CA). Each structure was energy-minimized using the Tripos force field with the default convergence criteria.

2-Phenyl-1*H*-indole-5-ol (4) To a solution of **3** (670 mg, 3.00 mmol) in 5 ml of CH₂Cl₂, BBr₃ (1.0 M in CH₂Cl₂; 9.0 ml, 9.0 mmol) was added dropwise at –78 °C, and then the mixture was stirred at room temperature for 13 h. The reaction was quenched with ice-water and extracted with AcOEt. The organic layer was washed with water and brine, dried and concentrated. The residue was recrystallized from EtOH to give **4** (459 mg, 73%). ¹H-NMR (DMSO-*d*₆) δ : 6.63 (d, 1H, *J*=2 Hz), 6.71 (dd, 1H, *J*=9, 2 Hz), 6.95 (d, 1H, *J*=2 Hz), 7.2–7.3 (m, 2H), 7.40 (t, 2H, *J*=8 Hz), 7.76 (d, 2H, *J*=8 Hz), 8.26 (br s, 1H), 10.55 (br s, 1H); EI-MS *m/z* 209 (M⁺).

3-Chloro-4-[(2-phenyl-1*H*-indol-5-yl)oxy]benzonitrile (5) A mixture of **4** (450 mg, 2.15 mmol), 3-chloro-4-fluorobenzonitrile (334 mg, 2.15 mmol) and K₂CO₃ (442 mg, 3.20 mmol) in 3 ml of DMSO was stirred at room temperature for 6 h. After dilution with AcOEt, the reaction mixture was washed with water and brine, dried and concentrated. The residue was recrystallized from AcOEt–EtOH to give **5** (595 mg, 80%). ¹H-NMR (CDCl₃) δ : 6.80–6.82 (m, 2H), 6.93 (dd, 1H, *J*=9, 2 Hz), 7.3–7.5 (m, 6H), 7.67 (d, 2H, *J*=8 Hz), 7.74 (d, 1H, *J*=2 Hz), 8.46 (br s, 1H); EI-MS *m/z* 344 (M⁺).

3-Chloro-4-[(2-phenyl-1*H*-indol-5-yl)oxy]benzoic Acid (2a) To a solution of **5** (190 mg, 0.55 mmol) in 5 ml of EtOH, 1.5 ml of 8 N KOH aqueous solution was added, and the mixture was refluxed for 4 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 AcOEt to give **2a** (150 mg, 75%); mp 268–270 °C. ¹H-NMR (DMSO-*d*₆) δ : 6.84 (d, 1H, *J*=9 Hz), 6.89–6.92 (m, 2H), 7.3–7.4 (m, 3H), 8.02 (d, 1H, *J*=2 Hz), 11.72 (s, 1H), 13.07 (br s, 1H); EI-MS *m/z* 363 (M⁺).

Table 4. Inhibitory Activities of 5 α -Reductase in Human and Rat Prostate Homogenates for Indole Derivatives

Compound No.	R	IC ₅₀ (nM)	
		Human	Rat
1		0.87	>1000
2d		0.44	>300
2m		2.1	73
Finasteride		4.1	13
($\pm$)-ONO-3805		538	2.6

Fig. 2. Optimized Structures of **2b**–**2d** Calculated by the Molecular Modeling Program Sybyl (Version 6.3)

3-Chloro-4-[(1-methyl-2-phenyl-1*H*-indol-5-yl)oxy]benzoic Acid (2b) A mixture of **5** (207 mg, 0.60 mmol), methyl iodide (170 mg, 1.2 mmol) and KOH (67 mg, 1.2 mmol) in 1.0 ml of DMSO was stirred at room temperature for 1.5 h. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with water and brine, dried and concentrated to give 3-chloro-4-[(1-methyl-2-phenyl-1*H*-indol-5-yl)oxy]benzonitrile (**6**) (211 mg). This crude oil **6** (190 mg) was dissolved in 2.5 ml of EtOH, then 1.0 ml of 8*N* KOH aqueous solution was added, and the mixture was refluxed for 5 h. The reaction mixture was acidified with 2*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 AcOEt to give **2b** (144 mg, 72%); mp 260–263 °C. ¹H-NMR (DMSO-*d*₆) δ: 3.79 (s, 3H), 6.59 (s, 1H), 6.81 (d, 1H, *J*=9 Hz), 7.01 (dd, 1H, *J*=9, 2 Hz), 7.35 (d, 1H, *J*=2 Hz), 7.4–7.6 (m, 6H), 7.81 (dd, 1H, *J*=8, 2 Hz), 8.03 (d, 1H, *J*=2 Hz); EI-MS *m/z* 377 (M⁺).

5-Benzyloxy-1-methyl-1*H*-indol-3-yl Phenyl Ketone (8) A mixture of 5-benzyloxy-1*H*-indole (**7**) (4.02 g, 18.0 mmol), methyl iodide (5.11 g, 36.0 mmol) and KOH (2.0 g, 36.0 mmol) in 15 ml of DMSO was stirred at room temperature for 30 min. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with water and brine, dried and concentrated to give 5-benzyloxy-1-methyl-1*H*-indole (4.06 g). To a solution of 1-benzoylpyrrolidine (1.05 g, 6.0 mmol) in 6 ml of 1,2-dichloroethane, 1.68 ml of POCl₃ was added dropwise under refluxing. Stirring was continued for 40 min at this temperature, then the crude 5-benzyloxy-1-methyl-1*H*-indole (1.42 g) in 4 ml of 1,2-dichloroethane was added, and the mixture was refluxed for 6 h. The reaction was quenched with water, then neutralized with 1*N* NaOH aqueous solution and extracted with AcOEt. The organic layer was washed with water and a saturated aqueous solution of NaCl, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane:AcOEt=2:3) to give **8** (1.33 g, 65%). ¹H-NMR (CDCl₃) δ: 3.81 (s, 3H), 5.18 (s, 2H), 7.07 (dd, 1H, *J*=9, 2 Hz), 7.26 (s, 1H), 7.32 (t, 1H, *J*=7 Hz), 7.40 (dd, 2H, *J*=8, 7 Hz), 7.5–7.6 (m, 5H), 7.80 (d, 2H, *J*=8 Hz), 8.08 (d, 1H, *J*=2 Hz); EI-MS *m/z* 341 (M⁺).

3-Benzyl-1-methyl-1*H*-indol-5-ol (9) To a solution of **8** (478 mg, 1.40 mmol) in 16 ml of AcOEt–EtOH–AcOH (10:3:3), 10% Pd–C (50 mg) was added. The flask was then placed under a hydrogen atmosphere and stirred at room temperature for 2 d. The catalyst was removed by filtration and the filtrate was concentrated. The residue was purified by silica gel column chromatography (eluent; hexane:AcOEt=4:1) to give **9** (155 mg, 47%). ¹H-NMR (CDCl₃) δ: 3.69 (s, 3H), 4.03 (s, 2H), 6.74 (s, 1H), 6.78 (dd, 1H, *J*=8, 2 Hz), 6.88 (d, 1H, *J*=2 Hz), 7.1–7.3 (m, 6H); EI-MS *m/z* 237 (M⁺).

4-[(3-Benzyl-1-methyl-1*H*-indol-5-yl)oxy]-3-chlorobenzoic Acid (2c) Essentially the same procedure as described above for the preparation of **2a** afforded **2c**; mp 220–222 °C. ¹H-NMR (DMSO-*d*₆) δ: 3.77 (s, 3H), 3.99 (s, 2H), 6.71 (d, 1H, *J*=9 Hz), 6.93 (dd, 1H, *J*=9, 2 Hz), 7.15 (m, 1H), 7.2–7.3 (m, 6H), 7.48 (d, 1H, *J*=9 Hz), 7.77 (dd, 1H, *J*=9, 2 Hz), 8.00 (d, 1H, *J*=2 Hz), 13.06 (br s, 1H); EI-MS *m/z* 391 (M⁺).

Method A. 3-Chloro-4-(1*H*-indol-5-yloxy)benzonitrile (11) A mixture of **7** (4.47 g, 20.0 mmol), 10% Pd–C (500 mg) and ammonium formate (10.09 g, 160 mmol) in 100 ml of EtOH was stirred at room temperature for 3 h. The catalyst was removed by filtration and the filtrate was concentrated to give 1*H*-indole-5-ol (**10**) (2.89 g). This crude oil **10** (2.89 g) was dissolved in 50 ml of *N,N*-dimethylformamide (DMF), then 3-chloro-4-fluorobenzonitrile (3.27 g, 21.2 mmol) and K₂CO₃ (6.91 g, 50.0 mmol) were added, and the mixture was heated at 80 °C for 16 h. The reaction was quenched with water and 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:Et₂O=5:1) to give **11** (1.08 g, 29%). ¹H-NMR (CDCl₃) δ: 6.55 (t, 1H, *J*=1 Hz), 6.91–6.94 (m, 1H), 7.29 (t, 1H, *J*=3 Hz), 7.33–7.37 (m, 2H), 7.42 (d, 1H, *J*=9 Hz), 7.72 (d, 1H, *J*=2 Hz), 8.37 (br s, 1H); EI-MS *m/z* 268 (M⁺).

3-Chloro-4-[(1-(5-phenylpentyl)-1*H*-indol-5-yl)oxy]benzonitrile (13f) To a suspension of KOH (292 mg, 5.21 mmol) in DMSO (3 ml), **11** (350 mg, 1.30 mmol) was added and the mixture stirred at room temperature for 40 min, and 5-phenylpentyl bromide (355 mg, 1.56 mmol) in DMSO (1 ml) was added dropwise at room temperature. Stirring was continued for 2 h at this temperature. The reaction was quenched with water and 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:Et₂O=95:5) to give **13f** (337 mg, 62%). ¹H-NMR (CDCl₃) δ: 1.32–1.43 (m, 2H), 1.61–1.72 (m, 2H), 1.82–1.93 (m, 2H), 2.56–2.63 (m, 2H), 3.43–3.52 (m, 2H), 4.08–4.16 (m, 2H), 6.46 (d, 1H, *J*=3 Hz), 6.77 (d, 1H, *J*=9 Hz), 6.92 (dd, 1H, *J*=9, 2 Hz), 7.11–7.38 (m, 9H), 7.73 (d, 1H, *J*=2 Hz); EI-MS *m/z* 414 (M⁺).

3-Chloro-4-[(1-(5-phenylpentyl)-1*H*-indol-5-yl)oxy]benzoic Acid (2f) Compound **13f** was hydrolyzed in the same manner as described for compound **2a** to give **2f**; mp 144–145 °C. ¹H-NMR (DMSO-*d*₆) δ: 1.33–1.46 (m, 2H), 1.61–1.73 (m, 2H), 1.83–1.95 (m, 2H), 2.60 (t, 2H, *J*=8 Hz), 4.08–4.17 (m, 2H), 6.46 (d, 1H, *J*=3 Hz), 6.78 (d, 1H, *J*=8 Hz), 6.96 (dd, 1H, *J*=9, 2 Hz), 7.11–7.22 (m, 4H), 7.24–7.38 (m, 4H), 7.83 (dd, 1H, *J*=9, 2 Hz), 8.20 (d, 1H, *J*=2 Hz); EI-MS *m/z* 433 (M⁺).

Method B. 1-Benzyl-1*H*-indol-5-ol (12d) To a suspension of KOH (13.5 g, 240 mmol) in DMSO (100 ml), **7** (13.4 g, 60.0 mmol) was added and the mixture stirred at room temperature for 50 min, and benzyl bromide (10.8 g, 63.0 mmol) in DMSO (50 ml) was added dropwise at room temperature. Stirring was continued for 3 h at this temperature and the reaction was then quenched with water and extracted with AcOEt. The organic layer was washed with water and brine, dried and concentrated, and the residue recrystallized from EtOH to give 1-benzyl-5-benzyloxy-1*H*-indole (16.0 g, 85%). To a suspension of 1-benzyl-5-benzyloxy-1*H*-indole (16.0 g, 51.0 mmol) in 300 ml of EtOH, 10% Pd–C (1.60 g) and ammonium formate (25.7 g, 408 mmol) was added, and the mixture was stirred at room temperature for 3 h. The catalyst was removed by filtration and the filtrate was concentrated. The residue was extracted with AcOEt and the extract was washed with 1*N* HCl aqueous solution, water and brine, then dried and concentrated to give **12d** (10.9 g, 96%). ¹H-NMR (CDCl₃) δ: 4.61 (s, 1H), 5.25 (s, 2H), 6.41 (d, 1H, *J*=2 Hz), 6.72 (dd, 1H, *J*=9, 2 Hz), 7.03 (d, 1H, *J*=2 Hz), 7.07–7.10 (m, 4H), 7.22–7.30 (m, 3H); EI-MS *m/z* 223 (M⁺).

4-[(1-Benzyl-1*H*-indol-5-yl)oxy]-3-chlorobenzonitrile (13d) A mixture of **12d** (3.35 g, 15.0 mmol), 3-chloro-4-fluorobenzonitrile (2.45 g, 15.8 mmol) and K₂CO₃ (3.11 g, 22.5 mmol) in 50 ml of DMF was stirred at 80 °C for 6 h. After dilution with AcOEt, the reaction mixture was washed with 1*N* NaOH aqueous solution, water and brine, then dried and concentrated to give **13d** (5.67 g, quant). ¹H-NMR (CDCl₃) δ: 5.33 (s, 2H), 6.53 (d, *J*=3 Hz), 6.76 (d, 1H, *J*=8 Hz), 6.88 (dd, 1H, *J*=9, 2 Hz), 7.12–7.14 (m, 2H), 7.21 (d, 1H, *J*=3 Hz), 7.25–7.37 (m, 6H), 7.71 (d, 1H, *J*=2 Hz); EI-MS *m/z* 358 (M⁺).

4-[(1-Benzyl-1*H*-indol-5-yl)oxy]-3-chlorobenzoic Acid (2d) Compound **13d** was hydrolyzed in the same manner as described for compound **2a** to give **2d**; mp 158–159 °C. ¹H-NMR (CDCl₃) δ: 5.31 (s, 2H), 6.52 (d, 1H, *J*=3 Hz), 6.76 (d, 1H, *J*=9 Hz), 6.90 (dd, 1H, *J*=9, 2 Hz), 7.12 (d, 2H, *J*=7 Hz), 7.18 (d, 1H, *J*=3 Hz), 7.22–7.35 (m, 5H), 7.81 (dd, 1H, *J*=9, 2 Hz), 8.18 (d, 1H, *J*=2 Hz); EI-MS *m/z* 377 (M⁺).

Method C. Ethyl 5-Hydroxy-1*H*-indole-1-carboxylate (14) To a solution of **7** (30.0 g, 134 mmol) in DMF solution, 5.36 g (60% in oil, 134 mmol) of NaH was added at 0 °C and the mixture stirred at 60 °C for 1 h. Ethyl chloroformate (15.2 g, 140 mmol) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 15 h. The reaction was quenched with water and 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:CHCl₃=1:1) to give ethyl 5-benzyloxy-1*H*-indole-1-carboxylate (28.5 g, 72%). This intermediate (8.98 g, 30.4 mmol) was dissolved in EtOH (180 ml), then 10% Pd–C (0.9 g) and ammonium formate (15.3 g, 243 mmol) were added, and the mixture was stirred at room temperature for 45 min. The catalyst was removed by filtration and the filtrate concentrated, and the residue extracted with AcOEt. The extract was washed with 1*N* HCl aqueous solution, water and brine, then dried and concentrated. The residue was purified by silica gel column chromatography (eluent; CH₂Cl₂:ether=98:2) to give **14** (4.90 g, 79%). ¹H-NMR (CDCl₃) δ: 1.46 (t, 3H, *J*=7 Hz), 4.42–4.53 (m, 2H), 5.00 (s, 1H), 6.48 (d, 1H, *J*=4 Hz), 6.86 (dd, 1H, *J*=9, 2 Hz), 6.99 (d, 1H, *J*=2 Hz), 7.59 (d, 1H, *J*=3 Hz), 7.95–8.10 (br s, 1H); EI-MS *m/z* 205 (M⁺).

Ethyl 5-(2-Chloro-4-cyanophenoxy)-1*H*-indole-1-carboxylate (15) Essentially the same procedure as described above for the preparation of **13d** afforded **15**. ¹H-NMR (CDCl₃) δ: 1.44–1.53 (m, 3H), 4.47–4.56 (m, 2H), 6.58 (d, 1H, *J*=3 Hz), 6.80 (d, 1H, *J*=9 Hz), 7.06 (dd, 1H, *J*=9, 2 Hz), 7.26 (d, 1H, *J*=2 Hz), 7.37–7.43 (m, 1H), 7.69 (d, 1H, *J*=3 Hz), 7.75 (d, 1H, *J*=2 Hz), 8.23 (d, 1H, *J*=9 Hz); EI-MS *m/z* 205 (M⁺).

3-Chloro-4-(1*H*-indol-5-yloxy)benzoic Acid (16) Compound **15** was hydrolyzed in the same manner as described for compound **2a** to give **16**. ¹H-NMR (DMSO-*d*₆) δ: 6.45 (s, 1H), 6.80 (d, 1H, *J*=9 Hz), 6.90 (dd, 1H, *J*=9, 2 Hz), 7.31 (d, 1H, *J*=2 Hz), 7.43 (dd, 1H, *J*=3, 2 Hz), 7.48 (d, 1H, *J*=9 Hz), 7.80 (dd, 1H, *J*=9, 2 Hz), 8.02 (d, 1H, *J*=2 Hz), 11.26 (s, 1H), 13.05 (s, 1H); EI-MS *m/z* 287 (M⁺).

3-Chloro-4-[(1-(4-propylbenzyl)-1*H*-indol-5-yl)oxy]benzoic Acid (2j) To a suspension KOH (117 mg, 2.09 mmol) in DMSO (3.5 ml), **16** (150 mg, 0.52 mmol) was added and the mixture stirred at room temperature for 30 min, and 4-propylbenzyl bromide (133 mg, 0.62 mmol) in DMSO (0.5 ml)

was added dropwise at room temperature. Stirring was continued for 2 h at this temperature and the reaction was then quenched with water and 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; CHCl₃) and recrystallized from EtOH to give **2j** (113 mg, 52%): mp 168–171 °C. ¹H-NMR (CDCl₃) δ: 0.93 (t, 3H, *J*=7 Hz), 1.54–1.68 (m, 2H), 2.56 (t, 2H, *J*=8 Hz), 5.30 (s, 2H), 6.52 (d, 1H, *J*=3 Hz), 6.79 (d, 1H, *J*=8 Hz), 6.93 (dd, 1H, *J*=9, 2 Hz), 7.06 (d, 2H, *J*=8 Hz), 7.13 (d, 2H, *J*=8 Hz), 7.19 (d, 1H, *J*=3 Hz), 7.28–7.37 (m, 2H), 7.79–7.87 (m, 1H), 8.19 (d, 1H, *J*=2 Hz); EI-MS *m/z* 419 (*M*⁺).

Authentic Materials The 5α-reductase inhibitors finasteride^{60a} and (±)-ONO-3805^{7a} 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 [4-¹⁴C] 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, various concentrations of the test compounds were 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 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 N₂ gas and resuspended with 40 μl AcOEt. 20 μl of AcOEt was spotted on the TLC plate and separated twice by AcOEt/cyclohexane (1:1) as the developing solvent. The steroid standards were located by UV (254 nm) and by spraying with a 1% CeSO₄–10% H₂SO₄ 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 IC₅₀ values for 5α-reductase activity were obtained from the linear line drawn by least-squares fitting methods.

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