A Novel Class of Inhibitors for Human Steroid 5α-Reductase: Phenoxybenzoic Acid Derivatives. I

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In a search for novel nonsteroidal inhibitors of human prostatic 5α-reductase, we found a new series of phenoxybenzoic acid derivatives to be potent human prostatic 5α-reductase inhibitors. Among them, 4-(biphenyl-4-yloxy)benzoic acid derivatives (2n, YM-31758), 2o and 2s showed more potent inhibitory activities than finasteride with IC_{50} values of 0.87, 0.67 and 0.56 nM, respectively. The optimized structures for the phenoxybenzoic acid derivatives 2d—2i were calculated by molecular modeling analysis, and the favorable distance between the carbon of the carboxyl group and the centroid of the phenyl group (benzene ring C) was found to be in the 9—11 Å range.

Key words 5α-reductase inhibitor; benign prostatic hypertrophy; phenoxybenzoic acid derivative

Benign prostatic hyperplasia (BPH), skin disorders such as acne, male pattern baldness and hirsutism are androgen-related disorders, and are associated with elevated levels of dihydrotestosterone (DHT).[4] 5α-Reductase is an NADPH-dependent enzyme responsible for the conversion of testosterone to DHT. As a consequence, a 5α-reductase inhibitor is expected to provide a potential treatment for BPH. Several 5α-reductase inhibitors have been reported which might provide a novel therapeutic treatment for androgen-related disorders,[5,6] including both steroidal inhibitors, finasteride[5a] and epristeride,[5b] and a nonsteroidal inhibitor, ONO-3805[6a] (Fig. 1). Following the discovery of these inhibitors, the existence of two different 5α-reductase isozymes, called type 1 and type 2 5α-reductase, have been reported in humans and rats.[7—9] 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 have demonstrated clinical efficacy for the treatment of BPH; however, there is a possibility of adverse effects due to their steroidal structures.[10] 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)[11] for human prostatic 5α-reductase, whereas ONO-3805 showed only moderate activity (IC_{50}=538 nM)[11] 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 have reduced potential for side effects compared to steroidal inhibitors. To develop potent nonsteroidal 5α-reductase inhibitors, we began a chemical file screening program and found that the benzoic acid derivative **1** showed moderate inhibitory activity for 5α-reductase in HS27 human foreskin fibroblast cells[8] with an IC_{50} value of 317 nM. In order to increase the inhibitory activity against human prostatic 5α-reductase, we began modifications of the benzoic acid derivative **1**. This report describes the results of structure–activity relationships of the benzoic acid derivatives.

Chemistry Benzoic acids (**2a**—**2i**, **2l**—**2p**) were prepared by alkylation of phenol derivatives (**3**, **4**) and subsequent oxidation of the intermediate aldehydes or hydrolysis of the intermediate esters and nitrile as shown in Chart 1. *N*-Arylation of 4-aminobiphenyl with 4-fluorobenzonitrile in

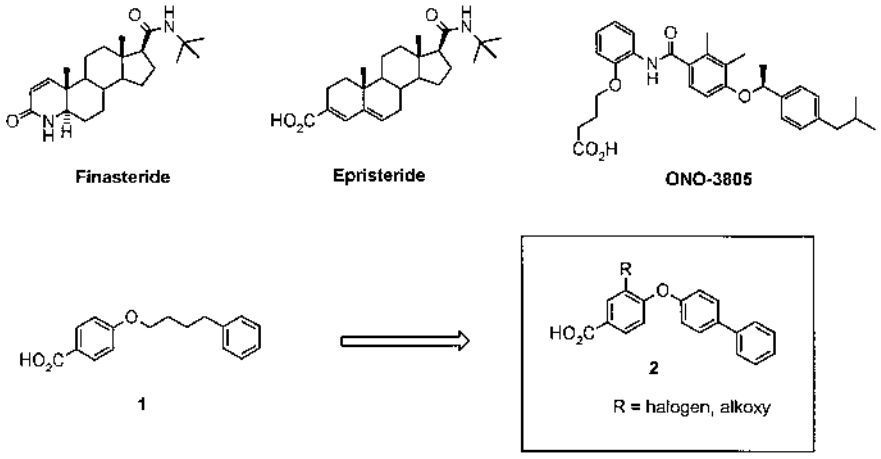
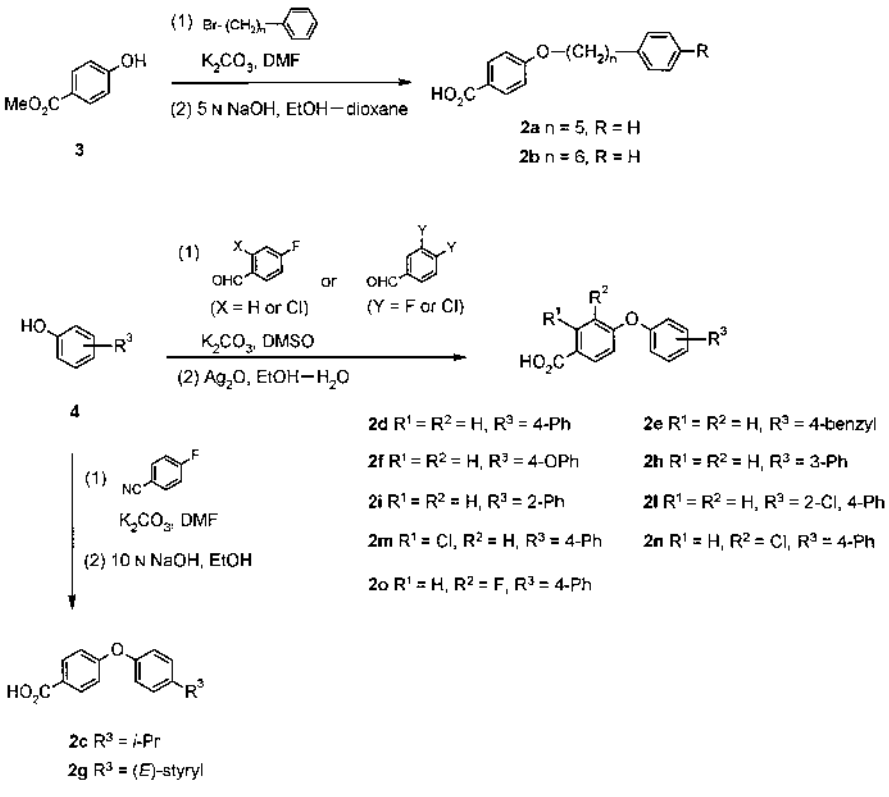


Fig. 1

* To whom correspondence should be addressed.



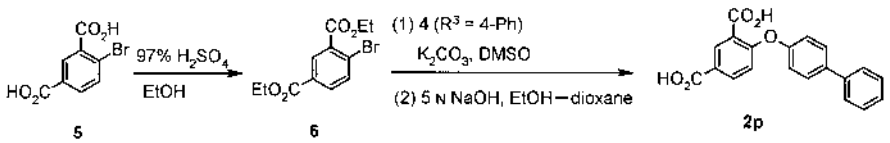


Chart 1

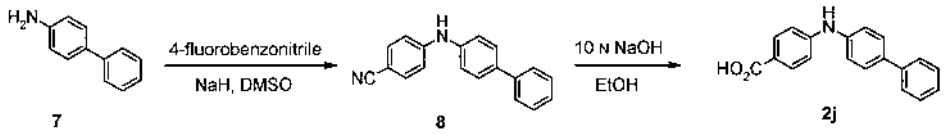


Chart 2

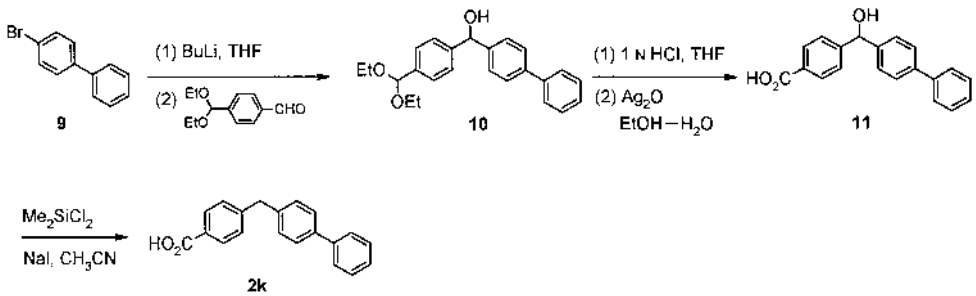


Chart 3

the presence of NaH followed by hydrolysis with NaOH gave **2j** (Chart 2). Compound **2k** was prepared as shown in Chart 3. Compound **10**, prepared by addition of biphenyl-4-yllithium to 4-diethoxymethylbenzaldehyde, was hydrolyzed with HCl, and the resulting aldehyde was converted to the carboxylic acid **11** by oxidation with Ag_2O. The dehydroxylation of **11** was performed using Me_2SiCl_2 and NaI to give **2k**.[12] 3-Nitrobenzoic acid derivative **2q** and 3-aminobenzoic acid derivative **2r** were prepared as shown in Chart 4. Compound **13** was hydrolyzed with NaOH to give the carboxylic acid **2q**. Compound **2r** was obtained by the reduction of **13** in the presence of Pd–C and subsequent hydrolysis with NaOH. Compounds **2s**, **2t** and **2u** were obtained by Ullmann condensations and subsequent conversion into carboxylic

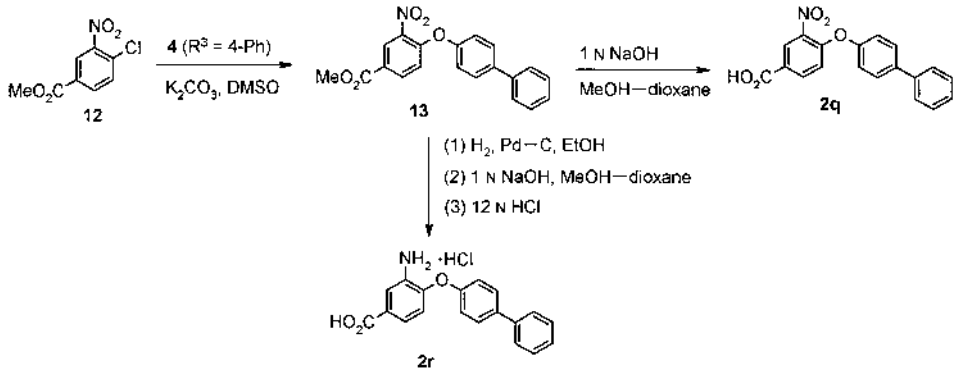


Chart 4

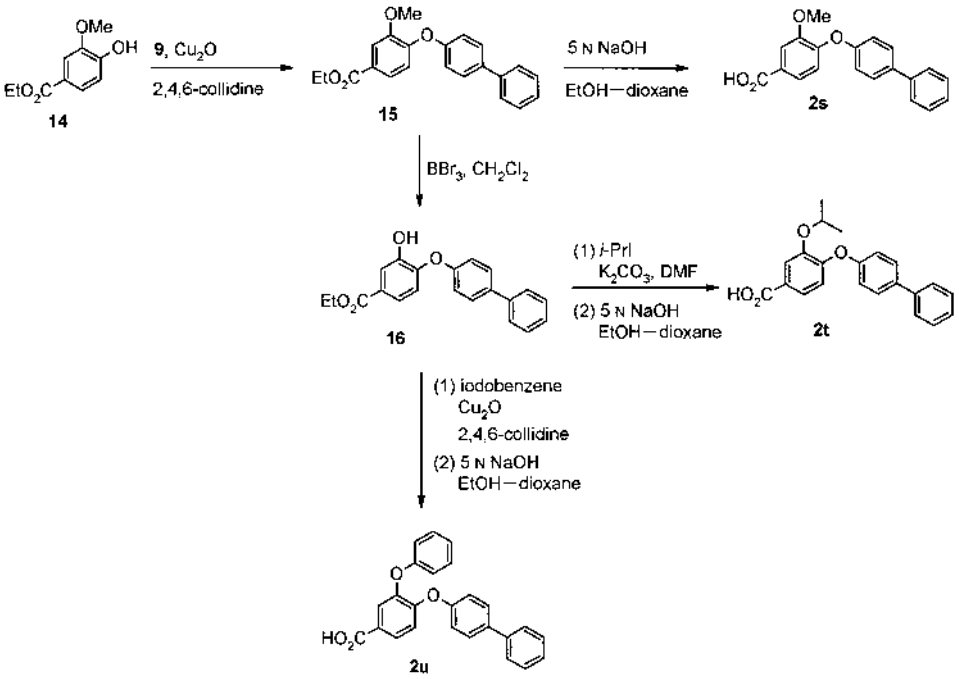


Chart 5

acids, as shown in Chart 5.

Results and Discussion

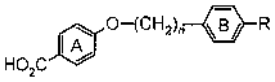
The inhibitory activities of the benzoic acid derivatives were evaluated on the basis of the ability to inhibit 5α-reductase in HS27 human foreskin fibroblast cells and human prostate at pH 5.5 which is optimal for the activity of the type 2 enzyme. The results are described in Tables 1—4.

We found the benzoic acid derivative **1**, which showed moderate inhibitory activity for 5α-reductase in HS27 by a chemical file screening program. Initially, the effects of the length of the alkylene moiety and substituents at the 4-position of the benzene ring B in compound **1** were investigated as described in Table 1. Elongation of the alkylene moiety (**2a**, **2b**) retained potency, while the phenoxy benzoic acid derivative **2c** which has no alkylene moiety resulted in a slight increase in the activity to 5α-reductase in HS27 with an IC_{50} value of 105 nM. Furthermore, replacement of the isopropyl group in compound **2c** with a phenyl group (**2d**) showed 40-fold greater inhibitory activity, with an IC_{50} value of 7.8 nM. These results suggest that an aryloxy group is more favorable than an alkoxy group as the substituent of benzene ring A for high potency. Since compound **2d** showed potent inhibitory activity for 5α-reductase in human prostate with an IC_{50} value of 1.1 nM, we next focused our efforts on the biphenyl derivative **2d** and modification of this compound was carried out in order to increase the inhibitory activity against human prostatic 5α-reductase.

Replacement of the phenyl group in compound **2d** with a benzyl group (**2e**), phenoxy group (**2f**), or (*E*)-styryl group (**2g**) resulted in a decrease in activity compared to compound **2d**, suggesting a phenyl group to be beneficial at this position.

Concerning the position of the phenyl group (benzene ring C) of compound **2d**, the 3-phenyl derivative **2h** resulted in a slight loss of activity with an IC_{50} value of 6.4 nM, while the 2-phenyl derivative **2i** resulted in a dramatic loss of activity, as described in Table 2. These results suggest that the distance between benzene ring C and the carboxyl group is important for potent inhibitory activity. In order to investigate the influence of distance, optimized structures for phenoxybenzoic acid derivatives **2d**—**2i** were calculated by a system-

Table 1. Physicochemical Data and Inhibitory Activities of 5α-Reductases in HS27 Foreskin Fibroblast Cells and Human Prostate Homogenates for Benzoic Acids



Compd. No.	n	R	mp (°C)	Formula	Analysis (%) Calcd (Found)		IC_{50} (nM)	
					C	H	HS27	Prostate
1	4	H	130—131	$C_{17}H_{18}O_3$	75.53 (75.60	6.71 6.77)	317	N.T.
2a	5	H	150—151	$C_{18}H_{20}O_3$	76.03 (76.02	7.09 7.13)	199	N.T.
2b	6	H	97—98	$C_{19}H_{22}O_3$	76.48 (76.48	7.43 7.61)	155	59
2c	0		126—127	$C_{16}H_{16}O_3$	74.98 (74.69	6.29 6.32)	105	N.T.
2d	0		237—239	$C_{19}H_{14}O_3$	78.61 (78.74	4.86 4.91)	7.8	1.1
2e	0		147—148	$C_{20}H_{16}O_3$	78.93 (78.95	5.30 5.27)	N.T.	13
2f	0		182—183	$C_{19}H_{14}O_4$	74.50 (74.54	4.61 4.62)	N.T.	4.1
2g	0		273—274	$C_{21}H_{16}O_3$	79.73 (79.61	5.10 5.17)	N.T.	15

N.T.: Not tested.

atic 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 benzene ring C were calculated from their optimized structures. The calculated data and the optimized structures of compounds **2d**—**2i** are shown in Table 3 and Fig. 2, respectively. Among them, the most potent compound **2d** is a linear compound, and the centroid of benzene ring C is positioned at a distance of around 10.5 Å from the carboxyl group through the benzene ring juncture. The corresponding distances of compounds **2e** and **2g** which showed 10 times less potent inhibitory activities than compound **2d**, were around 8.8 and 12.5 Å, respectively. On the other hand, compound **2i** is a non-linear compound, and the position of the benzene ring C is probably too close to the carboxyl group (C–centroid distance=8.062 Å) to show potent inhibitory activity. These data led us to speculate that the favorable distance between the carboxyl group and the centroid of benzene ring C was estimated to be in the 9—11 Å range and that there is a hydrophobic pocket interacting with benzene ring C in the enzyme.

Next, modification of the ether linker of compound **2d** was investigated. Substitution of the ether linker of compound **2d** with amine (**2j**) or methylene (**2k**) reduced inhibitory activities, with IC_{50} values of 25 and 10 nM, respectively, demonstrating the ether linker to be beneficial at this position.

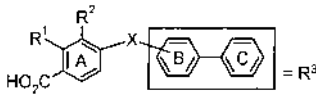
Finally, the effects of substituents on the benzene rings A and B were investigated. Introduction of a chloro group at the 2-position of benzene ring B (**2l**) or benzene ring A (**2m**) resulted in a slight loss of potency, while introduction of a chloro group at the 3-position of benzene ring A (**2n**) showed strong potency, with an IC_{50} value of 0.87 nM. Concerning substituents at the 3-position of benzene ring A, replacement with a fluoro (**2o**) or methoxy (**2s**) showed high potency with IC_{50} values of below 1 nM. Furthermore, the 3-nitrobenzoic acid derivative **2q** retained potency, while the 3-aminobenzoic acid derivative **2r** showed reduced potency and the isophthalic acid derivative **2p** resulted in a dramatic loss of activity. These findings demonstrated that the presence of a hydrophobic substituent at the 3-position of benzene ring A is favorable for potent inhibitory activity. Replacement of the methoxy group in compound **2s** with isopropoxy (**2t**) or phenoxy (**2u**) resulted in decreased inhibitory activity compared to compound **2s**, indicating that bulky substituents such as isopropoxy or phenoxy groups are unfavorable at this position.

Compounds **2n**, **2o** and **2s**, which showed potent inhibitory activity for human prostatic 5α-reductase, were evaluated for their rat prostatic 5α-reductase inhibitory activities. The results are summarized in Table 4. Finasteride and (±)-ONO-3805 exhibited inhibitory activities with IC_{50} values of 13 and 2.6 nM against the rat prostatic enzyme, respectively. The benzoic acid derivatives **2n**, **2o** and **2s** showed no measurable inhibition at 1 μM, suggesting these benzoic acid derivatives to be selective human prostatic 5α-reductase inhibitors. Possibly the low sequence homology between the human and rat enzymes is one reason.[7]

Conclusions

In order to develop more potent nonsteroidal inhibitors of human prostatic 5α-reductase than ONO-3805, we selected the benzoic acid derivative **1** as a lead compound and prepared mainly phenoxy benzoic acid derivatives. Consequently, we have identified a new series of biphenyl-4-yloxy benzoic acid derivatives as potent human prostatic 5α-reduc-

Table 2. Physicochemical Data and Inhibitory Activities of 5α-Reductase in Human Prostate Homogenates for Benzoic Acids



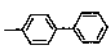
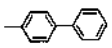
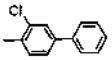
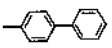
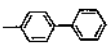
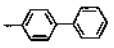
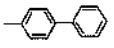
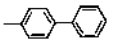
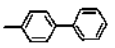
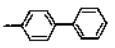
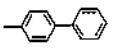
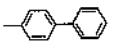
Conpd. No.	R^1	R^2	X	R^3	mp (°C)	Formula	Analysis (%) Calcd (Found)				IC_{50} (nM)
							C	H	N	Halogen	
2h	H	H	O		161—162	$C_{19}H_{14}O_3$	78.61 (78.71	4.86 4.92)			6.4
2i	H	H	O		155—156	$C_{19}H_{14}O_3$	78.61 (78.51	4.86 4.90)			>10000
2j	H	H	NH		240—241	$C_{19}H_{15}NO_2$	78.87 (78.85	5.23 5.26	4.84 4.48)		25
2k	H	H	CH_2		235—236	$C_{20}H_{16}O_2$	83.31 (83.17	5.59 5.63)			10
2l	H	H	O		191—192	$C_{19}H_{13}O_3Cl$	70.27 (70.36	4.03 4.15		10.92 11.15)	2.7
2m	Cl	H	O		219—220	$C_{19}H_{13}O_3Cl$	70.27 (70.38	4.03 4.01		10.92 10.84)	6.4
2n	H	Cl	O		204—205	$C_{19}H_{13}O_3Cl$	70.27 (70.22	4.03 3.87		10.92 11.09)	0.87
2o	H	F	O		226—227	$C_{19}H_{13}O_3F$	74.02 (74.01	4.25 4.40		6.16 5.98)	0.67
2p	H	CO_2H	O		274—275	$C_{20}H_{14}O_5$	71.85 (71.53	4.22 4.31)			>100
2q	H	NO_2	O		230—232	$C_{19}H_{13}NO_5$	68.06 (67.95	3.91 3.98	4.18 4.18)		1.2
2r	H	NH_2	O		193—194	$C_{19}H_{15}NO_3 \cdot HCl$	66.77 (66.83	4.72 4.90	4.10 3.86	10.37 10.09)	7.3
2s	H	OMe	O		201—203	$C_{20}H_{16}O_4$	74.99 (74.94	5.03 5.02)			0.56
2t	H	O(iso-Pr)	O		191—192	$C_{22}H_{20}O_4$	75.84 (75.75	5.79 5.89)			7.1
2u	H	OPh	O		203—204	$C_{25}H_{18}O_4$	78.52 (78.24	4.74 4.86)			355

Table 3. Distances between the Carbon of the Carboxyl Group and the Centroid of the Benzene Ring C for Compounds **2d**—**2i**

Compd. No.	C–Centroid distance (Å)	IC_{50} (nM)[a)]
2d	10.476	1.1
2e	8.819	13
2f	9.549	4.1
2g	12.524	15
2h	11.023	6.4
2i	8.062	>10000

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

tase inhibitors. In particular, compounds **2n** (YM-31758), **2o** and **2s** showed more potent inhibitory activity than finasteride. The favorable distance between the carbon of the carboxyl group and the centroid of benzene ring C was revealed to be in the 9—11 Å range by molecular modeling analysis. Further efforts to discover novel nonsteroidal inhibitors possessing more potent inhibitory activity for the human prostatic 5α-reductase are ongoing.

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 (MS) spectra 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 Na_2SO_4 or $MgSO_4$, 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).

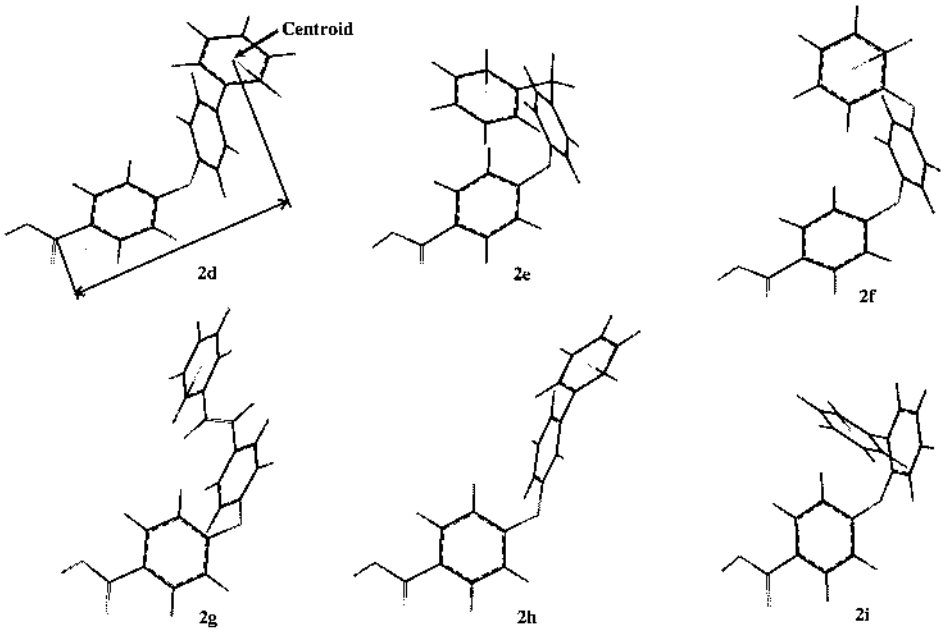
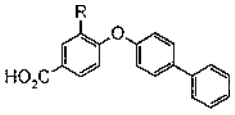


Fig. 2. Optimized Structures of **2d**—**2i** Calculated by the Molecular Modeling Program Sybyl (Version 6.3)

Table 4. Inhibition of 5α-Reductasses in Human and Rat Prostate Homogenates by Benzoic Acid Series



Compd. No.	R	IC_{50} (nM)	
		Human	Rat
2n	Cl	0.87	>1000
2o	F	0.67	>1000
2s	OMe	0.56	>1000
Finasteride		4.1	13
(±)-ONO-3805		538	2.6

Each structure was energy-minimized using the Tripos force field with the default convergence criteria.

4-(5-Phenylpentyloxy)benzoic Acid (2a) A mixture of **3** (300 mg, 1.97 mmol), 5-bromo-1-phenylpentane (500 mg, 2.20 mmol), KI (0.06 mmol, 10 mg) and K_2CO_3 (410 mg, 2.97 mmol) in *N*,*N*-dimethylformamide (DMF) (10 ml) was stirred at 100 °C for 8 h. 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 4-(5-phenylpentyloxy)benzoate (280 mg, 47%). This intermediate (260 mg, 0.87 mmol) was dissolved in 4 ml of EtOH–dioxane (3 : 1), then 5 N NaOH aqueous solution (4.0 ml) was added, and the mixture was heated at 40 °C for 30 min. The reaction mixture was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with brine, dried and concentrated, and the residue was recrystallized from EtOH to give **2a** (100 mg, 40%): mp 150—151 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.4—2.0 (m, 6H), 2.66 (t, J=7 Hz, 2H), 4.02 (t, J=6 Hz, 2H), 6.8—7.0 (m, 2H), 7.0—7.5 (m, 5H), 7.9—8.2 (m, 2H); FAB-MS *m*/*z* 285 (M^++H).

4-(4-Isopropylphenoxy)benzoic Acid (2c) A mixture of 4-isopropylphenol (2.72 g, 20.0 mmol), 4-fluorobenzonitrile (2.42 g, 20.0 mmol) and K_2CO_3 (4.15 g, 30.0 mmol) in DMF (40 ml) was stirred at 110 °C for 14 h. After dilution 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 4-(4-isopropylphenoxy)benzonitrile (4.49 g, 95%). This intermediate (4.05 g, 17.1 mmol) was dissolved in EtOH (49 ml), then 10 N NaOH aqueous solution (60 ml) was added, and the mixture was refluxed for 28 h. The reaction mixture was acidified with 3 N HCl aqueous solution, and extracted with AcOEt. The extract was washed with brine, dried and concentrated, and the residue was recrystallized from hexane to give **2c** (3.46 g, 79%): mp 126—127 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.27 (d, J=7 Hz, 6H), 2.94 (m, 1H), 6.9—7.1 (m, 4H), 7.2—7.4 (m, 2H), 7.9—8.2 (m, 2H); EI-MS *m*/*z* 256 (M^+).

4-(Biphenyl-4-yloxy)benzoic Acid (2d) A mixture of biphenyl-4-ol (1.70 g, 10.0 mmol), 4-fluorobenzaldehyde (1.24 g, 10.0 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) in dimethyl sulfoxide (DMSO) (20 ml) was stirred at 100 °C for 6.5 h. After dilution with AcOEt, the organic layer was washed with water and brine, dried and concentrated. The residue was recrystallized from EtOH to give 4-(biphenyl-4-yloxy)benzaldehyde (1.93 g, 70%). This intermediate (550 mg, 2.00 mmol) was dissolved in 22 ml of EtOH–water (1 : 1), then silver nitrate (710 mg, 4.18 mmol) and NaOH (320 mg, 8.00 mmol) were added, and the mixture was refluxed for 1 h. The precipitate was removed by filtration and the filtrate was concentrated. The residue was acidified with 1 N HCl aqueous solution and extracted with AcOEt. The extract was washed with brine, dried and concentrated, and the residue was recrystallized from EtOH to give **2d** (510 mg, 88%): mp 237—239 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.0—7.8 (m, 11H), 7.9—8.1 (m, 2H), 12.82 (m, 1H); EI-MS *m*/*z* 290 (M^+).

Diethyl 4-Bromoisophthalate (6) A mixture of **5** (4.90 g, 20.0 mmol) and 97% H_2SO_4 (2.0 ml) in EtOH (20 ml) was refluxed for 12 h and then concentrated. After dilution with AcOEt, the organic layer was washed with water, 1 N NaOH aqueous solution and brine, dried and concentrated to give **6** (5.17 g, 86%): ^{1}H-NMR ($CDCl_3$) δ: 1.4—1.5 (m, 6H), 4.3—4.5 (m, 4H), 7.73 (d, J=8 Hz, 1H), 7.96 (dd, J=8, 2 Hz, 1H), 8.40 (d, J=2 Hz, 1H); FAB-MS *m*/*z* 301 (M^++H).

4-(Biphenyl-4-yloxy)isophthalic Acid (2p) A mixture of **6** (4.00 g, 13.3 mmol), biphenyl-4-ol (2.26 g, 13.3 mmol) and K_2CO_3 (2.76 g, 20.0 mmol) in DMSO (13 ml) was stirred at 100 °C for 4 d. After dilution 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=19 : 1) to give diethyl 4-(biphenyl-4-yloxy)isophthalate (1.79 g, 39%). This intermediate (390 mg, 1.00 mmol) was dissolved in 4.5 ml of EtOH–dioxane (1 : 2), then 5 N NaOH aqueous solution (1.5 ml) was added, and the mixture was heated at 50 °C for 2 d and then concentrated. The residue was acidified with 3 N HCl aqueous solution and extracted with AcOEt. The extract was washed with brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; EtOH : AcOEt=2 : 3) and recrystallized from EtOH to give **2p** (215 mg, 64%): mp 274—275 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.1—7.2 (m, 3H), 7.36 (t, J=7 Hz, 1H), 7.47 (t, J=8 Hz, 2H), 7.66 (d, J=7 Hz, 2H), 7.71 (d, J=9 Hz, 2H), 8.09 (d, J=9 Hz, 1H), 8.40 (s, 1H), 13.16 (s, 2H); EI-MS *m*/*z* 334 (M^+).

4-(Biphenyl-4-ylamino)benzonitrile (8) To a solution of **7** (1.69 g, 10.0 mmol) in DMSO (7 ml), 60% NaH (440 mg, 11.0 mmol) was added,

and the mixture was stirred at room temperature for 1 h. A solution of 4-fluorobenzonitrile (610 mg, 5.0 mmol) in DMSO (5 ml) was added and the mixture was stirred at room temperature for 1 h. After 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 purified by silica gel column chromatography (eluent; hexane : CH_2Cl_2 : acetone=7 : 2 : 1) to give **8** (820 mg, 30%): ^{1}H-NMR ($CDCl_3$) δ: 7.0—7.9 (m, 13H), 9.01 (s, 1H); EI-MS *m/z* 270 (M^+).

4-(Biphenyl-4-ylamino)benzoic Acid (2j) To a solution of **8** (300 mg, 1.10 mmol) in EtOH (4 ml), 10 N NaOH aqueous solution (5 ml) was added, and the mixture was refluxed for 24 h. The reaction mixture was acidified with 1 N HCl aqueous solution and extracted with AcOEt. The extract was washed with brine, dried and concentrated, and the residue was recrystallized from AcOEt to give **2j** (150 mg, 47%): mp 240—241 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.0—8.0 (m, 13H), 8.83 (s, 1H), 12.29 (br s, 1H); EI-MS *m/z* 289 (M^+).

(Biphenyl-4-yl)(4-diethoxymethylphenyl)methanol (10) To a solution of **9** (1.40 g, 6.00 mmol) in tetrahydrofuran (THF) (30 ml), BuLi (1.6 M in hexane; 5.4 ml, 8.6 mmol) was added dropwise at −70 °C, and the mixture stirred at this temperature for 15 min, and a solution of 4-diethoxymethylbenzaldehyde (1.25 g, 6.00 mmol) in THF (5 ml) was added dropwise. Stirring was continued for 1.5 h at −70 °C and the reaction was then quenched with ice-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 : AcOEt=10 : 1) to give **10** (1.79 g, 82%): ^{1}H-NMR ($CDCl_3$) δ: 1.22 (t, *J*=7 Hz, 6H), 2.27 (d, *J*=4 Hz, 1H), 3.4—3.7 (m, 4H), 5.48 (s, 1H), 5.89 (d, *J*=4 Hz, 1H), 7.3—7.7 (m, 13H); EI-MS *m/z* 362 (M^+).

4-[(Biphenyl-4-yl)(hydroxy)methyl]benzoic Acid (11) To a solution of **10** (1.79 g, 4.90 mmol) in THF (50 ml), 1 N HCl aqueous solution (10 ml) was added, and the mixture was stirred at room temperature for 30 min and then concentrated. After dilution with AcOEt and water, the organic layer was washed with water and brine, dried and concentrated to give 4-[(biphenyl-4-yl)(hydroxy)methyl]benzaldehyde (1.32 g). This crude material (1.32 g, 4.60 mmol) was dissolved in 60 ml of EtOH–water (1 : 1), silver nitrate (2.33 g, 13.7 mmol) and NaOH (1.10 g, 27.5 mmol) were added, and the mixture was refluxed for 5 h. The precipitate was removed by filtration and the filtrate was concentrated. The residue was acidified with 1 N HCl aqueous solution and extracted with AcOEt. The extract was washed with brine, dried and concentrated, and the residue was recrystallized from 2-propanol to give **11** (1.08 g, 78%): ^{1}H-NMR (DMSO-d_6) δ: 5.83 (s, 1H), 6.10 (d, *J*=4 Hz, 1H), 7.3—7.6 (m, 11H), 7.7—7.9 (m, 2H), 12.94 (br s, 1H); EI-MS *m/z* 304 (M^+).

4-(Biphenyl-4-ylmethyl)benzoic Acid (2k) A mixture of **11** (640 mg, 2.10 mmol), NaI (1.26 g, 8.40 mmol) and Me_2SiCl_2 (0.51 ml, 4.20 mmol) in CH_3CN (10 ml) was stirred at room temperature for 18 h. After dilution with AcOEt, the reaction mixture was washed with water, 10% $Na_2S_2O_3$ aqueous solution and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=9 : 1) and recrystallization from 2-propanol to give **2k** (70 mg, 12%): mp 235—236 °C; ^{1}H-NMR (DMSO-d_6) δ: 4.06 (s, 2H), 7.2—8.0 (m, 13H), 12.80 (s, 1H); EI-MS *m/z* 288 (M^+).

Methyl 4-(Biphenyl-4-yloxy)-3-nitrobenzoate (13) A mixture of **12** (4.31 g, 20.0 mmol), biphenyl-4-ol (3.40 g, 20.0 mmol) and K_2CO_3 (6.22 g, 45.0 mmol) in DMSO (40 ml) was stirred at 100 °C for 19 h. After dilution with AcOEt, the reaction mixture was washed with 1 N NaOH aqueous solution, water and brine, dried and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane : AcOEt=95 : 5) to give **13** (4.18 g, 60%): ^{1}H-NMR ($CDCl_3$) δ: 3.95 (s, 3H), 7.06 (d, *J*=9 Hz, 1H), 7.18 (d, *J*=7 Hz, 2H), 7.37 (t, *J*=7 Hz, 1H), 7.46 (dd, *J*=8, 7 Hz, 2H), 7.58 (d, *J*=7 Hz, 2H), 7.65 (d, *J*=7 Hz, 2H), 8.14 (dd, *J*=9, 2 Hz, 1H), 8.62 (d, *J*=2 Hz, 1H); EI-MS *m/z* 349 (M^+).

4-(Biphenyl-4-yloxy)-3-nitrobenzoic Acid (2q) To a solution of **13** (420 mg, 1.20 mmol) in 10.5 ml of MeOH–dioxane (2.5 : 8), 1 N NaOH aqueous solution (2.5 ml) was added, and the mixture was stirred at room temperature for 40 min and then concentrated. The residue 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 **2q** (310 mg, 76%): mp 230—232 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.21 (d, *J*=9 Hz, 1H), 7.30 (d, *J*=9 Hz, 2H), 7.38 (t, *J*=7 Hz, 1H), 7.48 (t, *J*=8 Hz, 2H), 7.69 (d, *J*=7 Hz, 2H), 7.79 (d, *J*=9 Hz, 2H), 8.19 (dd, *J*=9, 2 Hz, 1H), 8.53 (d, *J*=2 Hz, 1H), 13.50 (s, 1H); EI-MS *m/z* 335 (M^+).

3-Amino-4-(biphenyl-4-yloxy)benzoic Acid Monohydrochloride (2r) To a solution of **13** (1.78 g, 5.10 mmol) in EtOH (70 ml), 10% Pd–C (100 mg) was added. The flask was then placed under hydrogen atmosphere and stirred at room temperature for 20 h. The reaction mixture was filtered to remove the catalyst, and the filtrate was concentrated to give methyl 3-amino-4-(biphenyl-4-yloxy)benzoate (1.62 g). This crude material (190 mg) was dissolved in 1.5 ml of MeOH–dioxane (1 : 2), then 1 N NaOH aqueous solution was added, and the mixture was stirred at 50 °C for 18 h. After the reaction mixture was concentrated, the residue was taken up in water (15 ml), and the pH was adjusted to 7 by the addition of 1 N HCl aqueous solution. The mixture was washed with AcOEt, and 12 N HCl (3 ml) aqueous solution was added. The resulting crystals were collected by filtration to give **2r** (180 mg, 96%): mp 193—194 °C; ^{1}H-NMR (DMSO-d_6) δ: 6.92 (d, *J*=9 Hz, 1H), 7.18 (d, *J*=9 Hz, 2H), 7.36 (t, *J*=7 Hz, 1H), 7.47 (dd, *J*=8, 7 Hz, 2H), 7.54 (dd, *J*=8, 2 Hz, 1H), 7.66 (d, *J*=7 Hz, 2H), 7.73 (d, *J*=9 Hz, 2H), 7.82 (d, *J*=2 Hz, 1H); EI-MS *m/z* 305 (M^+−HCl).

Ethyl 4-(Biphenyl-4-yloxy)-3-methoxybenzoate (15) A mixture of **14** (2.94 g, 15.0 mmol), **9** (3.50 g, 15.0 mmol) and Cu_2O (2.58 g, 18.0 mmol) in 2,4,6-collidine (6 ml) was stirred at 165 °C for 44 h. The reaction mixture was acidified with 2 N HCl aqueous solution, then extracted with AcOEt, and the insoluble material was removed by filtration. 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 **15** (2.47 g, 47%): ^{1}H-NMR ($CDCl_3$) δ: 1.41 (t, *J*=7 Hz, 3H), 3.94 (s, 3H), 4.39 (q, *J*=7 Hz, 2H), 6.96 (d, *J*=8 Hz, 1H), 7.06 (d, *J*=9 Hz, 2H), 7.33 (t, *J*=7 Hz, 1H), 7.43 (dd, *J*=8, 7 Hz, 2H), 7.5—7.6 (m, 4H), 7.64 (dd, *J*=8, 2 Hz, 1H), 7.69 (d, *J*=2 Hz, 1H); EI-MS *m/z* 348 (M^+).

Ethyl 4-(Biphenyl-4-yloxy)-3-hydroxybenzoate (16) To a solution of **15** (2.02 g, 5.80 mmol) in CH_2Cl_2 (6 ml), BBr_3 (1.0 M in CH_2Cl_2; 11.6 ml, 11.6 mmol) was added dropwise at −78 °C, and the mixture was stirred at 0 °C for 3 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–hexane to give **16** (1.60 g, 83%): ^{1}H-NMR (DMSO-d_6) δ: 1.31 (t, *J*=7 Hz, 2H), 4.29 (q, *J*=7 Hz, 2H), 7.01 (d, *J*=9 Hz, 2H), 7.06 (d, *J*=8 Hz, 1H), 7.34 (t, *J*=7 Hz, 1H), 7.4—7.5 (m, 3H), 7.6—7.7 (m, 5H), 10.03 (s, 1H); EI-MS *m/z* 334 (M^+).

4-(Biphenyl-4-yloxy)-3-methoxybenzoic Acid (2s) Compound **15** (312 mg, 0.90 mmol) was hydrolyzed in the same manner as described for compound **2a** to give **2s** (236 mg, 82%): mp 201—203 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.85 (s, 3H), 7.02 (dd, *J*=7, 2 Hz, 2H), 7.09 (d, *J*=8 Hz, 1H), 7.34 (t, *J*=7 Hz, 1H), 7.45 (dd, *J*=8, 7 Hz, 2H), 7.5—7.7 (m, 6H), 12.98 (s, 1H); EI-MS *m/z* 320 (M^+).

4-(Biphenyl-4-yloxy)-3-isopropoxybenzoic Acid (2t) Essentially the same procedure as described above for the preparation of **2a** afforded **2t**: mp 203—204 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.19 (d, *J*=6 Hz, 6H), 4.64 (m, 1H), 7.03 (dd, *J*=7, 2 Hz, 2H), 7.10 (d, *J*=8 Hz, 1H), 7.34 (t, *J*=7 Hz, 1H), 7.45 (dd, *J*=8, 7 Hz, 2H), 7.58 (dd, *J*=8, 2 Hz, 1H), 7.6—7.7 (m, 5H), 12.96 (s, 1H); EI-MS *m/z* 348 (M^+).

4-(Biphenyl-4-yloxy)-3-phenoxybenzoic Acid (2u) Essentially the same procedure as described above for the preparation of **2s** afforded **2u**: mp 203—204 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.02 (d, *J*=8 Hz, 1H), 7.1—7.2 (m, 3H), 7.19 (d, *J*=9 Hz, 1H), 7.3—7.5 (m, 5H), 7.56 (d, *J*=2 Hz, 1H), 7.6—7.7 (m, 4H), 7.78 (dd, *J*=9, 2 Hz, 1H), 13.05 (s, 1H); EI-MS *m/z* 382 (M^+).

Authentic Materials The 5α-reductase inhibitors finasteride[5a] and (±)-ONO-3805[6a] were synthesized in our company according to the methods described in the literature.

Biological Methods. Preparation of 5α-Reductase from HS27, Rat Prostate and Human Prostate Human genital skin fibroblast cell line HS27 (CRL1634) was obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 100 unit/ml penicillin and 100 μg/ml streptomycin. The cell monolayer was released from the culture flask using 0.25% trypsin and 0.02% EDTA. The cells were washed 3 times with saline and collected by centrifugation. The cell pellet was suspended in 10 mM Tris–HCl buffer (pH 7.0) and homogenized with a Teflon–glass homogenizer. The homogenate was sonicated for 30 s×3 times and centrifuged at 1000 ***g*** for 5 min. The supernatant was stored at −80 °C until use.

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 at pH 6.5. The suspension was stored at −80 °C until use.

Human prostatic tissues from BPH patients who had received 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.*[13] 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 HS27 and the human prostate enzyme) and the enzyme fractions. To identify the inhibitory effect of the drugs, various concentrations of 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 N_2 gas and resuspended with 40 μl AcOEt. 20 μl of AcOEt was spotted on a 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% $CeSO_4$–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 and 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 by the least-squares fitting method.[14]

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References and Notes

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