Design and Synthesis of 1,2,4-Oxadiazole Derivatives as Non-steroidal 5α-Reductase Inhibitors

Chih-Shiang Chang[a] (張誌祥), Wai Ming Kan[b] (簡偉明), Chiu-Liang Chen[b] (陳秋良), K. C. Wang[a] (王光昭) and Ji-Wang Chern[a]* (陳基旺)

[a]*School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan 100, R.O.C.*

[b]*Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan 701, R.O.C.*

The purpose of this study was to synthesize compounds in which the 1,2,4-oxadiazole moiety replaced the amide bond of ONO3805 and to evaluate its 5α-reductase inhibitory activity as a potential benign prostatic hyperplasia therapeutic target. Four 1,2,4-oxadiazole derivatives, **1**, **2**, **8**, and **20**, were evaluated *in vitro* against 5α-reductase of rat liver microsome. The prepared **1** and **2** possessed similar binding affinity (Ki) to that of ONO3805. Therefore, the use of 1,2,4-oxadiazole ring as surrogate of the amide bond in ONO3805 has a successful result in this study. It leads not only to enhance chemical stability but also to maintain meaningful inhibitory activity. The butyric acid moiety of these inhibitors is considered to play an important role in mimicing the phosphoric acid portion of coenzyme-NADPH in interacting with the active site of 5α-reductase.

INTRODUCTION

Steroid 5α-reductase is a membrane-associated enzyme, which catalyzes the reduction of testosterone to the biologically more active dihydrotestosterone (DHT) in the presence of NADPH.[1] Elevated DHT has been implicated as a causative factor of skin disorders and benign prostatic hyperplasia (BPH).[2] Therefore, inhibition of 5α-reductase is an attractive target for therapeutic intervention in disorders associated with elevated levels of DHT such as BPH,[3,4] acne,[5] male pattern baldness,[6] and hirsutism.[7] A number of 5α-reductase inhibitors have been reported, including both steroidal[8] and nonsteroidal inhibitors.[9] Starting our research program to find a new 5α-reductase inhibitor, we focused our attention on the development of novel nonsteroidal compounds, especially ONO3805. According to the bioisosterism,[10] some research groups have successfully replaced the ester or amide group of biologically active compounds by an 1,2,4-oxadiazole moiety to improve *in vivo* efficacy.[11] The intramolecular acid catalyzed the hydrolysis of the amide bond[12,13] even though the hydrolysis exhibits a half-life of 7 years under neutral conditions and at room temperature.[14] In addition, we found that the amide bond of ONO3805 was partially cleaved in a DMSO-d_6 solution at 37 °C in four months. Replacement of the amide bond with a 1,2,4-oxadiazole ring for increasing hydrolytic resistance,[15] metabolic stability, and improving pharmacokinetic performance[16] has been reported. Herein, derivatives with 1,2,4-oxadiazole surrogate of the amide bond in ONO3805 as potential 5α-reductase inhibitors were designed and synthesized (Fig. 1).

RESULTS AND DISCUSSION

The synthesis of target compound **1** is illustrated in

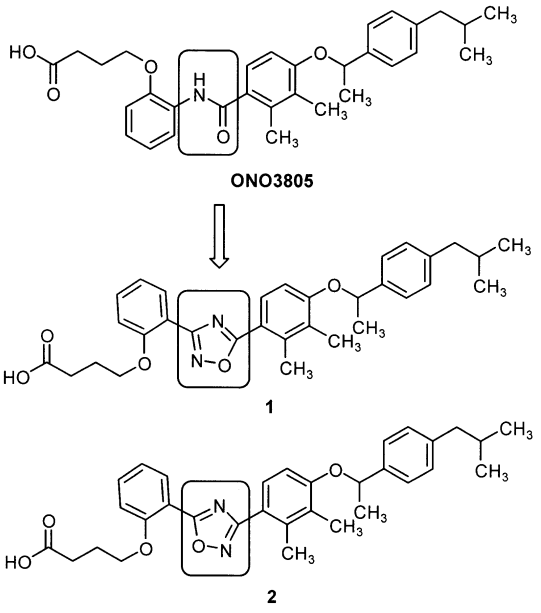
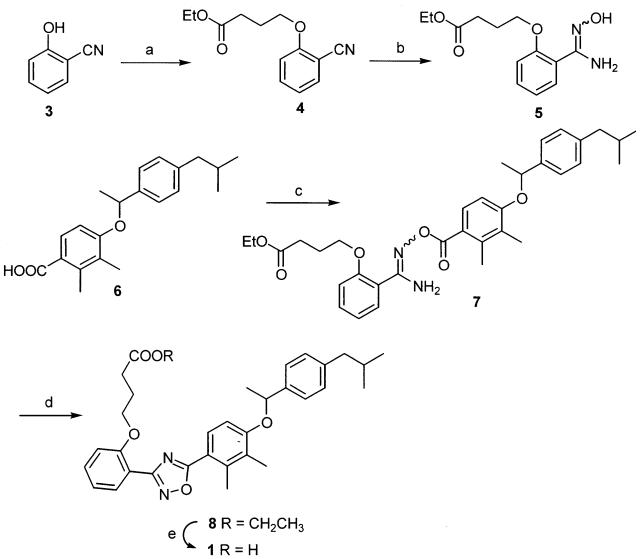


Fig. 1. The amide linkage of ONO3805 was replaced with 1,2,4-oxadiazole bioisostere.

Scheme I. The coupling of substituted amidoxime **5** and 4-[1-(4-isobutylphenyl)ethoxy]-2,3-dimethylbezoic acid (**6**) using 1,1′-carbonyldiimidazole in THF afforded **7**, which was followed by cyclization in diglyme at 110 °C to form the 1,2,4-oxadiazole derivative **8**.[17] Finally, treatment of **8** with alkaline solution gave 89% yield of **1**. The required **5** was prepared from 2-cyanophenol by alkylation with ethyl γ-bromobutyrate and subsequent treatment with hydroxylamine. The other fragment **6** was prepared by a modified procedure from Harris.[18] The oxadiazole isomer **2** was prepared by initial treatment of substituted amidoxime **14** and diester **16** with NaH in THF to afford ethyl butyrate **17** and a mixture containing **18** and **19**. Finally, hydrolysis of **17** and the mixture of **18** and **19** with aqueous lithium hydroxide afforded 1,2,4-oxadiazole **2** and **20**, respectively (Scheme III). The required amidoxime **14** was prepared as shown in Scheme II. Firstly, the treatment of 2,3-dimethyl-*p*-anisaldehyde with hydroxylamine-*o*-sulfonic acid in a mixed solvent of water and acetonitrile (1:1) to produce benzonitrile **10**,[19] which was demethylation with BBr_3 to give **11**. Then, **11** coupled with 1-(4-isobutylphenyl)ethanol by Mitsunobu reaction to furnish benzonitrile **12**.[20] Subsequent treatment of **12** with hydroxylamine in Et_3N yielded amide **13** (48%) and amidoxime **14** (45%). The other fragment **16** was prepared from methyl salicylate by alkylation with ethyl γ-bromobutyrate.

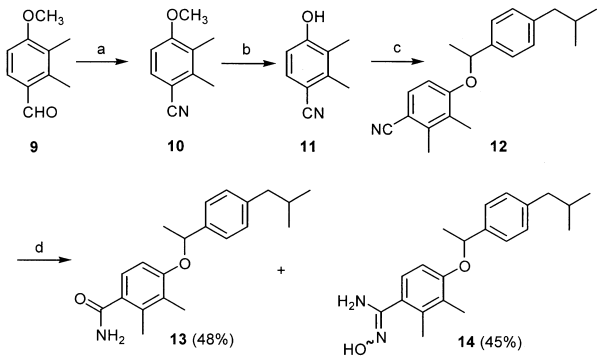
The prepared 1,2,4-oxadiazole derivatives, **1**, **2**, **8**, and **20**, were subjected against 5α-reductase inhibition assay (Table 1). The 1,2,4-oxadiazole isomers **1** and **2** possessed high

Scheme I



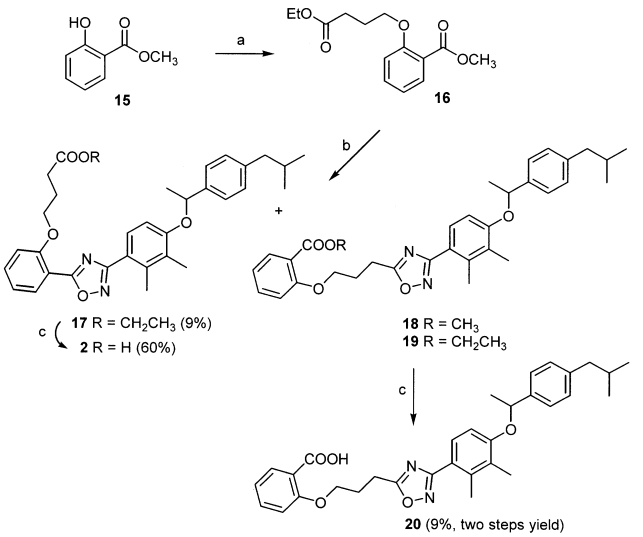
Reagents: (a) $EtOOC(CH_2)_3Br$, K_2CO_3, CH_3CN, reflux, 92%; (b) NH_2OH-HCl, K_2CO_3, absolute EtOH, reflux, 47%; (c) **5**, CDI, THF, rt, 40%; (d) diglyme, 110 °C, 95%; (e) 10% NaOH in MeOH, rt, 89%.

Scheme II



Reagents: (a) HOSA, CH_3CN, H_2O, 60 °C, 96%; (b) BBr_3, CH_2Cl_2, reflux, 98%; (c) 1-(4-isobutylphenyl)ethanol, Ph_3P, DEAD, THF, rt, 49%; (d) NH_2OH-HCl, Et_3N, absolute EtOH, reflux.

Scheme III



Reagents: (a) $EtOOC(CH_2)_3Br$, K_2CO_3, CH_3CN, reflux, 98%; (b) **14**, NaH, THF, 4 Å MS, 60 °C; (c) 1N LiOH, dioxane, 80 °C.

inhibitory activity to 5α-reductase with Ki = 15.63 and 22.36 nM, respectively, which is comparable with that of ONO3805 (Ki = 8.46 nM). These results demonstrated that the 1,2,4-oxadiazole moiety is as useful as the amide bioisostere in ONO3805 chemical stability. Holt et al.[21] proposed that the butanoic acid moiety of ONO3805 was localized in the region of the phosphate groups of NADPH, and the lipophilic part was oriented in the region of the steroidal rings C and D to occupy the hydrophobic pocket of 5α-reductase. Interestingly, compound **8** lost activity against 5α-reductase (Ki > 50 μM). This result might indicate that **8** could not fit into the negative-ionizable region because the carboxylic function was masked by an ethyl group. Surprisingly, compound **20**

Table 1. Binding Affinity Against 5α-Reductase of Rat Liver Microsome

Compounds	Binding Affinity Ki ± SD (nM)[a]
1	15.63 ± 9.69
2	22.36 ± 6.30
8	NA[b]
20	NA[b]
ONO3805	8.46 ± 5.92

[a] Values are in four experiments.
[b] Not active at concentration of 50 μM.

did not show any inhibitory activity. The reason might be that the benzoic acid moiety is unable to function as the butanoic acid moiety in **1** and **2** in fitting into the negative-ionizable region as proposed.

The initial result showed that the 1,2,4-oxadiazole bioisosteres, **1** and **2**, could successfully retain inhibitory activity of 5α-reductase by comparison with the amide linkage of ONO3805. The oxadiazole **8** lost its activity when butanoic acid moiety was masked by an ethyl group, indicating that the carboxylic acid group was important to mimic the phosphoric acid portion of NADPH. Moreover, the 2-alkoxybenzoic acid moiety also could not fit the active site of 5α-reductase. Therefore, the negative ionizable region of 5α-reductase is considered to play an important role in its catalytic activity of reducing testosterone to DHT.

EXPERIMENTAL SECTION

All of the solvents and chemicals were reagent grade. Normal phase silica gels of 9385 (230-400 mesh) for column chromatography, and TLC plates of 573 (Si60 with F_{254}, 0.25 mn) were purchased from E. Merck A. G., Darmstadt, Germany. [4-^{14}C]-Testosterone (2.10 GBq/mmol) was purchased from NEN Life Science Products, Inc., Boston, U.S.A. Melting points measured on a Buchi 510 melting point apparatus were uncorrected. IR spectra were recorded on a Jasco A-100 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on Bruker DPX-200 or Bruker AMX-400 spectrophotometers using the solvent peaks as reference standards. EIMS spectra were recorded on Finnigan Mat GCQ^{TM} GC/MS and HREIMS on JEOL JMS-HX-100 mass spectrometer. Elemental analyses were carried out on a Perkin-Elmer 240 elemental analyzer and the results were within ±0.4% of the theoretical values.

Ethyl 4-(2-cyanophenoxy)butyrate (4)

To a stirred solution of 2-cyanophenol (20 g, 0.17 mol) and K_2CO_3 (25.84 g, 0.19 mol) dissolved in dried acetonitril (500 mL) was added γ-bromo-n-butyric acid ethyl ester (32.8 g, 0.17 mol) under nitrogen. The mixture was heated at reflux for 11 h, filtered, and concentrated in vacuo. The residue was subjected to chromatography (silica gel, n-hexane/ethyl acetate = 9/1, R_f = 0.19) to afford **4** (36.4 g, 92 %) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42-7.51 (m, 2H, ArH), 6.90-6.98 (m, 2H, ArH), 4.03-4.14 (m, 4H, CH_2), 2.52 (t, 2H, J = 7.2 Hz, $COCH_2$), 2.04-2.17 (m, 2H, CH_2), 1.20 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 160.3, 134.2, 133.6, 120.7, 116.2, 112.1, 101.8, 69.5, 60.4, 30.2, 24.0, 14.0; MS (EI) *m/z* 233 (M^+); Anal. Calcd. for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.88; H, 6.46; N, 5.98.

Ethyl 4-[2-[amino(hydroxyimino)methyl]phenoxy]-butyrate (5)

To a stirred solution of hydroxylamine hydrochloride (2.98 g, 42.9 mmol) in absolute ethanol (250 mL) was added K_2CO_3 (5.92 g, 42.86 mmol) at room temperature under nitrogen for 10 min, and then added a solution of **4** (5.0 g, 21.4 mmol) in absolute ethanol (10 mL). The mixture was heated at reflux for 11 h and then evaporated in vacuo. The residue was subjected to chromatography (silica gel, $CHCl_3$, R_f = 0.15) to give **5** (2.69 g, 47%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.61 (dd, 1H, J = 7.63 Hz, ArH), 7.26-7.31 (m, 1H, ArH), 6.87-6.98 (m, 2H, ArH), 5.37 (br s, 2H, NH_2), 4.01-4.16 (m, 4H, CH_2), 2.48 (t, 2H, J = 7.2 Hz, CH_2), 2.08-2.14 (m, 2H, CH_2), 1.21 (t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.9, 156.3, 152.2, 130.8, 129.7, 121.1, 120.9, 112.5, 67.6, 60.5, 30.7, 24.4, 14.1; MS (EI) *m/z* 266 (M^+); Anal. Calcd. for $C_{13}H_{18}N_2O_4$: C, 58.64; H, 6.81; N, 10.52. Found: C, 58.60; H, 6.58; N, 10.30.

4-[2-[4-[1-(4-Isobutylphenyl)ethoxy]-2,3-dimethyl-N-benzoyloxy]carbamimidoylphenoxy]butanoic acid ethyl ester (7)

To a stirred solution of **6** (300 mg, 0.92 mmol) and 1,1′-carbonyldiimidazole (193.9 mg, 1.20 mmol) dissolved in dried THF (30 mL) was added **5** (318.2 mg, 1.19 mmol) at room temperature. The mixture was stirred for 18 h under nitrogen. After the mixture was reacted completely (indicated by phosphomolybdic acid on TLC plate displayed an orange spot), purified by chromatography (silica gel, n-hexane/ethyl acetate = 7/3, R_f = 0.17) to afford **7** which was recrystallized from ether to give a white solid (210 mg, 40%). mp 103-104

°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.87 (dd, 1H, *J* = 7.2; 1.3 Hz, ArH), 6.89-7.54 (m, 8H, ArH), 6.59 (d, 1H, *J* = 8.7 Hz, ArH), 5.58 (br s, 2H, NH_2), 5.33 (q, 1H, *J* = 6.3 Hz, CH), 4.04-4.15 (m, 4H, OCH_2), 2.51 (s, 3H, CH_3), 2.41-2.47 (m, 4H, -$COCH_2$; $ArCH_2$), 2.28 (s, 3H, CH_3), 2.05-2.18 (m, 2H, CH_2), 1.75-1.89 (m, 1H, CH), 1.63 (d, 3H, *J* = 6.5 Hz, CH_3), 1.20 (t, 3H, *J* = 7.1 Hz, CH_3), 0.87 (d, 6H, *J* = 6.6 Hz, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.0, 165.8, 158.2, 156.7, 156.3, 141.0, 140.1, 139.4, 131.8, 130.8, 129.3, 128.3, 127.0, 125.1, 122.7, 121.3, 119.6, 112.5, 109.9, 109.6, 76.1, 67.8, 60.6, 45.1, 30.9, 30.1, 24.5, 24.5, 22.4, 17.2, 14.2. 12.2; MS (EI) *m/z* 574.5 (M^+); HRMS (EI) Calcd. for $C_{34}H_{42}N_2O_6$ (M^+): 574.3042. Found: 574.3035.

4-[2-[5-[4-[1-(4-Isobutylphenyl)ethoxy]-2,3-dimethylphenyl]-[1,2,4]oxadiazol-3-yl]phenoxy]butanoic acid ethyl ester (8)

A so lu tion of **7** (220 mg, 0.38 mmol) dis solved in dried 2-methoxyethyl ether (diglyme, 10 mL) was heated at 110 °C un der ni tro gen for 10 h. Af ter cool ing, the so lu tion was con-cen trated in vacuo, pu ri fied by chro ma tog ra phy (sil ica gel, n-hexane/ethyl ac e tate = 9/1) to give **8** (R_f = 0.27), and fol-lowed by recrystallization from eth a nol to fur nish a white solid (200 mg, 95%). mp 81.5-82.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.05 (dd, 1H, *J* = 7.7; 1.8 Hz, ArH), 7.76 (d, 1H, *J* = 8.8 Hz, ArH), 7.0-7.39 (m, 7H, ArH), 6.69 (d, 1H, *J* = 8.8 Hz, ArH), 5.37 (q, 1H, *J* = 6.4 Hz, CH), 4.04-4.20 (m, 4H, CH_2), 2.67 (s, 3H, CH_3), 2.64 (t, 2H, *J* = 7.0 Hz, -$COCH_2$), 2.43 (d, 2H, *J* = 7.2 Hz, $ArCH_2$), 2.33 (s, 3H, CH_3), 2.10-2.27 (m, 2H, CH_2), 1.76-1.89 (m, 1H, CH), 1.65 (d, 3H, *J* = 6.4 Hz, CH_3), 1.20 (t, 3H, *J* = 7.1 Hz, CH_3), 0.87 (d, 6H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.7, 173.4, 166.9, 158.5, 157.3, 141.0, 139.9, 138.9, 131.9, 131.3, 129.3, 128.9, 127.1, 125.1, 120.6, , 116.7, 116.4, 112.7, 110.6, 76.1, 67.5, 60.3, 45.0, 30.5, 30.1, 24.4, 24.4, 22.3, 17.5, 14.2, 12.3; MS (EI) *m/z* 556.5 (M^+), 511, 396 (base peak); HRMS (EI) Calcd. for $C_{34}H_{40}N_2O_5$ (M^+): 556.2937. Found 556.2928.

4-[2-[5-[4-[1-(4-Isobutylphenyl)ethoxy]-2,3-dimethylphenyl]-[1,2,4]oxadiazol-3-yl]phenoxy]butanoic acid (1)

To a stirred sus pen sion of **8** (500 mg, 0.89 mmol) in meth a nol (150 mL) was added 10% NaOH (15 mL) at room tem per a ture. And the mix ture was stirred for 5 days. Af ter cool ing in an ice-bath, the mix ture was acid i fied with 6 N HCl to pH 3-4 un der vig or ous stir ring, fil tered, and washed with wa ter. The solid was recrystallized from eth a nol to af-ford **1** as a white solid (408 mg, 89%). mp 143-144 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.03 (dd, 1H, *J* = 7.7 Hz, ArH), 7.73 (d, 1H, *J* = 8.7 Hz, ArH), 7.39-7.47 (m, 1H, ArH), 7.22-7.26 (m, 2H, ArH), 6.99-7.11 (m, 4H, ArH), 6.68 (d, 1H, *J* = 8.9 Hz, ArH), 5.36 (q, 1H, *J* = 6.1 Hz, CH), 4.18 (t, 2H, *J* = 5.8 Hz, O-CH_2), 2.70 (t, 2H, *J* = 7.1 Hz, -$COCH_2$), 2.64 (s, 3H, CH_3), 2.42 (d, 2H, *J* = 7.1 Hz, $ArCH_2$), 2.32 (s, 3H, CH_3). 2.10-2.22 (m, 2H, CH_2), 1.71-1.91 (m, 1H, CH), 1.64 (d, 3H, *J* = 6.7 Hz, CH_3), 0.86 (d, 6H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.9, 176.0, 166.7, 158.7, 157.1, 141.1, 139.9, 139.0, 132.2, 131.4, 129.4, 129.0, 127.2, 125.1, 120.9, 116.4, 116.2, 112.6, 110.7, 76.4, 66.7, 45.1, 30.6, 30.1, 24.4, 24.4, 22.4, 17.5, 12.3; MS (EI) *m/z* 528.3 (M^+), 368.1, 161.1 (base peak); HRMS (EI) Calcd. for $C_{32}H_{36}N_2O_5$ (M^+): 528.2624. Found 528.2636.

2,3-Dimethyl-4-methoxybenzonitrile (10)

To a stirred so lu tion of 2,3-dimethyl-p-anisaldehyde (5 g, 30.5 mmol) dis solved in acetonitrile (200 mL) was added dropwise an aque ous so lu tion (200 mL) of hydroxylamine-o- sulfonic acid (5.17 mg, 45.7 mmol) at room tem per a ture dur ing 30 min pe riod, and then the mix ture was heated at 60 °C for 18 h. Af ter cool ing, the mix ture was neu tral ized with 10% NaOH and evap o rated in vacuo. The res i due was ex-tracted with $CHCl_3$ (5 × 60 mL), dried over Na_2SO_4, fil tered, con cen trated, chromatographed (sil ica gel, n-hexane/ethyl ac e tate = 96/4, R_f = 0.32), and recrystallized from acetonitrile to fur nish **10** as a white solid (4.69 g, 96%). mp 40 °C; IR (KBr): 2950, 2840, 2225, 1590, 1580 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 7.41 (d, 1H, *J* = 8.6 Hz, ArH), 6.71 (d, 1H, *J* = 8.6 Hz, ArH), 3.84 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3), 2.11 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 160.5, 141.1, 131.4, 126.3, 119.3, 107.9, 104.7, 55.6, 18.1, 11.7; MS (EI) *m/z* 161 (M^+), 146; Anal. Calcd. for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.63; H, 7.16; N, 8.40.

2,3-Dimethyl-4-hydroxybenzonitrile (11)

To a stirred so lu tion of **10** (4.69 g, 29.1 mmol) dis-solved in dry di chloro methane (200 mL) was added BBr_3 (21.9 g, 87.3 mmol) at 0 °C un der ni tro gen. The re ac tion mix-ture was warmed up to 50 °C for 6 h, and then poured into crushed-ice while stir ring vig or ously. The re sulted mix ture was ex tracted with $CHCl_3$ (4 × 150 mL), dried over Na_2SO_4, filtered, decoloured by active char coal, and fol lowed by recrystallization from $CHCl_3$ to give **11** as a white solid (4.21 g, 98%). mp 134 °C; IR (KBr): 3285, 2227 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, 1H, *J* = 8.4 Hz, ArH), 6.73 (d, 1H, *J* = 8.4 Hz, ArH). 6.19 (s, 1 H, OH). 2.44 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 157.8, 142.5, 131.3, 124.4, 119.3, 113.3, 104.4, 18.3, 11.7; MS (EI) *m/z* 147 (M^+); Anal. Calcd. for C_9H_9NO: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.10; H, 6.34; N, 9.31.

2,3-Dimethyl-4-[1-(4-isobutylphenyl)ethoxy]benzonitrile (12)

To a stirred soution of **11** (300 mg, 2.04 mmol), 1-(4-isobutylphenyl)ethanol (472.8 mg, 2.65 mmol) and triphenyl phosphine (802.6 mg, 3.06 mmol) dissolved in THF (30 mL) was added diethylazodicarboxylate (532.9 mg, 3.06 mmol) at room temperature under nitrogen. After 8 h, the colour of solution was changed from color less to blackish green, concentrated, triturated with ether, filtered, and then the filtrate was purified by chromatography (silica gel, n-hexane, R_f = 0.1), followed by recrystallization from n-hexane to give **12** as a white solid (305 mg, 49%). mp 66 °C; IR (KBr): 2960, 2930, 2875, 2225, 1590 cm^{-1}. ^1H NMR (200 MHz, CD_3OD) δ 7.09-7.27 (m, 5H, ArH), 6.60 (d, 1H, *J* = 8.7 Hz, CH), 5.33 (q, 1H, *J* = 6.4 Hz, CH), 2.44 (d, 2H, *J* = 7.1 Hz, CH_2), 2.44 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 1.77-1.91 (m, 1H, CH), 1.65 (d, 3H, *J* = 6.4 Hz, CH_3), 0.88 (d, 6H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.9, 141.2, 141.1, 139.5, 131.0, 129.3, 126.8, 125.0, 119.2, 110.8, 104.5, 76.3, 45.0, 30.0, 24.3, 22.2, 18.2, 12.0; MS (EI) *m/z* 307 (M^+); Anal. Calcd. for $C_{21}H_{25}NO$: C, 82.04; H, 8.20; N, 4.56. Found: C, 81.71; H, 8.21; N, 4.51.

4-[1-(4-Isobutylphenyl)ethoxy]-2,3-dimethylbenzamide (13) and 4-[1-(4-Isobutylphenyl)ethoxy]-2,3-dimethylbenzamidoxime (14)

To a stirred solution of hydroxylamine hydrochloride (2.03 g, 29.28 mmol) and triethylamine (2.96 g, 29.28 mmol) dissolved in absolute ethanol (200 mL) was added **12** (0.90 g, 2.93 mmol). The mixture was heated at reflux for 8 days under nitrogen. The mixture was then evaporated in vacuo and the residue was subjected to chromatography (silica gel, n-hexane/ethyl acetate = 7/3). The fast-moving fraction was collected to give **13** (R_f = 0.33) which was recrystallized from $CHCl_3$ to afford a white solid (0.32 g, 48%). mp 125-126 °C; IR (KBr): 3383, 3193, 2920, 1706, 1646, 1596, 1464 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.23 (d, 2H, *J* = 8.1 Hz, ArH), 7.07 (d, 2H, *J* = 8.1 Hz, ArH), 7.04 (d, 1H, *J* = 8.5 Hz, ArH), 6.64 (d, 1H, *J* = 8.5 Hz, ArH), 5.39 (q, 1H, *J* = 6.4 Hz, CH), 4.63 (br s, 2H, NH_2), 2.41 (d, 2H, *J* = 7.2 Hz, CH_2), 2.31 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 1.74-1.87 (m, 1H, CH), 1.60 (d, 3H, *J* = 6.4 Hz, CH_2), 0.86 (d, 6H, *J* = 4.7 Hz, CH_3); ^{13}C NMR (50 MHz, CD_3OD) δ 175.7, 157.1, 141.1, 140.9, 135.6, 129.3, 126.7, 125.5, 125.2, 110.6, 76.1, 45.1, 39.5, 23.8, 21.7, 16.1, 11.2; LCMS (ESI, acetic acid) *m/z* 326.2 (MH^+); Anal. Calcd. for $C_{21}H_{27}NO_2$: C, 77.50; H, 8.36; N, 4.30. Found: C, 77.55; H, 8.56; N, 4.24. The low-moving fraction was collected to give **14** (R_f = 0.18) which was recrystallized from acetonitrile to give a white solid (0.31 g, 45%). mp 140-141 °C; IR (KBr): 3490, 3381, 3250, 2927, 1640, 1595, 1494 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.23 (d, 2H, *J* = 8.0 Hz, ArH), 7.07 (d, 2H, *J* = 8.0 Hz, ArH), 6.90 (d, 1H, *J* = 8.5 Hz, ArH), 6.55 (d, 1H, *J* = 8.5 Hz, ArH), 5.25 (q, 1H, *J* = 6.4 Hz, OCH), 4.69 (br, 2H, NH_2), 2.42 (d, 2H, *J* = 7.1 Hz, CH_2), 2.28 (s, 3H, CH_3), 1.88 (s, 3H, CH_3), 1.75-1.88 (m, 1H, CH), 1.55 (d, 3H, *J* = 6.4 Hz, CH_3), 0.86 (d, 3H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 156.5, 153.8, 140.8, 140.4, 136.7, 129.3, 126.8, 126.4, 125.3, 125.2, 110.5, 76.0, 45.1, 30.2, 24.4, 22.4, 16.9, 12.2; MS (ESI, acetic acid) *m/z* 341.1 (MH^+); Anal. Calcd. for $C_{21}H_{28}N_2O_2$: C, 74.08; H, 8.29; N, 8.23. Found: C, 74.12; H, 8.10; N, 7.98.

2-(3-Ethoxycarbonylpropoxy)benzoic acid methyl ester (16)

To a stirred suspension of **15** (10 g, 65.72 mmol) and K_2CO_3 (18.2 g, 131.44 mmol) in dried acetonitrile (300 mL) was added γ-bromobutyric acid ethyl ester (12.8 g, 65.72 mmol) at reflux for 24 h under nitrogen. The hot mixture was filtered, washed with acetonitrile, and concentrated. The resulting residue was subjected to chromatography (silica gel, n-hexane/ethyl acetate = 95/5) to obtain **16** (17.1 g, 98%) as a yellowish oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (dd, 1H, *J* = 1.8, 7.62 Hz, ArH), 7.37-7.41 (m, 1H, ArH), 6.90-6.94 (m, 2H, ArH), 4.09 (q, 2H, *J* = 7.2 Hz, CH_2), 4.05 (t, 2H, *J* = 6.1 Hz, CH_2). 3.84 (s, 3H, CH_3), 2.54 (t, 2H, *J* = 7.3 Hz, CH_2), 2.07-2.13 (m, 2H, CH_2), 1.21 (t, 3H, *J* = 7.3 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.2, 166.7, 158.2, 133.3, 131.6, 120.2, 120.2, 113.1, 67.4, 60.3, 51.8, 30.4, 24.4, 14.1. Anal. Calcd. for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.30; H, 6.78.

4-[2-[3-[4-[1-(Isobutylphenyl)ethoxy]-2,3-dimethylphenyl]-[1,2,4]oxadiazol-5-yl]phenoxy]butyric acid (2) and 2-[3-[3-[4-[1-(Isobutylphenyl)ethoxy]-2,3-dimethylphenyl]-[1,2,4]oxadiazol-5-yl]propoxy]benzoic acid (20)

To a mixture solution of **14** (1 g, 2.94 mmol) dissolved in anhydrous THF (20 mL) containing 4 Å powdered molecular sieves (3 g) was stirred at room temperature for 30 min under nitrogen, and then sodium hydride was added (17.54 mg of 80% dispersion in oil, 3.23 mmol). The mixture was then heated at 60 °C for 1 h. After cooling, to the mixture was added a solution of **16** (2 g, 7.51 mmol) in THF (10 mL) and the resulting mixture was then heated at 60°C for 3 h. The solution was evaporated in vacuo and the residue was subjected to chromatography (silica gel, n-hexane/$CHCl_3$ = 6/4). The fast-moving band was collected to afford **17** (R_f = 0.28) as a yellowish oil (145 mg, 9%). IR (neat): 2955, 1734, 1595, 1546, 1464 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.11 (dd, 1H, *J* = 1.8; 8.0 Hz, ArH), 7.62 (d, 1H, *J* = 8.6 Hz, ArH),

7.44-7.53 (m, 1H, ArH), 7.26 (d, 2H, *J* = 6.2 Hz, ArH), 7.01-7.11 (m, 4H, ArH), 6.68 (d, 1H, *J* = 8.7 Hz, ArH), 5.35 (q, 1H, *J* = 6.3 Hz, OCH), 4.04-4.20 (m, 4H, CH_2), 2.64 (t, 2H, *J* = 7.2 Hz, CH_2), 2.55 (s, 3H, CH_3), 2.43 (d, 2H, *J* = 7.1 Hz, CH_2), 2.32 (s, 3H, CH_3), 2.12-2.26 (m, 2H, CH_2), 1.74-1.89 (m, 1H, CH), 1.65 (d, 3H, *J* = 6.3 Hz, CH_3), 1.20 (t, 3H, *J* = 7.1 Hz, CH_3), 0.88 (d, 6H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.8, 173.2, 169.4, 157.7, 157.3, 140.9, 140.3, 137.9, 133.8, 131.6, 129.3, 128.5, 126.8, 125.2, 120.7, 119.2, 113.9, 113.0, 110.7, 76.0, 67.6, 60.3, 45.1, 30.5, 30.1, 24.5, 24.3, 22.4, 17.7, 14.2, 12.3. Compound **17** was used for the next reaction without further purification. Subsequently, to the solution of **17** (60 mg, 0.11 mmol) dissolved in dioxane (8 mL) was added 3 mL of 1 N LiOH at 80°C for 30 min under vigorous stirring. After cooling, the mixture was acidified with 4 N HCl to pH 3-4, evaporated, and subjected to chromatography (silica gel, $CHCl_3$/CH_3OH = 97/3) to give **2** which was recrystallized from acetonitrile to afford a white soild (35 mg, 60%). mp 103-104 °C; IR (KBr): 2955, 1709, 1595, 1547, 1463, 1339, 1265 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 8.10 (dd, 1H, *J* = 1.61; 6.91 Hz, ArH), 7.61 (d, 1H, *J* = 8.66 Hz, ArH), 7.44-7.52 (m, 1H, ArH), 7.26 (d, 2H, *J* = 7.4 Hz, ArH), 6.99-7.10 (m, 4H, ArH), 6.69 (d, 1H, *J* = 8.7 Hz, ArH), 5.35 (q, 1H, *J* = 6.3 Hz, OCH), 4.17 (t, 2H, *J* = 5.9 Hz, OCH_2), 2.71 (t, 2H, *J* = 7.1 Hz, CH_2), 2.53 (s, 3H, CH_3), 2.42 (d, 2H, *J* = 7.2 Hz, CH_2), 2.31 (s, 3H, CH_3), 2.12-2.27 (m, 2H, CH_2), 1.76-1.89 (m, 1H, CH), 1.63 (d, 3H, *J* = 6.3 Hz, CH_3), 0.87 (d, 6H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.1, 173.8, 169.4, 157.6, 157.4, 140.9, 140.3, 137.9, 133.8, 131.6, 129.3, 128.5, 126.8, 125.3, 120.9, 119.2, 113.8, 113.0, 110.8, 76.0, 67.3, 45.1, 30.9, 30.1, 24.3, 24.3, 22.4, 17.7, 12.3; LCMS (ESI, Et_3N) *m/z* 527.3 (M-1), 441.3 (base peak); HRMS (ESI) Calcd. for $C_{32}H_{35}N_2O_5$ (M^+): 528.2624. Found: 528.2618.

The slow-moving band was collected to give a mixture of **18** (R_f = 0.15) and **19** (R_f = 0.17) as a yellowish oil (0.7 g) and then it was hydrolyzed with 1 N LiOH for 1 h at 60 °C. After it was acidified and evaporated, the solution was eluted by a mixture of $CHCl_3$/CH_3OH (97/3) to afford **20** (R_f = 0.14) as a yellow ish oil (132 mg, two steps gave 9% yield). IR (neat): 2954, 1694, 1600, 1460 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 8.13 (dd, 1H, *J* = 1.7; 7.8 Hz, ArH), 7.47-7.55 (m, 2H, ArH), 7.23 (d, 2H, *J* = 8.0 Hz, ArH), 7.0-7.14 (m, 4H, ArH), 6.65 (d, 1H, *J* = 6.4 Hz, ArH), 5.32 (q, 1H, *J* = 6.4 Hz, OCH), 4.37 (t, 2H, *J* = 6.4 Hz, OCH_2), 3.14 (t, 2H, *J* = 7.1 Hz, CH_2), 2.46 (s, 3H, CH_3), 2.42 (d, 2H, *J* = 7.1 Hz, CH_2), 2.36-2.53 (m, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.75-1.88 (m, 1H, CH), 1.63 (d, 3H, *J* = 6.4 Hz, CH_3), 0.86 (d, 6H, *J* = 6.6 Hz, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.2, 169.9, 165.8, 158.0, 157.6, 141.4, 140.7, 138, 135.4, 134.2, 129.7, 128.9, 127.4, 125.6, 122.8, 119.0, 118.6, 113.1, 111.1, 110.0, 76.5, 68.8, 45.5, 30.6, 26.2, 24.8, 23.2, 22.8, 18.2, 12.8; LCMS (ESI, Et_3N) *m/z* 527.4 (M-1); HRMS (FAB) Calcd. for $C_{32}H_{37}O_5N_2$ (MH^+): 529.2701. Found: 529.2702.

Rat 5α-Reductase Inhibition Assay

The inhibition assay was performed according to that reported by Russell et. al[22] with some minor modifications. Briefly, in 200 μL of 20 mM sodium phosphate buffer (pH 6.5), containing 5μM [4-^{14}C]-testosterone (2.10GBq/mmol), 200 μM of NADPH and 0.05% Tween-80, various amounts of the tested compound was added. The reaction was initiated with the addition of 20 μg of rat liver microsome and incubated at 37 °C for 30 minutes. After incubation, the reaction was terminated with the addition of 1 mL of acetone. The supernatant was removed and dried. The residue was spotted on a TLC plate (silica gel) and developed with dichloromethane/ether 1:1. Radioactivities were recorded with a Bioscan γ-ray detector. The amount of product formed was calculatedcorrespondingly.

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Testosterone; Dihydrotestosterone; 5α-Reductase; Benign prostatic hyperplasia; Oxadiazole.

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