Synthesis and Pharmacological Evaluation of Novel Arylpiperazine Derivatives as Nonsteroidal Androgen Receptor Antagonists

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The search for novel antiandrogens by high-throughput screening (HTS) of the Yamanouchi chemical library led to the discovery of the lead compound (5), which possesses an arylmorpholine moiety. Through the optimization of the lead compound (5), we have found a series of novel arylpiperazine derivatives. Among them, 4-[4-cyano-(3-trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)piperazine-1-carboxamide (22; YM-92088) exhibited a potent AR antagonistic activity with an IC_{50} value of 0.47 μM in the reporter assay, which is more potent than bicalutamide (4) which has an IC_{50} value of 0.89 μM.

Key words androgen receptor; antagonist; antiandrogen; prostate cancer

The androgen receptor (AR) is a member of the nuclear receptor superfamily and acts as a ligand-dependent intracellular transcription factor.[1—3] The AR is also responsible for mediating the physiological actions of the androgens, namely testosterone and 5α-dihydrotestosterone (DHT). Androgens play a critical role in normal male development and the subsequent maintenance of secondary male characteristics such as muscle mass, bone mass, strength, fat distribution, and spermatogenesis.[4—6] The male sexual accessory organs, such as the prostate, are stimulated to grow and are maintained in size and secretory function by the continued actions of androgens. It is well established that androgens play a major role in human prostate disorders.[7] Thus compounds that block the action or synthesis of androgens have been proven useful in the treatment of diseases such as prostate cancer, benign prostatic hypertrophy, hirsutism, and acne.[8—11]

AR antagonists,[12,13] including cyproterone acetate (**1**),[14] flutamide (**2**),[15—17] nilutamide (**3**),[18] and bicalutamide (**4**),[19—22] have been used clinically for the treatment of prostate cancer (Fig. 1). Cyproterone acetate (**1**), which is classified as a steroidal AR antagonist, possesses progestational activity and suppresses the secretion of gonadotrophins, both of which are unwanted side effects. A number of nonsteroidal AR antagonists have been reported in the literature[23—30] and three of these, flutamide (**2**), nilutamide (**3**), and bicalutamide (**4**), are used clinically in conjunction with gonadotropin-releasing hormone (GnRH) agonists.[31] These nonsteroidal AR antagonists exhibit some adverse effects such as mastodynia, gynaecomastia and hepatotoxicity[15—22]; therefore potent AR antagonists with less adverse effects are highly desirable.

To identify novel AR antagonists, we conducted an HTS of the Yamanouchi chemical library using a reporter assay, which measured the inhibitory activity of test compounds on androgen-induced activation of AR. Compound **5** was discovered as an HTS hit with an associated IC_{50} value of 3.1 μM. Through optimization of the lead compound (**5**), we have found a series of novel arylpiperazine derivatives. Herein, we describe the results of our studies on the syntheses and structure–activity relationships of these arylpiperazine derivatives as novel nonsteroidal AR antagonists.

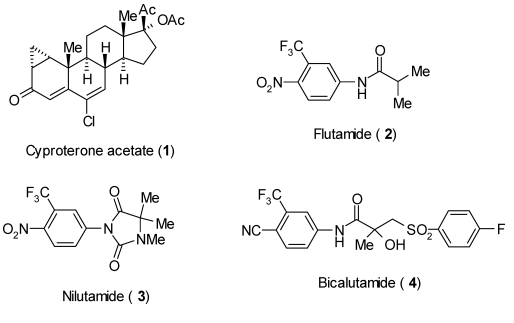
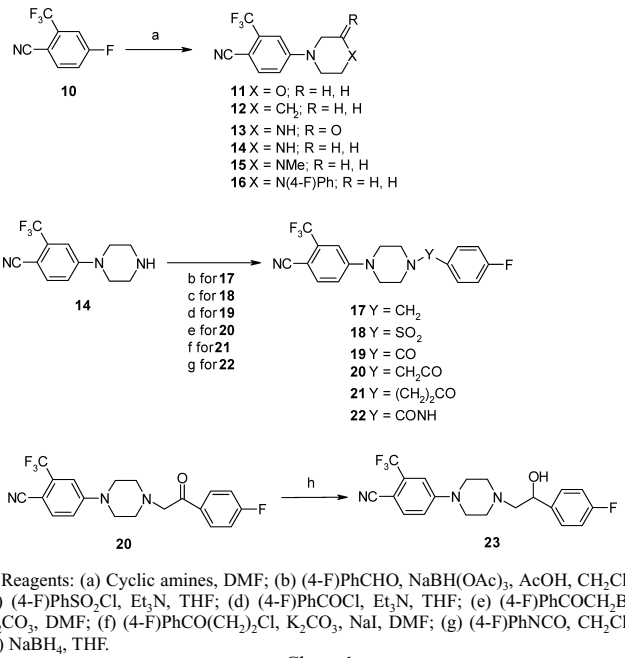


Fig. 1. Structures of Androgen Antagonists Used in Therapeutics

Chemistry

Compounds selected for biological evaluation were



Reagents: (a) Cyclic amines, DMF; (b) (4-F)PhCHO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2; (c) (4-F)$PhSO_2Cl$, Et_3N, THF; (d) (4-F)PhCOCl, Et_3N, THF; (e) (4-F)$PhCOCH_2Br$, K_2CO_3, DMF; (f) (4-F)$PhCO(CH_2)_2Cl$, K_2CO_3, NaI, DMF; (g) (4-F)PhNCO, CH_2Cl_2; (h) $NaBH_4$, THF.

Chart 1

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prepared as described in Chart 1. Compounds **11**—**16** were prepared by the *ipso* substitution of 4-fluoro-2-trifluoromethylbenzonitrile with the corresponding cyclic amines; good yields were observed. Synthesis of the benzyl derivative (**17**) was achieved by the reductive amination of the intermediate piperazine (**14**) with 4-fluorobenzaldehyde. The piperazine (**14**) was treated with 4-fluorophenylsulfonyl chloride or 4-fluorobenzoyl chloride to afford **18** and **19** respectively, in excellent yields. Alkylation of the piperazine (**14**) with 4-fluorophenacyl bromide or 3-chloro-4′-fluoropropiophenone with sodium iodide afforded **20** and **21**, respectively. Treatment of **14** with 4-fluorophenyl isocyanate provided the urea derivative (**22**). Reduction of the carbonyl group of **20** with sodium borohydride gave the corresponding secondary alcohol analogue (**23**).

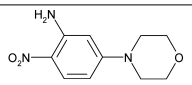
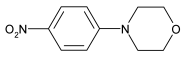
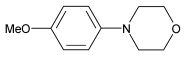
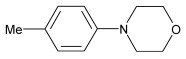
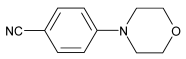
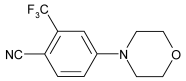
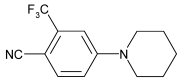
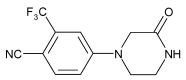
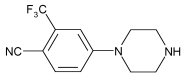
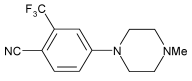
Results and Discussion

All the analogues prepared were evaluated for their AR antagonistic activity using a reporter assay; the resulting IC_{50} values are listed in Tables 1 and 2.

We selected compound **5**, which was discovered from HTS with an IC_{50} value of 3.1 μM, as the lead compound and started to make modifications to compound **5**. Firstly, we investigated the effect of the substituents on the phenyl ring. The removal of the amino group of **5** resulted in a slight decrease in the AR antagonistic activity (compound **6**). Furthermore we try to replace the toxicity concerned nitro group by other groups. As shown in compounds **7** and **8**, introduction of an electron-donating group afforded very deleterious effects on the inhibitory activity. Although the cyano analogue **9** showed weak activity, the introduction of an additional trifluoromethyl group (**11**), having a similar moiety as compared to bicalutamide (**4**), led to a 6-fold increase in the AR antagonistic activity relative to compound **5**. These results suggested that the electron-withdrawing effects and/or the hydrophobicity of the trifluoromethyl group may be very important for AR antagonistic activity. Subsequently, we carried out the replacement of the morpholine ring with other cyclic amines. Among these cyclic amine analogues (**12**—**15**), only the *N*-methyl-piperazine derivative (**15**) exhibited a potent inhibitory activity. Therefore we carried out further modifications of the substituents on the piperazine framework (Table 2).

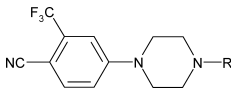
The 4-fluorophenyl derivative (**16**) exhibited a 2-fold enhancement in inhibitory activity over compound **15** (IC_{50}=0.54, 1.0 μM, respectively). Insertion of a methylene linkage between the piperazine and the 4-fluorophenyl ring, such as **17**, resulted in a 3-fold decrease in its inhibitory activity relative to **16**. Compound **18**, which contained a 4-fluorophenylsulfonyl moiety like bicalutamide, was less active than **17**. On the other hand, the corresponding amide derivative (**19**) exhibited more potent AR antagonistic activity than compounds **17** and **18**. These results suggested that the conformation of the piperazine framework may be important. Insertion of a methylene group into compound **19**, such as **20** and **21**, led to a decrease in potency indicating that the long distance between the 4-fluorophenyl ring and piperazine framework was unfavorable. Reduction of the carbonyl group (**23**) resulted in a slight increase in inhibitory activity relative to **20**. Interestingly, the urea derivative (**22**) exhibited the most potent AR antagonistic activity in this series with an IC_{50} value of 0.47 μM, which is more potent than bicalu-

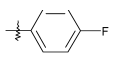
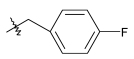
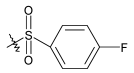
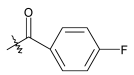
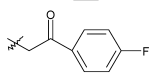
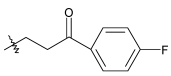
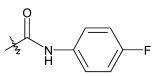
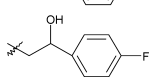
Table 1. AR Antagonistic Activities of Arylmorpholine and Arylpiperazine Derivatives

Compound	Structure	IC_{50} (μM)[a]
5		3.1
6		4.3
7		NE[b]
8		10%[b]
9		43%[b]
11		0.51
12		48%[b]
13		NE[b]
14		18%[b]
15		1.0
4		0.89

a) Compounds were tested for their ability to inhibit AR mediated transcriptional activation using a reporter assay. *b*) Percent inhibition at 10 μM. NE: No effect.

Table 2. AR Antagonistic Activities of Arylpiperazine Derivatives



Compound	Structure	IC_{50} (μM)[a]
15	Me	1.0
16		0.54
17		1.8
18		2.6
19		0.81
20		2.3
21		51%[b]
22 (YM-92088)		0.47
23		1.5
4		0.89

a, *b*) Refer to Table 1.

tamide (**4**) with an IC_{50} value of 0.89 μM.

Conclusion

We have succeeded in the identification of a novel lead compound as an AR antagonist through an HTS. The original lead **5** inhibited the AR mediated transcriptional activity with an IC_{50} value of 3.1 μM. Modification of the lead **5** resulted in the identification of the arylpiperazine derivatives with improved AR antagonistic activity.

Compound **22** exhibited the most potent AR antagonistic activity with an IC_{50} value of 0.47 μM in the reporter assay, which is more potent than bicalutamide (**4**) which has an IC_{50} value of 0.89 μM. Compound **22** was also found to possess potent AR binding affinity (Ki=68 nM for rat prostate AR against ^{3}H-mibolerone)[32] and no intrinsic AR agonistic activity at concentrations up to 10 μM. Furthermore, compound **22** significantly inhibited the growth of prostate weights in rats (data not shown). These results suggest that compound **22** (YM-92088) has potential as a novel nonsteroidal AR antagonist. We will report the details in due course.

Experimental

In general, all reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. Melting points were determined on a Yanaco MP-500D micro melting point apparatus without correction. ^{1}H-NMR spectra were measured with a JMN-EX400 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description, s=singlet, d=doublet, t=triplet, m=multiplet and br=broad peak). MS spectra were determined with a JEOL JMS-LX2000 spectrometer. Elemental analysis was performed with a Yanaco MT-5 microanalyzer (C, H, N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of theoretical values.

Compounds **5**—**9** were prepared according to literatures.[33—37]

4-Morpholino-2-trifluoromethylbenzonitrile (11) To a solution of 4-fluoro-2-(trifluoromethyl)benzonitrile (**10**, 600 mg, 3.17 mmol) in *N*,*N*-dimethylformamide (DMF, 10 ml) was added morpholine (1.1 g, 12.7 mmol) at ambient temperature and stirred at 80 °C for 21 h. The reaction mixture was diluted with H_2O. The precipitate was filtered off and washed with H_2O to give the title compound (775 g, 95%) as a colorless solid. mp 200 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.43 (4H, t, J=4.9 Hz), 3.73 (4H, t, J=4.9 Hz), 7.25 (1H, dd, J=8.8, 2.5 Hz), 7.32 (1H, d, J=2.5 Hz), 7.86 (1H, d, J=8.8 Hz). FAB-MS m/z: 257 (M+H$^+$). *Anal.* Calcd for $C_{12}H_{11}N_2OF_3$: C, 56.25; H, 4.33; N, 10.93; F, 22.24. Found: C, 56.23; H, 4.26; N, 11.01; F, 22.39.

The following compounds **12**—**16** were prepared using a procedure similar to described for **11** from the fluoride **10** and the corresponding amines.

4-Piperidino-2-trifluoromethylbenzonitrile (12) Compound **12** was prepared from **10** and piperidine in 70% yield, colorless solid. mp 68—69 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.48—1.68 (6H, m), 3.40—3.52 (4H, m), 7.20 (1H, dd, J=9.3, 2.4 Hz), 7.26 (1H, d, J=2.4 Hz), 7.78 (1H, d, J=9.3 Hz). FAB-MS m/z: 255 (M+H$^+$). *Anal.* Calcd for $C_{13}H_{13}N_2F_3$: C, 61.41; H, 5.15; N, 11.02; F, 22.42. Found: C, 61.34; H, 5.10; N, 11.04; F, 22.60.

4-(3-Oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (13) Compound **13** was prepared from **10** and 3-oxopiperazine in 33% yield, pale yellow solid. mp 205—207 °C (AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 3.29—3.37 (2H, m), 3.61—3.68 (2H, m), 3.98 (2H, s), 7.17 (1H, dd, J=9.1, 2.7 Hz), 7.24 (1H, d, J=2.7 Hz), 7.85 (1H, d, J=9.1 Hz), 8.26 (1H, br). FAB-MS m/z: 270 (M+H$^+$). *Anal.* Calcd for $C_{12}H_{10}N_3OF_3$: C, 53.54; H, 3.74; N, 15.61; F, 21.17. Found: C, 53.42; H, 3.51; N, 15.65; F, 21.12.

4-Piperazin-1-yl-2-(trifluoromethyl)benzonitrile Hydrochloride (14) Compound **14** was prepared from **10** and piperazine in 73% yield, colorless solid. mp 269—271 °C (MeOH/AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 3.16—3.24 (4H, m), 3.67—3.77 (4H, m), 7.31 (1H, dd, J=8.8, 2.4 Hz), 7.40 (1H, d, J=2.4 Hz), 7.91 (1H, d, J=8.8 Hz), 9.45 (1H, br). FAB-MS m/z: 256 (M+H$^+$). *Anal.* Calcd for $C_{12}H_{12}N_3F_3\cdot$HCl: C, 49.41; H, 4.49; N, 14.41; Cl, 12.15; F, 19.54. Found: C, 49.21; H, 4.47; N, 14.46; Cl, 12.04; F, 19.63.

4-(4-Methylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile Hydrochloride (15) Compound **15** was prepared from **10** and 1-methylpiperazine in 76% yield, colorless solid. mp 226—228 °C (EtOH/AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 2.78 (3H, s), 3.10 (2H, br), 3.35 (4H, br), 4.22 (2H, br), 7.34 (1H, dd, J=8.8, 2.4 Hz), 7.43 (1H, d, J=2.4 Hz), 7.93 (1H, d, J=8.8 Hz), 11.29 (1H, br). FAB-MS m/z: 270 (M+H$^+$). *Anal.* Calcd for $C_{13}H_{14}N_3F_3\cdot$HCl$\cdot$0.1H_2O: C, 50.77; H, 4.98; N, 13.66; Cl, 11.53; F, 18.53. Found: C, 50.61; H, 4.95; N, 13.74; Cl, 11.48; F, 18.62.

4-[4-(4-Fluorophenyl)piperazin-1-yl]-2-(trifluoromethyl)benzonitrile (16) Compound **16** was prepared from **10** and 1-fluorophenylpiperazine in 78% yield, pale yellow solid. mp 104—105 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 3.19—3.26 (4H, m), 3.57—3.67 (4H, m), 6.97—7.12 (4H, m), 7.31 (1H, dd, J=8.8, 2.4 Hz), 7.38 (1H, d, J=2.4 Hz), 7.87 (1H, d, J=8.8 Hz). FAB-MS m/z: 350 (M+H$^+$). *Anal.* Calcd for $C_{18}H_{15}N_3F_4$: C, 61.89; H, 4.33; N, 12.03; F, 21.75. Found: C, 61.69; H, 4.22; N, 12.03; F, 21.96.

4-[4-(4-Fluorobenzyl)piperazin-1-yl]-2-(trifluoromethyl)benzonitrile Hydrochloride (17) To a solution of **14** (1.5 g, 5.88 mmol) in AcOH (0.34 ml, 5.88 mmol) and CH_2Cl_2 (20 ml) was added benzaldehyde (0.63 ml, 5.88 mmol) and $NaBH(OAc)_3$ (1.87 g, 8.82 mmol). After stirring at ambient temperature for 4 h, the solution was concentrated *in vacuo*. The residue was diluted with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=50/1) to give the free base of the title compound (2.08 g, 97%) as a colorless oil. The free base of **17** (470 mg, 1.29 mmol) was dissolved in AcOEt and the solution was treated with 4 M solution of HCl in AcOEt. The precipitate was filtered off and washed with AcOEt to give the title compound (408 mg, 79%) as a colorless solid. mp 221—223 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.02—3.20 (2H, m), 3.25—3.40 (2H, m), 3.45—3.60 (2H, m), 4.14—4.30 (2H, m), 4.32—4.45 (2H, m), 7.26—7.35 (3H, m), 7.41 (1H, d, J=2.0 Hz), 7.67—7.78 (2H, m), 7.92 (1H, d, J=8.8 Hz), 11.99 (1H, br). FAB-MS m/z: 364 (M+H$^+$). *Anal.* Calcd for $C_{19}H_{17}N_3F_4\cdot$HCl: C, 57.08; H, 4.54; N, 10.51; Cl, 8.87; F, 19.01. Found: C, 57.14; H, 4.32; N, 10.54; Cl, 8.77; F, 19.29.

4-{4-[(4-Fluorophenyl)sulfonyl]piperazin-1-yl}-2-(trifluoromethyl)-benzonitrile (18) To a mixture of **14** (300 mg, 1.18 mmol) and Et_3N (232 mg, 2.29 mmol) in THF (5 ml) was added 4-fluorobenzenesulfonyl chloride (274 mg, 1.41 mmol) and stirred at room temperature for 2 h. The reaction mixture was added saturated aqueous $NaHCO_3$, stirred for 5 h, extracted with AcOEt, and washed with H_2O. The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and crystallized from AcOEt/*n*-hexane to give the title compound (400 mg, 82%) as a colorless solid. mp 162—163 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.98—3.10 (4H, m), 3.49—3.64 (4H, m), 7.16—7.26 (1H, m), 7.30 (1H, d, J=2.0 Hz), 7.45—7.56 (2H, m), 7.79—7.91 (3H, m). FAB-MS m/z: 414 (M+H$^+$). *Anal.* Calcd for $C_{18}H_{15}N_3O_2F_4S$: C, 52.30; H, 3.66; N, 10.16; F, 18.38; S, 7.76. Found: C, 52.31; H, 3.61; N, 10.26; F, 18.77; S, 7.74.

4-[4-(4-Fluorobenzoyl)piperazin-1-yl]-2-(trifluoromethyl)benzonitrile (19) To a mixture of **14** (300 mg, 1.18 mmol) and Et_3N (178 mg, 1.96 mmol) in THF (5 ml) was added 4-fluorobenzoyl chloride (223 mg, 1.41 mmol) and stirred at room temperature for 1 h. The reaction mixture was added saturated aqueous $NaHCO_3$, stirred for 30 min, extracted with AcOEt, and washed with H_2O. The organic layer was dried over Na_2SO_4, concentrated *in vacuo* and crystallized from AcOEt/*n*-hexane to give the title compound (308 mg, 69%) as a colorless solid. mp 171—173 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.40—3.84 (8H, m), 7.23 (1H, dd, J=8.8, 2.4 Hz), 7.27—7.34 (3H, m), 7.50—7.57 (2H, m), 7.87 (1H, d, J=8.8 Hz). FAB-MS m/z: 378 (M+H$^+$). *Anal.* Calcd for $C_{19}H_{15}N_3OF_4$: C, 60.48; H, 4.01; N, 11.14; F, 20.14. Found: C, 60.49; H, 4.02; N, 11.19; F, 20.20.

4-{4-[2-(4-Fluorophenyl)-2-oxoethyl]piperazin-1-yl}-2-(trifluoromethyl)benzonitrile (20) To a mixture of **14** (500 mg, 1.96 mmol) and K_2CO_3 (300 mg, 2.16 mmol) in DMF (10 ml) was added 4-fluorophenacyl bromide (425 mg, 1.96 mmol) and stirred at room temperature for 1 h. The reaction mixture was diluted with H_2O, extracted with AcOEt, and washed with H_2O. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=50/1) and crystallized from AcOEt/*i*-Pr_2O to give the title compound (709 mg, 92%) as a colorless solid. mp 162 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.65 (4H, d, J=4.8 Hz), 3.47 (4H, d, J=4.8 Hz), 3.93 (2H, s), 7.24 (1H, dd, J=8.8, 2.4 Hz), 7.31 (1H, d, J=2.4 Hz), 7.33—7.39 (2H, m), 7.83 (1H, d, J=8.8 Hz), 8.05—8.13 (2H, m). FAB-MS m/z: 392 (M+H$^+$). *Anal.* Calcd for $C_{20}H_{17}N_3OF_4$: C, 61.38; H, 4.38; N, 10.74; F, 19.42. Found: C, 61.49; H, 4.15; N, 10.64; F, 19.54.

4-{4-[3-(4-Fluorophenyl)-3-oxopropyl]piperazin-1-yl}-2-(trifluoromethyl)benzonitrile (21) To a mixture of **14** (280 mg, 1.10 mmol), NaI (1.65 g, 11.0 mmol) and K_2CO_3 (230 mg, 1.65 mmol) in DMF (10 ml) was added 3-chloro-4′-fluoropropiophenone (230 mg, 1.21 mmol) and stirred at 80 °C for 14 h. The reaction mixture was diluted with H_2O. The precipitate

was filtered off and washed with H_2O, and recrystallized from AcOEt/*i*-Pr_2O to give the title compound (334 mg, 75%) as a colorless solid. mp 144—145 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.45—2.85 (6H, m), 3.15—3.55 (6H, m), 7.18—7.42 (4H, m), 7.83 (1H, d, J=8.3 Hz), 8.02—8.15 (2H, m). FAB-MS *m/z*: 406 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{19}N_3OF_4$: C, 62.22; H, 4.72; N, 10.37; F, 18.75. Found: C, 61.94; H, 4.75; N, 10.34; F, 19.13.

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)piperazine-1-carboxamide (22) To a solution of **14** (780 mg, 2.64 mmol) in CH_2Cl_2 (10 ml) was added 4-fluorophenyl isocyanate (0.36 ml, 3.17 mmol) and stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=1/3) and crystallized from MeOH/Et_2O to give the title compound (472 mg, 46%) as a colorless solid. mp 214—217 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.4—3.68 (8H, m), 7.03—7.12 (2H, m), 7.27 (1H, dd, J=8.8, 1.9 Hz), 7.34 (1H, d, J=1.9 Hz), 7.43—7.52 (2H, m), 7.87 (1H, d, J=8.8 Hz), 8.66 (1H, br). FAB-MS *m/z*: 393 ($M+H^+$). *Anal.* Calcd for $C_{19}H_{16}N_4OF_4$: C, 58.16; H, 4.11; N, 14.28; F, 19.37. Found: C, 58.11; H, 4.06; N, 14.33; F, 19.40.

4-{4-[2-(4-Fluorophenyl)-2-hydroxyethyl]piperazin-1-yl}-2-(trifluoromethyl)benzonitrile Hydrochloride (23) To a solution of **20** (330 mg, 0.84 mmol) in THF (10 ml) was added sodium borohydride (35 mg, 0.84 mmol) and stirred at room temperature for 19 h. The reaction mixture was added saturated aqueous NH_4Cl and stirred for 20 min and extracted with AcOEt and washed with H_2O. The organic layer was dried over Na_2SO_4, concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=30/1) to give the free base of the title compound (170 mg, 51%) as a colorless oil. The free base of **23** was dissolved in AcOEt and the solution was treated with 4 M solution of HCl in AcOEt. The precipitate was filtered off and washed with AcOEt to give the title compound (183 mg, 51%) as a colorless solid. mp 229 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.17—3.40 (4H, m), 3.45—3.60 (2H, m), 3.65—3.83 (2H, m), 4.15—4.35 (2H, m), 5.28 (1H, dd, J=9.6, 2.7 Hz), 6.38 (1H, br), 7.19—7.27 (2H, m), 7.36 (1H, dd, J=8.6, 2.1 Hz), 7.42—7.52 (3H, m), 7.93 (1H, d, J=8.6 Hz), 11.03 (1H, br). FAB-MS *m/z*: 394 (M^++1). *Anal.* Calcd for $C_{20}H_{19}N_3OF_4 \cdot HCl \cdot 0.2H_2O$: C, 55.42; H, 4.74; N, 9.69; Cl, 8.18; F, 17.53. Found: C, 55.38; H, 4.70; N, 9.44; Cl, 8.08; F, 17.49

Evaluation of Transcriptional Activity for Human Androgen Receptor (a) Establishment of CHO cells stably transfected with human androgen receptor gene and MMTV-luciferase reporter gene or SV40-luciferase gene;

Chinese hamster ovary (CHO) cells were maintained in Alpha-modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). The culture medium of neomycin-resistant clone cells was supplemented with 10% dextran-coated charcoal-stripped FBS (DCC-FBS) and 500 μg/ml of neomycin. The CHO cells were transfected at 40—70% confluence in 10-cm petri dishes with a total of 20 μg DNA (pMAMneoLUC; MMTV-luciferase reporter plasmid and pSG5-hAR; human androgen receptor expression plasmid, or SV40-LUC; SV40-luciferase reporter plasmid containing neomycin resistant gene) by calcium phosphate mediated transfection. The stable transfected cells were selected in the culture medium supplemented with neomycin. The selected clone was designated as AR/CHO#3 (human AR gene and MMTV-luciferase reporter gene integrated CHO cell) or SV/CHO#10 (SV-40-luciferase reporter gene integrated CHO cell), respectively;

(b) Activities of the tested compounds to inhibit androgen receptor mediated transcription induced by DHT (AR antagonistic activity).

The stable transfected AR/CHO#3 or SV/CHO#10 cells were plated onto 96 well luminoplates (Packard) at a density of 2×10^4 cells/well, respectively. Four to 8 h later, the medium was changed to the medium containing DMSO, 0.3 nM of DHT, or 0.3 nM of DHT and the tested compound. At the end of incubation, the medium was removed and then cells were lysed with 20 μl of lysis buffer [25 mM Tris–HCl (pH 7.8), 2 mM dithiothreitol, 2 mM 1,2-cyclohexanediamine-tetraacetic acid, 10% glycerol and 1% TritonX-100]. Luciferase substrate [20 mM Tris–HCl (pH 7.8), 1.07 mM $(MgCO_3)_4 Mg(OH)_2 \cdot 5H_2O$, 2.67 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM EDTA, 33.3 mM dithiothreitol, 0.27 mM coenzyme A, 0.47 mM luciferin, 0.53 mM ATP] was added and luciferase activity was measured with a ML3000 luminometer (Dynatech Laboratories). AR antagonistic activities were calculated by formula below.

AR antagonistic activity (%)=$100(I-X)/(I-B)$

I: (luciferase activity of AR/CHO#3)/(luciferase activity of SV/CHO#10) in the presence of 0.3 nM of DHT

B: (luciferase activity of AR/CHO#3)/(luciferase activity of SV/CHO#10) in the presence of DMSO

X: (luciferase activity of AR/CHO#3)/(luciferase activity of SV/CHO#10) in the presence of 0.3 nM of DHT and the tested compound

The concentration of compounds showing 50% of AR antagonistic activity, IC_{50} values, were obtained by nonlinear analysis using statistical analysis system (SAS).

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