N-Arylpiperazine-1-carboxamide Derivatives: a Novel Series of Orally Active Nonsteroidal Androgen Receptor Antagonists

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A novel series of *N*-arylpiperazine-1-carboxamide derivatives was synthesized and their androgen receptor (AR) antagonist activities and *in vivo* antiandrogenic properties were evaluated. Reporter assays indicated that *trans*-2,5-dimethylpiperazine derivatives are potent AR antagonists, and in this series *trans*-*N*-4-[4-cyano-3-(trifluoromethyl)phenyl]-*N*-(2,4-difluorophenyl)-2,5-dimethylpiperazine-1-carboxamide (18g, YM-175735) exhibited the most potent antiandrogenic activity. Compared to bicalutamide, YM-175735 is an approximately 4-fold stronger AR antagonist and has slightly increased antiandrogenic activity, suggesting that YM-175735 may be useful in the treatment of prostate cancer.

Key words androgen receptor; antagonist; antiandrogen; prostate cancer

Prostate cancer has become the most common cancer among men, and the second leading cause of male cancer deaths in the United States.[1)] Testosterone and 5α-dihydrotestosterone are androgens that are required for the development of both the normal prostate and prostate cancer.[2)] Androgens act through the androgen receptor (AR), which belongs to the steroid-receptor superfamily of ligand-dependent transcription factors.[3,4)] Both steroidal and nonsteroidal antiandrogens are available, and these molecules are of clinical utility as chemotherapeutic agents for prostate cancer (Fig. 1).[5,6)] Cyproterone acetate (CPA: **1**) is a typical steroidal AR antagonist.[7)] It was one of the earliest of these drugs to be administered orally, but CPA shows agonistic activity and overlapping effects with other hormonal systems, leading to a range of unpleasant side effects. A number of nonsteroidal AR antagonists have been reported in the literature[8—17)] and three of these, flutamide (**2**),[18—20)] nilutamide (**3**)[21)] and bicalutamide (**4**)[22—25)] (Fig. 1), are pure antiandrogens used in the treatment of prostate cancer.[26)] However, these nonsteroidal AR antagonists exhibit adverse effects such as mastodynia, gynaecomastia and hepatotoxicity[18—25)]; and therefore potent AR antagonists with fewer adverse effects are highly desirable. Moreover, flutamide therapy requires administration three times each day, and bicalutamide is taken once a day. Therefore, from a quality of life perspective, it would be desirable for new generation AR antagonists to have a longer duration of action, at least equal to that of bicalutamide.

In a previous paper,[27)] we reported a new series of *N*-arylpiperazine derivatives as potent nonsteroidal AR antagonists. Among these derivatives, YM-92088 (**5**) was shown to be a more potent than bicalutamide as an *in vitro* AR antagonist (**4**). However, the *in vivo* antiandrogenic activity of **5** was lower than that of bicalutamide. Hence, to find AR antagonists with greater oral potency, we have conducted further modification of **5**, and in this paper we describe the results of our studies on the synthesis and pharmacological evaluation of a series of *N*-arylpiperazine-1-carboxamide derivatives as AR antagonists.

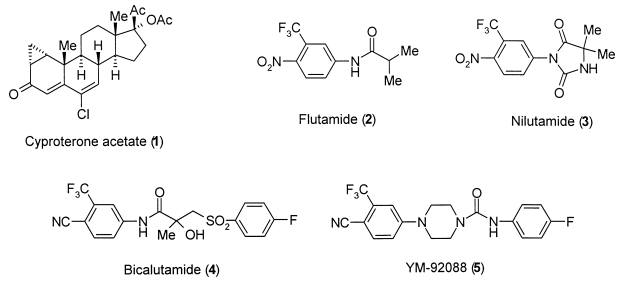

Fig. 1. Structures of Androgen Antagonists

Chemistry

Compounds selected for biological evaluation were prepared as described in Charts 1—4. All synthesized compounds were characterized by ^{1}H-NMR, mass spectrometry and elemental analysis.

As shown in Chart 1, compounds **7**—**12** and **19** were prepared in good yields by *ipso* substitution of 4-fluoro-2-(trifluoromethyl)benzonitrile (**6**) with the corresponding cyclic amines and, in the case of compound **9**, by subsequent deprotection of the Boc group. Treatment of **7**—**12** with 4-fluorophenyl isocyanate afforded the urea derivatives **13**—**18a**. The amide derivative (**20**) was obtained from compound **19** by hydrolysis followed by conventional amidation. Compound **22** was obtained by coupling *N*-Boc piperidinone with 4-bromo-2-(trifluoromethyl)benzonitrile, which was prepared by a Sandmeyer reaction with **21**, followed by dehydration using $POCl_3$. Hydrogenation of the dihydropyridine moiety of **22** gave the piperidine (**23**) in good yields. After deprotection of the Boc groups of **22** and **23**, the piperidines were treated with 4-fluorophenyl isocyanate to give compounds **24** and **25**, respectively (Chart 2). A Pd-C catalyzed Suzuki coupling[28,29)] between 4-bromo-2-(trifluoromethyl)benzonitrile and 4-carboxyphenylboronic acid provided the biphenyl **26**, which was then converted to compound **27** in a similar manner to that described for compound **20** (Chart 3). Compound **28** was obtained by *ipso* substitution of **6** with excess ethylenediamine, followed by reductive amination with benzaldehyde using $NaBH(OAc)_3$. The piperazine framework was constructed in moderate yield by treatment of **28** with glyoxal in aqueous conditions,[30)] and subsequent removal of the benzyl group by hydrogenolysis gave compound **29**. Com-

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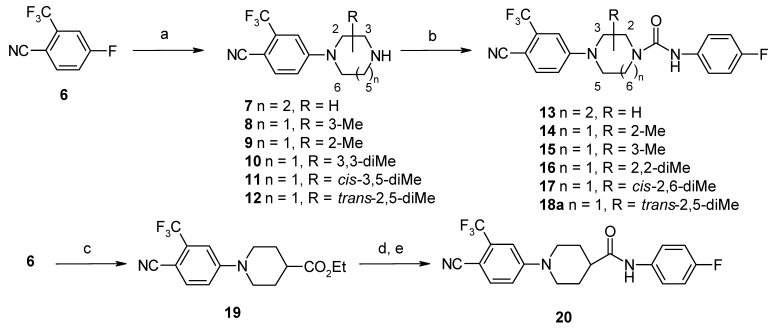
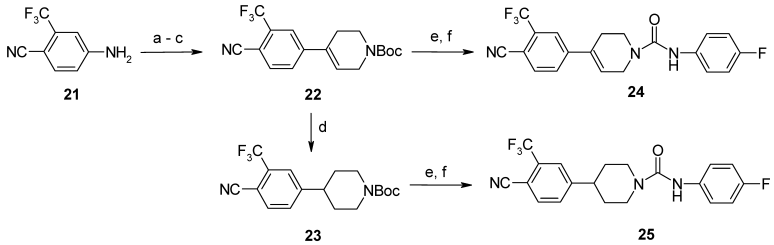


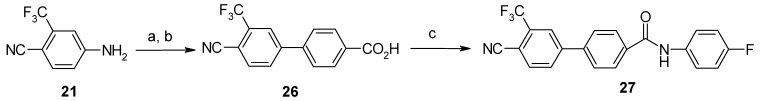
Reagents: (a) Homopiperazine or substituted piperazines, DMF (then TFA for **9**); (b) (4-F)PhNCO, CH_2Cl_2; (c) ethyl piperidine-4-carboxylate, K_2CO_3, DMF; (d) 1M NaOH, EtOH; (e) (i) $(COCl)_2$, cat.DMF, CH_2Cl_2; (ii) (4-F)$PhNH_2$, CH_2Cl_2.

Chart 1



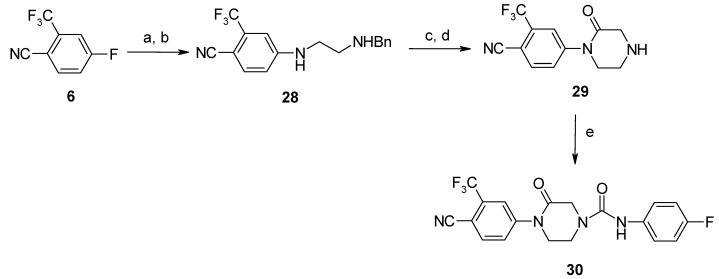
Reagents: (a) $NaNO_2$, HBr aq. then CuBr, HBr aq.; (b) n-BuLi, THF, *t*-butyl 4-oxopiperidine-1-carboxylate; (c) $POCl_3$, pyridine; (d) H_2, 10% Pd-C, MeOH; (e) TFA, 1,4-dioxane; (f) (4-F)PhNCO, CH_2Cl_2.

Chart 2



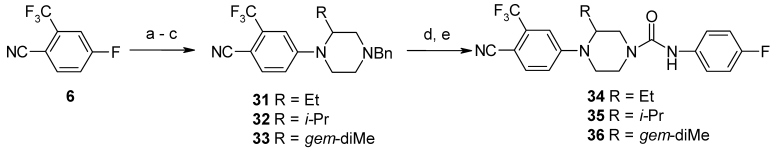
Reagents: (a) $NaNO_2$, HBr aq. then CuBr, HBr aq.; (b) 4-carboxyphenylboronic acid, 10% Pd-C, Na_2CO_3, EtOH; (c) (i) $(COCl)_2$, cat.DMF, CH_2Cl_2; (ii) (4-F)$PhNH_2$, CH_2Cl_2.

Chart 3



Reagents: (a) Ethylenediamine; (b) PhCHO, $NaBH(OAc)_3$, AcOH; (c) $(CHO)_2$, THF, H_2O; (d) H_2, 10% Pd-C, AcOH; (e) (4-F)PhNCO, CH_2Cl_2.

Chart 4



Reagents: (a) 1-Benzylpiperazin-2-one, Et_3N, DMF; (b) LDA, THF then RX; (c) borane THF complex, THF; (d) H_2, 10% Pd-C, MeOH; (e) (4-F)PhNCO, CH_2Cl_2.

Chart 5

pound **30** was synthesized by treatment of **29** with 4-fluorophenyl isocyanate (Chart 4). Chart 5 shows the synthesis of compounds with bulky groups at the α position on the piperazine framework, such as compound **33**. Introduction of the alkyl groups on the piperazine framework was achieved by alkylation of the arylpiperazinone with the corresponding

alkyl halides, using lithium diisopropylamide (LDA). Reduction of the amide with a borane-tetrahydrofuran (THF) complex gave the corresponding amine derivatives (**31**—**33**). Syntheses of compounds **34**—**36** were similar to that for compound **30**.

Results and Discussion

All the analogues were evaluated for their AR antagonistic activity using a reporter assay; the resulting IC_{50} values are listed in Tables 1—3. As described in the introduction, YM-92088 (**5**) shows less potent *in vivo* antiandrogenic activity than bicalutamide (**4**); the reason is unclear, but we speculate that the piperazine framework of **5** is easily metabolized. In fact, compound **5** has been found to be metabolically unstable in human liver microsomes (54% remaining after 1 h), and therefore to find more potent and orally active AR antagonists, we concentrated our efforts on further modification of **5**, focusing mainly on the piperazine framework.

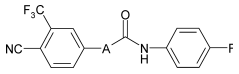
Firstly, we converted the piperazine ring of **5** into alternative cyclic amines, such as homo-piperazine and piperidine (Table 1). Ring expansion of the piperazine (compound **13**) resulted in an approximately 3-fold decrease in the inhibitory activity. Replacement of the sp^2-like urea nitrogen atom on the piperazine ring with an sp^3 carbon atom in compound **20** led to a substantial reduction in potency, relative to **5**. The piperidine derivative (**25**) also exhibited a weaker inhibitory activity, probably due to the change from the sp^2-like aniline nitrogen to an sp^3 carbon. However, introduction of the sp^2 carbon atom into the piperidine ring in compound **24** provided a 4-fold improvement in potency, compared to **25**. Moreover, biphenyl derivative (**27**) was approximately equipotent with **5**. These results suggest that both sp^2-like nitrogen atoms in the piperazine ring were important for potency, and that the piperazine framework of **5** plays a spatial role as a linker with planar geometry at the N atoms. Consequently, we selected the *N*-arylpiperazine-1-carboxamide as an optimal scaffold, and introduced further substituents onto the piperazine framework of **5**.

Next, we introduced an alkyl group onto the piperazine ring. As shown in Table 2, methyl substitution at the 2-position caused an approximately 3-fold increase in the potency (IC_{50}=0.18 and 0.47 μM for **14** and **5**, respectively). Since addition of a methyl group at the 3-position (**15**) was preferred over the 2-position, we further introduced another alkyl group at the 3-position. Although the ethyl derivative (**34**) exhibited comparable inhibitory activity, introduction of an isopropyl group (**35**) resulted in 8-fold reduction in potency, compared to **15**, indicating that increased bulkiness at this position may be unfavorable for AR antagonism. Introduction of an oxo group onto the piperazine ring at the 3-position resulted in particularly deleterious effects on the inhibitory activity (compound **30**). Subsequently, we synthesized di-substituted derivatives for further investigation of the substituent effects on the piperazine framework. The 3,3-dimethyl derivative (**36**) showed a slight decrease in inhibitory activity, but the 2,2-dimethyl derivative (**16**) was significantly less active relative to the corresponding monomethyl derivative (**14**). As shown by compound **17**, introduction of 2,6-*cis*-dimethyl substituents was also detrimental to AR antagonism, probably due to an unfavorable conformation by the interference of free rotation around the urea bond. Interestingly, the 2,5-*trans*-dimethyl derivative (**18a**) exhibited comparable activity to the monomethyl derivatives (**14**, **15**) (IC_{50}=0.13, 0.18 and 0.10 μM, respectively). These results suggest that introduction of specific methyl group(s) may lead to a preferred conformation of the piperazine ring that increases the AR antagonist activity.

Lastly, we conducted further modification of the 4-fluorophenyl group of the 2,5-*trans*-dimethyl derivative (**18a**). Replacement of the fluorine atom with another halogen, such as a chlorine or bromine, at the *para* position resulted in only a small reduction in potency, and other derivatives (**18d**—**g**) exhibited comparable inhibitory activity to **18a** (Table 3).

The 2,5-*trans*-dimethyl derivatives (**18a**—**g**) were also evaluated for *in vivo* antiandrogenic activity, based on their inhibition of ventral prostate growth in testosterone propionate-treated castrated rats, using once daily oral administration for 5 d (Table 3). The 2,5-*trans*-dimethyl derivative (**18a**) exhibited increased *in vivo* antiandrogenic activity compared to the unsubstituted derivative (**5**) (64% and 32% inhibition, respectively, compared to the control), and showed improved

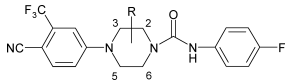
Table 1. AR Antagonistic Activities of Arylpiperazine, Arylpiperidine and Biphenyl Derivatives



Compound	A	IC_{50} (μM)[a]
5		0.47
13		1.6
20		7.2
25		4.9
24		1.2
27		0.61
4		0.89

a) Compounds were tested for their ability to inhibit AR mediated transcriptional activation using a reporter assay. IC_{50} values were determined by a single experimental run in triplicate.

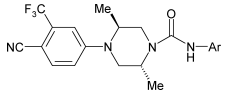
Table 2. AR Antagonistic Activities of the *N*-Arylpiperazine-1-carboxamide Derivatives

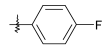
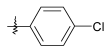
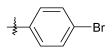
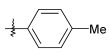
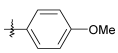
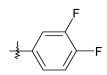
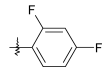


Compound	R	IC_{50} (μM)[a]
5	H	0.47
14	2-Methyl	0.18
15	3-Methyl	0.10
34	3-Ethyl	0.14
35	3-Isopropyl	0.77
30	3-Oxo	17%[b]
36	3,3-Dimethyl	0.25
16	2,2-Dimethyl	8.5
17	*cis*-2,6-Dimethyl	5.0
18a	*trans*-2,5-Dimethyl	0.13
4		0.89

a) Refer to Table 1. *b*) Percent inhibition at 10 μM.

Table 3. *In Vitro* and *in Vivo* Activities of the *trans*-2,5-Dimethylpiperazine Derivatives



Cpd.	Ar	IC_{50} (μM)[a]	% inhibition[b]
5		0.47	32%
18a		0.13	64%**
18b		0.24	33%
18c		0.57	60%**
18d		0.27	27%
18e		0.11	17%
18f		0.11	62%**
18g		0.20	85%** ED_{50}=1.1 mg/kg
4		0.89	75%** ED_{50}=1.6 mg/kg

a) Refer to Table 1. *b*) The mean percent changes from the respective control value of ventral prostate weight after oral administration in testosterone propionate-treated castrated rats (10 mg/kg/d for 5 d, n=5 or 6). ** $p<0.01$ *versus* control by Dunnett's multiple comparison test.

metabolic stability in human liver microsomes, compared to compound **5** (72% and 54% remaining, respectively, after 1 h). Although there may be some species difference in metabolic stability, the relative antiandrogenic activities suggest that the metabolic stability of **18a** in rat is also better than that of **5**. Interestingly, different substituents on the phenyl ring produced various *in vivo* results. Hence, the activity of the bromine derivative (**18c**) was comparable to **18a**, but the chlorine (**18b**) and methyl (**18d**) derivatives were less active than **18a**. Although the methoxy derivative (**18e**) was a potent *in vitro* AR antagonist, with an IC_{50} value of 0.11 μM, its *in vivo* potency was very weak. Surprisingly, the 2,4-difluoro derivative (**18g**) strongly inhibited the growth of rat prostate by 85% at a dose of 10 mg/kg, whereas the 3,4-difluoro derivative (**18f**) had an effect comparable to that of the 4-fluoro derivative (**18a**). These results indicate that introduction of an additional fluorine atom at the 2-position on the phenyl ring may be important for *in vivo* activity. Compound **18g** showed dose-dependent inhibition of the growth of rat prostate, and its ED_{50} value was 1.1 mg/kg, making it more potent than bicalutamide (ED_{50}=1.6 mg/kg) and suggesting that **18g** (YM-175735) has potential as a novel nonsteroidal AR antagonist.

Conclusion

A novel series of *N*-arylpiperazine-1-carboxamide derivatives were synthesized and their androgen receptor (AR) antagonist activities and *in vivo* antiandrogenic effects were evaluated. Reporter assays indicated that *trans*-2,5-dimethylpiperazine derivatives were potent AR antagonists, and in this series, *trans*-*N*-4-[4-cyano-3(trifluoromethyl)-phenyl]-*N*-(2,4-difluorophenyl)-2,5-dimethylpiperazine-1-carboxamide (**18g**, YM-175735) exhibited the most potent antiandrogenic activity. Compared to bicalutamide, YM-175735 showed an approximately 4-fold stronger activity as an AR antagonist, and showed a slightly increase in *in vivo* antiandrogenic activity, suggesting that YM-175735 may be useful for the treatment of prostate cancer.

Experimental

In general, all reagents and solvents were 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-LA300 or 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.

4-(1,4-Diazepan-1-yl)-2-(trifluoromethyl)benzonitrile (7) To a solution of 4-fluoro-2-(trifluoromethyl)benzonitrile (**6**, 5.0 g, 26.4 mmol) in *N*,*N*-dimethylformamide (DMF, 50 ml) was added 1,4-diazepane (10.6 g, 105.8 mmol) at ambient temperature and stirred at 80 °C for 21 h. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=10/1) to give the title compound (4.55 g, 64%) as a colorless solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.68—1.80 (2H, m), 2.60—2.66 (2H m), 2.82—2.90 (2H, m), 3.54—3.70 (4H, m), 7.00—7.06 (2H, m), 7.74 (1H, d, J=8.4 Hz). FAB-MS m/z: 270 ($M+H^+$).

(±)-4-(3-Methylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (8) The title compound was prepared from 2-methylpiperazine in a manner similar to that described for compound **7** as a colorless solid (quant.). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.03 (3H, d, J=6.3 Hz), 2.36—2.48 (1H, m), 2.62—2.85 (3H, m), 2.90—2.99 (1H, m), 3.79—3.93 (2H, m), 7.16—7.31 (2H, m), 7.79 (1H, d, J=9.3 Hz). FAB-MS m/z: 270 ($M+H^+$).

(±)-4-(2-Methylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (9) *tert*-Butyl 4-[4-cyano-3-(trifluoromethyl)phenyl]-3-methylpiperazine-1-carboxylate was prepared from *tert*-butyl 3-methylpiperazine-1-carboxylate[31] in a manner similar to that described for compound **7** (65%) as a colorless powder. A mixture of the intermediate (1.2 g, 3.25 mmol) and TFA (6 ml) was stirred at 0 °C for 30 min and the solution was concentrated *in vacuo*. The residue was diluted with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was dried and concentrated under reduced pressure to give **9** (920 mg, quant.) as a pale yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.11 (3H, d, J=6.6 Hz), 2.56—2.70 (1H, m), 2.74—2.89 (2H, m), 2.90—3.05 (2H, m), 3.54—3.67 (1H, m), 4.05—4.23 (1H, m), 7.11—7.24 (2H, m), 7.80 (1H, d, J=9.0 Hz). FAB-MS m/z: 270 ($M+H^+$).

4-(3,3-Dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (10) The title compound was prepared from 2,2-dimethylpiperazine[32] in a manner similar to that described for compound **7** as a colorless solid (55%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.05 (6H, s), 2.77—2.90 (2H, m), 3.21 (2H, s), 3.29—3.39 (2H, m), 7.14—7.29 (2H, m), 7.76 (1H, d, J=8.7 Hz). FAB-MS m/z: 284 ($M+H^+$).

(±)-*cis*-4-(3,5-Dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (11) The title compound was prepared from *cis*-2,6-dimethylpiperazine in a manner similar to that described for **7** as a colorless solid (81%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.03 (6H, d, J=6.3 Hz), 2.21—2.41 (2H, m), 2.65—2.83 (2H, m), 3.82—3.94 (2H, m), 7.16—7.32 (2H, m), 7.80 (1H, d, J=8.8 Hz). FAB-MS m/z: 284 ($M+H^+$).

(±)-*trans*-4-(2,5-Dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (12) The title compound was prepared from *trans*-2,5-dimethylpiperazine in a manner similar to that described for compound **7** as a yellow oil (quant.). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.16—1.24 (6H, m), 2.67—2.77 (1H, m), 3.06—3.18 (1H, m), 3.25—3.41 (3H, m), 3.70—3.83 (1H, m), 6.96 (1H, dd, J=8.7, 2.4 Hz), 7.12 (1H, d, J=2.4 Hz), 7.62 (1H, d, J=8.7 Hz). EI-MS m/z: 283 (M^+).

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-1,4-diazepane-1-carboxamide (13) To a solution of 4-(1,4-diazepan-1-yl)-2-(trifluoromethyl)benzonitrile (**7**, 500 mg, 1.86 mmol) in CH_2Cl_2 (10 ml) was added 4-fluorophenyl isocyanate (0.23 ml, 2.04 mmol) at ambient temperature and stirred for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by silica gel column chromatography

($CHCl_3$/MeOH=50/1). The resulting solid was further purified by recrystallization from AcOEt to give the title compound (550 mg, 73%) as a colorless crystalline solid. mp 179—180 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.80—1.95 (2H, m), 3.39—3.50 (2H, m), 3.60—3.85 (6H, m), 6.97—7.15 (4H, m), 7.29—7.37 (2H, m), 7.76 (1H, d, J=8.8 Hz), 8.34 (1H, s). FAB-MS m/z: 407 ($M+H^+$). *Anal.* Calcd for $C_{20}H_{18}N_4OF_4$: C, 59.11; H, 4.46; N, 13.79; F, 18.70. Found: C, 58.93; H, 4.45; N, 13.82; F, 18.60.

(±)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-2-methylpiperazine-1-carboxamide (14) The title compound was prepared from (±)-4-(3-methylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**8**) in a manner similar to that described for **13** as a colorless solid (84%). mp 211—216 °C (AcOEt/iPr$_2$O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.16 (3H, d, J=6.9 Hz), 3.06—3.19 (1H, m), 3.29—3.41 (2H, m), 3.84—4.01 (3H, m), 4.35—4.48 (1H, m), 7.03—7.12 (2H, m), 7.25 (1H, dd, J=8.8, 2.5 Hz), 7.31 (1H, d, J=2.5 Hz), 7.42—7.52 (2H, m), 7.84 (1H, d, J=8.8 Hz), 8.55 (1H, s). FAB-MS m/z: 407 ($M+H^+$). *Anal.* Calcd for $C_{20}H_{18}N_4OF_4$: C, 59.11; H, 4.46; N, 13.79; F, 18.70. Found: C, 59.38; H, 4.61; N, 13.85; F, 18.59.

(±)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-3-methylpiperazine-1-carboxamide (15) The title compound was prepared from (±)-4-(2-methylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**9**) in a manner similar to that described for compound **13** as a colorless solid (77%). mp 197—199 °C (AcOEt/iPr$_2$O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.10 (3H, d, J=6.8 Hz), 3.11—3.32 (3H, m), 3.76—3.86 (1H, m), 3.95—4.14 (2H, m), 4.29—4.40 (1H, m), 7.04—7.12 (2H, m), 7.19—7.30 (2H, m), 7.44—7.51 (2H, m), 7.86 (1H, d, J=8.7 Hz), 8.61 (1H, s). FAB-MS m/z: 405 ($M-H^-$). *Anal.* Calcd for $C_{20}H_{18}N_4OF_4$: C, 59.11; H, 4.46; N, 13.79; F, 18.70. Found: C, 58.94; H, 4.45; N, 13.71; F, 18.97.

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-2,2-dimethylpiperazine-1-carboxamide (16) The title compound was prepared from 4-(3,3-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**10**) in a manner similar to that described for compound **13** as a colorless solid (60%). mp 197—201 °C (AcOEt). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.43 (6H, s), 3.54—3.61 (2H, m), 3.67 (2H, s), 3.75—3.86 (2H, m), 7.02—7.09 (2H, m), 7.11 (1H, dd, J=8.8, 2.5 Hz), 7.17 (1H, d, J=2.5 Hz), 7.35—7.45 (2H, m), 7.83 (1H, d, J=8.8 Hz), 8.45 (1H, s). FAB-MS m/z: 421 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OF_4$: C, 60.00; H, 4.80; N, 13.33; F, 18.08. Found: C, 59.98; H, 4.72; N, 13.34; F, 18.19.

(±)-*cis*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-2,6-dimethylpiperazine-1-carboxamide (17) The title compound was prepared from (±)-*cis*-4-(3,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**11**) in a manner similar to that described for compound **13** as a colorless solid (79%). mp 205 °C (AcOEt). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.29 (6H, d, J=6.4 Hz), 3.18—3.31 (2H, m), 3.97—4.14 (2H, m), 4.30—4.45 (2H, m), 7.02—7.17 (2H, m), 7.30—7.39 (2H, m), 7.44—7.54 (2H, m), 7.84 (1H, d, J=8.6 Hz), 8.45 (1H, s). FAB-MS m/z: 421 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OF_4$: C, 60.00; H, 4.80; N, 13.33; F, 18.08. Found: C, 59.91; H, 4.78; N, 13.34; F, 18.34.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-2,5-dimethylpiperazine-1-carboxamide (18a) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**12**) and 4-fluoropheny isocyanate in a manner similar to that described for compound **13** as a colorless solid (74%). mp 200—203 °C (EtOH). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J=6.5 Hz), 1.18 (3H, d, J=6.7 Hz), 3.31—3.48 (2H, m), 3.66—3.79 (1H, m), 3.82—3.95 (1H, m), 4.29—4.42 (1H, m), 4.43—4.57 (1H, m), 7.03—7.13 (2H, m), 7.22—7.33 (2H, m), 7.43—7.54 (2H, m), 7.84 (1H, d, J=8.9 Hz), 8.60 (1H, s). FAB-MS m/z: 421 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OF_4$: C, 60.00; H, 4.80; N, 13.33; F, 18.08. Found: C, 59.91; H, 4.97; N, 13.26; F, 17.98.

(±)-*trans*-*N*-(4-Chlorophenyl)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazine-1-carboxamide (18b) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)-benzonitrile (**12**) and 4-chlorophenyl isocyanate in a manner similar to that described for compound **13** as a colorless solid (66%). mp 196 °C (EtOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, d, J=6.3 Hz), 1.18 (3H, d, J=6.6 Hz), 3.30—3.47 (2H, m), 3.67—3.78 (1H, m), 3.82—3.93 (1H, m), 4.29—4.41 (1H, m), 4.43—4.56 (1H, m), 7.22—7.33 (4H, m), 7.48—7.57 (2H, m), 7.84 (1H, d, J=9.0 Hz), 8.69 (1H, s). FAB-MS m/z: 437 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OClF_3$: C, 57.74; H, 4.61; N, 12.82; Cl, 8.12; F, 13.05. Found: C, 57.42; H, 4.50; N, 12.79; Cl, 8.42; F, 12.82.

(±)-*trans*-*N*-(4-Bromophenyl)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazine-1-carboxamide (18c) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)-benzonitrile (**12**) and 4-bromophenyl isocyanate in a manner similar to that described for compound **13** as a colorless solid (84%). mp 192 °C (CH_2Cl_2). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.09 (3H, d, J=6.6 Hz), 1.17 (3H, d, J=6.6 Hz), 3.30—3.46 (2H, m), 3.67—3.78 (1H, m), 3.82—3.92 (1H, m), 4.29—4.41 (1H, m), 4.43—4.56 (1H, m), 7.20—7.32 (2H, m), 7.37—7.52 (4H, m), 7.84 (1H, d, J=9.0 Hz), 8.69 (1H, s). FAB-MS m/z: 483, 481 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OBrF_3$: C, 52.40; H, 4.19; N, 11.64; Br, 16.60; F, 11.84. Found: C, 52.15; H, 4.24; N, 11.56; Br, 16.31; F, 11.67.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-(4-methylphenyl)piperazine-1-carboxamide (18d) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**12**) and *p*-tolyl isocyanate in a manner similar to that described for compound **13** as a colorless solid (61%). mp 188 °C (AcOEt). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, d, J=6.6 Hz), 1.17 (3H, d, J=6.6 Hz), 2.23 (3H, s), 3.30—3.45 (2H, m), 3.66—3.77 (1H, m), 3.82—3.92 (1H, m), 4.28—4.41 (1H, m), 4.43—4.56 (1H, m), 7.05 (2H, d, J=8.4 Hz), 7.22—7.39 (4H, m), 7.84 (1H, d, J=9.0 Hz), 8.46 (1H, s). FAB-MS m/z: 417 ($M+H^+$). *Anal.* Calcd for $C_{22}H_{23}N_4OF_3$: C, 63.45; H, 5.57; N, 13.45; F, 13.69. Found: C, 63.24; H, 5.51; N, 13.42; F, 13.74.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-methoxyphenyl)-2,5-dimethylpiperazine-1-carboxamide (18e) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**12**) and 4-methoxyphenyl isocyanate in a manner similar to that described for compound **13** as a colorless solid (61%). mp 195 °C (AcOEt/Et$_2$O). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, d, J=6.6 Hz), 1.16 (3H, d, J=6.6 Hz), 3.31—3.45 (2H, m), 3.66—3.77 (1H, m), 3.71 (3H, s), 3.81—3.92 (1H, m), 4.28—4.41 (1H, m), 4.42—4.55 (1H, m), 6.79—6.87 (2H, m), 7.22—7.39 (4H, m), 7.84 (1H, d, J=9.0 Hz), 8.40 (1H, s). FAB-MS m/z: 433 ($M+H^+$). *Anal.* Calcd for $C_{22}H_{23}N_4O_2F_3$: C, 61.10; H, 5.36; N, 12.96; F, 13.18. Found: C, 60.84; H, 5.23; N, 13.05; F, 13.05.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(3,4-difluorophenyl)-2,5-dimethylpiperazine-1-carboxamide (18f) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**12**) and 3,4-difluorophenyl isocyanate in a manner similar to that described for compound **13** as a colorless solid (50%). mp 185 °C (MeOH). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, d, J=6.6 Hz), 1.53 (3H, d, J=6.6 Hz), 3.28—3.47 (2H, m), 3.67—3.78 (1H, m), 3.82—3.92 (1H, m), 4.29—4.55 (2H, m), 7.21—7.37 (4H, m), 7.58—7.70 (1H, m), 7.85 (1H, d, J=8.7 Hz), 8.76 (1H, s). FAB-MS m/z: 439 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{19}N_4OF_5$: C, 57.53; H, 4.37; N, 12.78; F, 21.67. Found: C, 57.51; H, 4.52; N, 12.74; F, 21.40.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(2,4-difluorophenyl)-2,5-dimethylpiperazine-1-carboxamide (18g) The title compound was prepared from (±)-*trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**12**) and 2,4-difluorophenyl isocyanate in a manner similar to that described for compound **13** as a colorless solid (82%). mp 169—171 °C (AcOEt/*n*-hexane). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.11 (3H, d, J=6.6 Hz), 1.18 (3H, d, J=6.6 Hz), 3.30—3.48 (2H, m), 3.67—3.88 (2H, m), 4.28—4.51 (2H, m), 6.96—7.07 (1H, m), 7.18—7.43 (4H, m), 7.84 (1H, d, J=8.7 Hz), 8.39 (1H, s). FAB-MS m/z: 439 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{19}N_4OF_5$: C, 57.53; H, 4.37; N, 12.78; F, 21.67. Found: C, 57.51; H, 4.35; N, 12.99; F, 21.38.

Ethyl 1-[4-Cyano-3-(trifluoromethyl)phenyl]piperidine-4-carboxylate (19) A mixture of 4-fluoro-2-(trifluoromethyl)benzonitrile (**6**, 1.0 g, 5.29 mmol), ethyl piperidine-4-carboxylate (0.92 ml, 5.82 mmol) and K_2CO_3 (1.1 g, 7.94 mmol) in DMF (50 ml) was stirred at ambient temperature for 17 h. The mixture was poured into water and the precipitate was filtered and washed with water to give the title compound (1.51 g, 88%) as a colorless solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.19 (3H, t, J=7.1 Hz), 1.52—1.66 (2H, m), 1.85—1.97 (2H, m), 2.59—2.71 (1H, m), 3.01—3.17 (2H, m), 3.92—4.02 (2H, m), 4.08 (2H, q, J=7.1 Hz), 7.24 (1H, dd, J=8.8, 2.5 Hz), 7.30 (1H, d, J=2.5 Hz), 7.81 (1H, d, J=8.8 Hz). EI-MS m/z: 326 (M^+).

1-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)piperidine-4-carboxamide (20) A mixture of ethyl 1-[4-cyano-3-(trifluoromethyl)-phenyl]piperidine-4-carboxylate (**19**, 1.41 g, 4.32 mmol) and 1 M NaOH (5.4 ml, 5.40 mmol) in EtOH (50 ml) and THF (20 ml) was stirred at ambient temperature for 4 d. The reaction mixture was concentrated *in vacuo* and the residue was diluted with saturated aqueous NH_4Cl and was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give 1-[4-cyano-3-(trifluoromethyl)phenyl]piperidine-4-carboxylic acid (1.23 g, 95%) as a colorless solid. To a solution of 1-[4-cyano-3-(trifluoromethyl)phenyl]piperidine-4-carboxylic acid (500 mg,

1.68 mmol) in CH_2Cl_2 (25 ml) was added oxalyl chloride (0.3 ml, 3.35 mmol) and DMF (1 drop). After stirring at ambient temperature for 1 h, the solution was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 ml) and added to a cooled solution of 4-fluoroaniline (0.48 ml, 5.04 mmol) in CH_2Cl_2 (10 ml). After stirring at ambient temperature for 1 h, the precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=1/1). The resulting solid was further purified by recrystallization from AcOEt to give the title compound (371 mg, 56%) as a colorless crystalline solid. mp 185—187 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.60—1.76 (2H, m), 1.83—1.96 (2H, m), 2.59—2.70 (1H, m), 2.96—3.13 (2H, m), 4.05—4.19 (2H, m), 7.09—7.18 (2H, m), 7.23—7.29 (1H, m), 7.30—7.35 (1H, m), 7.58—7.67 (2H, m), 7.82 (1H, d, J=8.8 Hz), 10.01 (1H, s). 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, 61.42; H, 4.30; N, 10.72; F, 19.60.

***tert*-Butyl 4-[4-Cyano-3-(trifluoromethyl)phenyl]-3,6-dihydropyridine-1(2*H*)-carboxylate (22)** A suspension of 4-amino-2-(trifluoromethyl)benzonitrile (**21**, 10.0 g, 53.72 mmol) in 48% HBr (50 ml) was cooled in an ice-salt bath to 0 °C. A solution of sodium nitrite (3.71 g, 53.72 mmol) in water (10 ml) was added dropwise at such a rate that the temperature of the reaction mixture was under 5 °C. After stirring for 1 h, the reaction mixture was poured into a solution of copper(I) bromide (7.71 g, 53.72 mmol) in 48% HBr (55 ml). The mixture was stirred at ambient temperature for 2 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$, H_2O and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=10/1) to give 4-bromo-2-(trifluoromethyl)benzonitrile (11.37 g, 85%) as a pale brown oil. A solution of *n*-BuLi in *n*-hexane (2.2 ml, 1.59 M, 3.48 mmol) was added dropwide to a solution of 4-bromo-2-(trifluoromethyl)benzonitrile (790 mg, 3.16 mmol) in dry THF (30 ml) at −78 °C. The reaction mixture was stirred for 15 min, and a solution of *tert*-butyl 4-oxopiperidine-1-carboxylate (693 mg, 3.48 mmol) in dry THF (3 ml) was added to the reaction mixture at −78 °C. After stirring at ambient temperature for 1 h, the reaction mixture was poured into ice-water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=50/1) to give *tert*-butyl 4-[4-cyano-3-(trifluoromethyl)phenyl]-4-hydroxypiperidine-1-carboxylate (380 mg, 32%) as a pale brown solid. To a cooling solution of *tert*-butyl 4-[4-cyano-3-(trifluoromethyl)phenyl]-4-hydroxypiperidine-1-carboxylate (1.27 g, 3.43 mmol) in pyridine (25 ml) was added phosphoryl chloride (3.2 ml, 34.3 mmol) at 0 °C. After stirring at ambient temperature for 1 d, the reaction mixture was quenched with saturated $NaHCO_3$ and the resultant precipitate was filtered off and washed with H_2O to give the title compound (973 mg, 81%) as a pale brown solid. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.43 (9H, s), 2.47—2.58 (2H, m), 3.51—3.59 (2H, m), 4.02—4.10 (2H, m), 6.52—6.60 (1H, m), 7.90—8.00 (2H, m), 8.15 (1H, d, J=8.7 Hz). FAB-MS *m*/*z*: 353 ($M+H^+$).

***tert*-Butyl 4-[4-Cyano-3-(trifluoromethyl)phenyl]piperidine-1-carboxylate (23)** A mixture of *tert*-butyl 4-[4-cyano-3-(trifluoromethyl)phenyl]-3,6-dihydropyridine-1(2*H*)-carboxylate (**22**, 724 mg, 2.05 mmol) and 10% Pd-C (36 mg) in MeOH (20 ml) was stirred under H_2 at ambient temperature for 4 h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=1/1) to give the title compound (550 mg, 76%) as a yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.42 (9H, s), 1.50—1.65 (2H, m), 1.72—1.85 (2H, m), 2.71—3.01 (3H, m), 3.99—4.17 (2H, m), 7.77—7.85 (1H, m), 7.88—7.93 (1H, m), 8.10 (1H, d, J=8.1 Hz). FAB-MS *m*/*z*: 355 ($M+H^+$).

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-3,6-dihydropyridine-1(2*H*)-carboxamide (24) To a cooling solution of *tert*-butyl 4-[4-cyano-3-(trifluoromethyl)phenyl]-3,6-dihydropyridine-1(2*H*)-carboxylate (**22**, 400 mg, 1.14 mmol) in 1,4-dioxane (10 ml) was added trifluoroacetic acid (3 ml). After stirring at ambient temperature for 5 h, the solution was concentrated *in vacuo*. The residue was diluted with saturated aqueous $NaHCO_3$ and was extracted with AcOEt. The organic layer was washed with H_2O, dried and concentrated to give 4-(1,2,3,6-tetrahydropyridin-4-yl)-2-(trifluoromethyl)benzonitrile (290 mg, quant.) as a brown solid. The title compound was prepared from 4-(1,2,3,6-tetrahydropyridin-4-yl)-2-(trifluoromethyl)benzonitrile in a manner similar to that described for compound **13** as a colorless powder (47%). mp 194—196 °C (AcOEt). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.56—2.65 (2H, m), 3.64—3.73 (2H, m), 4.17—4.27 (2H, m), 6.62—6.70 (1H, m), 7.04—7.13 (2H, m), 7.45—7.53 (2H, m), 7.93—8.04 (2H, m), 8.16 (1H, d, J=8.3 Hz), 8.62 (1H, s). FAB-MS *m*/*z*: 390 ($M+H^+$). *Anal.* Calcd for $C_{20}H_{15}N_3OF_4$: C, 61.70; H, 3.88; N, 10.79; F, 19.52. Found: C, 61.53; H, 3.94; N, 10.76; F, 19.52.

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)piperidine-1-carboxamide (25) The title compound was prepared from *tert*-butyl 4-[4-cyano-3-(trifluoromethyl)phenyl]piperidine-1-carboxylate (**23**) in a manner similar to that described for compound **24** as a colorless powder (2 steps 22%). mp 161—162 °C (EtOH/*i*Pr_2O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.56—1.72 (2H, m), 1.77—1.89 (2H, m), 2.81—2.93 (2H, m), 2.96—3.07 (1H, m), 4.24—4.35 (2H, m), 7.02—7.11 (2H, m), 7.43—7.52 (2H, m), 7.81—7.86 (1H, m), 7.91—7.95 (1H, m), 8.12 (1H, d, J=7.8 Hz), 8.55 (1H, s). 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, 61.26; H, 4.28; N, 10.78; F, 19.75.

4′-Cyano-3′-(trifluoromethyl)biphenyl-4-carboxylic Acid (26) Synthesis of 4-bromo-2-(trifluoromethyl)benzonitrile was described as above (compound **22**). A mixture of 4-bromo-2-(trifluoromethyl)benzonitrile (1.88 g, 7.52 mmol), 4-carboxyphenylboronic acid (1.37 g, 8.27 mmol), Na_2CO_3 (3.2 g, 30.08 mmol) and 10% Pd-C (320 mg) in EtOH (80 ml) was refluxed for 2 h. After cooling, the reaction mixture was poured into water. The precipitate was filtered off, mother liquid was diluted with aqueous citric acid and extracted with AcOEt. The organic layer was dried and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=30/1) to give the title compound (1.80 g, 82%) as a colorless solid. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.95—8.40 (7H, m), 13.16 (1H, br). FAB-MS *m*/*z*: 290 ($M-H^-$).

4′-Cyano-*N*-(4-fluorophenyl)-3′-(trifluoromethyl)biphenyl-4-carboxamide (27) The title compound was prepared from 4′-cyano-3′-(trifluoromethyl)biphenyl-4-carboxylic acid (**26**) in a manner similar to that described for compound **20** as a colorless powder (46%). mp 191—193 °C (AcOEt/*i*Pr_2O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.17—7.26 (2H, m), 7.77—7.87 (2H, m), 8.02—8.15 (4H, m), 8.28—8.36 (3H, m), 10.43 (1H, br). FAB-MS *m*/*z*: 385 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{12}N_2OF_4$: C, 65.63; H, 3.15; N, 7.29; F, 19.77. Found: C, 65.83; H, 3.04; N, 7.24; F, 19.63.

4-{[2-(Benzylamino)ethyl]amino}-2-(trifluoromethyl)benzonitrile (28) 4-[(2-Aminoethyl)amino]-2-(trifluoromethyl)benzonitrile was prepared from ethylenediamine in a manner similar to that described for compound **7** (95%). To a solution of 4-[(2-aminoethyl)amino]-2-(trifluoromethyl)benzonitrile (1.15 g, 5.0 mmol) in AcOH (30 ml) was added benzaldehyde (640 mg, 6.0 mmol) and $NaBH(OAc)_3$ (2.23 g, 10.0 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 (AcOEt) to give the title compound (920 mg, 58%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.89—2.98 (2H, m), 3.16—3.27 (2H, m), 3.81 (2H, s), 5.07—5.22 (1H, m), 6.60—6.69 (1H, m), 6.85 (1H, d, J=2.2 Hz), 7.23—7.38 (5H, m), 7.54 (1H, d, J=8.5 Hz). FAB-MS *m*/*z*: 320 ($M+H^+$).

4-(2-Oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (29) A mixture of 4-{[2-(benzylamino)ethyl]amino}-2-(trifluoromethyl)benzonitrile (**28**, 800 mg, 2.5 mmol) and 40% aqueous glyoxal (0.57 ml, 5.0 mmol) in THF (10 ml) and H_2O (5 ml) was stirred at ambient temperature for 14 h. The solution was diluted with H_2O and was extracted with AcOEt. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=3/2) to give 4-(4-benzyl-2-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (590 mg, 66%) as a colorless powder. A mixture of 4-(4-benzyl-2-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (580 mg, 1.6 mmol) and 10% Pd-C (59 mg) in AcOH (30 ml) was stirred under H_2 at ambient temperature for 3 h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=33/1) to give the title compound (340 mg, 79%) as a yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.28 (2H, t, J=5.5 Hz), 3.76 (2H, s), 3.79 (2H, t, J=5.5 Hz), 7.69—7.76 (1H, m), 7.81—7.90 (2H, m). FAB-MS *m*/*z*: 270 ($M+H^+$).

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-3-oxopiperazine-1-carboxamide (30) The title compound was prepared from 4-(2-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**29**) in a manner similar to that described for compound **13** as a colorless powder (83%). mp 174—175 °C (AcOEt/*i*Pr_2O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.82—3.89 (2H, m), 3.90—3.99 (2H, m), 4.34 (2H, s), 7.05—7.14 (2H, m), 7.46—7.54 (2H, m), 7.94 (1H, dd, J=8.4, 2.0 Hz), 8.14 (1H, d, J=2.0 Hz), 8.24 (1H, d, J=8.4 Hz), 8.67 (1H, s). FAB-MS *m*/*z*: 407 ($M+H^+$). *Anal.* Calcd for $C_{19}H_{14}N_4O_2F_4$: C, 56.16; H, 3.47; N, 13.79; F, 18.70. Found: C, 55.98; H, 3.30; N, 13.90; F, 18.91.

(±)-4-(4-Benzyl-2-ethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (31) 4-(4-Benzyl-3-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile was prepared from 1-benzylpiperazin-2-one in a manner similar to that described for compound **7** as a colorless solid (73%). A solution of 4-(4-benzyl-3-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (2.0 g, 5.57 mmol) in dry THF (20 ml) was added dropwise to a solution of LDA (738 mg, 6.68 mmol) in dry THF (10 ml) at −78 °C. The reaction mixture was stirred for 20 min, and iodoethane (0.67 ml, 8.36 mmol) was added to the reaction mixture at −78 °C. The cold bath was removed and the reaction mixture was poured into saturated NH_4Cl at −10 °C and extracted with AcOEt. The organic layer was washed with H_2O and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=2/1) to give 4-(4-benzyl-2-ethyl-3-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (1.50 g, 72%) as a pale yellow form. A solution of borane THF complex in THF (6.10 ml, 1.0 M, 6.10 mmol) was added dropwise to a solution of 4-(4-benzyl-2-ethyl-3-oxopiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (1.47 g, 3.80 mmol) in dry THF (30 ml) at 0 °C. After stirring at 0 °C for 5 h, the reaction mixture was quenched with MeOH (10 ml) and 1 M HCl (38 ml, 38 mmol) and concentrated *in vacuo*. The residue was neutralized by saturated $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with H_2O and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt=1/1) to give the title compound (670 mg, 47%) as a pale yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.67—0.77 (3H, m), 1.40—1.57 (1H, m), 1.76—1.92 (1H, m), 2.02—2.17 (2H, m), 2.77—2.97 (2H, m), 3.08—3.20 (1H, m), 3.40 (1H, d, J=13.2 Hz), 3.60 (1H, d, J=13.2 Hz), 3.73—3.86 (1H, m), 3.94—4.08 (1H, m), 7.12—7.42 (7H, m), 7.79 (1H, d, J=8.7 Hz). FAB-MS m/z: 374 ($M+H^+$).

(±)-4-(4-Benzyl-2-isopropylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (32) The title compound was prepared from 2-iodopropane in a manner similar to that described for compound **31** as a colorless oil (3 steps 56%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.66 (3H, d, J=6.6 Hz), 0.81 (3H, d, J=6.6 Hz), 1.91—2.13 (2H, m), 2.47—2.63 (1H, m), 2.81—2.95 (2H, m), 3.16—3.40 (2H, m), 3.57 (1H, d, J=13.2 Hz), 3.75—3.93 (2H, m), 7.14—7.39 (7H, m), 7.73 (1H, d, J=9.0 Hz). FAB-MS m/z: 388 ($M+H^+$).

4-(4-Benzyl-2,2-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (33) The title compound was prepared using 2 equivalents of LDA and iodomethane in a manner similar to that described for compound **31** as a colorless oil (3 steps 43%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.17 (6H, s), 2.30 (2H, s), 2.47—2.57 (2H, m), 3.23—3.33 (2H, m), 3.51 (2H, s), 7.22—7.39 (5H, m), 7.46—7.52 (2H, m), 7.95 (1H, d, J=9.0 Hz). FAB-MS m/z: 374 ($M+H^+$).

(±)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-3-ethyl-*N*-(4-fluorophenyl)piperazine-1-carboxamide (34) A mixture of (±)-4-(4-benzyl-2-ethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**31**, 650 mg, 1.74 mmol) and 10% Pd-C (65 mg) in MeOH (20 ml) was stirred under 1 atm H_2 gas at ambient temperature for 7 h. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=33/1) to give 4-(2-ethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (460 mg, 93%) as a pale yellow oil. The title compound was prepared from (±)-4-(2-ethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile in a manner similar to that described for compound **13** as a colorless powder (70%). mp 180—182 °C (AcOEt/iPr$_2$O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.87 (3H, t, J=7.3 Hz), 1.42—1.65 (2H, m), 3.13—3.32 (3H, m), 3.78—3.88 (1H, m), 4.01—4.19 (3H, m), 7.04—7.12 (2H, m), 7.17—7.23 (1H, m), 7.26 (1H, d, J=2.4 Hz), 7.43—7.51 (2H, m), 7.83 (1H, d, J=9.3 Hz), 8.61 (1H, s). FAB-MS m/z: 421 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OF_4$: C, 60.00; H, 4.80; N, 13.33; F, 18.08. Found: C, 59.69; H, 4.71; N, 13.11; F, 17.97.

(±)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-3-isopropylpiperazine-1-carboxamide (35) The title compound was prepared from (±)-4-(4-benzyl-2-isopropylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**32**) in a manner similar to that described for compound **34** as a colorless amorphous (2 steps 64%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.72 (3H, d, J=6.8 Hz), 1.02 (3H, d, J=6.8 Hz), 2.03—2.17 (1H, m), 3.05—3.17 (2H, m), 3.22—3.31 (1H, m), 3.86—3.97 (2H, m), 3.99—4.09 (1H, m), 4.18—4.28 (1H, m), 7.03—7.11 (2H, m), 7.22—7.32 (2H, m), 7.40—7.49 (2H, m), 7.79 (1H, d, J=8.8 Hz), 8.61 (1H, s). FAB-MS m/z: 435 ($M+H^+$). *Anal.* Calcd for $C_{22}H_{22}N_4OF_4$: C, 60.82; H, 5.10; N, 12.90; F, 17.49. Found: C, 60.81; H, 5.02; N, 12.79; F, 17.22.

4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-fluorophenyl)-3,3-dimethylpiperazine-1-carboxamide (36) The title compound was prepared from 4-(4-benzyl-2,2-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**33**) in a manner similar to that described for compound **34** as a colorless powder (2 steps 21%). mp 179—180 °C (AcOEt/iPr$_2$O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.31 (6H, s), 3.52—3.61 (4H, m), 3.62—3.70 (2H, m), 7.03—7.12 (2H, m), 7.32—7.42 (2H, m), 7.44—7.53 (2H, m), 7.89 (1H, d, J=8.3 Hz), 8.46 (1H, s). FAB-MS m/z: 421 ($M+H^+$). *Anal.* Calcd for $C_{21}H_{20}N_4OF_4$: C, 60.00; H, 4.80; N, 13.33; F, 18.08. Found: C, 59.94; H, 4.91; N, 13.30; F, 18.19.

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 eight hours 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 $(MgCO_3)_4Mg(OH)_2\cdot5H_2O$, 2.67 mM $MgSO_4\cdot7H_2O$, 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;

$$\text{AR antagonistic activity (\%)} = 100(I-X)/(I-B)$$

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).

Evaluation of Antiandrogenic Activities in Castrated Immature Rats Treated with Andorgen Male Wistar rats were supplied by Charles River Japan Inc. (Atsugi). Prepubertal male rats aged 3 weeks were castrated by the scrotal route under ether anesthesia. Three days after the castration, testosterone propionate (TP, 0.5 mg/kg, s.c.) was administered once daily for 5 d alone or in combination with the tested compound (10—30 mg/kg, *p.o.*). TP was dissolved in cotton seed oil containing 5% ethanol. The tested compound was suspended with 0.5% methylcellulose. The rats were sacrificed by excessive chloroform anesthesia 6 h after final dosing, and both ventral prostates and seminal vesicles+coagulate glands were removed and weighed. The antiandrogenic activity was expressed as a percentage of inhibition of the TP effect (TP-treated rats were arbitrarily assigned a value of 0% and vehicle-treated rats a value of 100%).

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