Bioorganic & Medicinal Chemistry 17 (2009) 64–73

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Potent and orally bioavailable CCR4 antagonists: Synthesis and structure–activity relationship study of 2-aminoquinazolines

Kazuhiro Yokoyama *, Noriko Ishikawa, Susumu Igarashi, Noriyuki Kawano, Naoyuki Masuda, Wataru Hamaguchi, Shingo Yamasaki, Yohei Koganemaru, Kazuyuki Hattori, Takahiro Miyazaki, Shin-ichi Ogino, Yuzo Matsumoto, Makoto Takeuchi, Mitsuaki Ohta

Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

ARTICLE INFO

Article history:
Received 16 September 2008
Revised 7 November 2008
Accepted 8 November 2008
Available online 17 November 2008

Keywords:
Chemokine receptor 4 (CCR4) antagonists
2-Aminoquinazolines
Inflammatory disease

ABSTRACT

Starting with a series of CC chemokine receptor-4 (CCR4) antagonists developed in a previous study, the potency was improved by replacing the pyrrolidine moiety of *N*-(4-chlorophenyl)-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidin-1-yl)quinazolin-4-amine **2** with a 3-(hydroxymethyl)piperidine. The resulting compound (1′-{4-[(4-chlorophenyl)amino]-6,7-dimethoxyquinazolin-2-yl}-1,4′-bipiperidin-3-yl)methanol **8ic** was a strong inhibitor of human/mouse chemotaxis. Oral administration of **8ic** showed anti-inflammatory activity in a murine model of acute dermatitis (oxazolone-induced contact hypersensitivity test) in a dose-dependent manner.

1. Introduction

Chemokines are relatively small (~8 to 14 kDa), mostly basic proteins that are involved in various physiological and pathological processes.[1,2] CC chemokine receptor 4 (CCR4) was originally cloned from T-lymphocyte and thymus cells, where it is highly expressed.[3–5] Almost all skin-homing cells expressing the cutaneous lymphocyte antigen in blood carry CCR4.[6] Thymus and activation-regulated chemokine (TARC, CCL17) as well as macrophage-derived chemokine (MDC, CCL22) are CC chemokines that are both highly specific biological ligands for CCR4.[7,8] In vivo studies of CCL17 and CCL22 antibodies have indicated their utility in preventing several immunological responses.[9–14] Therefore, the use of CCR4 antagonists would be a novel, therapeutic method of intervention for disease in which CCR4 participates.

In a previous manuscript,[15] *N*-cycloheptyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidin-1-yl)quinazolin-4-amine **1a** was identified as a potent CCR4 antagonist with potent in vitro (GTPγS binding) activity (Fig. 1). Optimization of compound **1a** led to the discovery of 2-(1,4′-bipiperidin-1′-yl)-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **1b**, which potently inhibited functional chemotaxis in mice (IC_{50} = 39 nM), although the compound also had a high intrinsic clearance value (human liver microsomes). Additional optimization to identify a CCR4 antagonist with improved metabolic stability led to the discovery of *N*-(4-chlorophenyl)-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidin-1-yl)quinazolin-4-amine **2**, which had a low intrinsic clearance value (human liver microsomes), although the functional inhibition of chemotaxis in mice remained moderate.[16] The potency of these compounds, **1a**, **1b**, and **2**, was evident after subcutaneous administration in the murine oxazolone-induced contact hypersensitivity test, a known model of acute skin inflammation. When these compounds were administered orally, however, very little inhibition was observed (Table 5). In this paper, further efforts to circumvent these problems via synthesis are described. In the previous report, the metabolic liability of the cycloheptylamine substituent was identified as a factor that might affect the high clearance of compound **1a** in human liver microsomes. The previous structure–activity relationship (SAR) study on compound **2** also suggested that the introduction of an aminoalcohol at the 4-position of the 2-piperidine ring produced a favorable effect on the inhibition of human chemotaxis. Based on these findings, a series of compounds was synthesized where the cycloheptylamine and piperidine of **2** were replaced with various substituents.

2. Chemistry

Novel aminoalcohol **5** was prepared from *tert*-butyl 4-oxopiperidine-1-carboxylate **3** in 2 or 3 steps, as summarized in Scheme 1. The reductive amination of compound **3** with an appropriate amine afforded the corresponding *N*-Boc-protected diamines **4a**, **4c**, and **4e**. The *O*-methylated compounds **4b** and **4d** were obtained from **4a** and **4c**, respectively, by treatment with sodium hydride

* Corresponding author. Tel.: +81 29 863 6773; fax: +81 29 854 1519.
E-mail address: kazuhiro.yokoyama@jp.astellas.com (K. Yokoyama).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.11.020

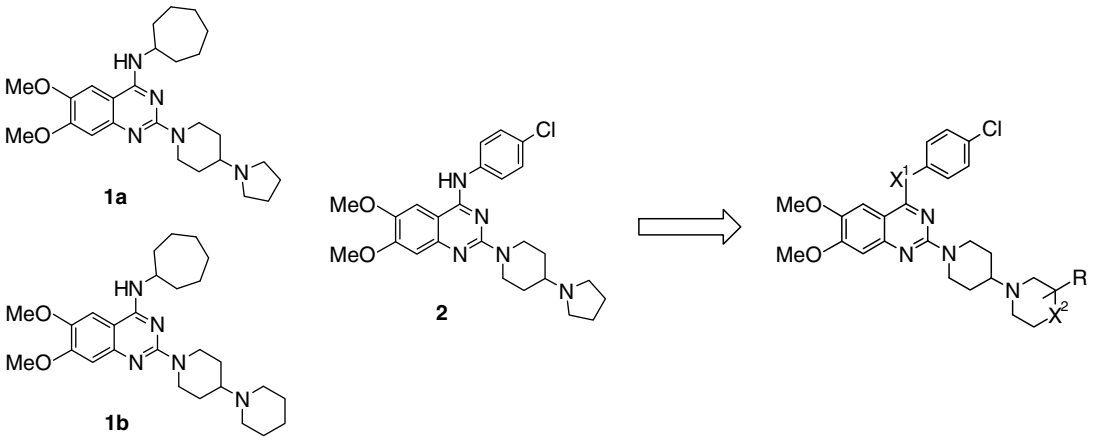
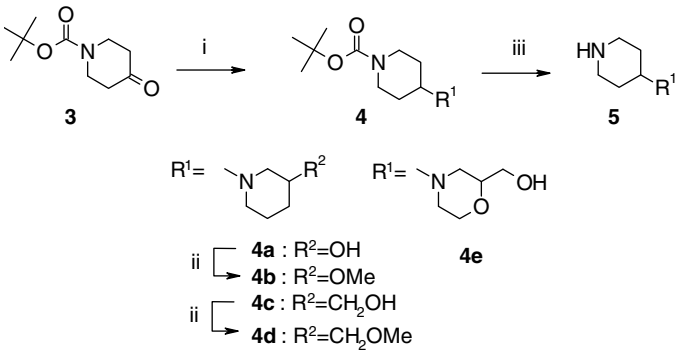


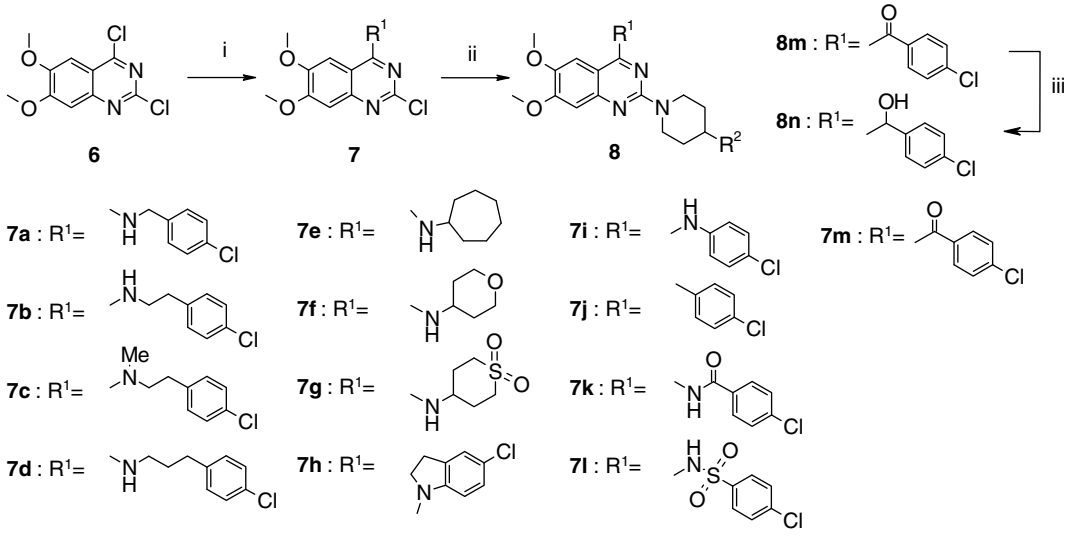
Figure 1. CCR4 inhibitors **1a**, **1b**, **2** discovered previously, and 2-aminoquinazolines evaluated in this article.



Scheme 1. Reagents and conditions: (i) Amines, 10% Pd–C, MeOH; (ii) NaH, MeI, DMF; (iii) 4-M HCl, AcOEt.

and methyl iodide. Deprotection of the Boc group of compounds **4a–e** proceeded readily under acidic conditions to yield the desired amines **5a–e**.

The preparation of 2-aminoquinazolines **8** is summarized in Scheme 2. Selective substitutions at the 4-position of quinazoline **6** with various amines proceeded smoothly to yield the corresponding 4-amino-2-chloro-quinazolines **7a–h**, even though this procedure did not work well with the reaction of 4-chloroaniline. The coupling of 4-chloroaniline and quinazoline **6** occurred in the mixture of aqueous hydrogen chloride and ethanol to yield an inseparable mixture of the desired 4-amino-2-chloro-quinazoline **7i** and 2,4-disubstituted quilazoline (ca. 9–10:1), which was brought to the next reaction. Introduction of the 4-chlorophenyl moiety was achieved under Suzuki-coupling conditions to yield **7j**. Couplings of 4-chlorobenzamide/4-chlorosulfonamide with **6** were performed under basic (NaH/DMF and *t*-BuOK/DMF, respectively) conditions to afford the corresponding benzamide **7k** and sulfonamide **7l**. Aroylation of **6** with 4-chlorobenzaldehyde proceeded via treatment with NaH/1,3-dimethylimidazolium iodide in refluxing dioxane to yield benzoylquinazoline **7m**.[17] Subsequent substitutions at the 2-position of quinazolines **7a–m** with piperidines readily proceeded in the presence of base (Hunig's base or DBU) to yield the corresponding 2-aminoquinazolines **8a–m**. Benzylalcohol **8n** was prepared by the reduction of the ketone **8m**.



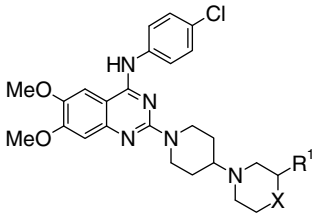
Scheme 2. Reagents and conditions: (i) For **7a–h**, amines, Hunig's base, DMF; For **7i**, 4-chloroaniline, 1-M HCl, EtOH; For **7j**, 4-chlorophenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3 aq., toluene; For **7k**, 4-chlorobenzene amide, NaH, DMF; For **7l**, 4-chlorobenzene sulfoneamide, *t*-BuOK, DMF; For **7m**, 4-chlorobenzaldehyde, 1,3-dimethylimidazolium iodide, NaH, dioxane; (ii) $4R^1$-piperidine, *n*-BuOH, Hunig's base or DBU; (iii) $NaBH_4$, MeOH.

3. Results and discussion

The CCR4 antagonist activities of the synthesized compounds were determined by measuring the degree to which human CCL22-derived [^{35}S]GTPγS was prevented from binding to the receptor. The CCR4-antagonism of these compounds was also confirmed using the chemotaxis assay. The results are summarized in Tables 1–3.

We first explored the substituents at the 4-position of piperidine (Table 1). The 3-hydroxypiperidine analogue **8ia** was slightly less active than the parent compounds **1a**, **1b**, and **2** in the [^{35}S]GTPγS-binding assay, but was a potent chemotactic factor for human cells. *O*-Methylation of the hydroxyl group deteriorated its activity in the [^{35}S]GTPγS-binding and human chemotaxis assays (**8ib**). The results of the 3-(hydroxymethyl)piperidine analogue **8ic** were comparable to those of the parent compounds in the [^{35}S]GTPγS-binding and mouse chemotaxis assays, and also showed more potent activity than the parent compounds in the human chemotaxis assay. *O*-Methylation of the hydroxyl group of **8ic** resulted in the loss of potency in all three assays (**8id**). This indicates that the hydroxyl group at this position is important, especially for the inhibition of chemotaxis. Introduction of oxygen into the piperidine ring resulted in significant loss in all three assays (**8ie**).

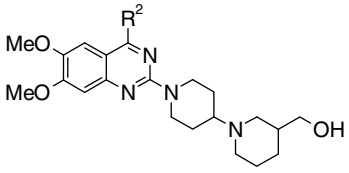
Table 1
CCR4 inhibitory activities of 4-substituted piperidine derivatives

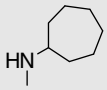
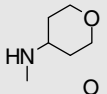
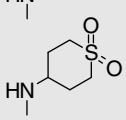
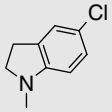


Compound	X	R^1	[^{35}S]GTPγS IC_{50} (μM) Human[a]	Chemotaxis IC_{50} (μM) Human[a]	Chemotaxis IC_{50} (μM) Mouse[a]
8ia	$-CH_2-$	–OH	0.048	0.085	0.43
8ib	$-CH_2-$	–OMe	0.10	0.51	0.42
8ic	$-CH_2-$	$-CH_2OH$	0.019	0.023	0.058
8id	$-CH_2-$	$-CH_2OMe$	0.052	0.064	0.23
8ie	–O–	$-CH_2OH$	0.10	0.62	50% at 1 μM
1a			0.019	0.20	0.13
1b			0.018	0.14	0.039
2			0.024	0.11	0.13

[a] See Section 6.2, pharmacology.

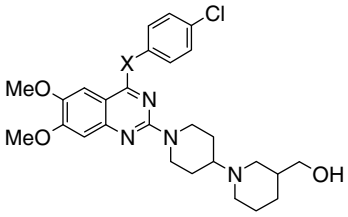
Table 2
CCR4 inhibitory activities of 4-substituted quinazoline derivatives



Compound	R^2	[^{35}S]GTPγS IC_{50} (μM) Human[a]	Chemotaxis IC_{50} (μM) Human[a]	Chemotaxis IC_{50} (μM) Mouse[a]
8e		0.030	0.10	0.019
8f		4% at 1 μM	NT[b]	NT[b]
8g		0% at 1 μM	NT[b]	NT[b]
8h		40% at 1 μM	NT[b]	NT[b]
8ic		0.019	0.023	0.058

[a] See Section 6.2, pharmacology.
[b] Not tested.

Table 3
The effect of the linker between the 4-chlorobenzene and the quinazoline



Compound	X	[^{35}S]GTPγS IC_{50} (μM) Human[a]	Chemotaxis IC_{50} (μM) Human[a]	Chemotaxis IC_{50} (μM) Mouse[a]
8a	$-NHCH_2-$	0.087	0.45	0.14
8b	$-NHCH_2CH_2-$	0.061	43% at 1 μM	0.39
8c	$-N(CH3)CH_2CH_2-$	23% at 1 μM	NT[b]	NT[b]
8d	$-NHCH_2CH_2CH_2-$	0.18	NT[b]	NT[b]
8j	none	>1 μM	NT[b]	NT[b]
8k	–NHCO–	11% at 1 μM	NT[b]	NT[b]
8l	$-NHSO_2-$	5% at 1 μM	NT[b]	NT[b]
8m	–CO–	42% at 1 μM	NT[b]	NT[b]
8n	–CHOH–	7% at 1 μM	NT[b]	NT[b]
8ic	–NH–	0.019	0.023	0.058
2		0.024	0.11	0.13

[a] See Section 6.2, pharmacology.
[b] Not tested.

With the finding of this most promising 3-(hydroxymethyl)-piperidine moiety, the focus of the modification was shifted to the substituent at the 4-position of compound **8ic** (Table 2). First, the cycloheptylamine moieties were introduced to verify that the SAR discovered in a previous report[15] would be applicable. The results obtained with compound **8e** were almost comparable to those with **8ic**, and indicated that the SAR at this position would be similar to that of the derivatives with a 4-(1-pyrrolidinyl)-piperidine moiety at the 2-position of the quinazoline core. The introduction of tetrahydro-*2H*-pyran-4-amine (**8f**) and tetrahydro-*2H*-thiopyran-4-amine 1,1-dioxide (**8g**) resulted in a complete loss of potency. These results clearly showed that the hydrophobic moieties are needed for potent activity. Another more rigid 5-chloroindoline substituent was also introduced (**8h**), but it was not effective.

The effect of the linker between the 4-chlorobenzene and the quinazoline was investigated last (Table 3). One to three methylene linkers were introduced between benzene and nitrogen, but these modifications (**8a**, **8b**, **8d**) did not result in improved potency. *N*-Methylation of the nitrogen atom to the corresponding *N*-methyl derivative (**8c**) led to considerable loss of potency. Removal of the linker (**8j**) or replacement of the nitrogen with an amide (**8k**), sulfonamide (**8l**), ketone (**8m**), or hydroxymethyl (**8n**) all reduced the potency. These findings indicated that an amino, NH linker is necessary for optimal potency.

Before the evaluation of in vivo pharmacology, the metabolic stabilities of **8e** and **8ic** in human liver microsomes were further

Table 4
The intrinsic clearance values of compounds **1a**, **1b**, **2**, **8e**, and **8ic**

Compound	Intrinsic clearance values (ml/min/kg)
1a	6600
1b	17377
2	2532
8e	9449
8ic	5047

Table 5
The inhibitory effects of **1a**, **b**, **2**, **8e**, and **8ic** in vivo

Compound	Oxazolone-induced contact hypersensitivity	
	Dose	% Inhibition of ear swelling[a]
1a	30 mg/kg *s.c.*, sid[b]	39**,d
	100 mg/kg, *p.o.*, bid[c]	8
1b	30 mg/kg, *p.o.*, bid[c]	2
2	30 mg/kg, *s.c.*, bid[c]	43**,d
	30 mg/kg, *p.o.*, bid[c]	2
8e	30 mg/kg, *p.o.*, bid[c]	11
8ic	30 mg/kg, *s.c.*, sid[b]	44**,d
	10 mg/kg, *p.o.*, sid[b]	13
	30 mg/kg, *p.o.*, sid[b]	17
	100 mg/kg, *p.o.*, sid[b]	39**,d

[a] See Section 6.2, pharmacology.
[b] Administered once daily.
[c] Administered twice daily.
[d] $p < 0.01$ versus vehicle, Dunnett's multiple range test.

characterized to verify the effect of the hydroxymethyl piperidine moiety of these compounds on the metabolic factor (Table 4). Interestingly, introduction of the hydroxymethyl group to the piperidine ring improved the clearance value (**8e**, CL_{int} = 9499 mL/h/kg) of the compound **1b** (CL_{int} = 17,377 mL/h/kg). The clearance value of compound **8e** was further improved by replacing the cycloheptyl ring with the 4-chrolobenzene ring (**8ic**, CL_{int} = 5,047 mL/h/kg). These data suggest that the hydroxymethyl group somewhat impedes the metabolism of the piperidine ring. Although the hydroxymethylpiperidine moiety is slightly more vulnerable than the pyrrolidine moiety (**8e** vs **1a** and **8ic** vs **2**), compounds **8ic** and **2** are thought to be metabolically stable enough, and these clearance values are comparable to those of several commercial drugs.[18]

4. In vivo pharmacology

Since several compounds (**1a, 1b, 2, 8e, 8ic**) were identified as potent inhibitors of mouse chemotaxis, their efficacy was evaluated in vivo (murine oxazolone-induced contact hypersensitivity test, Table 5). Compounds **1a**, **2**, and **8ic** were administered subcutaneously to mice previously sensitized to oxazolone. Potent inhibition (39–44%) of ear swelling was observed at the 30 mg/kg level (one or two doses). These results were almost comparable to those of anti-inflammatories (steroids and non-steroidal anti-inflammatory drugs) reported in the literature.[19] Compounds **1a** and **2**, which have a piperidinopyrrolidine moiety at the 2-position of the quinazoline core, were also administered orally, but very little inhibition was observed. Given that these compounds are efficacious when administered subcutaneously, their poor oral bioavailability is probably due to poor intestinal absorption. In contrast, oral administration of compounds **8e** and **8ie**, which have a bipiperidinylmethanol moiety, yielded more favorable results than that of compounds **1a** and **2**, and oral administration of **8ic** at a 100 mg/kg dose was found to be almost as efficacious as the 30 mg/kg subcutaneous dose. These results indicated that the bipiperidinylmethanol moiety had a good effect on the oral bioavailability of the compounds compared to the piperidinopyrrolidine moiety.

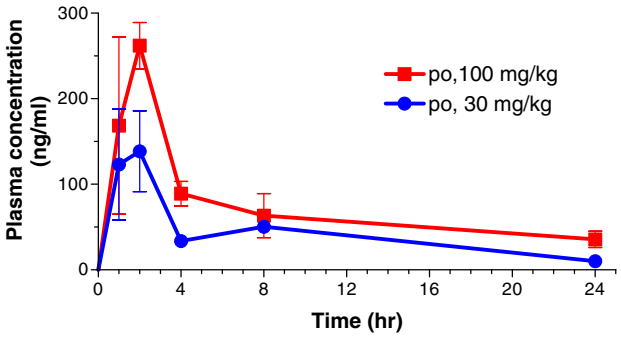


Figure 2. Pharmacokinetic profile of compound **8ic**.

Table 6
Pharmacokinetic parameters of compound **8ic** in plasma after a single oral administration to mice

Parameter	Dose (mg/kg)	
	30	100
T_{max} (h)	2	2
C_{max} (ng/mL)	139	262
$AUC_{0\rightarrow 24h}$ (ng/h/mL)	1014	1749

The plasma concentration of compound **8ic** was also measured after oral administration of 30 mg/kg and 100 mg/kg for PK-PD correlation (Fig. 2 and Table 6). The maximum plasma concentration (C_{max}) reached 139 ng/mL (30 mg/kg dose) and 262 ng/mL (100 mg/kg dose), which are both well above the mouse chemotaxis IC_{50} value (30 ng/mL). A good relationship between the maximum plasma concentration and the rate of ear swelling inhibition was also noted. These results suggested that the level of concentration of the compound obtained by the 100 mg/kg dose is required to obtain a potent activity in the acute dermatitis model.

5. Conclusion

Starting with CCR4 antagonists **1a**, **1b**, and **2** (reported previously), potency was improved by replacing the pyrrolidine moiety of **2** with a 3-(hydroxymethyl)piperidine. The resulting compound (**8ic**) was a strong inhibitor of human (IC_{50} = 23 nM) and mouse (IC_{50} = 58 nM) cell chemotaxis. Also, **8ic** was found to be an orally bioavailable inhibitor, and its oral administration resulted in dose-dependent anti-inflammatory activity in a murine model of acute dermatitis. The potency obtained at the 100 mg/kg dose was almost comparable to those of other anti-inflammatory drugs, including steroids. These results indicated that this quinazoline-based CCR4 antagonist has the potential to treat inflammatory skin diseases. Further efforts to improve the pharmacokinetic parameters and potency related to this series will be reported in due course.

6. Experimental

6.1. Chemistry

In general, reagents and solvents were used as purchased without further purification. Melting points were determined with a Yanaco MP-500D melting point apparatus and left uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer. Chemical shifts were expressed in δ(ppm) values with tetramethylsilane as an internal standard

(NMR description: s, singlet; d, doublet; t, triplet; m, multiplet; and br, broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of the theoretical values.

6.1.1. *tert*-Butyl 3-hydroxy-1,4′-bipiperidine-1′-carboxylate (4a)

3-Hydroxypiperidine (2.02 g, 20 mmol) and 10% palladium on carbon (1.0 g) was added to the solution of *tert*-butyl 4-oxopiperidine-1-carboxylate (3.98 g, 20 mmol) in MeOH (100 ml), and the mixture was stirred at room temperature under 1 atm of hydrogen gas for 18 h, and then filtered through a pad of Celite. The filtrate was concentrated to yield crude *tert*-butyl 3-hydroxy-1,4′-bipiperidine-1′-carboxylate **4a** (5.75 g) as a yellow oil.

6.1.2. *tert*-Butyl 3-methoxy-1,4′-bipiperidine-1′-carboxylate (4b)

NaH (60% in oil, 1.11 g, 27.8 mmol) was added to the solution of crude **4a** (4.15 g) in DMF (70 ml), and the mixture was stirred at room temperature for 30 min. Methyl iodide (1.56 ml, 25.1 mmol) was added to the reaction mixture, and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by adding ice water, and the resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was purified via column chromatography (chloroform/MeOH/NH_4OH) to yield *tert*-butyl 3-methoxy-1,4′-bipiperidine-1′-carboxylate **4b** (1.98 g, 6.63 mmol, 2 steps, 46%) as a yellow oil.

MS (FAB+) *m/z* 299 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.20–1.29 (1H, m), 1.46 (9H, s), 1.69–1.78 (4H, m), 1.92–1.96 (1H, m), 2.13–2.26 (2H, m), 2.40–2.48 (1H, m), 2.66–2.68 (3H, m), 2.93–2.95 (1H, m), 3.00–3.12 (1H, m), 3.25–3.31 (1H, m), 3.36 (3H, s), 3.70–3.83 (1H, m), 4.14 (2H, br).

6.1.3. *tert*-Butyl 3-(hydroxymethyl)-1,4′-bipiperidine-1′-carboxylate (4c)

3-Piperidinemethanol (4.43 g, 38 mmol) and 10% palladium on carbon (700 mg) was added to the solution of *tert*-butyl 4-oxo-1-piperidine carboxylate (7.66 g, 38 mmol) in MeOH (200 ml). The mixture was stirred at room temperature under 1 atm of hydrogen gas for 2 days, and then filtered through a pad of Celite. The filtrate was concentrated in vacuo. The residue was purified via column chromatography (chloroform/MeOH/NH_4OH) to yield *tert*-butyl 3-(hydroxymethyl)-1,4′-bipiperidine-1′-carboxylate **4c** (9.98 g, 81%) as a pale yellow oil.

MS (FAB+) *m/z* 299 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.17–1.26 (1H, m), 1.45 (9H, s), 1.51–1.62 (2H, m), 1.65–1.84 (6H, m), 2.23–2.27 (1H, m), 2.34–2.47 (2H, m), 2.64–2.67 (3H, m), 2.85 (1H, m), 3.54–3.58 (1H, m), 3.67–3.71 (1H, m), 4.14 (2H, s, br).

6.1.4. *tert*-Butyl 3-(methoxymethyl)-1,4′-bipiperidine-1′-carboxylate (4d)

Compound **4d** (1.3 g, 4.2 mmol, 84%) was obtained as a colorless oil from *tert*-butyl 3-(hydroxymethyl)-1,4′-bipiperidine-1′-carboxylate **4c** (1.49 g, 5 mmol) using procedures similar to those described for the synthesis of **4b**.

MS (FAB+) *m/z* 313 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92–1.00 (1H, m), 1.37–1.44 (1H, m), 1.45 (9H, s), 1.51–1.59 (1H, m), 1.61–1.77 (5H, m), 1.84–1.94 (2H, m), 2.11–2.17 (1H, m), 2.37–2.44 (1H, m), 2.63–2.70 (2H, m), 2.79–2.82 (1H, m), 2.92–2.94 (1H, m), 3.18–3.30 (2H, m), 3.31 (3H, s), 4.13 (2H, s, br).

6.1.5. 1,4′-Bipiperidin-3-ol (5a)

4-M HCl ethyl acetate solution (10 ml) was added to the solution of crude **4a** (1.6 g) in ethyl acetate (20 ml), and the resulting mixture was stirred at room temperature for 3 days. The reaction mixture was concentrated in vacuo, and the residue was washed with ethyl acetate to yield 1,4′-bipiperidin-3-ol hydrochloride salt **5a** (973 mg, 3.8 mmol, 2 steps, 68%) as a colorless solid.

MS (FAB+) *m/z* 185 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.27–1.36 (1H, m), 1.58–1.69 (1H, m), 1.84–2.30 (6H, m), 2.55–2.63 (1H, m), 2.81–2.90 (2H, m), 2.99–3.17 (1H, m), 3.22–3.28 (2H, m), 3.38–3.50 (3H, m), 3.98–4.08 (1H, m), 9.14 (1H, br), 9.33 (1H, br), 11.38 (1H, s, br).

Compounds **5b–d** were prepared using procedures similar to those described for the synthesis of **5a**.

6.1.6. 3-Methoxy-1,4′-bipiperidine (5b)

The hydrochloride salt of **5b** (1.38 g, 5.1 mmol, 77%) was obtained as a colorless solid from *tert*-butyl 3-methoxy-1,4′-bipiperidine-1′-carboxylate **4b** (1.98 g).

MS (FAB+) *m/z* 199 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.23–1.1.35 (1H, m), 1.55–1.72 (1H, m), 1.89–2.34 (6H, m), 2.64–2.72 (1H, m), 2.87–2.90 (3H, m), 2.97–3.17 (1H, m), 3.23–3.3.35 (1H, m), 3.31 (3H, s), 3.38–3.49 (5H, m), 3.72–3.77 (1H, m), 9.34–9.37 (1H, br), 9.50 (1H, s, br), 11.50 (1H, s, br).

6.1.7. 1,4′-Bipiperidin-3-ylmethanol (5c)

The hydrochloride salt of **5c** (5.08 g, 94%) was obtained as a pale yellow solid from *tert*-butyl 3-(hydroxymethyl)-1,4′-bipiperidine-1′-carboxylate **4c** (6.23 g).

^1H NMR (400 MHz, CD_3OD) δ: 1.20–1.34 (1H, m), 1.70–1.77 (1H, m), 1.80–2.07 (4H, m), 2.10–2.25 (1H, m), 2.28–2.40 (2H, m), 2.74–2.82 (1H, m), 2.87–2.96 (1H, m), 3.00–3.09 (2H, m), 3.23 (3H, s), 3.27–3.33 (2H, m), 3.45–3.54 (5H, m).

6.1.8. 3-(Methoxymethyl)-1,4′-bipiperidine (5d)

Compound **5d** (662 mg, 3.12 mmol, 75%) was obtained as a pale yellow oil from *tert*-butyl 3-(methoxymethyl)-1,4′-bipiperidine-1′-carboxylate **4d** (1.3 g, 4.2 mmol).

^1H NMR (400 MHz, $CDCl_3$) δ: 1.20–1.34 (1H, m), 1.70–1.77 (1H, m), 1.80–2.07 (4H, m), 2.10–2.25 (1H, m), 2.28–2.40 (2H, m), 2.74–2.82 (1H, m), 2.87–2.96 (1H, m), 3.00–3.09 (2H, m), 3.23 (3H, s), 3.27–3.33 (2H, m), 3.45–3.54 (5H, m).

6.1.9. 2-Chloro-*N*-(4-chlorobenzyl)-6,7-dimethoxyquinazolin-4-amine (7a)

4-Chlorobenzylamine (547 mg, 3.80 mmol) was added to a solution of 2,4-dichloro-6,7-dimethoxyquinazoline **6** (500 mg, 1.93 mmol) in DMF (70 ml). The resulting mixture was stirred at room temperature for 29 h, and then concentrated in vacuo. 1-M NaOH aqueous solution (2 ml) was added to the residue, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was purified via column chromatography (hexane/ethyl acetate) to yield 2-chloro-*N*-(4-chlorobenzyl)-6,7-dimethoxyquinazolin-4-amine **7a** (762 mg, quant.) as a white solid.

MS (ESI+) *m/z* 364 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.88 (3H, s), 3.97 (3H, s), 4.73 (2H, d, J = 5.7 Hz), 7.10 (1H, s), 7.40 (4H, br s), 7.67 (1H, s), 7.95 (1H, s), 8.31 (1H, s), 8.88 (1H, t, J = 5.7 Hz).

Compounds **7b–h** were prepared using procedures similar to those described for the synthesis of **7a**.

6.1.10. 2-Chloro-*N*-[2-(4-chlorophenyl)ethyl]-6,7-dimethoxyquinazolin-4-amine (7b)

Compound **7b** (955 mg, quant.) was obtained as a white amorphous from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (500 mg, 1.93 mmol), 4-chlorophenethylamine (300 mg, 1.93 mmol) and Hunig's base (300 mg, 2.32 mmol).

MS (FAB+) *m/z* 378 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 2.94–2.98 (2H, m), 3.67–3.72 (2H, m), 3.87 (3H, s), 3.89 (3H,s), 7.08 (1H, s), 7.30 (1H, d, J = 8.2 Hz), 7.36 (1H, d, J = 8.2 Hz), 7.59 (1H, s), 7.95 (1H, s), 8.43 (1H, t, J = 5.2 Hz).

6.1.11. 2-Chloro-*N*-[2-(4-chlorophenyl)ethyl]-6,7-dimethoxy-*N*-methylquinazolin-4-amine (7c)

Compound **7c** (239 mg, 32%) was obtained as a grayish solid from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (500 mg, 1.93 mmol), 2-(4-chlorophenyl)-*N*-methylethanamine (327 mg, 1.93 mmol) and Hunig's base (301 mg, 2.32 mmol).

MS (FAB+) *m/z* 392 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.01–3.05 (2H, m), 3.38 (3H, s), 3.86 (3H, s), 3.89–3.90 (2H, m), 3.91 (3H, s), 7.11 (1H, s), 7.31–7.36 (5H, m).

6.1.12. 2-Chloro-*N*-[3-(4-chlorophenyl)propyl]-6,7-dimethoxyquinazolin-4-amine (7d)

Compound **7d** (414 mg, 68%) was obtained as a yellowish solid from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (400 mg, 1.54 mmol), 3-(4-chlorophenyl)propan-1-amine (262 mg, 1.54 mmol) and Hunig's base (240 mg, 1.85 mmol).

MS (FAB+) *m/z* 392 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.92–2.00 (2H, m), 2.63 (2H, t, J = 7.6 Hz) 3.48–3.53 (2H, m), 3.886 (3H, s), 3.889 (3H,s), 7.07 (1H, s), 7.28–7.34 (4H, m), 7.60 (1H, s), 8.32 (1H, t, J = 5.2 Hz).

6.1.13. 2-Chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine (7e)

Compound **7e** (2.15 g, 79%) was obtained as a colorless solid from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (2.11 g, 8.14 mmol) and cycloheptylamine (2.02 g, 17.9 mmol).

MS (ESI+) *m/z* 336 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.44–1.76 (10H, m), 1.92–2.01 (2H, m), 3.88 (3H, s), 3.90 (3H, s), 4.22–4.33 (1H, m), 7.05 (1H, s), 7.66 (1H, s), 7.98 (1H, d, J = 9.5 Hz).

6.1.14. 2-Chloro-6,7-dimethoxy-*N*-(tetrahydro-2*H*-pyran-4-yl)quinazolin-4-amine (7f)

Compound **7f** (114 mg, 0.35 mmol, 23%) was obtained as a yellow solid from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (389 mg, 1.5 mmol), tetrahydro-2H-pyran-4-amine[20] (152 mg, 1.5 mmol) and Hunig's base (0.52 ml).

MS (ESI+) *m/z* 324 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.60–1.70 (2H, m), 2.13–2.16 (2H, m), 3.58–3.65 (2H, m), 3.98 (3H, s), 4.02 (3H, s), 4.04–4.07 (2H, m), 4.49–4.52 (1H, m), 5.33 (1H, s, br), 6.80 (1H, s), 7.15 (1H, s).

6.1.15. 2-Chloro-*N*-(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)-6,7-dimethoxyquinazolin-4-amine (7g)

Compound **7g** (147 mg, 0.40 mmol, 26%) was obtained as a yellow solid from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (389 mg, 1.5 mmol), tetrahydro-*2H*-thiopyran-4-amine 1,1-dioxide[21] (279 mg, 1.5 mmol) and Hunig's base (0.52 ml).

MS (ESI+) *m/z* 372 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ: 2.30–2.39 (2H, m), 2.52–2.55 (2H, m), 3.15–3.19 (2H, m), 3.26–3.32 (2H, m), 3.99 (3H, s), 4.02 (3H, s), 4.63–4.66 (1H, m), 5.87 (1H, s, br), 6.91 (1H, s), 7.16 (1H, s).

6.1.16. 2-Chloro-4-(5-chloro-2,3-dihydro-1*H*-indol-1-yl)-6,7-dimethoxyquinazoline (7h)

Compound **7h** (1.26 g, 3.4 mmol, 49%) was obtained as a white solid from 2,4-dichloro-6,7-dimethoxyquinazoline **6** (1.8 g, 6.8 mmol), 5-chloroindoline (1.04 g, 6.8 mmol) and Hunig's base (1.06 g).

MS (ESI+) *m/z* 376 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.21 (2H, t, J = 8.0 Hz), 3.85 (3H, s), 3.96 (3H, s), 4.52 (2H, t, J = 8.0 Hz), 7.23 (1H, d, J = 8.4 Hz), 7.26 (1H, s), 7.31 (1H, s), 7.40 (1H, s), 7.41 (1H, t, J = 8.4 Hz)

6.1.17. 2-Chloro-*N*-(4-chlorophenyl)-6,7-dimethoxyquinazolin-4-amine (7i)

4-Chloroaniline (2.55 g, 20 mmol) and 1-M HCl aqueous solution (15 ml) was added to the solution of 2,4-dichloro-6,7-dimethoxyquinazoline (5.18 g, 20 mmol) in EtOH (150 ml), and the resulting mixture was stirred at 60 °C for 10 h. The reaction mixture was neutralized with 1-M NaOH aqueous solution, and the resulting precipitate was filtered to yield crude **7i** (6.92 g), which was used in subsequent reaction without further purification.

6.1.18. 2-Chloro-4-(4-chlorophenyl)-6,7-dimethoxyquinazoline (7j)

4-Chlorophenyl boronic acid (810 mg, 5.2 mmol) was added to the solution of 2,4-dichloro-6,7-dimethoxyquinazoline (1.04 g, 4 mmol) in toluene (8 ml), 1-M Na_2CO_3 aqueous solution (4 ml) and $Pd(PPh_3)_4$ (230 mg, 0.2 mmol), and the resulting mixture was stirred under reflux for 6 h. Ethyl acetate and water was added to the reaction mixture. The resulting precipitate was filtered and washed with ethyl acetate to yield crude 2-chloro-4-(4-chlorophenyl)-6,7-dimethoxyquinazoline **7j** (950 mg), which was used in subsequent reaction without further purification.

6.1.19. 4-Chloro-*N*-(2-chloro-6,7-dimethoxyquinazolin-4-yl)-benzamide (7k)

NaH (101 mg, 2.31 mmol) was added to the solution of 4-chlorobenzamide (300 mg, 1.93 mmol) in DMF (20 ml), and the mixture was stirred at room temperature for 30 min. The solution of 2,4-dichloro-6,7-dimethoxyquinazoline (500 mg, 1.93 mmol) in DMF (20 ml) was added to the reaction mixture, and the resulting mixture was stirred at 80 °C for 8 h. The reaction mixture was concentrated in vacuo. Water was added to the residue, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was washed with chloroform–acetonitrile solution to yield 4-chloro-*N*-(2-chloro-6,7-dimethoxyquinazolin-4-yl)benzamide **7k** (238 mg, 0.63 mmol, 33%) as a white solid.

MS (ESI+) *m/z* 378 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.83 (3H, s), 3.88 (3H, s), 6.98 (1H, s), 7.56 (1H, br s), 7.50 (2H, d, J = 8.8 Hz), 7.97(2H, d, J = 8.8 Hz).

6.1.20. 4-Chloro-*N*-(2-chloro-6,7-dimethoxyquinazolin-4-yl)-benzenesulfonamide (7l)

Potassium *tert*-butoxide (238 mg, 2.12 mmol) was added to the solution of 4-chlorobenzesulfonamide (370 mg, 1.93 mmol) in DMF (20 ml), and the mixture was stirred at room temperature for 30 min. The solution of 2,4-dichloro-6,7-dimethoxyquinazoline (500 mg, 1.93 mmol) in DMF (10 ml) was added to the reaction mixture, and the resulting mixture was stirred at 80 °C for 20 h. The reaction mixture was concentrated in vacuo. Water was added to the residue, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was washed with chloroform–acetonitrile solution to yield 4-chloro-*N*-(2-chloro-6,7-dimethoxyquinazolin-4-yl)benzenesulfonamide **7l** (154 mg, 0.37 mmol, 19%) as a white solid.

MS (ESI+) *m/z* 414 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.86 (3H, s), 3.87 (3H, s), 6.98 (1H, s), 7.50 (1H, br s), 7.50 (2H, d, J = 8.8 Hz), 7.97 (2H, d, J = 8.8 Hz).

6.1.21. (2-Chloro-6,7-dimethoxyquinazolin-4-yl)(4-chlorophenyl)methanone (7m)

4-Chlorobenzaldehyde (1.2 g, 8.8 mmol) was added to the solution of 2,4-dichloro-6,7-dimethoxyquinazoline (2.08 g, 8.0 mmol) in dioxane (50 ml), 1,3-dimethylimidazolium iodide (448 mg, 2.0 mmol) and NaH (55% in oil, 384 mg, 8.8 mmol), and the mixture was stirred under reflux for 3 h. The reaction mixture was

poured into ice water, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was recrystallized from EtOH to yield (2-chloro-6,7-dimethoxyquinazolin-4-yl)(4-chlorophenyl)methanone **7m** (1.83 g, 5.0 mmol, 63%) as a yellow solid.

MS (ESI+) *m/z* 363 $[M+H]^+$, ^1H NMR (400 MHz, DMSO-d_6) δ: 3.86 (3H, s), 4.04 (3H, s), 7.32 (1H, s), 7.52 (1H, s), 7.67 (2H, d, *J* = 8.4 Hz), 7.98 (2H, d, *J* = 8.4 Hz).

6.1.22. (1′-{4-[(4-Chlorobenzyl)amino]-6,7-dimethoxyquinazolin-2-yl}-1,4′-bipiperidin-3-yl)methanol (8a)

1,4′-Bipiperidin-3-ylmethanol dihydrochloride **5c** (476 mg, 1.75 mmol) and Hunig's base (910 mg, 7.02 mmol) was added to a solution of the 2-chloro-*N*-(4-chlorobenzyl)-6,7-dimethoxyquinazolin-4-amine **7a** (639 mg) in *n*-BuOH (20 ml), and the resulting mixture was stirred at 110 °C for 1 day. The reaction mixture was cooled to room temperature and concentrated in vacuo, and the residue was purified via column chromatography (chloroform/MeOH/NH_4OH) to yield (1′-{4-[(4-chlorobenzyl)amino]-6,7-dimethoxyquinazolin-2-yl}-1,4′-bipiperidin-3-yl)methanol **8a** (561 mg, 1.1 mmol, 61%) as a wine-red amorphous. The residue was treated with 4-M HCl ethyl acetate solution (0.6 ml), and the precipitate was washed with ethanol to yield the hydrochloride salt of **8a** (259 mg, 28%).

Mp (dec.) 216–226 °C (EtOH); MS (FAB+) *m/z* 526 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.11–1.20 (1H, m), 1.69–1.71 (3H, m), 1.81–1.84 (1H, m), 1.89–1.96 (1H, m), 2.04–2.22 (3H, m), 2.49–2.51 (1H, m), 2.55–2.63 (1H, m), 3.06–3.11 (2H, m), 3.23–3.39 (4H, m), 3.48–3.51 (1H, m), 3.88 (3H, s), 3.89 (3H, s), 4.75–4.76 (2H, m), 4.81 (2H, br s), 7.42 (2H, d, *J* = 8.6 Hz), 7.50 (2H, d, *J* = 8.6 Hz), 7.65 (1H, s), 7.98 (1H, s), 10.24 (1H, s), 10.82 (1H, s), 12.54 (1H, s).

Anal. calcd. for $C_{28}H_{36}ClN_5O_3$ 2HCl 0.8H_2O: C, 54.83, H, 6.51, N, 11.42, Cl, 17.34. Found: C, 54.67, H, 6.48, N, 11.47, Cl, 17.57.

Compounds **8b–m** were prepared using procedures similar to those described for the synthesis of **8a**.

6.1.23. 1′-(4-{[2-(4-Chlorophenyl)ethyl]amino}-6,7-dimethoxyquinazolin-2-yl)-1,4′-bipiperidin-3-yl]methanol (8b)

Compound **8b** (500 mg, 1.01 mmol, 74%) was obtained as a colorless amorphous from 2-chloro-*N*-[2-(4-chlorophenyl)ethyl]-6,7-dimethoxyquinazolin-4-amine **7b** (522 mg, 1.38 mmol), 1,4′-bipiperidin-3-ylmethanol dihydrochloride **5c** (374 mg, 1.38 mmol) and Hunig's base (716 mg). This compound **8b** was treated with 4-M HCl ethyl acetate solution (0.6 ml), and the precipitate was washed with ethanol to yield the hydrochloride salt of **8b** (343 mg, 60%) as a colorless solid.

Mp (dec.) 220–221 °C (EtOH); MS (FAB-) *m/z* 538 $[M-H]^-$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.12–1.20 (1H, m), 1.69–2.04 (5H, m), 2.14 (1H, m), 2.28–2.30 (2H, m), 2.61–2.70 (1H, m), 2.84–2.87 (1H, m), 2.98–3.01 (2H, m), 3.11–3.18 (2H, m), 3.24–3.45 (4H, m), 3.49–3.60 (1H, m), 3.72–3.82 (2H, m), 3.87 (3H, s), 3.88 (3H, s), 4.80–4.90 (2H, br d), 7.30 (2H,d, *J* = 8.3 Hz), 7.37 (2H, d, *J* = 8.3 Hz), 7.66 (1H, s), 7.85 (1H, s), 9.65 (1H, s), 10.85 (1H, s), 12.50 (1H, s).

Anal. calcd. for $C_{29}H_{38}ClN_5O_3$ 2HCl 0.8H_2O: C, 55.51, H, 6.68, N, 11.16, Cl, 16.95. Found: C, 55.57, H, 6.63, N, 11.19, Cl, 16.92.

6.1.24. [1′-(4-{[2-(4-Chlorophenyl)ethyl](methyl)amino}-6,7-dimethoxyquinazolin-2-yl)-1,4′-bipiperidin-3-yl]methanol dihydrochloride (8c)

1,4′-Bipiperidin-3-ylmethanol dihydrochloride **5c** (158 mg, 0.58 mmol) and potassium carbonate (242 mg) was added to a solution of the 2-chloro-*N*-[2-(4-chlorophenyl)ethyl]-6,7-dimethoxy-*N*-methylquinazolin-4-amine **7c** (229 mg, 0.58 mmol) in *n*-BuOH (20 ml), and the resulting mixture was stirred at 110 °C for 1 day. The reaction mixture was cooled to room temperature and concentrated in vacuo, and the residue was purified via column chromatography (chloroform/MeOH/NH_4OH) to yield **8c** (58 mg, 0.10 mmol, 18%). This compound **8c** was treated with 4-M HCl ethyl acetate solution, and the precipitate was washed with isopropanol and ethyl acetate to yield the hydrochloride salt of **8c** (46 mg, 70%) as a white solid.

MS (FAB+) *m/z* 554 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.07–1.24 (1H, m), 1.60–2.05 (5H, m), 2.08–2.35 (3H, m), 2.58–2.70 (1H, m), 2.79–2.92 (1H, m), 3.00–3.07 (2H, m), 3.09–3.20 (2H, m), 3.23–3.45 (4H, m), 3.56 (3H, s), 3.85 (3H, s), 3.91 (3H, s), 3.95–4.05 (2H, m), 4.74–4.90 (2H, br d), 7.30–7.45 (5H, s), 7.78 (1H, s), 10.90 (1H, s), 12.68 (1H, s).

Anal. calcd. for $C_{30}H_{40}ClN_5O_3$ 2HCl 2.2H_2O 0.4NH_4Cl: C, 52.37, H, 7.03, N, 10.99, Cl, 17.52. Found: C, 52.49, H, 6.73, N, 11.20, Cl, 17.73.

6.1.25. [1′-(4-{[3-(4-Chlorophenyl)propyl]amino}-6,7-dimethoxyquinazolin-2-yl)-1,4′-bipiperidin-3-yl]methanol (8d)

Compound **8d** (652 mg, quant.) was obtained as a colorless solid from 2-chloro-*N*-[3-(4-chlorophenyl)propyl]-6,7-dimethoxyquinazolin-4-amine **7d** (332 mg, 0.85 mmol), 1,4′-bipiperidin-3-ylmethanol dihydrochloride **5c** (230 mg, 0.85 mmol) and DBU (387 mg). This compound **8d** was treated with 4-M HCl ethyl acetate solution, and the precipitate was washed with isopropanol, ethanol and methanol to yield the hydrochloride salt of **8d** (211 mg, 33%) as a white solid.

MS (FAB+) *m/z* 554 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.10–1.23 (1H, m), 1.60–1.83 (4H, m), 1.83–2.03 (3H, m), 2.09–2.30 (3H, m), 2.58–2.66 (1H, m), 2.66–2.72 (2H, m), 2.77–2.88 (1H, m), 3.00–3.15 (2H, m), 3.23–3.44 (4H, m), 3.48–3.60 (3H, m), 3.87 (6H, s), 4.68–4.85 (2H, br d), 7.28 (2H,d, *J* = 8.4 Hz), 7.35 (2H, d, *J* = 8.4 Hz), 7.64 (1H, s), 7.87 (1H, s), 9.57 (1H, s), 10.87 (1H, s), 12.44 (1H, s).

Anal. calcd. for $C_{30}H_{40}ClN_5O_3$ 2HCl 1.5H_2O 0.3C_3H_8O: C, 55.22, H, 7.11, N, 10.42, Cl, 15.82. Found: C, 55.34, H, 7.20, N, 10.50, Cl, 15.82.

6.1.26. {1′-[4-(Cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]-1,4′-bipiperidin-3-yl}methanol (8e)

Compound **8e** (370 mg, 0.74 mmol, 27%) was obtained as a yellow solid from 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **7e** (940 mg, 2.8 mmol) and 1,4′-bipiperidin-3-ylmethanol dihydrochloride **5c** (1.11 g, 5.6 mmol). This compound **8e** was treated with 4-M HCl ethyl acetate solution, and the precipitate was washed with ethyl acetate to yield the hydrochloride salt of **8e** (300 mg) as a colorless solid.

Mp (dec.) 197–199 °C (AcOEt); MS (FAB+) *m/z* 498 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.08–1.22 (1H, s), 1.44–1.86 (14H, m), 1.90–2.04 (3H, m), 2.07–2.20 (1H, m), 2.22–2.34 (2H, m), 2.57–2.69 (1H, m), 2.78–2.92 (1H, m), 3.06–3.20 (2H, m), 3.22–3.44 (4H, m), 3.48–3.60 (1H, m), 3.87 (3H, s), 3.89 (3H, s), 4.24–4.34 (1H, m), 4.29 (1H, br), 4.80–4.90 (2H, br), 7.65 (1H, s), 7.89 (1H, s), 9.01 (1H, d, *J* = 6.0 Hz), 10.86 (1H, s), 12.45 (1H, s).

Anal. calcd. for $C_{28}H_{43}N_5O_3$ 2HCl 1.5H_2O: C, 56.27, H, 8.10, N, 11.72, Cl, 11.86. Found: C, 56.31, H, 8.23, N, 11.83, Cl, 12.07.

6.1.27. {1′-[6,7-Dimethoxy-4-(tetrahydro-2*H*-pyran-4-ylamino)-quinazolin-2-yl]-1,4′-bipiperidin-3-yl}methanol (8f)

Compound **8f** (90 mg, 0.19 mmol, 53%) was obtained as a yellow solid from 2-chloro-6,7-dimethoxy-*N*-(tetrahydro-*2H*-pyran-4-yl)quinazolin-4-amine **7f** (112 mg, 0.35 mmol), 1,4′-bipiperidin-3-ylmethanol **5c** (99 mg, 0.38 mmol) and DBU (0.19 ml). This compound **8f** was treated with 4-M HCl dioxane solution, and the precipitate was washed with acetonitrile to yield the hydrochloride salt of **8f** (50 mg, 45%) as a brown amorphous solid.

MS (FAB+) *m/z* 486 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.08–1.24 (1H, s), 1.63–1.98 (9H, m), 2.08–2.21 (1H, br, s), 2.23–2.34 (2H, m), 2.57–2.70 (1H, m), 2.78–2.92 (1H, m), 3.09–3.20 (2H, m), 3.22–3.60 (7H, m), 3.88 (3H, s), 3.89 (3H, s), 3.93–4.01 (2H, m), 4.32–4.46 (1H, m), 4.78–4.92 (2H, br), 7.66 (1H, s), 7.93 (1H, s), 9.12 (1H, d, *J* = 7.2 Hz), 10.86 (1H, s), 12.45 (1H, s).

Anal. calcd. for $C_{28}H_{43}N_5O_3$ 2HCl 2.6H_2O: C, 51.58, H, 7.69, N, 11.57, Cl, 11.71. Found: C, 51.58, H, 7.82, N, 11.74, Cl, 11.71.

6.1.28. (1′-{4-[(1,1-Dioxidotetrahydro-*2H*-thiopyran-4-yl)-amino]-6,7-dimethoxyquinazolin-2-yl}-1,4′-bipiperidin-3-yl)-methanol (8g)

Compound **8g** (125 mg, 0.23 mmol, 60%) was obtained as a yellow solid from 2-chloro-*N*-(1,1-dioxidotetrahydro-*2H*-thiopyran-4-yl)-6,7-dimethoxyquinazolin-4-amine **7g** (145 mg, 0.39 mmol), 1,4′-bipiperidin-3-ylmethanol **5c** (112 mg, 0.43 mmol) and DBU (0.20 ml). This compound **8g** was treated with 4-M HCl dioxane solution, and the precipitate was washed with acetonitrile to yield the hydrochloride salt of **8g** (115 mg, 75%) as a beige solid.

MS (FAB+) *m/z* 534 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.08–1.24 (1H, s), 1.65–2.00 (5H, m), 2.08–2.40 (7H, m), 2.58–2.70 (1H, m), 2.75–2.92 (1H, m), 3.07–3.62 (11H, m), 3.88 (6H, s), 4.58–4.68 (1H, m), 4.84–5.00 (2H, br), 7.65 (1H, s), 7.85 (1H, s), 9.13 (1H, d, *J* = 7.6 Hz), 10.84 (1H, s), 12.51 (1H, s).

Anal. calcd. for $C_{26}H_{39}N_5O_5S$ 2.1HCl 2.6H_2O: C, 47.52, H, 7.10, N, 10.67, Cl, 11.33, S, 4.88. Found: C, 47.77, H, 7.41, N, 10.88, Cl, 11.43, S, 4.53.

6.1.29. {1′-[4-(5-Chloro-2,3-dihydro-1*H*-indol-1-yl)-6,7-dimethoxyquinazolin-2-yl]-1,4′-bipiperidin-3-yl}methanol (8h)

8h (476 mg, 0.88 mmol, 71%) was obtained from 2-chloro-4-(5-chloro-2,3-dihydro-1*H*-indol-1-yl)-6,7-dimethoxyquinazoline **7h** (468 mg, 1.2 mmol), 1,4′-bipiperidin-3-ylmethanol dihydrochloride **5c** (337 mg, 1.2 mmol) and DBU (568 mg). This compound **8h** (438 mg) was treated with 4-M HCl dioxane solution, and the precipitate was washed with methanol to yield the hydrochloride salt of **8h** (176 mg, 35%) as a white solid.

MS (FAB+) *m/z* 538 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.08–1.22 (1H, m), 1.62–2.00 (5H, m), 2.04–2.18 (1H, m), 2.19–2.32 (2H, m), 2.58–2.70 (1H, m), 2.79–2.90 (1H, m), 3.07–3.60 (9H, m), 3.86 (3H, s), 3.94 (3H, s), 4.55–4.90 (4H, m), 7.25–7.50 (3H, m), 7.72 (2H, br, s), 10.70 (1H, br, s), 13.05 (1H, br, s).

Anal. calcd. for $C_{29}H_{36}ClN_5O_3$ 2HCl 3H_2O: C, 52.37, H, 6.67, N, 10.53, Cl, 15.99. Found: C, 52.50, H, 6.69, N, 10.45, Cl, 15.75.

6.1.30. 1′-{4-[(4-Chlorophenyl)amino]-6,7-dimethoxyquinazolin-2-yl}-1,4′-bipiperidin-3-ol (8ia)

Compound **8ia** (329 mg, 0.66 mmol, 66%) was obtained as a brown solid from crude 2-chloro-*N*-(4-chlorophenyl)-6,7-dimethoxyquinazolin-4-amine hydrochloride **7i** (350 mg) and 1,4′-bipiperidin-3-ol dihydrochloride **5a** (257 mg, 1.0 mmol). This compound **8ia** (325 mg) was treated with 4-M HCl ethyl acetate solution, and the precipitate was recrystallized from ethanol to yield the hydrochloride salt of **8ia** (254 mg, 68%) as a colorless solid.

Mp (dec.) 250–260 °C (EtOH); MS (FAB+) *m/z* 498 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.24–2.30 (8H, m), 2.52–2.87 (1H, m), 3.00–3.20 (3H, m), 3.40–3.55 (3H, m), 3.66–3.76 (1H, m), 3.89 (3H, s), 3.94 (3H, s), 4.68–4.88 (2H, br), 7.52 (2H, d, *J* = 8.8 Hz), 7.73–7.80 (3H, m), 8.18–8.28 (1H, br), 11.11 (2H, br).

Anal. calcd. for $C_{26}H_{32}ClN_5O_3$ 2.1HCl H_2O: C, 52.70, H, 6.14, N, 11.82, Cl, 18.55. Found: C, 52.88, H, 6.34, N, 11.97, Cl, 18.31.

6.1.31. *N*-(4-chlorophenyl)-6,7-dimethoxy-2-(3-methoxy-1,4′-bipiperidin-1′-yl)quinazolin-4-amine (8ib)

Compound **8ib** (452 mg, 0.88 mmol, 88%) was obtained as a brown solid from crude 2-chloro-*N*-(4-chlorophenyl)-6,7-dimethoxyquinazolin-4-amine hydrochloride **7i** (387 mg), 3-methoxy-1,4′-bipiperidine dihydrochloride **5b** (271 mg, 1.0 mmol) and DBU (609 mg). This compound **8ib** (450 mg) was treated with 4-M HCl ethyl acetate solution, and the precipitate was recrystallized from ethanol to yield the hydrochloride salt of **8ib** (243 mg, 47%) as a colorless solid.

Mp (dec.) 213–223 °C (EtOH); MS (FAB+) *m/z* 512 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.23–2.30 (8H, m), 2.58–2.70 (1H, m), 2.90–3.18 (3H, m), 3.30 (3H, s), 3.40–3.55 (3H, m), 3.66–3.76 (1H, m), 3.91 (3H, s), 3.94 (3H, s), 4.69–4.85 (2H, br), 7.50–7.56 (2H, m), 7.71–7.78 (3H, m), 8.19 (1H, s), 11.02–11.20 (2H, br).

Anal. calcd. for $C_{27}H_{34}ClN_5O_3$ 2HCl 2H_2O: C, 52.22, H, 6.49, N, 11.28, Cl, 17.13. Found: C, 52.14, H, 6.56, N, 11.30, Cl, 17.02.

6.1.32. (1′-{4-[(4-Chlorophenyl)amino]-6,7-dimethoxyquinazolin-2-yl}-1,4′-bipiperidin-3-yl)methanol (8ic)

Compound **8ic** (390 mg, 66%) was obtained as a colorless amorphous from 2-chloro-*N*-(4-chlorophenyl)-6,7-dimethoxyquinazolin-4-amine hydrochloride **7i** (550 mg), 1,4′-bipiperidin-3-ylmethanol **5c** (230 mg, 1.16 mmol) and Hunig's base (220 mg, 1.74 mmol). This compound **8ic** was treated with 4-M HCl ethyl acetate solution, and the precipitate was recrystallized from Et_2O to yield the hydrochloride salt of **8ic** (330 mg, 0.56 mmol, 74%) as a colorless solid.

Mp (dec.) 221–223 °C (Et_2O); MS (FAB+) *m/z* 512 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.10–1.22 (1H, s), 1.60–2.00 (5H, m), 2.05–2.30 (3H, m), 2.58–2.69 (1H, m), 2.78–2.91 (1H, m), 3.04–3.18 (2H, m), 3.22–3.58 (5H, m), 3.90 (3H, s), 3.94 (3H, s), 4.68–4.84 (2H, br), 7.53 (2H, d, *J* = 8.8 Hz), 7.70–7.78 (3H, m), 8.19 (1H, s), 10.78 (1H, s), 11.06 (1H, s), 12.86 (1H, s).

Anal. calcd. for $C_{27}H_{34}N_5O_3Cl$ 2HCl 2H_2O: C, 52.22, H, 6.49, N, 11.28, Cl, 17.13. Found: C, 52.38, H, 6.50, N, 11.23, Cl, 16.75.

6.1.33. *N*-(4-Chlorophenyl)-6,7-dimethoxy-2-[3-(methoxymethyl)-1,4′-bipiperidin-1′-yl]quinazolin-4-amine (8id)

Compound **8id** (525 mg, 77%) was obtained as a yellow oil from crude 2-chloro-*N*-(4-chlorophenyl)-6,7-dimethoxyquinazolin-4-amine hydrochloride **7i** (452 mg), 3-(methoxymethyl)-1,4′-bipiperidine **5d** (328 mg, 1.54 mmol) and Hunig's base (268 μl, 1.54 mmol). This compound **8id** was treated with 4-M HCl dioxane solution, and the precipitate was recrystallized from ethanol to yield the hydrochloride salt of **8id** (460 mg, 78%) as a white solid.

Mp (dec.) 229–230 °C (EtOH); MS (FAB+) *m/z* 526 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.12–1.25 (1H, s), 1.64–2.01 (5H, m), 2.17–2.39 (3H, m), 2.63–2.74 (1H, m), 2.79–2.91 (1H, m), 3.06–3.21 (2H, m), 3.23 (3H, s), 3.32–3.58 (5H, m), 3.90 (3H, s), 3.94 (3H, s), 4.70–4.83 (2H, m), 7.52 (2H, d, *J* = 8.4 Hz), 7.72–7.80 (3H, m), 8.20 (1H, s), 10.9 (1H, s), 11.1 (1H, s), 12.9 (1H, s).

Anal. calcd. for $C_{28}H_{36}N_5O_3$ 2HCl H_2O: C, 54.51, H, 6.53, N, 11.35, Cl, 17.24. Found: C, 54.31, H, 6.16, N, 11.29, Cl, 17.17.

6.1.34. [4-(1-{4-[(4-Chlorophenyl)amino]-6,7-dimethoxyquinazolin-2-yl}piperidin-4-yl)morpholin-2-yl]methanol (8ie)

Crude (4-piperidin-4-ylmorpholin-2-yl)methanol **5e** (1.64 g) was obtained as a colorless solid from *tert*-butyl 4-oxopiperidine-1-carboxylate (940 mg, 4.7 mmol) and morpholin-2-ylmethanol (550 mg, 4.7 mmol) using procedures similar to those described for the syntheses of **4a** and **5a**. The hydrochloride salt of **8ie** (59 mg, 9%) was obtained as a yellow solid from crude 2-chloro-*N*-(4-chlorophenyl)-6,7-dimethoxyquinazolin-4-amine hydrochloride **7i** (387 mg), crude (4-piperidin-4-ylmorpholin-2-yl)methanol dihydrochloride salt **5e** (410 mg) and DBU (0.45 ml, 3 mmol) using procedures similar to those described for the synthesis of **8id**.

MS (FAB+) *m/z* 514 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.76–1.84 (2H, m), 2.23–2.32 (2H, m), 2.78–2.89 (1H, m), 2.96–3.18 (3H, m), 3.24–3.60 (6H, m), 3.91 (3H, s), 3.94 (3H, s),

3.95–4.05 (2H, m), 4.67–4.78 (2H, m), 7.54 (2H, d, J = 8.8 Hz), 7.70 (1H, s), 7.73 (2H, d, J = 6.8 Hz), 8.17 (1H, s), 11.03 (1H, s), 11.65 (1H, s), 12.79 (1H, s).

Anal. calcd. for $C_{26}H_{32}ClN_5O_4$ 2HCl $3H_2O$: C, 48.72, H, 6.29, N, 10.93, Cl, 16.59. Found: C, 48.92, H, 6.24, N, 11.06, Cl, 16.58.

6.1.35. {1′-[4-(4-Chlorophenyl)-6,7-dimethoxyquinazolin-2-yl]-1,4′-bipiperidin-3-yl}methanol (8j)

Compound **8j** (390 mg, 0.78 mmol, 68%) was obtained as a yellow solid from 2-chloro-4-(4-chlorophenyl)-6,7-dimethoxyquinazoline **7j** (390 mg, 1.16 mmol), 1,4′-bipiperidin-3-ylmethanol **5c** (380 mg, 1.4 mmol) and DBU (880 mg, 5.80 mmol). This compound **8j** was treated with 4-M HCl ethyl acetate solution, and the precipitate was washed with Et_2O to yield the hydrochloride salt of **8j** (390 mg, 88%) as a yellowish solid.

Mp (dec.) 171–172 °C (Et_2O); MS (FAB+) m/z 497 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.06–1.22 (1H, s), 1.61–2.00 (5H, m), 2.05–2.30 (3H, m), 2.58–2.70 (1H, m), 2.78–2.91 (1H, m), 3.05–3.15 (2H, m), 3.21–3.75 (5H, m), 3.96 (6H, s), 4.96–5.12 (2H, br), 7.13 (1H, s), 7.56 (1H, s), 7.69 (2H, d, J = 8.3 Hz), 7.87 (2H, d, J = 8.3 Hz), 10.64 (1H, s).

Anal. calcd. for $C_{27}H_{33}N_4O_3Cl$ 2HCl $2.5H_2O$: C, 52.73, H, 6.56, N, 9.11, Cl, 17.29. Found: C, 52.73, H, 6.69, N, 9.15, Cl, 17.45.

6.1.36. 4-chloro-*N*-{2-[3-(hydroxymethyl)-1,4′-bipiperidin-1′-yl]-6,7-dimethoxyquinazolin-4-yl}benzamide (8k)

1,4′-Bipiperidin-3-ylmethanol dihydrochloride **5c** (136 mg, 0.50 mmol) and Hunig's base (196 mg) was added to a solution of the 4-chloro-*N*-(2-chloro-6,7-dimethoxyquinazolin-4-yl) benzamide **7k** (190 mg, 0.50 mmol) in *n*-BuOH (20 ml), and the resulting mixture was stirred at 110 °C for 1 day. The reaction mixture was cooled to room temperature and concentrated in vacuo, and the residue was purified via column chromatography (chloroform/MeOH/NH_4OH) to yield **8k** (83 mg, 0.15 mmol, 31%) as a red amorphous. This compound **8k** was treated with 4-M HCl ethyl acetate solution, and the precipitate was washed with isopropanol to yield the hydrochloride salt of **8k** (45 mg, 48%) as a white solid.

MS (FAB+) m/z 540 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.12–1.20 (1H, m), 1.68–1.92 (6H, m), 2.07 (2H, m), 2.73–2.90 (2H, m), 3.23–3.28 (2H, m), 3.35–3.75 (5H, m), 3.88 (3H, s), 3.93 (3H, s), 4.81 (2H, br d), 7.59 (1H, s), 7.61 (2H, d, J = 8.3 Hz), 7.75 (1H, s), 7.86 (2H, d, J = 8.3 Hz), 10.44 (1H, s), 11.41 (1H, s), 12.35 (1H, s).

Anal. calcd. for $C_{28}H_{34}ClN_5O_4$ 1.9HCl $3.6H_2O$ $0.1NH_4Cl$: C, 49.49, H, 6.45, N, 10.51, Cl, 15.65. Found: C, 49.09, H, 6.19, N, 10.82, Cl, 15.36.

6.1.37. 4-Chloro-*N*-{2-[3-(hydroxymethyl)-1,4′-bipiperidin-1′-yl]-6,7-dimethoxyquinazolin-4-yl}benzenesulfonamide (8l)

Compound **8l** (102 mg, 0.18 mmol, 54%) was obtained as a brown amorphous from 4-chloro-*N*-(2-chloro-6,7-dimethoxyquinazolin-4-yl)benzenesulfonamide **7l** (137 mg, 0.33 mmol), 1,4′-bipiperidin-3-ylmethanol dihydrochloride **5c** (90 mg, 0.33 mmol) and Hunig's base (129 mg). This compound **8l** was treated with 4-M HCl ethyl acetate solution, and the precipitate was washed with methanol to yield the hydrochloride salt of **8l** (27 mg, 20%) as a white solid.

MS (FAB+) m/z 576 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.14–1.16 (1H, m), 1.41 (2H, br s), 1.70–1.73 (1H, m), 1.86 (2H, br s), 2.09–2.11 (3H, m), 2.51–2.61 (1H, m), 2.72–2.82 (1H, m), 2.87–3.00 (2H, m), 3.20–3.52 (5H, m), 3.87 (6H, s), 4.49 (2H, d, J = 12 Hz), 7.39 (1H, s), 7.56 (1H, s), 7.60 (2H, d, J = 8.6 Hz), 7.83 (2H, d, J = 8.6 Hz), 10.49 (1H, s).

Anal. calcd. for $C_{27}H_{34}ClN_5O_5S$ 1.9HCl $2.2H_2O$: C, 47.34, H, 5.93, N, 10.22, Cl, 15.01, S, 4.68. Found: C, 47.44, H, 6.13, N, 9.91, Cl, 14.84, S, 4.53.

6.1.38. (4-Chlorophenyl){2-[3-(hydroxymethyl)-1,4′-bipiperidin-1′-yl]-6,7-dimethoxyquinazolin-4-yl}methanone (8m)

Compound **8m** (321 mg, 0.61 mmol, 67%) was obtained as a orange oil from (2-chloro-6,7-dimethoxyquinazolin-4-yl)(4-chlorophenyl) methanone **7m** (331 mg, 0.91 mmol), 1,4′-bipiperidin-3-ylmethanol **5c** (297 mg, 1.1 mmol) and Hunig's base (0.57 ml, 3.27 mmol). This compound **8m** was treated with 4-M HCl ethyl acetate solution, and the precipitate was recrystallized from isopropanol to yield the hydrochloride salt of **8m** (162 mg, 44%) as a yellow solid.

Mp (dec.) 180–181 °C (*i*PrOH); MS (FAB+) m/z 525 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.06–1.20 (1H, s), 1.60–1.95 (5H, m), 2.05–2.25 (3H, m), 2.58–2.68 (1H, m), 2.76–2.88 (1H, m), 2.89–3.04 (2H, m), 3.22–3.29 (1H, m), 3.32–3.55 (4H, m), 3.96 (6H, s), 4.72–4.90 (2H, br), 7.06 (1H, s), 7.19 (1H, s), 7.67 (2H, d, J = 8.4 Hz), 7.98 (2H, d, J = 8.8 Hz), 10.47 (1H, br).

Anal. calcd. for $C_{28}H_{33}ClN_4O_4$ 2HCl $0.8H_2O$: C, 54.92, H, 6.02, N, 9.15, Cl, 17.37. Found: C, 55.03, H, 6.03, N, 9.08, Cl, 17.23.

6.1.39. (4-Chlorophenyl){2-[3-(hydroxymethyl)-1,4′-bipiperidin-1′-yl]-6,7-dimethoxyquinazolin-4-yl}methanol (8n)

$NaBH_4$ (70 mg, 1.85 mmol) was added to the solution of (4-chlorophenyl){2-[3-(hydroxymethyl)-1,4′-bipiperidin-1′-yl]-6,7-dimethoxyquinazolin-4-yl}methadone **8m** (807 mg, 1.54 mmol) in MeOH (15 ml), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with water, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was washed with Et_2O to yield (4-chlorophenyl){2-[3-(hydroxymethyl)-1,4′-bipiperidin-1′-yl]-6,7-dimethoxyquinazolin-4-yl}methanol **8n** (663 mg, 1.26 mmol, 82%) as a green solid. This compound **8n** (221 mg) was treated with 4-M HCl ethyl acetate solution, and the precipitate was recrystallized from isopropanol to yield the hydrochloride salt of **8n** (127 mg, 0.21 mmol, 53%) as a yellow solid.

Mp (dec.) 204–205 °C (*i*PrOH); MS (FAB+) m/z 527 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.10–1.22 (1H, s), 1.63–2.00 (5H, m), 2.07–2.31 (3H, m), 2.58–2.70 (1H, m), 2.80–2.87 (1H, m), 3.02–3.14 (2H, m), 3.22–3.29 (1H, m), 3.34–3.45 (3H, m), 3.47–3.58 (1H, m), 3.87 (3H, s), 3.90 (3H, s), 5.00–5.12 (2H, br), 6.31 (1H, s), 7.40 (2H, d, J = 8.4 Hz), 7.45–7.58 (3H, m), 7.63 (1H, s), 10.72 (1H, br).

Anal. calcd. for $C_{28}H_{35}ClN_4O_4$ 2HCl $2.1H_2O$: C, 52.73, H, 6.51, N, 8.78, Cl, 16.68. Found: C, 52.80, H, 6.51, N, 8.72, Cl, 16.42.

6.2. Pharmacology

6.2.1. Human and murine CCR4-expressing cells

Cells from the mouse pre-B cell line B300-19 were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 50 μM 2-mercaptethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin. The expression vector pEF-BOS-Neo[22], carrying full-length human CCR4 cDNA (X85740; GenBank) or mouse CCR4 cDNA, (X90862; GenBank) was transfected into B300–19 cells via electroporation to isolate stable G418-resistant stable transformants.

6.2.2. [^{35}S]GTPγS-binding assay

Human CCR4-expressing cell membranes (1 μg/well protein) were incubated at 25 °C for 1.5 h with 150 pM [^{35}S]GTPγS (GE Healthcare), 5 mg/mL wheat germ agglutinin SPA beads (GE Healthcare), 2 μM GDP, and 3 nM MDC with various concentrations of test compounds in 200 μl of binding buffer [20 mM HEPES–NaOH (pH 7.05), 100 mM NaCl, 5 mM $MgCl_2$, and 0.2% (w/v) BSA]. Radioactivity was counted using a TopCount scintillation counter. Control wells, in the absence of either test compound (for total counts) or CCL22

(non-specific), were used to calculate the percent of total inhibition for each set of compounds. Assays were performed in duplicate at four different concentrations for each test compound, and the value represents an average of usually two determinations.

In our studies, CCL22 was used as the main CCR4 ligand because it was shown to have higher affinity and efficacy than CCL17 for the [^{35}S]GTPγS-binding reaction and cell chemotaxis.[23] In addition, our test compounds were found to inhibit responses induced by CCL22 and CCL17 equally (data not shown).

6.2.3. Chemotaxis assay

Chemotaxis assays were performed using 96-well chemotaxis chambers (Neuro Probe)[24] that had 5-μm pores, polycarbonate filters, and were polyvinylpyrrolidone-free. The chambers were incubated for 3 h at 37 °C in a humidified 5% CO_2 atmosphere. Human or mouse CCR4-expressing cells were suspended at 5×10^6 cells/mL in RPMI 1640 supplemented with 0.1% (w/v) BSA and treated with various concentrations of test compounds. The cell suspension (200 μl) was placed in the upper wells, and 3 nM human or 1 nM mouse CCL22 in the lower wells. The number of cells migrating to the lower chambers was quantified using a bioluminescent assay (ATP-Lite; PerkinElmer). Control wells containing no test compound (for total migrating cells) or CCL22 (non-specific) were used to calculate the percent of total inhibition for each set of compounds. Assays were performed in triplicate at four different concentrations for each test compound.

6.2.4. Oxazolone-induced contact hypersensitivity (CHS)

Female BALB/c mice (7 weeks; $n = 5$; Charles River Laboratories Japan) were sensitized with 150 μl of 3% oxazolone (Sigma) in ethanol by having it brushed onto their shaved abdomens. Mice brushed with ethanol only were used as the control (non-sensitized). Six days later, 10 μl of 1% oxazolone was applied to both sides of the right ear for immunological challenge. Ear thickness was measured using a dial thickness gauge (Mitsutoyo) 20 h after the challenge. For the dose given twice, the tested compounds were administered 30 min before and 8 h after the challenge. For the single dose, the tested compounds were administered only after the challenge (8 h for **1a** and 12h for **8ic**). The degree of ear swelling in each group was estimated from the difference in pre- and post-challenge ear thickness. The ear swelling in the control and vehicle groups was used to calculate the percent inhibition for tested compounds.

6.3. Data analysis

The concentration causing 50% inhibition (IC_{50}) was determined by nonlinear curve fitting using the SAS system (SAS Institute, Cary, NC, USA).

Acknowledgement

The authors express their deep gratitude to Dr. Ryo Naito, Dr. Hideki Kubota, Ms. Diana Fleig and Ms. Takako Furukawa for their helpful support during the preparation of this manuscript. The authors are also grateful to the staff of the Division of Analytical Science Laboratories for their assistance with elemental analysis and spectral measurement.

References and notes

1. (a) Rossi, D.; Zlotnik, A. *Annu. Rev. Immunol.* **2000**, *18*, 217; (b) Owen, C. *Pulm. Pharmacol. Ther.* **2001**, *12*, 121.
2. (a) Murphy, M. P.; Baggiolini, M.; Charo, F. I.; Hebert, A. C.; Horuk, R.; Matsushima, K.; Miller, H. L.; Oppenheim, J. J.; Power, A. C. *Pharmacol. Rev.* **2000**, *52*, 145; (b) Zlotnik, A.; Yoshie, O. *Immunity* **2000**, *12*, 121.
3. Hoogewerf, J. A.; Black, D.; Proudfoot, I. E. A.; Wells, C. N. T.; Power, A. C. *Biochem. Biophys. Res. Comm.* **1996**, *218*, 337.
4. Sallusto, F.; Lenig, D.; Mackay, R. C.; Lanzavecchia, A. *J. Exp. Med.* **1998**, *187*, 875.
5. (a) Andrew, P. D.; Ruffing, N.; Kim, H. C.; Miao, W.; Heath, H.; Li, Y.; Murphy, K.; Campbell, J. J.; Butcher, C. E.; Wu, L. *J. Immunol.* **2001**, *166*, 103; (b) Kim, H. C.; Rott, L.; Kunkel, J. E.; Genovese, C. M.; Andrew, P. D.; Lijun, Y.; Butcher, C. E. *J. Clin. Invest.* **2001**, *108*, 1331; (c) Katschke, J. K., Jr.; Rottman, B. J.; Ruth, H. J.; Qin, S.; Wu, L.; LaRosa, G.; Ponath, P.; Park, C. C.; Pope, M. R.; Koch, E. A. *Arthritis Rheum.* **2001**, *44*, 1022.
6. Campbell, J. J.; Haraldsen, G.; Pan, J.; Rottman, J.; Qin, S.; Panath, P.; Andrew, P. D.; Warnke, R.; Ruffig, N.; Kassam, N.; Wu, L.; Butcher, C. E. *Nature* **1999**, *400*, 776.
7. (a) Imai, T.; Baba, M.; Nishimura, M.; Kakizaki, M.; Takagi, S.; Yoshie, O. *J. Biol. Chem.* **1997**, *272*, 15036; (b) Imai, T.; Yoshida, T.; Baba, M.; Nishimura, M.; Kakizaki, M.; Yoshie, O. *J. Biol. Chem.* **1996**, *271*, 21514; (c) Godiska, R.; Chantry, D.; Raport, J. C.; Sozzani, S.; Allavena, P.; Leviten, D.; Mantovani, A.; Gray, W. P. *J. Exp. Med.* **1997**, *185*, 1595.
8. Mantrovani, A. *Immunol. Today* **1999**, *20*, 254.
9. Papina-Bordignon, P.; Papi, A.; Mariani, M.; Lucia, D. P.; Casoni, G.; Bellettato, C.; Buonsanti, C.; Miotto, D.; Mapp, C.; Villa, A.; Arrigoni, G.; Fabbri, M. L.; Sinigaglia, F. *J. Clin. Invest.* **2001**, *107*, 1357.
10. Vestergaard, C.; Bang, K.; Gesser, B.; Yoneyama, H.; Matsushima, K.; Larsen, G. C. *J. Invest. Dermat.* **2000**, *115*, 640.
11. Rottman, B. J.; Smith, L. T.; Ganley, G. K.; Kikuchi, T.; Krueger, G. J. *Lab. Invest.* **2001**, *81*, 335.
12. Ruth, H. J.; Rottman, B. J.; Katschke, J. K., Jr.; Qin, S.; Wu, L.; LaRosa, G.; Panath, P.; Pope, M. R.; Koch, E. A. *Arthritis Rheum.* **2001**, *44*, 2750.
13. Jo, Y.; Matsumoto, T.; Yada, S.; Fujisawa, K.; Esaki, M.; Onai, N.; Matsushima, K.; Iida, M. *Clin. Exp. Immunol* **2003**, *132*, 332.
14. (a) Gonzalo, J. A.; Pan, Y.; Lloyd, C. M.; Jia, G. Q.; Yu, G.; Dussault, B.; Powers, C. A.; Proudfoot, A. E.; Coyle, A. J.; Gearing, D.; Gutierrzez-Ramos, J. C. *J. Immunol.* **1999**, *163*, 403; (b) Kawasaki, S.; Takizawa, H.; Yoneyama, H.; Nakayama, T.; Fujisawa, R.; Izumizaki, M.; Imai, T.; Yoshie, O.; Momma, I.; Yamamoto, K.; Matsushima, K. *J. Immunol.* **2001**, *166*, 2055.
15. Yokoyama, K.; Ishikawa, N.; Igarashi, S.; Kawano, N.; Hattori, K.; Miyazaki, T.; Ogino, S.; Matsumoto, Y.; Takeuchi, M.; Ohta, M. *Bioorg. Med. Chem.* **2008**, *16*, 7021.
16. Yokoyama, K.; Ishikawa, N.; Igarashi, S.; Kawano, N.; Masuda, N.; Hattori, K.; Miyazaki, T.; Ogino, S.; Orita, M.; Matsumoto, Y.; Takeuchi, M.; Ohta, M. *Bioorg. Med. Chem.* **2008**, *16*, 7968.
17. Miyashita, A.; Matsuda, H.; Iijima, C.; Higashino, T. *Chem. Pharm. Bull.* **1990**, *38*, 1147.
18. Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350.
19. (a) Tarayre, P. J.; Barbara, M.; Aliga, M.; Tisne-Versailles, J. *Drug Res.* **1990**, *40*, 1125; (b) Ottosen, R. E.; Sørensen, D. M.; Björkling, F.; Skak-Nielseen, T.; Fjording, S. M.; Aaes, H.; Binderup, L. *J. Med. Chem.* **2003**, *46*, 5651. Prednisolone was administered subcutaneously to mice previously sensitized to oxazolone in accordance with our method described above (see Section 6.2.4). A 50% inhibition in ear swelling was observed at the 10 mg/kg dose..
20. Allegretti, M.; Berdini, V.; Cesta, C. M.; Curti, R.; Nicolini, L.; Topai, A. *Tetrahedron Lett.* **2001**, *42*, 4257.
21. Barkenbus, C.; Wuellner, A. J. *J. Am. Chem. Soc.* **1955**, *77*, 3866.
22. Mizuhara, S.; Nagata, S. *Nucleic Acids Res.* **1990**, *18*, 11.
23. Imai, T.; Chantry, D.; Raport, CJ.; Wood, CL.; Nishimura, M.; Godiska, R.; Yoshie, O.; Gray, PW. *J. Biol. Chem.* **1998**, *273*, 1764.
24. Boyden, S. *J. Exp. Med.* **1962**, *115*, 453.