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Discovery of potent CCR4 antagonists: Synthesis and structure–activity relationship study of 2,4-diaminoquinazolines

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ABSTRACT

A new series of quinazolines that function as CCR4 antagonists were discovered during the screening of our corporate compound libraries. Subsequent compound optimization elucidated the structure–activity relationships and led the identification of 2-(1,4′-bipiperidine-1′-yl)-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **14a**, which showed potent inhibition in the [^{35}S]GTPγS-binding assay (IC_{50} = 18 nM). This compound also inhibited the chemotaxis of human and mouse CCR4-expressing cells (IC_{50} = 140 nM, 39 nM).

1. Introduction

Chemokines are a group of structurally related chemoattractant cytokine molecules that regulate leukocyte trafficking. They are relatively small (~8–14 kDa) and consist of mostly basic proteins that are involved in various physiological and pathological processes, such as immune response, viral infection, angiogenesis/angiostasis, and more fundamentally organogenesis and homeostasis.[1] They are classified based on the position of the first two of four conserved cysteines, and there are two main subclasses, CXC (or α) and CC (or β) chemokines. Chemokines exert their biological activity through interactions with a subset of G-coupled seven transmembrane receptors. These are named according to the type of chemokines they interact with, that is, CXC receptors (CXCRs) and CC receptors (CCRs).[2]

CC chemokine receptor 4 (CCR4) was originally cloned from T-lymphocytes and the thymus, where CCR4 is highly expressed.[3] CCR4 was reported to be expressed predominantly on Th2-lymphocytes,[4] but recent studies revealed that CCR4 is also widely expressed on monocytes, macrophages, dendritic cells, and natural killer cells.[5] Thymus and activation-regulated chemokine (TARC, CCL17) as well as macrophage-derived chemokine (MDC, CCL22) are CC chemokines, and are both highly specific biological ligands for CCR4.[6] These two chemokines are constitutive chemokines that are responsible for normal leucocyte trafficking, but are also inducible chemokines that recruit leukocytes in response to inflammatory signals.[7] The involvement of CCR4 and its ligands CCL17 and CCL22 is indicated in a wide range of diseases, including asthma,[8] atopic dermatitis,[9] psoriasis,[10] rheumatoid arthritis,[11] and chronic inflammatory bowel disease.[12]In vivo studies of CCL17 and CCL22 antibodies have indicated their usefulness for preventing several immunological responses.[13] Therefore, therapy using CCR4 antagonists would be a novel intervention method for diseases in which CCR4 participates.

Several small molecule CCR4 antagonists have been reported in the literature (Fig. 1).[14] During the screening of our corporate compound libraries using the [^{35}S]GTPγS-binding assay,[15] 2,4-diamino 6,7-dimethoxyquinazoline derivative **5** was located in a quinazoline-based diversity library (Fig. 2) and was found to have potent inhibitory activity, and thus designated as the lead compound.

2. Combinatorial synthesis and the initial SAR study

The lead compound **5** was synthesized from 2,4-dichloro-6,7-dimethoxyquinazoline using a two-step, selective, sequential substitution of amines.[16] An additional quinazoline-templated combinatorial library was prepared to obtain the initial SAR of the lead compound **5**. Numerous commercially available alkylamines (anilines were not employed due to their poor reactivity) were introduced at the 2- and 4-positions of 6,7-dimethoxyquinazoline derivatives, and the synthesized compounds were evaluated in the [^{35}S] GTPγS-binding assay and [^{121}I]CCL22-binding assay at the concentration of 1 μM. Among the substituents at the 4-position of quinazoline (Table 1), the cycloalkylamines were found to

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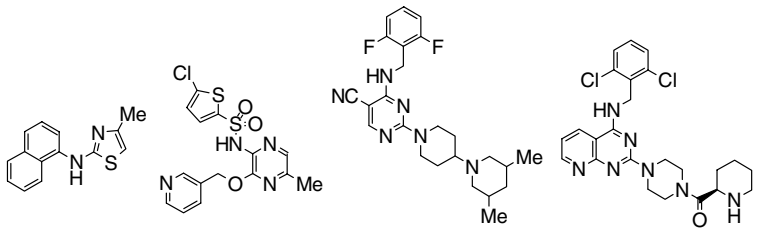
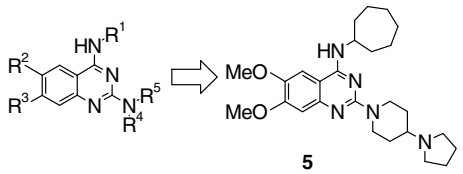


1 2 3 4

Figure 1. Small molecule CCR4 antagonists.



5

Figure 2. Discovery of compound **5** from quinazoline-based diversity library.

yield potent activity, while cycloheptylamine was the most potent. In general, a subtle change in the 4-substituents resulted in a considerable loss of potency. Only cycloalkylamines caused more than 50% inhibition at the 1 μM concentration; all other alkylamines inhibited less than 50%. Exploration of substituents at the 2-position included the testing of diamines such as piperazine and 4-aminosubstituted piperidine, which were required to yield potent activity (Table 2). In contrast to the SAR for the 4-position substituents, however, a subtle stereochemical change in the 2-position substituents did not affect the potency, and several types of the diamine substituents were found to have potent activity. Based on these data, further optimization of the quinazoline derivatives was performed using 4-cycloheptylamine analogues.

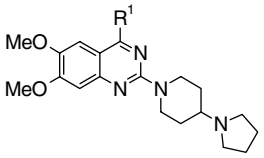
3. Chemistry

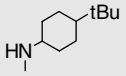
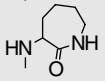
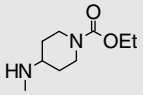
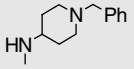
The novel amines **8** and **10** were synthesized as summarized in Scheme 1. Diamine **8** was prepared via reductive amination of the protected piperidone **6** with the 4-hydroxypiperidine, followed by the fluorination of the alcohol group using DAST, and subsequent deprotection of the benzyloxycarbonyl group. Compound **10a** was also prepared from the piperidone **6** by reductive amination with cyclopentylamine, followed by deprotection of the protecting group. Compound **10b** was synthesized by methylation of the secondary amine **9a** and subsequent deprotection.

The preparation of 2,4-diaminoquinazolines **14** is summarized in Scheme 2. The anthranic acid **11** was obtained commercially or prepared in-house (see Section 6). The reaction of **11** with urea, followed by treatment with phosphorous oxychloride under reflux, provided 2,4-dichloroquinazolines **12**. Selective substitution of the chlorine at the 4-position with cycloheptylamine yielded the corresponding 4-amino-2-chloro-quinazolines **13**, which was subsequently coupled with commercially available amines or prepared amines **8** and **10** at the 2-position under reflux in isopropanol to yield 2,4-diaminoquinazolines **14**.

N-Cycloheptyl-2-(1-cyclohexylpiperidine-4-yl)-6,7-dimethoxyquinazolin-4-amine **18** was prepared as shown in Scheme 3. The condensation of 1-[(benzyloxy)carbonyl]piperidine-4-carboxylic acid **15** with 2-amino-4, 5-dimethoxybenzonitrile, followed by treatment with H_2O_2 under reflux, yielded the cyclized product **16**. Treatment of **16** with phosphorous oxychloride under reflux, followed by the substitution of cycloheptylamine, provided 4-aminoquinazolines **17**. The removal of the benzyloxycarbonyl (CBz) group was readily accomplished by hydrogenolysis to yield the secondary amine, which was alkylated with iodocyclohexane in the presence of sodium carbonate to yield compound **18**.

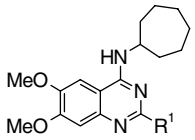
Table 1
CCR4 inhibitory activities of 4-substituted quinazoline derivatives

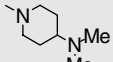
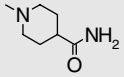
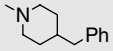
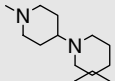
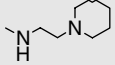
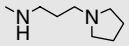
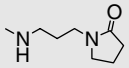
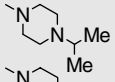
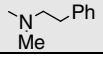


Compound	R^1	[^{35}S]GTPγS inh.% at 1 μM[a]	[^{125}I]CCL22 inh.% at 1 μM[a]
C-001		10	<0
C-002		7	4
C-003		3	<0
C-004		18	17
C-005		71 (IC_{50} = 480 nM)	54 (IC_{50} = 1900 nM)
C-006 (5)		93	100
C-007		11	<0
C-008		71 (IC_{50} = 200 nM)	54 (IC_{50} = 1200 nM)
C-009		16	16
C-010		10	6
C-011		4	<0
C-012		<0	<0
C-013		8	19
C-014		<0	2

[a] See Section 6, pharmacology.

Table 2
CCR4 inhibitory activities of 2-substituted quinazoline derivatives



Compound	R^1	[^{35}S]GTPγS[a] inh.% at 1 μM[a]	[^{125}I]CCL22 inh.% at 1 μM[a]
C-015		7	11
C-016		65 (IC_{50} = 260 nM)	77 (IC_{50} = 730 nM)
C-017		12	15
C-018		<0	3
C-019		17	<0
C-020		1	<0
C-021 (14a)		92	100
C-022		1	18
C-023		35	16
C-024		8	8
C-025		13	<0
C-026		68 (IC_{50} = 220 nM)	68 (IC_{50} = 660 nM)
C-027		7	7
C-028		<0	8
C-029		<0	6
C-030		<0	4
C-031		13	<0
C-032		0	<0
C-033		16	<0
C-034		<0	<0

[a] See Section 6, pharmacology.

4. 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 by chemotaxis assay. The results are summarized in Tables 3–5.

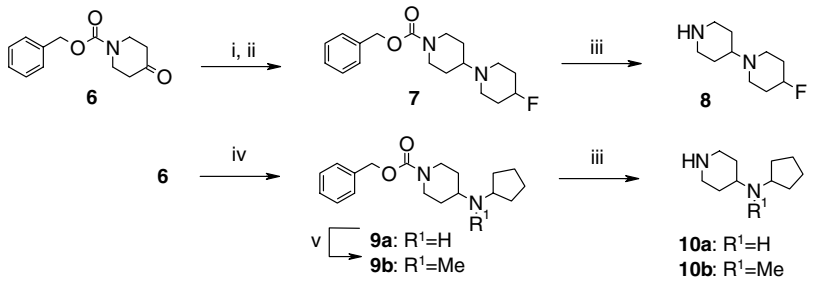
The parent compound 5 showed potent inhibition in the [^{35}S]GTPγS-binding assay (IC_{50} = 19 nM, Table 3). This compound also inhibited the chemotaxis of human and mouse CCR4-expressing cells (IC_{50} = 200 nM, 130 nM). The cytotoxicity of this compound **5** was also evaluated. The results showed that the minimum cytotoxic dose (3 μM, see Section 6) was far from the concentration that inhibited chemotaxis, which indicates that the observed inhibition was due to receptor antagonism, not cytotoxicity. To improve the potency of this compound, the substituents at the 4-position of the piperidine were first examined. From the results of combinatorial study mentioned above it was concluded that an aryl or alkyl substituent, like phenyl or cyclohexyl, would result in a significant loss of potency. In addition, the nitrogen atom of the pyrrolidine in compound **5** appeared to be important for potent activity in the [^{35}S]GTPγS-binding assay. Replacement of the pyrrolidine with a piperidine did not significantly change [^{35}S]GTPγS-binding or the human chemotactic factor, but there was a threefold increase in the inhibition of mouse cell chemotaxis compared to the parent compound **5**. Compounds **5** and **14a** were further evaluated by determining the [^{125}I]CCL22-binding inhibition. Both showed potent activity that was almost equivalent. The introduction of a fluoro substituent at the 4-position of the piperidine (**14b**) in compound **14a** also did not result in any significant loss in the [^{35}S]GTPγS-binding assay or the inhibition of human cell chemotaxis, but a 15-fold loss of potency was observed in the inhibition of mouse chemotaxis compared to compound **14a**. These findings indicate that the trend in SAR seemed to differ between human and mouse cell chemotaxis; mouse cell chemotaxis was very sensitive to subtle changes in this part of the template. Cyclopentylamine (**14c**) and *N*-methylcyclopentylamine (**14d**) were also hindered in this position. These substituents were tolerated, but their presence resulted in slight decreases in the inhibition of mouse cell chemotaxis.

More significant changes were then introduced to the right-hand portion of the structure (Table 4). A shift in the position of the nitrogen attached to piperidine at the 4-position was speculated to affect inhibitory activity, but the results of piperazine derivative **14e** were almost comparable to those of all three parent compound assays. This result showed that the positional shift of this basic nitrogen did not affect its potent inhibitory activity. The effect of the other piperazine nitrogen attached to the quinazoline core was also investigated. Removal of this nitrogen (**20**) produced a 10-fold loss of potency in the binding assay, which clearly showed that this nitrogen needs to be in its original position.

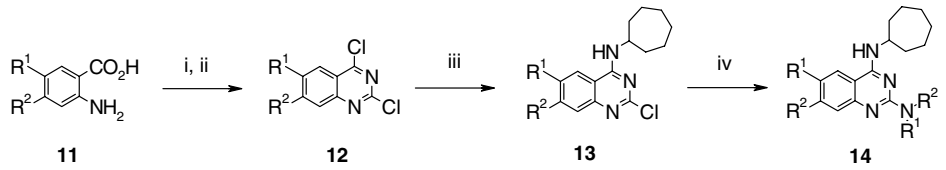
Finally, the SAR of the methoxy substituents at the 6- and 7-positions of quinazoline was investigated (Table 5). While removal of both of these methoxy groups (**14h**) resulted in significant loss of potency, the 6-methoxy analogue (**14f**) showed only a slight decrease of potency, and the 7-methoxy analogue (**14g**) maintained its activity compared to the parent compound **5** in all three assays. These results indicate that the 7-methoxy substituent is more important than 6-methoxy substituent for activity.

5. Conclusion

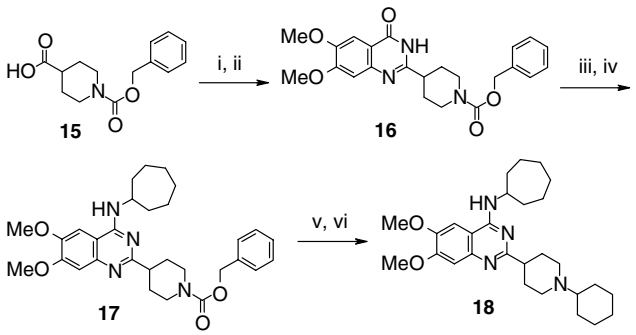
The screening of a corporate library of compounds yielded a new series of potent competitive CCR4 antagonists, which were then subjected to chemical optimization. Among this series, compound **14a** yielded the most potent inhibition in the [^{35}S]GTPγS-binding assay. This compound also potently inhibited the chemotaxis of human and mouse CCR4-expressing cells. The most interesting part of the SAR study was the stereochemical tolerance at the 4-position of the piperidine, and the increase in potency caused by the conversion



Scheme 1. Reagents and conditions: (i) 4-hydroxypiperidine, $Ti(Oi\text{-}Pr)_4$, 80 °C, then $NaBH_4$, EtOH; (ii) DAST, CH_2Cl_2; (iii) 10% Pd–C, EtOH; (iv) cyclopentylamine, $NaBH(OAc)_3$, AcOH; (v) 36%HCHO aq, HCO_2H, 70 °C.



Scheme 2. Reagents and conditions: (i) urea, 200 °C; (ii) $POCl_3$; (iii) cycloheptylamine, DMF; (iv) amines, *n*-BuOH, reflux.



Scheme 3. Reagents and conditions: (i) oxalylchloride, CH_2Cl_2, DMF (drops), then 2-amino-4,5-dimethoxybenzonitrile, pyridine; (ii) NaOH, 30%H_2O_2 aq, EtOH, reflux; (iii) $POCl_3$, reflux; (iv) cycloheptylamine, DMF; (v) 10% Pd–C, MeOH; (vi) iodocyclohexane, Na_2CO_3, DMF.

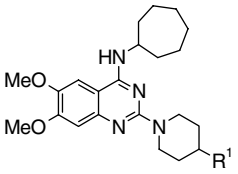
of pyrrolidine to piperidine. Therefore, it was shown that intensive synthetic conversions can lead to additional improvement of potency. Further developments related to the optimization of this series will be reported in due course.

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 the internal standard (NMR description key: s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. High-resolution mass spectra were recorded on a Waters QTOF Premier spectrometer; the results were within ±0.2 mDa of the theoretical values. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C,H,N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens); the results were within ±0.4% of the theoretical values.

Table 3
CCR4 inhibitory activities of 4-substituted piperidine derivatives



Compound	R^1	[^{35}S]GTPγS[a] (μM)	Chemotaxis (μM) human[a]	Chemotaxis (μM) mouse[a]	[^{125}I]CCL22[a] (μM)
5		0.019	0.20	0.13	0.070
14a		0.018	0.14	0.039	0.073
14b		0.032	0.31	0.60	NT
14c		0.013	0.10	0.29	NT
14d		0.018	0.12	0.35	NT

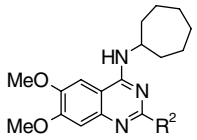
[a] See Section 6, pharmacology.

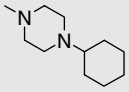
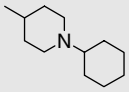
6.1.1. *N*-Cycloheptyl-6,7-dimethoxy-2-(4-pyrrolidine-1-ylpiperidine-1-yl)quinazolin-4-amine (5)

4-Pyrrolidin-1-ylpiperidine (930 mg, 6.0 mmol) was added to a solution of 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** (1.01 g, 3.0 mmol) in *n*-BuOH (10 mL), and the mixture was stirred under reflux for 3 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue in chloroform was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was purified using column chromatography (chloroform/MeOH/NH_4OH) to yield *N*-cycloheptyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine **5** (500 mg, 37%).

Mp (dec) 205–206 °C (Et_2O); MS (FAB$^+$) *m*/*z* 454 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.36 (2H, m), 1.45–1.75 (14H,

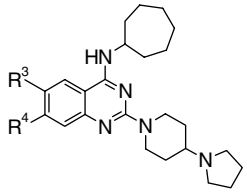
Table 4
CCR4 inhibitory activities of 2-substituted quinazoline derivatives



Compound	R^2	[^{35}S]GTPγS (μM)[a]	Chemotaxis (μM) human[a]	Chemotaxis (μM) mouse[a]	[^{125}I]CCL22[a] (μM)
14e		0.055	0.23	0.21	NT
20		0.51	12% at 1 μM	NT	NT
5		0.019	0.20	0.13	0.070

[a] See Section 6, pharmacology.

Table 5
CCR4 inhibitory activities of 6,7-substituted quinazoline derivatives



Compound	R^3	R^4	[^{35}S]GTPγS (μM)[a]	Chemotaxis (μM) human[a]	Chemotaxis (μM) mouse[a]	[^{125}I]CCL22[a] (μM)
14f	MeO–	H	0.031	0.41	0.80	NT
14g	H–	MeO–	0.013	0.12	0.22	NT
14h	H	H	0.20	1.4	NT	NT
5	MeO–	MeO–	0.019	0.20	0.13	0.070

[a] See Section 6, pharmacology.

m), 1.82–1.89 (2H, m), 1.93–2.02 (2H, m), 2.12–2.26 (1H, m), 2.47–2.53 (2H, m), 2.81–2.95 (2H, m), 3.25–3.40 (2H, m), 3.80 (3H, s), 3.81 (3H, s), 4.13–4.24 (1H, m), 4.54–4.64 (2H, m), 6.71 (1H, s), 7.24 (1H, d, J = 7.2 Hz), 9.45 (1H, s). Anal. Calcd for $C_{26}H_{39}N_5O_2$: C, 68.84; H, 8.67; N, 15.44. Found: C, 68.61; H, 8.92; N, 15.26.

6.1.2. 4-Fluoro-1,4′-bipiperidine (8)

The mixture of 4-hydroxypiperidine **6** (1.01 g, 10 mmol), benzyl 4-oxo-1-piperidine carboxylate (2.33 g, 10 mmol), and Ti(O*i*-Pr)$_4$ (5.69 g, 20 mmol) was stirred at 80 °C for 5 h. The reaction mixture was cooled on ice. Ethanol (100 mL) and sodium borohydride (757 mg, 20 mmol) were added, and the resulting mixture was stirred at room temperature for 1 h. The organic layer was concentrated in vacuo. The residue was partitioned between chloroform and 1 M-NaOH aqueous solution, and an insoluble solid was removed by filtration on Celite. The organic layer was then washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via silica gel column chromatography (chloroform/MeOH) to yield benzyl 4-hydroxy-1,4′-bipiperidine-1′-carboxylate (1.46 g, 4.6 mol, 46%).

A solution of benzyl 4-hydroxy-1,4′-bipiperidine-1′-carboxylate dissolved in methylenechloride (20 mL) was added slowly to a solution of DAST [(diethylamino)sulfur trifluoride] in methylenechloride (20 mL) over 1 h at 4 °C. The resulting mixture was stirred at room temperature for 4 h. The reaction was carefully quenched with saturated sodium bicarbonate aqueous solution, while maintaining the temperature of the reaction mixture under 15 °C. The reaction mixture was extracted with chloroform, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via column chromatography (chloroform/MeOH) to yield benzyl 4-fluoro-1,4′-bipiperidine-1′-carboxylate **7** (1.11 g, 3.47 mol, 75%).

MS (ESI$^+$) m/z 321 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23–1.36 (2H, m), 1.58–1.72 (4H, m), 1.74–1.90 (2H, m), 2.32–2.49 (5H, m), 2.69–2.86 (2H, m), 3.96–4.06 (2H, m), 4.52–4.72 (1H, m), 5.01 (2H, s), 7.28–7.40 (5H, m).

Palladium on carbon (10%,100 mg) was added to a solution of benzyl 4-fluoro-1,4′-bipiperidine-1′-carboxylate**7** (1.11 g) in ethanol (30 mL). The resulting mixture was stirred at room temperature under 1 atm of hydrogen gas for 6 h, and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to yield 4-fluoro-1,4′-bipiperidine **8** (469 mg) as a colorless oil, which was used in a subsequent reaction without further purification.

6.1.3. Benzyl 4-(cyclopentylamino)piperidine-1-carboxylate (9a)

Benzyl 4-oxo-1-piperidine carboxylate **6** (3.5g, 15 mmol) was added to a solution of cyclopentylamine (850 mg, 10 mmol) in acetic acid (100 mL). The resulting mixture was stirred at room temperature for 1 h. Sodium acetoxyborohydride (5.3 g, 25 mmol) was added to the reaction mixture, and the mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo. Potassium carbonate aqueous solution was added to the residue, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via column chromatography (chloroform/MeOH/ NH_4OH) to

yield benzyl 4-(cyclopentylamino)piperidine-1-carboxylate **9a** (2.15 g, 7.11 mol, 71%).

MS (ESI$^+$) m/z 303 [M+H]$^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22–1.30 (4H, m), 1.47–1.58 (2H, m), 1.63–1.73 (2H, m), 1.82–1.88 (4H, m), 2.63–2.70 (1H, m), 2.83–2.89 (2H, m), 3.16–3.23 (1H, m), 4.12 (2H, br), 5.12 (2H, s), 7.28–7.38 (5H, m).

6.1.4. Benzyl 4-[cyclopentyl(methyl)amino]piperidine-1-carboxylate (9b)

A mixture of benzyl 4-(cyclopentylamino)piperidine-1-carboxylate **9a** (364 mg, 1.2 mmol), 36% formaldehyde aqueous solution (1 mL), and formic acid (2 mL) was stirred at 70 °C for 1 h. Formaldehyde aqueous solution (36%, 2mL) and formic acid (1 mL) were added to the reaction mixture, which was then stirred at 90 °C for 18 h. The reaction mixture was concentrated in vacuo. Potassium carbonate aqueous solution was added to the residue, and the resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified via column chromatography (chloroform/MeOH/NH_4OH) to yield benzyl 4-[cyclopentyl(methyl)amino]piperidine-1-carboxylate **9b** (369 mg, 1.17 mol, 97%).

MS (ESI$^+$) m/z 317 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.40 (4H, m), 1.43–1.53 (2H, m), 1.54–1.66 (4H, m), 1.70–1.80 (2H, m), 2.09 (3H, s, br), 2.75 (2H, m, br), 2.89 (1H, m, br), 3.26–3.30 (1H, m), 4.01–4.10 (2H, br), 5.06 (2H, s), 7.28–7.40 (5H, m).

6.1.5. *N*-Cyclopentylpiperidine-4-amine (10a)

Palladium on carbon (10%, 50 mg) was added to a solution of benzyl 4-(cyclopentylamino)piperidine-1-carboxylate **9a** (493 mg) in ethanol (10 mL). The resulting mixture was stirred at room temperature under 1 atm of hydrogen gas for 8 h, and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to yield crude *N*-cyclopentylpiperidine-4-amine **10a** (279 mg) as a colorless oil, which was used in a subsequent reaction without further purification.

6.1.6. *N*-Cyclopentyl-*N*-methylpiperidine-4-amine (10b)

Palladium on carbon (10%, 40 mg) was added to a solution of benzyl 4-[cyclopentyl(methyl)amino]piperidine-1-carboxylate **9b** (369 mg, 1.17 mol) in ethanol (10 mL). The resulting mixture was stirred at room temperature under 1 atm of hydrogen gas for 8 h, and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to yield crude *N*-cyclopentyl-*N*-methylpiperidine-4-amine **10b** (187 mg) as a white solid, which was used in a subsequent reaction without further purification.

6.1.7. 2,4-Dichloro-6-methoxyquinazoline (12b)

A mixture of 2-amino-5-methoxybenzoic acid **11a** (5.13 g, 31 mmol) and urea (9.22 g, 153 mmol) was stirred at 200 °C for 1 h. The resulting mixture was treated with 2 M-NaOH aqueous solution, and the insoluble material was removed by filtration. The filtrate was acidified with concd HCl aqueous solution, and the resulting precipitate was filtered and washed with EtOH/H_2O (1:1) to yield crude 6-methoxyquinazoline-2,4-diol (3.93 g) as a grayish solid. $POCl_3$ (50 mL) was added to this crude compound and stirred under reflux for 6 h. The reaction mixture was concentrated in vacuo, and the resulting residue was added to a solution of potassium carbonate aqueous solution. The resulting mixture was then extracted with chloroform, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. This residue was purified via column chromatography (chloroform/MeOH) to yield 2,4-dichloro-6-methoxyquinazoline **12b** (2.81 g, 40% from **11a**).

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.00 (3H, s), 7.49 (1H, d, J = 2.4 Hz), 7.80–7.83 (1H, m), 7.99 (1H, d, J = 9.2 Hz).

6.1.8. 2,4-Dichloro-7-methoxyquinazoline (12c)

Palladium on carbon (10%, 400 mg) was added to the solution of 4-methoxy-2-nitrobenzoic acid (4.02 g, 20.4 mmol) in ethanol (100 mL). The resulting mixture was stirred at room temperature under 1 atm of hydrogen gas for 1 h, and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to yield crude 2-amino-4-methoxybenzoic acid **11b** (3.18 g) as a grayish solid, which was used in a subsequent reaction without further purification. Compound **12c** (660 mg, 14% from 4-methoxy-2-nitrobenzoic acid) was prepared from crude **11b** (3.17 g) using a procedure similar to that described for the synthesis of **12b**.

^{1}H NMR (300 MHz, DMSO-d_6) δ 4.00 (3H, s), 7.44–7.52 (2H, m), 8.18 (1H, d, J = 9.2 Hz).

6.1.9. 2-Chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine (13a)

Cycloheptylamine (2.02 g, 17.9 mmol) was added to a solution of 2,4-dichloro-6,7-dimethoxyquinazoline **12a** (2.11 g, 8.14 mmol) in DMF (20 mL), and the resulting mixture was stirred at room temperature for 2 h. Water was added to the reaction mixture, and the resulting solution was extracted with ethyl acetate. The organic layer was washed with 5% citric acid aqueous solution and brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was washed with diethyl ether to yield 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** as a colorless solid (2.15 g, 79%).

MS (ESI$^+$) m/z 336 [M+H]$^+$. ^{1}H 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).

Compounds **13b–d** were prepared using procedures similar to those described for the synthesis of **13a**.

6.1.10. 2-Chloro-*N*-cycloheptyl-6-methoxyquinazolin-4-amine (13b)

13b (1.13 g, 58%) was obtained as a colorless solid from 2,4-dichloro-6-methoxyquinazoline **12b** (1.46 g, 6.4 mmol).

MS (ESI$^+$) m/z 306 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45–1.76 (10H, m), 1.92–2.02 (2H, m), 3.90 (3H, s), 4.26–4.33 (1H, m), 7.41 (1H, dd, J = 9.2 Hz, 2.8 Hz), 7.54 (1H, d, J = 8.8 Hz), 7.75 (1H, d, J = 2.4 Hz), 8.19 (1H, d, J = 7.6 Hz).

6.1.11. 2-Chloro-*N*-cycloheptyl-7-methoxyquinazolin-4-amine (13c)

13c (590 mg, 89%) was obtained as a colorless solid from 2,4-dichloro-6-methoxyquinazoline **12c** (500 mg, 2.18 mmol).

MS (ESI$^+$) m/z 306 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42–1.76 (10H, m), 1.88–1.98 (2H, m), 3.87 (3H, s), 4.20–4.31 (1H, m), 7.02 (1H, d, J = 2.4 Hz), 7.11 (1H, dd, J = 9.2, 2.8 Hz), 8.18 (1H, d, J = 8.0 Hz), 8.26 (1H, d, J = 9.6 Hz).

6.1.12. 2-Chloro-*N*-cycloheptylquinazolin-4-amine (13d)

Crude **13d** was obtained from 2, 4-dichloroquinazoline[15] (1.50 g, 7.54 mmol) using a procedure similar to that described for the synthesis of **13a**. This crude solid was used in a subsequent reaction without further purification.

Compounds **14a–h** were prepared using procedures similar as described for the synthesis of **5**.

6.1.13. 2-(1,4′-Bipiperidine-1′-yl)-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine (14a)

14a (310 mg, 22%) was obtained as a yellowish solid from 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** (1.02 g, 3.0 mmol), and 4-piperidinopiperidine (1.14 g, 6.1 mmol).

Mp (dec) 184–186 °C (Et_2O); MS (FAB$^+$) m/z 468 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.40 (4H, m), 1.42–1.80 (16H, m), 1.92–2.02 (2H, m), 2.40–2.55 (3H, m), 2.65–2.77 (2H, m),

3.27–3.35 (2H, m), 3.806 (3H, s), 3.813 (3H, s), 4.12–4.24 (1H, m), 4.74–4.84 (2H, m), 6.71 (1H, s), 7.24 (1H, d, J = 7.6 Hz), 7.45 (1H, s). Anal. Calcd for $C_{26}H_{39}N_5O_2.0.5H_2O$: C, 68.04; H, 8.88; N, 14.69. Found: C, 68.27; H, 9.13; N, 14.68.

6.1.14. *N*-Cycloheptyl-2-(4-fluoro-1,4′-bipiperidine-1′-yl)-6,7-dimethoxyquinazolin-4-amine (14b)

14b (682 mg, 94%) was obtained as a white solid from 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** (504 mg, 1.5 mmol), Hunig's base (233 mg), and crude 4-fluoro-1,4′-bipiperidine **8** (279 mg). **14b** (682 mg) was treated with 4 M-HCl-dioxane, and the mixture was concentrated. The residue was recrystallized from isopropanol to yield the hydrochloride salt (440 mg, 0.79 mmol, 56%) as a white solid.

Mp (dec) 225–226 °C (*i*-PrOH); MS (FAB$^+$) m/z 486 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44–1.68 (6H, m), 1.71–1.90 (6H, m), 1.92–2.02 (2H, m), 2.03–2.40 (6H, m), 3.04–3.20 (4H, m), 3.35–3.50 (2H, m), 3.52–3.62 (1H, m), 3.87 (3H, s), 3.89 (3H, s), 4.26–4.32 (1H, m), 4.82–4.90 (2H, m), 4.93–5.10 (1H, m), 7.62–7.70 (1H, m), 7.90 (1H, s), 8.99–9.10 (1H, m), 11.28 (1H, m), 12.42–12.56 (1H, m). Anal. Calcd for $C_{27}H_{40}FN_5O_2{\cdot}2HCl{\cdot}1.7H_2O{\cdot}0.1C_3H_8O$: C, 55.09; H, 7.82; N, 11.77; Cl, 11.91; F, 3.19. Found: C, 54.99; H, 7.88; N, 11.75; Cl, 12.05; F, 3.19.

6.1.15. *N*-Cycloheptyl-2-[4-(cyclopentylamino)piperidine-1-yl]-6,7-dimethoxyquinazolin-4-amine (14c)

14c (660 mg, 87%) was obtained from 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** (548 mg, 1.63 mmol), Hunig's base (253 mg), and crude *N*-cyclopentylpiperidine-4-amine **10a** (275 mg). **14c** (660 mg) was treated with 4 M-HCl-dioxane, and the mixture was concentrated. The resulting residue was washed with Et_2O to yield the hydrochloride salt (660 mg, 87%) as a white solid.

Mp (dec) 248 °C (Et_2O); MS (FAB$^+$) m/z 468 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.40–1.80 (18H, m), 1.95–2.05 (4H, m), 2.20–2.28 (2H, m), 3.15–3.24 (2H, m), 3.58–3.62 (1H, m), 3.88 (6H, s), 4.24–4.36 (1H, m), 4.66–4.76 (2H, m), 7.52 (1H, s), 7.84 (1H, s), 8.96 (1H, br), 9.16 (2H, s), 12.30 (1H, s). Anal. Calcd for $C_{27}H_{41}N_5O_2{\cdot}2HCl{\cdot}1.4H_2O{\cdot}0.4C_4H_8O_2$: C, 57.15; H, 8.22; N, 11.65; Cl, 11.80. Found: C, 57.05; H, 8.18; N, 11.65; Cl, 11.96.

6.1.16. *N*-Cycloheptyl-2-{4-[cyclopentyl(methyl)amino]piperidine-1-yl}-6,7-dimethoxyquinazolin-4-amine (14d)

14d (185 mg, 37%) was obtained from 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** (344 mg, 1.03 mmol), Hunig's base (160 mg), and crude *N*-cyclopentyl-*N*-methylpiperidine-4-amine **10b** (187 mg). **14d** (185 mg) was treated with 4 M-HCl-dioxane, and the mixture was concentrated. The resulting residue was washed with Et_2O and EtOH to yield the hydrochloride salt (229 mg) as a white solid.

Mp (dec) 265–266 °C (EtOH); MS (FAB$^+$) m/z 482 [M+H]$^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 1.55–1.94 (17H), 1.95–2.32 (7H, m), 2.79 (3H, s), 3.29–3.31 (1H, m), 3.83–3.87 (2H, m), 3.88 (6H, s), 4.35–4.44 (1H, m), 4.84–4.86 (2H, m), 7.25 (1H, s), 7.70 (1H, s). Anal. Calcd for $C_{28}H_{43}N_5O_2{\cdot}2HCl{\cdot}0.8\ H_2O$: C, 59.10; H, 8.25; N, 12.31; Cl, 12.46. Found: C, 58.71; H, 8.28; N, 12.22; Cl, 12.85.

6.1.17. *N*-Cycloheptyl-2-(4-cyclohexylpiperazine-1-yl)-6,7-dimethoxyquinazolin-4-amine (14e)

14e (780 mg, 67%) was obtained as a colorless solid from 2-chloro-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine **13a** (840 mg, 5 mmol) and 1-cyclohexylpiperazine (860 mg). **14e** (700 mg) was treated with 4 M-HCl-dioxane, and the mixture was concentrated. The residue was washed with Et_2O to yield the hydrochloride salt (760 mg, 94%) as a colorless solid.

Mp (dec) 203–204 °C (Et_2O); MS (FAB$^+$) m/z 468 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04–1.17 (1H, m), 1.20–1.33 (2H, m), 1.37–1.69 (10H, m), 1.70–1.88 (6H, m), 1.90–2.00 (2H, m), 2.07–2.19 (2H, m), 3.52–3.65 (4H, m), 3.72–3.84 (2H, m), 3.87 (3H, s), 3.90 (3H, s), 4.26–4.38 (1H, m), 4.82–4.94 (2H, m), 7.70 (1H, s), 7.97 (1H, s), 9.21 (1H, d, J = 6.8 Hz), 11.53 (1H, s), 12.90 (1H, s). Anal. Calcd for $C_{27}H_{41}N_5O_2{\cdot}2HCl{\cdot}1.6H_2O$: C, 56.95; H, 8.18; N, 12.30; Cl, 12.45. Found: C, 56.81; H, 8.30; N, 12.21; Cl, 12.80.

6.1.18. *N*-Cycloheptyl-6-methoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine-*N*-cycloheptyl-6-methoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (14f)

14f (480 mg, 57%) was obtained as a colorless solid from 2-chloro-*N*-cycloheptyl-6-methoxyquinazolin-4-amine **13b** (610 mg, 2 mmol) and 4-pyrrolidin-1-ylpiperidine (620 mg). **14f** (450 mg) was treated with 4 M-HCl-ethylacetate and the mixture was concentrated. The resulting residue was washed with Et_2O to yield the hydrochloride salt (500 mg, 95%) as a colorless solid.

Mp (dec) 198–199 °C (Et_2O); MS (ESI$^+$) m/z 424 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46–1.69 (6H, m), 1.70–2.02 (13H, m), 2.18–2.28 (2H, m), 3.02–3.22 (4H, m), 3.42–3.52 (2H, m), 3.88 (3H, s), 4.26–4.38 (1H, m), 4.78–4.88 (2H, m), 7.41–7.48 (1H, m), 7.92–8.01 (2H, br), 9.25 (1H, s), 11.39 (1H, s), 12.49 (1H, s). Anal. Calcd for $C_{25}H_{37}N_5O{\cdot}2HCl\ H_2O$: C, 58.36; H, 8.03; N, 13.61; Cl, 13.78. Found: C, 58.52; H, 8.30; N, 13.72; Cl, 13.43.

6.1.19. *N*-Cycloheptyl-7-methoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (14g)

14g (470 mg, 94%) was obtained as a colorless amorphous from 2-chloro-*N*-cycloheptyl-7-methoxyquinazolin-4-amine **13c** (360 mg, 1.18 mmol) and 4-pyrrolidin-1-ylpiperidine (360 mg). **14g** (470 mg) was treated with 4 M-HCl-ethylacetate and the mixture was concentrated. The resulting residue was washed with Et_2O to yield the hydrochloride salt (370 mg, 67%) as a yellowish solid.

Mp (dec) 164–166 °C (Et_2O); MS (ESI$^+$) m/z 424 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42–1.76 (13H, m), 1.78–2.04 (6H, m), 2.06–2.20 (2H, m), 2.85–3.12 (4H, m), 3.40–3.55 (2H, m), 3.83 (3H, s), 4.14–4.28 (1H, m), 4.78–4.92 (2H, m), 6.65–7.10 (2H, br), 8.00–8.20 (1H, s), 10.86 (1H, br), 12.35 (1H, br). Anal. Calcd for $C_{25}H_{37}N_5O{\cdot}1.4HCl{\cdot}2H_2O$: C, 58.80; H, 8.37; N, 13.71; Cl, 9.72. Found: C, 59.04; H, 8.72; N, 13.79; Cl, 9.48.

6.1.20. *N*-Cycloheptyl-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (14h)

14h (2.20 g) was obtained from crude 2-chloro-*N*-cycloheptylquinazolin-4-amine**13d**, Hunig's base (1.3 mL), and 4-pyrrolidin-1-ylpiperidine (1.16 g). **14h** (2.20 g) was treated with 4 M-HCl-dioxane, and the mixture was concentrated. The resulting residue was recrystallized from Et_2O/MeCN to yield the hydrochloride salt (1.51 g, 3.2 mmol, 43% from 2, 4-dichloroquinazoline) as a slightly orange solid.

MS (FAB$^+$) m/z 394 [M+H]$^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44–2.03 (19H, m), 2.18–2.28 (2H, m), 2.98–3.25 (4H, m), 3.44–3.52 (2H, m), 4.25–4.36 (1H, m), 4.87–4.98 (2H, m), 7.38–7.45 (1H, m), 7.76–7.82 (1H, m), 8.10 (1H, d, J = 8.3 Hz), 8.50 (1H, d, J = 8.3 Hz), 9.32 (1H, d, J = 7.3 Hz), 11.55 (1H, s), 12.71 (1H, s). Anal. Calcd for $C_{24}H_{35}N_5{\cdot}2.1{\cdot}HCl{\cdot}1.8H_2O$: C, 57.36; H, 8.16; N, 13.94; Cl, 14.81. Found: C, 57.58; H, 8.41; N, 13.96; Cl, 14.57.

6.1.21. Benzyl 4-[4-(cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]piperidine-1-carboxylate (17)

DMF (3 drops) and oxalyl chloride (1.54 mL, 17.68 mmol) were added to a solution of 1-[(benzyloxy)carbonyl]piperidine-4-carboxylic acid **15** (4.43 g, 16.84 mmol) in CH_2Cl_2 (50 mL), and the

resulting mixture was stirred at room temperature for 2 h. A solution of 2-amino-4,5-dimethoxybenzonitrile (3 g, 16.84 mmol) in pyridine (30 mL) at 4 °C was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 18 h. Chloroform was added to the reaction mixture, and the solution was washed with 1 M HCl aqueous solution and brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was washed with diethyl ether to yield benzyl 4-{[(2-cyano-4,5-dimethoxyphenyl)amino]carbonyl}piperidine-1-carboxylate (5.6 g, 13.23 mmol, 79%) as a yellow solid. Benzyl 4-{[(2-cyano-4,5-dimethoxyphenyl) amino]carbonyl}piperidine-1-carboxylate (5.6 g) and 30% H_2O_2 aqueous solution (31 mL) were added to the mixture of NaOH (529 mg, 13.23 mmol) and EtOH (140 mL). The resulting mixture was stirred under reflux for 2 h, and the reaction mixture was concentrated in vacuo. HCl aqueous solution (1 M) was added to the solution of residue in chloroform, and the organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was washed with diethyl ether to yield benzyl 4-(6,7-dimethoxy-4-oxo-3,4-dihydroquinazolin-2-yl)piperidine-1-carboxylate **16** (5.4 g, 12.74 mmol, 96%) as a pale yellow solid. $POCl_3$ (50 mL) was added to this compound **16** (5.39 g) and stirred under reflux for 1 h. The reaction mixture was concentrated in vacuo, and potassium carbonate aqueous solution was added to the residue. The resulting mixture was extracted with chloroform, and the organic layer was washed with brine, dried over sodium sulfate, filtered, and evaporated in vacuo. The residue was purified via column chromatography to yield benzyl 4-(4-chloro-6,7-dimethoxyquinazolin-2-yl)piperidine-1-carboxylate (2.45 g, 5.55 mmol, 44%).

Benzyl 4-[4-(cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]piperidine-1-carboxylate **17** was prepared (1.02 g, 1.96 mmol, 78%) from benzyl 4-(4-chloro-6,7-dimethoxyquinazolin-2-yl)-piperidine-1-carboxylate (1.1 g, 2.5 mmol) using a procedure similar to that described for the synthesis of **13a**.

MS (ESI^+) m/z 519 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.42–1.79 (12H, m), 1.88–2.02 (4H, m), 2.81–2.93 (1H, m), 3.22–3.40 (2H, m), 3.88 (3H, s), 3.89 (3H, s), 4.04–4.12 (2H, m), 4.25–4.38 (1H, m), 5.10 (2H, s), 7.05 (1H, s), 7.29–7.40 (5H, m), 7.66 (1H, s).

6.1.22. *N*-Cycloheptyl-2-(1-cyclohexylpiperidine-4-yl)-6,7-dimethoxyquinazolin-4-amine (20)

Palladium on carbon (10%, 100 mg) stirred at room temperature under 1 atm of hydrogen gas for 4 h was added to a solution of benzyl 4-[4-(cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]piper-idine-1-carboxylate **17** (1.02 g, 1.96 mmol) in methanol (20 mL), and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to yield crude *N*-cycloheptyl-6,7-dimethoxy-2-piperidine-4-ylquinazolin-4-amine (721 mg) as a white solid, which was used in a subsequent reaction without further purification.

Sodium carbonate (239 mg, 2.26 mmol) and iodocyclohexane (613 mg) were added to the solution of the crude *N*-cycloheptyl-6,7-dimethoxy-2-piperidine-4-ylquinazolin-4-amine (721 mg) in DMF (20 mL). The resulting mixture was stirred at 80 °C for 5 h, and chloroform was added. The solution was then 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 *N*-cycloheptyl-2-(1-cyclohexylpiperidine-4-yl)-6,7-dimethoxyquinazolin-4-amine **20** (166 mg, 0.36 mmol, 19%) as a yellow oil. HCl ethyl acetate solution (4 M, 0.3 mL) was added to a solution of this oil **20** (166 mg) in methanol (10 mL). The resulting solution was stirred at room temperature for 1 h, and the reaction mixture was concentrated in vacuo. The residue was recrystallized from isopropanol to yield the hydrochloride salt (74 mg, 38%) as a white solid.

Mp (dec) 236–237 °C (*i*-PrOH); MS (FAB^+) m/z 467 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ 1.04–1.18 (1H, m), 1.20–1.34 (2H, m), 1.35–1.70 (10H, m), 1.70–1.88 (6H, m), 1.90–2.02 (2H, m), 2.05–2.45 (6H, m), 3.01–3.23 (3H, m), 3.48–3.58 (2H, m), 3.92 (3H, s), 3.94 (3H, s), 4.45–4.59 (1H, m), 7.42 (1H, s), 8.08 (1H, s), 9.62 (1H, s), 10.50 (1H, s). Anal. Calcd for $C_{28}H_{42}N_4O_2{\cdot}2HCl{\cdot}1.6H_2O$: C, 59.17; H, 8.37; N, 9.86; Cl, 12.47. Found: C, 58.67; H, 8.35; N, 9.90; Cl, 12.98.

6.1.23. *N*-Benzyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-001)

Benzylamine (21 mg, 0.2 mmol) was added to a solution of 2,4-dichloro-6,7-dimethoxyquinazoline **12a** (52 mg, 0.1 mmol) in DMF (0.1 mL), and the resulting mixture was stirred at room temperature for 10 h. Water was added to the reaction mixture, and the resulting solution was extracted with chloroform. The organic layer was evaporated in vacuo to yield crude *N*-benzyl-2-chloro-6,7-dimethoxyquinazolin-4-amine as an oil, which was used in a subsequent reaction without further purification. 4-pyrrolidin-1-ylpiperidine (31 mg, 0.2 mmol) was added to a solution of crude *N*-benzyl-2-chloro-6,7-dimethoxyquinazolin-4-amine in *n*-BuOH (0.1 mL), and the mixture was stirred under reflux for 10 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was purified using preparative HPLC to yield *N*-benzyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine **C-001** (30mg, 67%).

^1H NMR (400 MHz, $CDCl_3$) δ 1.61–1.69 (2H, m), 1.93–2.04 (6H, m), 2.78–2.96 (6H, m), 3.92 (3H, s), 3.94 (3H, s), 4.65 (2H, d, J = 5.4 Hz), 4.78 (2H, m), 7.04 (1H, s), 7.20–7.36 (6H, m), 8.10 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{26}H_{34}N_5O_2$: 448.2713. Found: 448.2694.

Compounds **C-002-034** were prepared using procedures similar to those described for the synthesis of **C-001**.

6.1.24. 6,7-Dimethoxy-*N*-(2-phenylethyl)-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-002)

27 mg, 58%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.71–1.80 (2H, m), 1.90–1.98 (6H, m), 2.05–2.07 (2H, m), 2.76–2.81 (2H, m), 2.94–3.01 (7H, m), 4.76–4.80 (2H, m), 6.99 (1H, s), 7.15 (1H, s), 7.20–7.36 (6H, m), 8.00 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{27}H_{36}N_5O_2$: 462.2869. Found: 462.2874.

6.1.25. *N*-Ethyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-003)

9 mg, 23%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, J = 7.1 Hz), 1.46–1.59 (2H, m), 1.78–1.80 (4H, m), 1.97–2.00 (2H, m), 2.19–2.26 (1H, m), 2.58–2.70 (4H, m), 2.87–2.94 (2H, m), 3.63 (2H, quartet, J = 7.3 Hz), 3.91 (3H, s), 3.92 (3H, s), 4.82–4.85 (2H, m), 6.74 (1H, s), 6.90 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{21}H_{32}N_5O_2$: 386.2556. Found: 386.2547.

6.1.26. *N*-Cyclopentyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-004)

23 mg, 54%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.60–1.85 (8H, m), 1.93–2.10 (4H, m), 2.07–2.14 (4H, m), 2.86–3.10 (7H, m), 3.92 (3H, s), 3.93 (3H, s), 4.30–4.35 (1H, m), 4.85–4.88 (2H, m), 7.03 (1H, s), 7.23 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{36}N_5O_2$: 426.2869. Found: 426.2855.

6.1.27. *N*-Cyclohexyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-005)

30 mg, 68%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.20–1.40 (5H, m), 1.68–1.80 (5H, m), 1.95–2.20 (8H, m), 2.87–3.01 (3H, m), 3.06–3.16 (4H, m), 3.89–4.11 (7H, m), 4.87–4.91 (2H, m), 7.12 (1H, s), 7.23 (1H, s),

7.75 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{25}H_{38}N_5O_2$: 440.3026. Found: 440.3015.

6.1.28. *N*-(4-*tert*-Butylcyclohexyl)-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-007)

29 mg, 59%.

^1H NMR (400 MHz, $CDCl_3$) δ 0.88–0.89 (9H, m), 0.96–1.40 (4H, m), 1.57–1.88 (5H, m), 1.95–2.05 (4H, m), 2.08–2.17 (4H, m), 2.79–3.20 (6H, m), 3.85–4.05 (6H, m), 4.34–4.43 (1H, m), 4.90–5.06 (2H, m), 6.06–6.07 (1H, m), 6.95 (1H, s), 7.08 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{29}H_{46}N_5O_2$: 496.3652. Found: 496.3649.

6.1.29. *N*-Cyclooctyl-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-008)

33 mg, 71%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.50–1.68 (8H, m), 1.70–1.84 (6H, m), 1.88–2.01 (6H, m), 2.05–2.15 (2H, m), 2.90–3.20 (6H, m), 3.93 (6H, s), 4.31–4.33 (1H, m), 4.93–4.96 (2H, m), 6.83–6.88 (1H, m), 7.15 (1H, s), 7.21 (1H, s), 8.14 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{27}H_{42}N_5O_2$: 468.3339. Found: 468.3325.

6.1.30. 3-{[6,7-Dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-yl]amino}azepan-2-one (C-009)

3.4 mg, 7%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.46–1.61 (4H, m), 1.65–2.01 (9H, m), 2.05–2.15 (1H, m), 2.22–2.27 (1H, m), 2.38–2.41 (1H, m). 2.55–2.70 (4H, m), 2.88–2.97 (2H, m), 3.20–3.48 (2H, m), 3.95 (3H, s), 3.96 (3H, s), 4.81–4.86 (3H, m), 6.07–6.10 (1H, m), 6.78–6.79 (1H, m), 6.88 (1H, s), 6.89 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{25}H_{37}N_6O_3$: 469.2927. Found: 469.2910.

6.1.31. Ethyl 4-{[6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-yl]amino}piperidine-1-carboxylate (C-010)

33 mg, 64%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.27 (3H, t, J = 6.8 Hz), 1.50–1.65 (2H, m), 1.78–1.90 (2H, m), 1.95–2.20 (8H, m), 2.85–3.20 (8H, m), 3.92 (3H, s), 3.94 (3H, s), 4.10–4.30 (5H, m), 4.89–4.93 (2H, m), 6.92–6.94 (1H, m), 7.08 (1H, s), 7.22 (1H, s), 8.58 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{27}H_{41}N_6O_4$: 513.3189. Found: 513.3171.

6.1.32. *N*-(1-Benzylpiperidine-4-yl)-6,7-dimethoxy-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-011)

39 mg, 73%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.74–1.88 (4H, m), 1.95–2.02 (4H, m), 2.05–2.15 (4H, m), 2.31–2.42 (2H, m), 2.75–2.92 (2H, m), 2.95–3.04 (1H, m), 3.06–3.21 (5H, m), 3.72 (2H, s), 3.93 (6H, s), 4.10–4.13 (1H, m), 4.88–4.91 (2H, m), 6.68–6.69 (1H, m), 7.02 (1H, s), 7.15 (1H, s), 7.30–7.43 (5H, m), 8.59 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{31}H_{43}N_6O_2$: 531.3448. Found: 531.3432.

6.1.33. 6,7-Dimethoxy-*N*,*N*-dimethyl-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazolin-4-amine (C-012)

18 mg, 47%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.79–1.89 (2H, m), 1.95–2.08 (4H, m), 2.10–2.18 (2H, m), 2.85–2.95 (2H, m), 3.09–3.28 (11H, m), 3.92 (3H, s), 3.99 (3H, s), 5.02–5.05 (2H, m), 7.15 (1H, s), 7.28 (1H, s), 8.53 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{21}H_{32}N_5O_2$: 386.2556. Found: 386.2546.

6.1.34. 6,7-Dimethoxy-4-piperidine-1-yl-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazoline (C-013)

26 mg, 61%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.68–1.90 (8H, m), 1.95–2.18 (6H, m), 2.79–2.95 (2H, m), 3.05–3.30 (5H, m), 3.45–3.65 (4H, m), 3.92 (3H, s), 3.98 (3H, s), 4.99–5.08 (2H, m), 6.99 (1H, s), 7.03 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{36}N_5O_2$: 426.2869. Found: 426.2852.

6.1.35. 6,7-Dimethoxy-4-morpholin-4-yl-2-(4-pyrrolidin-1-ylpiperidine-1-yl)quinazoline (C-014)

28 mg, 65%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.70–1.92 (2H, m), 2.00–2.22 (6H, m), 2.79–2.98 (2H, m), 3.05–3.38 (4H, m), 3.50–3.70 (4H, m), 3.85–3.91 (3H, m), 3.92 (3H, s), 3.98 (3H, s), 4.99–5.08 (2H, m), 6.96 (2H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{23}H_{34}N_5O_3$: 428.2662. Found: 428.2644.

6.1.36. *N*-Cycloheptyl-6,7-dimethoxy-2-piperidine-1-ylquinazolin-4-amine (C-015)

3.5 mg, 9%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.45–1.80 (14H, m), 1.85–2.20 (4H, m), 3.77–3.89 (4H, m), 3.92 (3H, s), 3.95 (3H, s), 4.20–4.35 (1H, m), 5.15–5.30 (1H, m), 6.73 (1H, s), 6.94 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{22}H_{33}N_4O_2$: 385.2604. Found: 385.2609.

6.1.37. *N*-Cycloheptyl-2-[4-(dimethylamino)piperidine-1-yl]-6,7-dimethoxyquinazolin-4-amine (C-016)

29 mg, 68%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.44–1.85 (12H m), 1.97–2.14 (4H, m), 2.56 (3H, s), 2.62 (3H, s), 2.89–3.10 (3H, m), 3.93 (6H, s), 4.10–4.25 (1H, m), 4.92–5.01 (2H, m), 6.73–6.75 (1H, m), 7.09 (1H, s), 7.14 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{38}N_5O_2$: 428.3026. Found: 428.3009.

6.1.38. *N*-Cycloheptyl-6,7-dimethoxy-2-(4-methylpiperidine-1-yl)quinazolin-4-amine (C-017)

6.2 mg, 16%.

^1H NMR (400 MHz, $CDCl_3$) δ 0.96 (3H, d, J = 6.4 Hz), 1.12–1.28 (2H, m), 1.40–1.82 (13H, m), 2.02–2.17 (2H, m), 2.82–2.95 (2H, m), 3.926 (3H, s), 3.933 (3H, s), 4.14–4.25 (1H, m), 4.70–4.80 (2H, m), 6.05 (1H, br), 6.93 (1H, s), 6.97 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{23}H_{35}N_4O_2$: 399.2760. Found: 399.2750.

6.1.39. 1-[4-(Cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]piperidine-4-ol (C-018)

23 mg, 57%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.50–1.80 (12H, m), 1.92–2.03 (2H, m), 2.08–2.18 (2H, m), 3.18–3.30 (2H, m), 3.85–4.00 (7H, m), 4.21–4.33 (1H, m), 4.50–4.62 (2H, m), 5.03–5.15 (1H, m), 6.71 (1H, s), 6.91 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{22}H_{33}N_4O_3$: 401.2553. Found: 401.2537.

6.1.40. 1-[4-(Cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]piperidine-4-carboxamide (C-019)

2 mg, 5%.

^1H NMR (400 MHz, DMSO-d_6) δ 1.39–1.78 (14H, m), 1.92–2.03 (2H, m), 2.29–2.39 (1H, m), 2.74–2.86 (2H, m), 3.81 (3H, s), 3.82 (3H s), 4.15–4.23 (1H, m), 4.68–4.77 (2H, m), 6.71–6.74 (2H, br), 7.26 (1H, s), 7.46 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{23}H_{34}N_5O_3$: 428.2662. Found: 428.2648.

6.1.41. 2-(4-Benzylpiperidine-1-yl)-*N*-cycloheptyl-6,7-dimethoxyquinazolin-4-amine (C-020)

24 mg, 51%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.18–1.35 (2H, m), 1.42–1.90 (13H, m), 2.00–2.19 (2H, m), 2.55 (2H, d, J = 6.8 Hz), 2.82–2.96

(2H, m), 3.90 (3H, s), 3.92 (3H, s), 4.07–4.18 (1H, m), 4.68–4.83 (2H, m), 7.01 (1H, s), 7.11–7.38 (6H, m). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{29}H_{39}N_4O_2$: 475.3073. Found: 475.3059.

6.1.42. N^4-Cycloheptyl-6,7-dimethoxy-N^2-(2-piperidine-1-ylethyl)quinazoline-2,4-diamine (C-022)

9.5 mg, 22%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.30–1.87 (16H, m), 2.00–2.20 (2H, m), 2.40–2.93 (6H, m), 3.60–3.76 (2H, m), 4.00 (6H, s), 4.30–4.47 (1H, m), 6.88 (1H, s), 7.03 (1H, br), 7.17 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{38}N_5O_2$: 428.3026. Found: 428.3015.

6.1.43. N^4-Cycloheptyl-6,7-dimethoxy-N^2-(3-pyrrolidin-1-ylpropyl)quinazoline-2,4-diamine (C-023)

13 mg, 30%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.40–1.80 (10H, m), 1.89–2.18 (8H, m), 2.98–3.20 (6H, m), 3.45–3.65 (2H, m), 3.94 (3H, s), 3.95 (3H, s), 4.25–4.38 (1H, m), 6.87 (1H, s), 7.05–7.17 (1H, br), 7.21 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{38}N_5O_2$: 428.3026. Found: 428.3018.

6.1.44. 1-(3-{[4-(Cycloheptylamino)-6,7-dimethoxyquinazolin-2-yl]amino}propyl)pyrrolidin-2-one (C-024)

4.9 mg, 11%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.49–1.82 (10H, m), 1.86–2.17 (6H, m), 2.36 (2H, t, J = 8.3 Hz), 3.36–3.55 (4H, m), 3.94 (3H, s), 3.97 (3H, s), 4.27–4.38 (1H, m), 6.30 (1H, br), 6.91 (2H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{36}N_5O_3$: 442.2818. Found: 442.2806.

6.1.45. N-Cycloheptyl-6,7-dimethoxy-2-(4-methylpiperazine-1-yl)quinazolin-4-amine (C-025)

21 mg, 53%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.43–1.84 (10H, m), 2.02–2.16 (2H, m), 2.41 (3H, s), 2.55–2.73 (4H, m), 3.91 (3H, s), 3.92 (3H, s), 3.93–4.00 (4H, m), 4.08–4.20 (1H, m), 6.99 (1H, s), 7.16 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{22}H_{34}N_5O_2$: 400.2713. Found: 400.2698.

6.1.46. N-Cycloheptyl-2-(4-isopropylpiperazine-1-yl)-6,7-dimethoxyquinazolin-4-amine (C-026)

22 mg, 51%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.16 (6H, d, J = 6.4 Hz), 1.46–1.80 (10H, m), 2.00–2.15 (2H, m), 2.70–2.88 (4H, m), 2.95–3.08 (1H, m), 3.920 (3H, s), 3.924 (3H, s), 3.97–4.08 (4H, m), 4.10–4.22 (1H, m), 6.70 (1H, br), 6.99 (1H, s), 7.10 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{38}N_5O_2$: 428.3026. Found: 428.3015.

6.1.47. N-Cycloheptyl-6,7-dimethoxy-2-(4-phenylpiperazine-1-yl)quinazolin-4-amine (C-027)

16 mg, 35%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.45–1.85 (10H, m), 2.07–2.19 (2H, m), 3.21–3.34 (4H, m), 3.938 (3H, s), 3.944 (3H, s), 4.02–4.10 (4H, m), 4.18–4.30 (1H, m), 6.36 (1H, br), 6.85–7.13 (5H, m), 7.25–7.35 (2H, m). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{27}H_{36}N_5O_2$: 462.2869. Found: 462.2861.

6.1.48. N-Cycloheptyl-6,7-dimethoxy-2-morpholin-4-ylquinazolin-4-amine (C-028)

1.7 mg, 4%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.50–1.80 (10H, m), 2.07–2.17 (2H, m), 3.77–3.90 (8H, m), 3.95 (3H, s), 3.96 (3H, s), 4.25–4.33 (1H, m), 5.02–5.10 (1H, m), 6.70 (1H, s), 6.91 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{21}H_{31}N_4O_3$: 387.2396. Found: 387.2406.

6.1.49. N^4-Cycloheptyl-N^2-isopropyl-6,7-dimethoxyquinazoline-2,4-diamine (C-029)

3.1 mg, 9%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (6H, d, J = 6.3 Hz), 1.50–1.85 (10H, m), 2.05–2.20 (2H, m), 3.94 (3H, s), 3.97 (3H, s), 4.19 (1H, tt, J = 6.8 Hz), 4.28–4.40 (1H, m), 6.15–6.29 (1H, m), 6.89 (1H, s), 6.96 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{20}H_{31}N_4O_2$: 359.2447. Found: 359.2442.

6.1.50. N^4-Cycloheptyl-N^2-cyclopentyl-6,7-dimethoxyquinazoline-2,4-diamine (C-030)

13 mg, 34%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.48–1.85 (16H, m), 1.93–2.13 (4H, m), 3.93 (3H, s), 3.96 (3H, s), 4.24–4.39 (2H, m), 6.92 (1H, s), 7.31 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{22}H_{33}N_4O_2$: 385.2604. Found: 385.2596.

6.1.51. N^2-Benzyl-N^4-cycloheptyl-6,7-dimethoxyquinazoline-2,4-diamine (C-031)

19 mg, 47%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.38–1.72 (10H, m), 1.91–2.03 (2H, m), 3.92 (3H, s), 3.93 (3H, s), 4.20–4.30 (1H, m), 4.62 (2H, d, J = 5.9 Hz), 6.88 (1H, s), 7.08 (1H, br), 7.15–7.36 (6H, m). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{24}H_{31}N_4O_2$: 407.2447. Found: 407.2433.

6.1.52. N^4-Cycloheptyl-6,7-dimethoxy-N^2-(2-thienylmethyl)quinazoline-2,4-diamine (C-032)

19 mg, 46%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.40–1.80 (10H, m), 1.98–2.12 (2H, m), 3.87 (3H, s), 3.90 (3H, s), 4.37–4.40 (1H, m), 4.78 (2H, d, J = 5.4 Hz), 6.79–6.93 (3H, m), 7.06–7.13 (1H, m), 7.33 (1H, s), 7.56 (1H, br). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{22}H_{29}N_4O_2S$: 413.2011. Found: 413.1996.

6.1.53. N^4-Cycloheptyl-6,7-dimethoxy-N^2-(2-phenylethyl)quinazoline-2,4-diamine (C-033)

20 mg, 48%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.52–1.85 (10H, m), 2.07–2.17 (2H, m), 2.91–3.01 (2H, m), 3.65–3.78 (2H, m), 3.95 (3H, s), 3.99 (3H, s), 4.38 (1H, br), 6.84 (1H, s), 6.96 (1H, s). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{25}H_{33}N_4O_2$: 421.2604. Found: 421.2597.

6.1.54. N^4-Cycloheptyl-6,7-dimethoxy-N^2-methyl-N^2-(2-phenylethyl)quinazoline-2,4-diamine (C-034)

24 mg, 55%.

^1H NMR (400 MHz, $CDCl_3$) δ 1.45–1.80 (10H, m), 2.05–2.18 (2H, m), 2.93 (2H, t, J = 7.3 Hz), 3.18 (3H, s), 3.82–4.00 (8H, m), 4.18–4.29 (1H, m), 6.93 (1H, br), 7.05 (1H, s), 7.12–7.33 (6H, m). High-resolution MS (ESI^+) m/z $[M+H]^+$. Anal. Calcd for $C_{26}H_{35}N_4O_2$: 435.2760. Found: 435.2741.

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[17], 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. [^{125}I]CCL22-binding assay

CCR4-receptor-binding assays were performed using a scintillation proximity assay (SPA). Human CCR4-expressing cells (2×10^5 cells/well) were incubated at 25 °C for 2 h with 25 pM [^{125}I]CCL22 (PerkinElmer Life Sciences) and 5 mg/mL wheat germ agglutinin SPA beads (GE Healthcare), with various concentrations of test compounds in 100 μl of binding buffer [50 mM Hepes (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.1% (w/v) bovine serum albumin (BSA)]. Radioactivity was counted using a TopCount scintillation counter (Packard Biosciences). Control wells free from test compound (for total counts) or containing excess unlabeled CCL22 (10 nM, 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 the average of two (usually) determinations.

6.2.3. [^{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 (Imai, T.; Chantry, D.; Raport, C. J.; Wood, C. L.; Nishimura, M.; Godiska, R.; Yoshie, O.; Gray, P. W. *J. Biol. Chem.* **1998**, *273*, 1764.). In addition, our test compounds were found to inhibit responses induced by CCL22 and CCL17 equally (data not shown).

6.2.4. Cytotoxicity assay

Cytotoxicity assays were performed using the Alamar Blue assay.[18] CCR4-expressing cells (5×10^4 cells/100 μl/well) were incubated in RPMI 1640 containing 10% FBS, 50 μM 2-mercaptethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO_2 atmosphere, and treated with 0.3–10 μM test compound. After 12 h, 10 μL of Alamar Blue dye (Biosource) was added to each well. After incubation for 4 h, the plates were read on a micro plate fluorescence reader (Spectra Max 190, Molecular Devices) at excitation and emission wavelengths of 570 and 600 nm, respectively. One hundred percent viable control wells containing no test compound were used to calculate the percent of total inhibition for each set of compounds. The minimum cytotoxic dose was designated as the concentration required to reduce the cell viability to less than 70%.

6.2.5. Chemotaxis assay

Chemotaxis assays were performed using 96-well chemotaxis chambers (Neuro Probe)[19] 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.

6.3. Data analysis

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

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