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Identification of 4-amino-2-cyclohexylaminoquinazolines as metabolically stable melanin-concentrating hormone receptor 1 antagonists

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Abstract—The optimization of the distance between two key pharmacophore features within our first hit compounds **1a** and **2a** led to the identification of a new class of potent non-peptidic antagonists for the MCH-R1, based around 4-amino-2-cyclohexylaminoquinazolines. In particular, ATC0065 (**2c**), N^2-[*cis*-4-({2-[4-Bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride, bound with high affinity to the MCH-R1 (IC_{50} value of 16 nM) and showed good metabolic stability in liver microsomes from human and rat.

1. Introduction

Melanin-concentrating hormone (MCH) was originally isolated from salmon pituitary as a cyclic 17 amino acid peptide.[1] Subsequently, rat MCH (rMCH) was identified and characterized as a cyclic 19 amino acid peptide.[2] In 1999, human MCH was identified as the endogenous ligand of the orphan G-protein-coupled receptor (GPCR), SLC-1 (subsequently termed MCH-R1). This pairing of the MCH ligand with its receptor, which has significant homology with the somatostatin receptors,[3] was made independently by several groups.[4–7] Subsequently, another human orphan receptor, SLT, originally identified from the human genomic sequence and cloned from a human hippocampus cDNA library, was identified as a second MCH receptor (MCH-R2).[8–12] Interestingly, MCH-R2 is found in humans, monkeys, dogs, and ferrets, whereas several lower species such as rats, mice, hamsters, guinea pigs, and rabbits either lack functional MCH-R2 or encode a non-functional MCH-R2 pseudogene.[13] Due to this species-specific expression of MCH-R2, potential physiological functions of the receptor have not been elucidated.

There has been significant interest in the pharmacology of MCH since the discovery of the MCH-R1.[14] Neurons containing MCH are located in the lateral hypothalamic area (LHA) and the rostromedial zona incerta, suggesting a role for MCH in feeding behavior and energy expenditure. Indeed, the intracerebroventricular (icv) injection of MCH in rats increases food intake.[15,16] Moreover, MCH mRNA has been reported to be upregulated in both normal and ob/ob mice, and to be increased in ob/ob mice relative to wild-type animals.[15] In contrast, prepro-MCH-knockout mice were found to be hypophagic and have reduced bodyweight and increased leanness relative to their wild-type counterparts.[17] Mice in which the MCH-R1 receptor has been knocked out have normal bodyweights but are lean and hyperphagic.[18,19] A peptide MCH-R1 antagonist when dosed icv reduced feeding and bodyweight gains over a period of 14 days.[20] These data suggest that a centrally acting MCH-R1 antagonist may reduce food intake and bodyweight, and hence may be useful for the treatment of obesity. This appears to be confirmed with a non-peptide antagonist (SNAP-7941) that when

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dosed twice daily ip (20 mg/kg) reduced feeding and bodyweight in diet induced obese rats.[21]

In addition, MCH was reported to induce anxiogenic effects when injected into the medial preoptic area in rats,[22] while icv injections of MCH increased the number of entries into the open arms in the elevated plus-maze test,[23] suggesting that MCH may also be implicated in the modulation of emotional states. This hypothesis is supported by a recent finding that SNAP-7941, a selective antagonist for the MCH-R1, exhibits anxiolytic and antidepressant effects in rodent models.[21]

Since the pioneering discovery of the small molecule MCH-R1 antagonist, T-226296 ((−)-enantiomer of compound **I**), which exhibits high affinity for MCH-R1 in receptor binding assays (IC_{50} value of 5.5 nM), a plethora of structurally diverse MCH-R1 selective antagonists have been reported (Fig. 1).[24] An alternative cyclization pattern and incorporation of a thieno[3,2-*d*]prymidin-4(3*H*)-one ring that served to lock the amide conformation led to the discovery of GW3430 (**II**) (pIC_{50} value of 9.3).[25] GW3430 is an orally active compound causing significant and dose-dependent weight loss in obese AKR mice over a 12-day period. GlaxoSmithKline has also reported the initiation of first clinical evaluation of an MCH-R1 antagonist with GW3430 for the treatment of obesity. Synaptic has reported an extensive series of substituted anilinic piperidines, showing that significant structural diversity in the left portion of their series maintained MCH-R1 antagonist activity. Among them, SNAP-7941 (**III**), featuring an *N*-acyl moiety in the *meta*-position, has been revealed as a competitive antagonist of MCH-R1 in a phosphoinositide accumulation assay (pA_2 value of 9.24).[21] By combining MCH-R1 modeling and structural input from dopamine D_2 receptor ligands, compound **IV** (IC_{50} value of 28 nM) was identified as the lead compound among a new series of MCH-R1 antagonists.[26] Based on reported structure–activity relationships (SAR), the antagonist activity of the phenoxyphenylurea derivatives appeared in general to exhibit a considerable tolerance around the amine in the right part of the molecule, whilst it was generally quite sensitive to structural modifications in the left part, which is somewhat distinct from the previously known series. The last structural motif is the substituted biaryl scaffold, which is similar to known acyl-CoA cholesterol acyltransferase (ACAT) inhibitors. Since the discovery of this motif, Schering has continued far-reaching investigations, claiming a number of MCH-R1 selective antagonists in diverse patent applications. One of the most noticeable structural modifications involves incorporating the trans-bicycloheptyl moiety in place of the biaryl structure to avoid potential mutagenicity of the biarylaniline in vivo; this modifica-

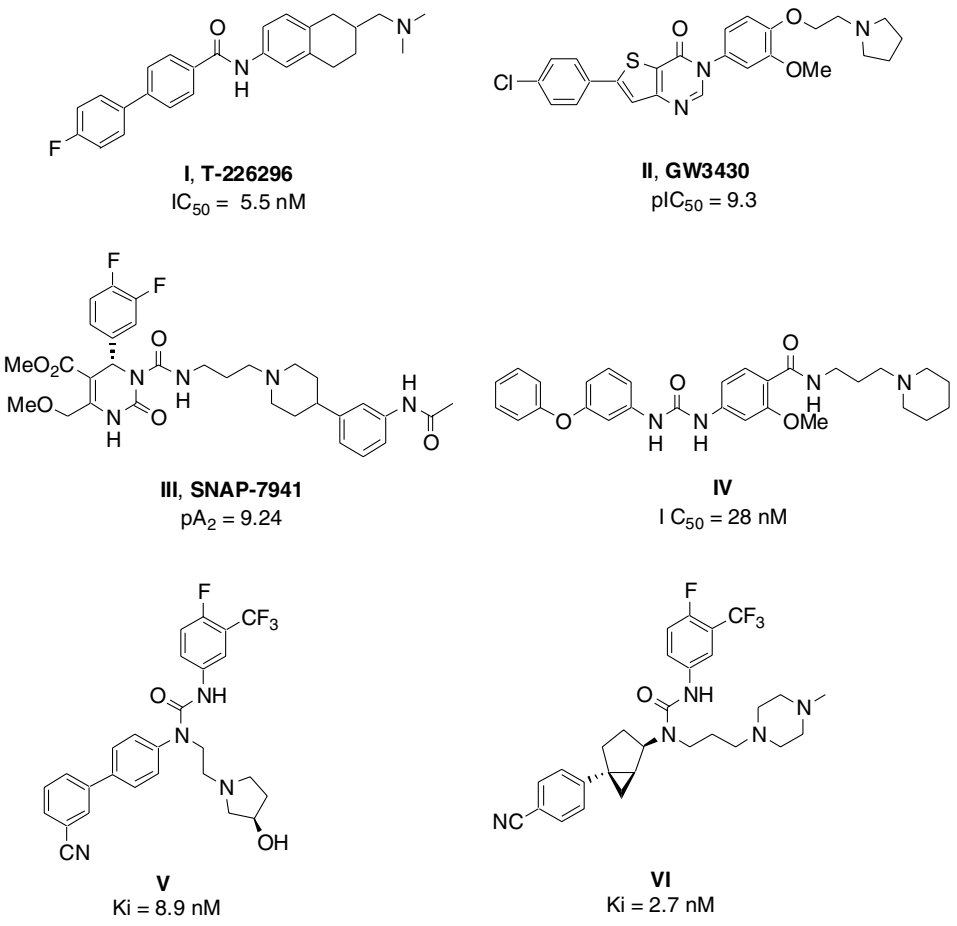
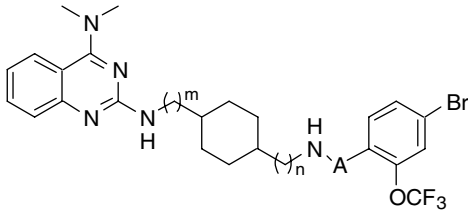


Figure 1. Non-peptidic MCH-R1 antagonists.

tion is exemplified by compounds **V** and **VI** (K_i values of 8.9 and 2.7 nM, respectively).[27]

As previously reported, high-throughput screening resulted in the identification of our lead compound AR129330 (Table 1).[28] During our initial exploration, we identified that both 4-amino-substitution of the quinazoline and the terminal phenyl group are critical for MCH-R1 antagonist activity and the quinazoline and the benzene rings form two key parts of the MCH-R1 pharmacophore. Subsequent systematic optimization of the spacer and the linker portions of our lead series identified a core 4-dimethylamino-2-cyclohexylamino-quinazoline structure, exemplified by compounds **1a** and **2a** (IC_{50} values of 4.9 and 35 nM, respectively).[29] Although these initial hit compounds showed potent affinity for the MCH-R1, in particular compound **1a**, compound **1a** displayed metabolic instability ($T_{1/2}$ values of 8.9 and 5.8 min for human and rat liver microsomes, respectively). We considered that the metabolic instability of compounds **1a** might be caused by the benzylic amine structure. Therefore, we believed that both compounds **1a** and **2a** had sufficient potency, as well as a pathway forward to improving metabolic stability, to deserve further optimization of the distance between the two key pharmacophore features. In this report, we disclose our efforts to investigate SAR at the MCH-R1 based on the 4-amino-2-cyclohexylaminoquinazoline series and to improve metabolic stability, leading to the identification of ATC0065, a potent and orally efficacious antagonist of MCH-R1 for the treatment of either obesity or, as previously reported, anxiety and depression.[30]

Table 1. Quinazoline derivatives as MCH-R1 antagonists

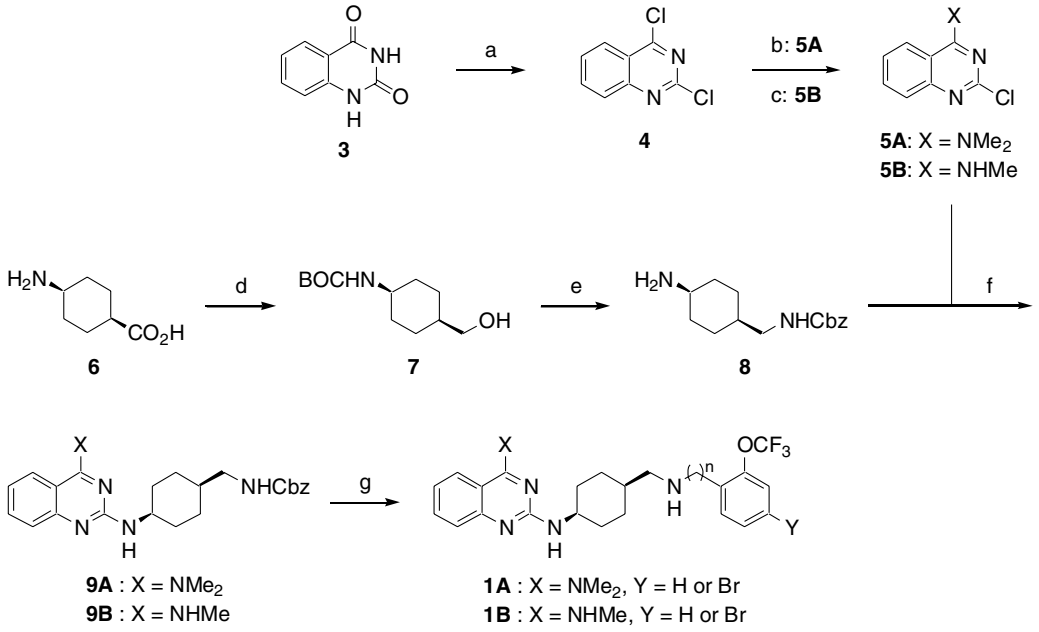


Compound	Conformation	*m*	*n*	A	MCH-R1 IC_{50}[a] (nM)
AR129330	*trans*	1	1	SO_2	160
1a	*cis*	0	1	CH_2	4.9
2a	*cis*	0	0	CH_2	35

[a] Compounds were evaluated for their ability to compete with [^{125}I](Phe^{13}, Tyr^{19})MCH at a human MCH-R1 stably expressed in HEK293 cells. Data represent means of 1–3 separate experiments performed from 5 concentrations in duplicate.

2. Chemistry

Preparation of the quinazoline derivatives with the *cis*-4-aminomethylcyclohexylamine is summarized in Scheme 1. Quinazoline cores **5A** or **5B** were synthesized in two steps from commercially available 1*H*,3*H*-quinazoline-2,4-dione **3**. The starting material was reacted with phosphorus oxychloride ($POCl_3$) under reflux in the presence of *N*,*N*-dimethylaniline to provide 2,4-dichloro-quinazoline **4**. Selective substitution of the chlorine at the 4-position with 50% aqueous dimethylamine or 40% aqueous methylamine gave the corresponding 4-amino-2-chloro-quinazoline **5A** or **5B**.[31] The mono-



Scheme 1. Reagents and conditions: (a) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 7 h, 86%; (b) aq 50% Me_2NH, THF, rt, 80 min, 94%; (c) aq 40% $MeNH_2$, THF, rt, 40 min, 94%; (d) i—$SOCl_2$, MeOH, rt, 4.5 h, ii—$(BOC)_2O$, Et_3N, $CHCl_3$, rt, 5 h, iii—LAH, Et_2O, rt, 2 h, 77% in three steps; (e) i—phthalimide, 40% DEAD in toluene, PPh_3, THF, rt, 2.5 days, ii—hydrazine hydrate, EtOH, reflux, 2.25 h, iii—CbzCl, Et_3N, $CHCl_3$, rt, 16 h, 91% in three steps, iv—4 M HCl in EtOAc, EtOAc, rt, 3 h, 95%; (f) compound **5A** or **5B**, isopropanol, reflux; (g) i—H_2, 5% Pd–C or 10% Pd–C, MeOH, 50 °C, ii—RCHO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt or RCHO, $NaBH_3CN$, AcOH, MeOH, rt, or i—H_2, 5% Pd–C or 10% Pd–C, MeOH, 50 °C, ii—RCOCl, iPr_2NEt, CH_2Cl_2, 4 °C, iii—1 M BH_3-THF in THF, THF, reflux.

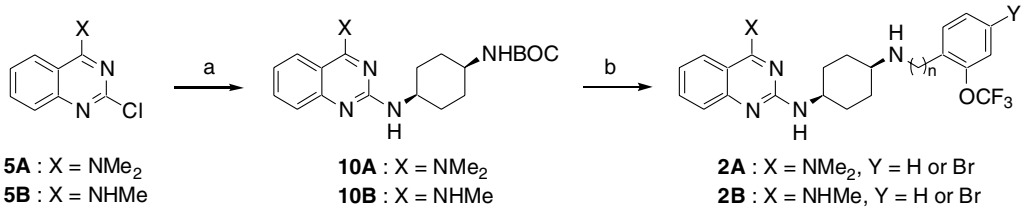
protected *cis*-1,4-cyclohexylmethyldiamine **8** was prepared in seven steps from commercially available 4-aminocyclohexane carboxylic acid **6**. Treatment of **6** with thionyl chloride in methanol provided the corresponding methyl ester. Protection of the amine as its *tert*-butyl carbamate (BOC) derivative followed by reduction of the ester group with lithium aluminum hydride (LAH) then produced alcohol **7**. Conversion of the alcohol **7** to the corresponding amine was accomplished via the phthalimide derivative, and the amine was then protected as benzyl carbamate (Cbz). Hydrogen chloride treatment readily removed the BOC-group of the di-protected diamine to afford amine **8**, which was then condensed with 4-amino-2-chloro-quinazoline **5A** or **5B** under reflux in isopropanol to give the coupling product **9A** or **9B**. Removal of the Cbz group by hydrogenolysis followed by reductive amination with appropriate aldehydes afforded the desired quinazoline derivatives **1A** or **1B**.

The general synthetic approach to the *cis*-cyclohexane-1,4-diamine derivatives **2A** or **2B** is outlined in Scheme 2. The coupling of **5A** or **5B** synthesized in Scheme 1 with commercially available *tert*-butyl (*cis*-4-aminocyclohexyl)carbamate was accomplished upon reflux in isopropanol to afford coupling product **10A** or **10B**. Deprotection of the BOC-group was achieved with hydrogen chloride followed by reductive amination with appropriate aldehydes to afford the desired quinazoline derivatives **2A** or **2B**.

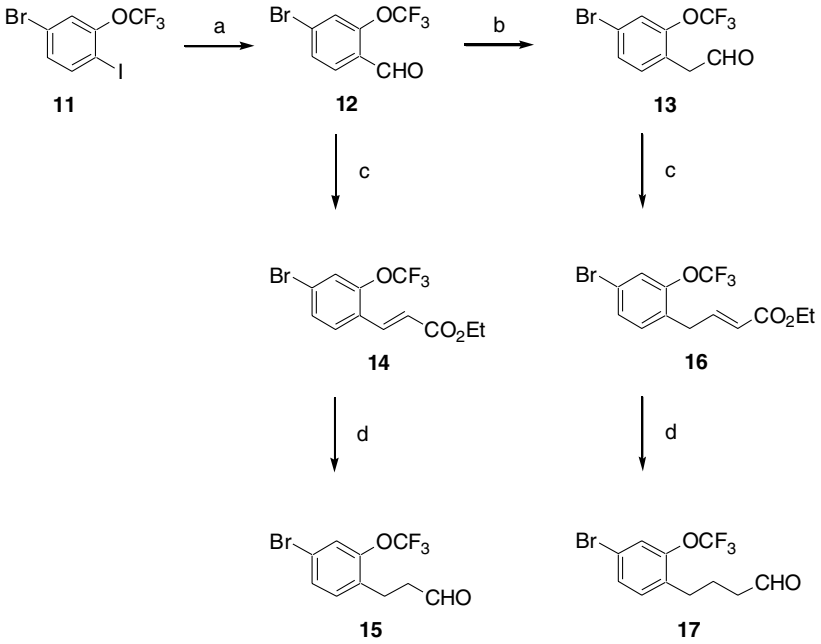
Scheme 3 illustrates the syntheses of the aldehydes with different chain lengths. Formylation of commercially available 4-bromo-1-iodo-2-(trifluoromethoxy)benzene **11** with *N*-formylmorpholine led to the corresponding benzaldehyde **12**.[32] One carbon homologation of **12** was achieved by Wittig reaction followed by hydrolysis under acidic conditions to afford phenyl acetaldehyde **13**. Treatment of **12** with Horner–Emmons reagent provided the α,β-unsaturated ester **14**. Reduction of **14** with LAH followed by oxidation afforded the C3-aldehyde **15**. C4-aldehyde **17** was synthesized from phenyl acetaldehyde **13** by a similar procedure.

3. Results and discussion

The SAR of the MCH-R1 binding of the 4-amino-2-cyclohexylaminoquinazolines is summarized in Table 2. Our initial efforts focused on finding the optimal

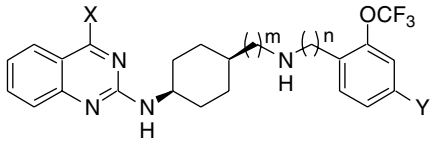


Scheme 2. Reagents and conditions: (a) *tert*-butyl (*cis*-4-aminocyclohexyl)carbamate, isopropanol, reflux; (b) i—4 M HCl in EtOAc, EtOAc, rt, ii—RCHO, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, or RCHO, $NaBH_3CN$, AcOH, MeOH, rt, or 4-bromo-1-iodo-2-(trifluoromethoxy)benzene, $Pd_2(dba)_3$, (R)-BINAP, NaOtBu, 18-C-6, THF, reflux.



Scheme 3. Reagents and conditions: (a) *N*-formylmorpholine, *n*-BuLi in hexane, THF, rt, 80 min, 77%; (b) $MeOCH{=}P(Ph)_3$, Et_2O, rt, 1 h; 10% H_2SO_4, AcOH, rt, 30 min, 67%; (c) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, 4 °C or rt; (d) i—LAH, Et_2O, rt, ii—PCC, Celite, CH_2Cl_2, rt.

Table 2. In vitro data of quinazolines with length of spacer modifications



Compound	*m*	*n*	X	Y	MCH-R1 IC_{50}[a] (nM)
1a	1	1	NMe_2	Br	4.9
1b	1	1	NMe_2	H	5.5
1c	1	2	NMe_2	Br	41
1d	1	2	NMe_2	H	42
1e	1	1	NHMe	Br	4.8
1f	1	1	NHMe	H	7.1
1g	1	2	NHMe	Br	45
1h	1	2	NHMe	H	79
2b	0	0	NMe_2	Br	520
2a	0	1	NMe_2	Br	35
2c (ATC0065)	0	2	NMe_2	Br	16
2d	0	2	NMe_2	H	8.7
2e	0	3	NMe_2	Br	11
2f	0	4	NMe_2	Br	16
2g	0	1	NHMe	Br	71
2h	0	2	NHMe	Br	16
2i	0	3	NHMe	Br	58

[a] See footnote a for Table 1.

distance between the two regions of the MCH-R1 pharmacophore in the *cis*-4-aminomethylcyclohexylamine derivatives. Compound **1c**, in which a methylene group was incorporated between the two features, was about 8-fold less potent at the MCH-R1 than our initial hit compound **1a**. Replacement of a bromine atom with hydrogen in the benzene ring led to compound **1b**, which maintained very potent affinity at the MCH-R1 (IC_{50} value of 5.5 nM). The lengthening of compound **1b** by a methylene group gave reduced interaction with the MCH-R1 (IC_{50} value of 42 nM for compound **1d**). Compound **1e**, in which the dimethylamino group at the 4-position of the quinazoline was replaced by a methylamino group, provided potency comparable to that of our initial hit compound **1a**. Elongation of the molecule showed the same tendency as that seen in the 4-dimethylamino-quinazoline series, whereby compound **1g** showed significantly decreased MCH-R1 affinity (IC_{50} value of 45 nM). Replacement of a bromine atom with hydrogen in the benzene ring demonstrated the same trend as that observed in the 4-dimethylamino-quinazoline series, with compound **1f** demonstrating potent MCH-R1 antagonist activity (IC_{50} value of 7.1 nM). One carbon homologation of compound **1f** provided compound **1h**, which displayed significantly reduced affinity for the MCH-R1 (IC_{50} value of 79 nM) as shown in the comparisons, compounds **1a** versus **1c**, compounds **1b** versus **1d**, and compounds **1e** versus **1g**.

We then examined the effects of changes in the distance between the two extremes of the pharmacophore among *cis*-1,4-cyclohexyldiamine derivatives. The aniline compound **2b** was essentially devoid of antagonist activity for the MCH-R1 (IC_{50} value of 520 nM), but lengthening of the molecule by incorporating methylene groups improved affinity at the MCH-R1 (IC_{50} values of 16, 11, and 16 nM for compound **2c** (ATC0065), **2e**, and **2f**, respectively) compared to the initial hit compound **2a**. Compound **2d**, wherein bromine was again replaced by hydrogen in the benzene ring, increased in MCH-R1 antagonist activity by a factor of about 2 (IC_{50} value of 8.7 nM). Among the 4-methylamino-quinazoline series, compounds **2g**, **2h**, and **2i**, compound **2h** showed optimal affinity at the MCH-R1 (IC_{50} value of 16 nM).

Based on the following comparisons, compounds **1b** versus **1f**, compounds **1d** versus **1h**, compounds **2a** versus **2g**, and compounds **2e** versus **2i**—we concluded that 4-dimethylamino-quinazoline derivatives were superior to 4-methylamino-quinazoline derivatives in terms of MCH-R1 antagonist activity. Replacing a bromine atom with hydrogen in the benzene ring among this series had virtually no effect on affinity for the MCH-R1. Furthermore, data for compounds **1a**, **1b**, **1e**, **1f**, **2c**, **2d**, and **2h**, which all had potent antagonist activity at the MCH-R1, suggested that the distance of three atoms was the optimal length from the cyclohexane ring to the benzene ring.

Although compounds **1b**, **1e**, and **1f** showed potent affinity for the MCH-R1, all of these compounds displayed metabolic instability in liver microsomes from both human and rat, probably due to the benzylic amine structure (data not shown). However, ATC0065 showed a significant improvement in metabolic stability in both of the liver microsomes ($T_{1/2}$ values of 73 and 30 min for human and rat, respectively) in comparison to our initial hit compound **1a** ($T_{1/2}$ values of 8.9 and 5.8 min for human and rat, respectively). We succeeded in the enhancement of metabolic stability with good MCH-R1 antagonist activity only by altering the position of a nitrogen atom, leading to the further investigation of ATC0065 in vivo pharmacology.[30]

4. Conclusion

The optimization of the distance between two key pharmacophore features within our first hit compounds **1a** and **2a** led to the identification of a new class of potent non-peptidic antagonists for the MCH-R1, based around 4-amino-2-cyclohexylaminoquinazolines. In particular, ATC0065 (**2c**), N^2-[*cis*-4-({2-[4-Bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride, bound with high affinity to the MCH-R1 (IC_{50} value of 16 nM) and showed good metabolic stability in liver microsomes from human and rat. Additionally, ATC0065 was shown to exhibit anxiolytic and antidepressant effects in various animal models of anxiety and depression.[30]

5. Experimental

5.1. Chemistry

All reagents and solvents were used as purchased from commercial sources. Moisture-sensitive reactions were

carried out under a nitrogen atmosphere. The progress of the reactions was followed by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F_{254} on glass plates or Fuji Silysia Chemical, NH-silica gel on glass plates). Flash column chromatography and medium-pressure liquid column chromatography were carried out using silica gel Wako Pure Chemical C-200 or NH-silica gel Fuji Silysia chromatorex DM1020. Melting points were measured on a Yanaco MP-500D melting point apparatus and are uncorrected. All compounds were characterized by mass spectra (MS) and proton nuclear magnetic resonance (^{1}H NMR). MS were obtained on a Shimadzu Profile (EI and CI), a JEOL JMS-SX102 (FAB), a Micromass Platform LC (ESI), or a Micromass Q-ToF2 (ESI). ^{1}H NMR spectra were recorded on a Varian Instruments Gemini 2000 (200 MHz) or a Varian Instruments INOVA 300 (300 MHz). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) are given in hertz (Hz) and multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), br s (broad), or m (multiplet). In vitro active compounds were further characterized by elemental analyses, which were performed on a Perkin-Elmer 2400 (carbon, hydrogen, and nitrogen) and Dionex DX-500 (fluorine, chlorine, bromine, and sulfur). The results were within 0.4% of theoretical values.

5.2. 2,4-Dichloroquinazoline (4)

To a suspension of quinazoline-2,4(1*H*,3*H*)-dione **3** (150 g, 925 mmol) in $POCl_3$ (549 mL, 5.89 mol) was added *N*,*N*-dimethylaniline (123 mL, 962 mmol). The mixture was stirred at reflux for 7 h and concentrated. The solution was then poured into ice water and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 50% $CHCl_3$ in hexane to 10% EtOAc in $CHCl_3$) to give **4** (159 g, 86%) as a pale yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.27 (dt, J = 8.3, 1.1 Hz, 1H), 7.95–8.04 (m, 2H), 7.71–7.81 (m, 1H); CI MS *m*/*z* 199 (M^++1, 100%).

5.3. 2-Chloro-*N*,*N*-dimethylquinazolin-4-amine (5A)

A solution of **4** (102 g, 530 mmol) in THF (1.2 L) was cooled to 4 °C and aqueous 50% Me_2NH (139 mL, 1.33 mol) was added. The mixture was stirred at room temperature for 80 min and concentrated. Saturated aqueous $NaHCO_3$ was poured into the residue and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was suspended in 50% Et_2O in hexane (250 mL) and stirred at room temperature for 30 min. The precipitate was collected by filtration, washed with 50% Et_2O in hexane, and dried at 80 °C to give **5A** (104 g, 94%) as a pale yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.00 (d, J = 8.4 Hz, 1H), 7.65–7.78 (m, 2H), 7.38 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 3.41 (s, 6H); ESI MS *m*/*z* 207 (M^+, 100%).

5.4. 2-Chloro-*N*-methylquinazolin-4-amine (5B)

A solution of **4** (125 g, 628 mmol) in THF (1 L) was cooled to 4 °C and 40% aqueous $MeNH_2$ (136 mL, 1.57 mol) was added. The mixture was stirred at room temperature for 40 min and concentrated. Saturated aqueous $NaHCO_3$ was poured into the residue and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The precipitate was collected by filtration, washed with H_2O and hexane, and dried at 80 °C to give **5B** (114 g, 94%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.22 (d, J = 4.8 Hz, 3H), 6.35 (br s, 1H), 7.39–7.50 (m, 1H), 7.64–7.78 (m, 3H); ESI MS *m*/*z* 194 (M^++1, 100%).

5.5. *tert*-Butyl [*cis*-4-(hydroxymethyl)cyclohexyl]carbamate (7)

A suspension of *cis*-4-aminocyclohexanecarboxylic acid **6** (244 g, 1.71 mol) in MeOH (2.45 L) was cooled to −8 °C. Thionyl chloride (440 mL, 6.03 mol) was then added dropwise. The resulting solution was stirred at room temperature for 4.5 h and concentrated to give a white solid. To a suspension of the above solid in $CHCl_3$ (3.00 L) were added triethylamine (261 mL, 1.88 mol) and $(BOC)_2O$ (409 g, 1.88 mol), successively. The mixture was stirred at room temperature for 5 h and poured into water. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 11% EtOAc in hexane to 10% MeOH in $CHCl_3$ and NH-silica gel, 33% EtOAc in hexane to 10% MeOH in $CHCl_3$) to give a colorless oil (531 g). After a suspension of lithium aluminum hydride (78.3 g, 2.06 mol) in Et_2O (7.9 L) was cooled to −4 °C, a solution of the above oil (531 g) in Et_2O (5.3 L) was added below 0 °C. The resulting suspension was stirred at room temperature for 2 h. The reaction mixture was cooled on an ice bath, quenched with cold water, and filtered through a pad of Celite. The filtrate was dried over $MgSO_4$, filtered, and concentrated. A suspension of the precipitate in hexane (300 mL) was stirred at room temperature for 1 h, filtered, washed with hexane, and dried at 70 °C to give **7** (301 g, 77%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 4.30–4.82 (m, 1H), 3.75 (br s, 1H), 3.51 (d, J = 6.2 Hz, 2H), 1.52–1.77 (m, 8H), 1.45 (s, 9H), 1.16–1.36 (m, 2H); ESI MS *m*/*z* 252 (M^++23, 100%).

5.6. Benzyl ({*cis*-4-[(*tert*-butoxycarbonyl)amino]cyclohexyl}methyl)carbamate (7–1)

To a solution of **7** (17.7 g, 77.2 mmol) in THF (245 mL) were added triphenylphosphine (20.2 g, 77.0 mmol) and phthalimide (11.4 g, 77.5 mmol), successively. The resulting suspension was cooled to 4 °C and 40% diethyl azodicarboxylate (DEAD) in toluene was added over 1 h. The mixture was stirred at room temperature for 2.5 days, concentrated, and purified by flash column chromatography (silica gel, 33% EtOAc in hexane) to give a white solid (27.5 g). To a suspension of the above solid in EtOH (275 mL) was added hydrazine hydrate

(5.76 g, 115 mmol). The mixture was stirred at reflux for 2.25 h and concentrated. The precipitate was dissolved in aqueous 10% sodium hydroxide (350 mL). The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. To a solution of the residue in $CHCl_3$ (275 mL) was added triethylamine (8.54 g, 84.4 mmol). The resulting solution was cooled to 0 °C and CbzCl (14.4 g, 84.4 mmol) was added below 5 °C. The mixture was stirred at room temperature for 16 h and poured into aqueous saturated $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 2% MeOH in $CHCl_3$) to give **7**–**1** (25.3 g, 91%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.27–7.38 (m, 5H), 5.09 (s, 2H), 4.76–4.92 (m, 1H), 4.42–4.76 (m, 1H), 3.72 (br s, 1H), 3.10 (t, J = 6.4 Hz, 2H), 1.48–1.75 (m, 7H), 1.44 (s, 9H), 1.13–1.31 (m, 2H); ESI MS m/z 385 (M^++23, 100%).

5.7. Benzyl [(*cis*-4-aminocyclohexyl)methyl]carbamate (8)

To a solution of **7**–**1** (12.9 g, 35.6 mmol) in EtOAc (129 mL) was added 4 M hydrogen chloride in EtOAc (129 mL). The mixture was stirred at room temperature for 3 h. The precipitate was collected by filtration, washed with EtOAc, and dried under reduced pressure. The solid was dissolved in aqueous saturated $NaHCO_3$ and the aqueous layer was extracted with $CHCl_3$ five times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and dried under reduced pressure to give **8** (8.88 g, 95%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.36 (s, 5H), 5.12 (br s, 3H), 2.96–3.32 (m, 3H), 1.36–1.98 (m, 9H); ESI MS m/z 263 (M^++1, 100%).

5.8. Benzyl [(*cis*-4-{[4-(dimethylamino)quinazolin-2-yl]amino}cyclohexyl)methyl]carbamate (9A)

A mixture of **5A** (2.02 g, 9.73 mmol) and **8** (3.07 g, 11.7 mmol) in isopropanol (10 mL) was stirred at reflux for 7 days. The reaction mixture was poured into aqueous saturated $NaHCO_3$ and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (NH-silica gel, 33% EtOAc in hexane) to give **9A** (3.55 g, 84%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.81 (d, J = 9.0 Hz, 1H), 7.26–7.52 (m, 7H), 7.01 (ddd, J = 8.2, 6.5, 1.7 Hz, 1H), 5.10 (s, 2H), 4.93–5.06 (m, 1H), 4.82–4.93 (m, 1H), 4.18–4.28 (m, 1H), 3.26 (s, 6H), 3.11 (t, J = 6.3 Hz, 2H), 1.80–1.93 (m, 2H), 1.52–1.73 (m, 5H), 1.23–1.40 (m, 2H); ESI MS m/z 434 (M^++1, 100%).

5.9. Benzyl [(*cis*-4-{[4-(methylamino)quinazolin-2-yl]amino}cyclohexyl)methyl]carbamate (9B)

The title compound was prepared from **5B** and **8** according to the experimental procedure for **9A** to provide a white solid (80% yield): ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.21–1.96 (m, 9H), 3.01–3.16 (m, 5H), 4.22–4.33 (m, 1H), 4.81–4.91 (m, 1H), 4.99–5.20 (m, 3H), 5.61–5.70 (m, 1H), 7.00–7.10 (m, 1H), 7.25–7.55 (m, 8H); ESI MS m/z 420 (M^++1, 100%).

5.10. N^2-[*cis*-4-({[4-Bromo-2-(trifluoromethoxy)benzyl]amino}methyl)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride (1a)

To a suspension of **9A** (4.57 g, 10.5 mmol) in MeOH (46 mL) was added 5% Pd–C (460 mg). The mixture was stirred at 50 °C under a hydrogen atmosphere for 2.5 days. The reaction mixture was filtered through a pad of Celite and concentrated to give a colorless foam. To a solution of the above foam (500 mg, 1.67 mmol) in CH_2Cl_2 (5 mL) were added **12** (449 mg, 1.67 mmol), AcOH (100 mg, 1.67 mmol), and $NaBH(OAc)_3$ (531 mg, 2.51 mmol), successively. The mixture was stirred at room temperature for 9 h and poured into aqueous saturated $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 25% EtOAc in hexane) to give a colorless oil (371 mg). After 4 M hydrogen chloride in EtOAc (5 mL) was added to a solution of the above oil (227 mg) in EtOAc (1 mL), the mixture was stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with Et_2O, and dried under reduced pressure to give **1a** (147 mg, 34%) as a white solid: mp 244.5–247.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 12.62 (s, 1H), 10.07 (br s, 2H), 8.66 (d, J = 7.6 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.52 (dd, J = 8.3, 1.8 Hz, 1H), 7.33–7.48 (m, 2H), 7.26 (t, J = 7.5 Hz, 1H), 4.11–4.36 (m, 3H), 3.51 (s, 6H), 2.76–2.97 (m, 2H), 1.51–2.28 (m, 10H); ESI MS m/z 552 [M(free)$^+$+1, 95%], 554 [M(free)$^+$+3, 100%]; Anal. Calcd for $C_{25}H_{29}BrF_3N_5O \cdot 2HCl$: C, 48.02; H, 5.00; N, 11.20; Br, 12.78; Cl, 11.34; F, 9.11. Found: C, 47.90; H, 5.03; N, 10.99; Br, 12.59; Cl, 11.28; F, 9.12.

5.11. N^4,N^4-Dimethyl-N^2-[*cis*-4-({[2-(trifluoromethoxy)benzyl]amino}methyl)cyclohexyl]quinazoline-2,4-diamine dihydrochloride (1b)

The title compound was prepared from **9A** and 2-(trifluoromethoxy)benzaldehyde according to the experimental procedure for **1a** to provide a white solid (36% yield): mp 255.0–259.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.41–2.28 (m, 9H), 2.70–2.94 (m, 2H), 3.50 (s, 6H), 4.15–4.30 (m, 1H), 4.31 (br s, 2H), 7.18–7.51 (m, 5H), 7.63 (t, J = 7.6 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 8.16–8.28 (m, 1H), 8.69 (d, J = 7.5 Hz, 1H), 9.81–10.22 (m, 2H), 12.81 (s, 1H); ESI MS m/z 474 [M(free)$^+$+1, 100%]; Anal. Calcd for $C_{25}H_{30}F_3N_5O \cdot 2HCl$: C, 54.95; H, 5.90; N, 12.82; Cl, 12.98; F, 10.43. Found: C, 54.63; H, 5.90; N, 12.62; Cl, 12.81; F, 10.31.

5.12. N^2-{*cis*-4-[({2-[4-Bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)methyl]cyclohexyl}-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride (1c)

The title compound was prepared from **9A** and **13** according to the experimental procedure for **1a** to provide a white solid (12% yield): mp 145.5–148.5 °C; ^{1}H NMR

(300 MHz, $CDCl_3$, δ): 1.56–2.10 (m, 9H), 2.92–3.30 (m, 4H), 3.32–3.62 (m, 8H), 4.30 (br s, 1H), 7.17–7.52 (m, 5H), 7.66 (t, J = 7.6 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 8.70 (d, J = 7.6 Hz, 1H), 9.06–9.82 (m, 2H), 12.45 (s, 1H); ESI HRMS m/z 566.1763 [M(free)$^+$+1], calcd for $C_{26}H_{32}BrF_3N_5O$ 566.1742.

5.13. N^4,N^4-Dimethyl-N^2-{*cis*-4-[({2-[2-(trifluoromethoxy)phenyl]ethyl}amino)methyl]cyclohexyl}quinazoline-2,4-diamine dihydrochloride (1d)

To a solution of [2-(trifluoromethoxy)phenyl]acetic acid (350 mg, 1.59 mmol) in CH_2Cl_2 (6 mL) were added DMF (1.0 μL, 15 μmol) and thionyl chloride (165 μL, 2.26 mmol). The mixture was stirred at reflux for 50 min and concentrated to give an acid chloride as a pale yellow oil. To a solution of N^2-[*cis*-4-(aminomethyl)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine (453 mg, 1.51 mmol) obtained by the same method as compound **1a** in CH_2Cl_2 (4 mL) was added iPr_2NEt (553 μL, 3.17 mmol). After cooling to 4 °C, the above acid chloride in CH_2Cl_2 (4 mL) was added and the mixture was stirred at 4 °C for 5 h and quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (NH-silica gel, 33–66% EtOAc in hexane) to give a colorless oil (396 mg). To a solution of the above oil (246 mg) in THF (3.5 mL) was added 1 M BH_3-THF in THF (3.95 mL, 3.95 mmol) and the mixture was stirred at reflux for 2.5 h and concentrated. To a solution of the residue in THF (3.5 mL) was added aqueous 1 M hydrochloric acid (4.41 mL, 4.41 mmol) and the mixture was stirred at reflux for 70 min and quenched with aqueous 2 M sodium hydroxide. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 50% EtOAc in hexane) to give a colorless oil. After 4 M hydrogen chloride in EtOAc (1.3 mL) was added to a solution of the above oil in EtOAc (6 mL), the mixture was stirred at room temperature for 1 h and concentrated. A suspension of the above oil in Et_2O (15 mL) was stirred at room temperature for 1 h. The precipitate was collected by filtration, washed with Et_2O, and dried under reduced pressure to give **1d** (81 mg, 30%) as a white solid: mp 231.5–235.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.50–2.01 (m, 8H), 2.10–2.20 (m, 1H), 2.92–3.07 (m, 2H), 3.11–3.30 (m, 2H), 3.38–3.60 (m, 8H), 4.22–4.35 (m, 1H), 7.15–7.32 (m, 4H), 7.42–7.54 (m, 2H), 7.66 (t, J = 7.7 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 8.72 (d, J = 7.7 Hz, 1H), 9.72 (br s, 2H), 12.56 (s, 1H); FAB MS m/z 488 [M(free)$^+$+1, 100%]; Anal Calcd for $C_{26}H_{32}F_3N_5O \cdot 2HCl \cdot 1.5H_2O$: C, 53.15; H, 6.35; N, 11.92; Cl, 12.07; F, 9.70. Found: C, 53.17; H, 6.20; N, 11.99; Cl, 11.99; F, 9.76.

5.14. N^2-[*cis*-4-({[4-Bromo-2-(trifluoromethoxy)benzyl]amino}methyl)cyclohexyl]-N^4-methylquinazoline-2,4-diamine dihydrochloride (1e)

To a solution of **9B** (2.73 g, 6.50 mmol) in MeOH (27 mL) was added 10% Pd–C (273 mg). The mixture was stirred at 50 °C under a hydrogen atmosphere for 14 h. The reaction mixture was filtered through a pad of Celite and concentrated to give a colorless foam. To a solution of the above foam (300 mg, 1.05 mmol) in MeOH (3 mL) were added **12** (283 mg, 1.05 mmol), AcOH (63 mg, 1.05 mmol), and $NaBH_3CN$ (99 mg, 1.58 mmol), successively. The mixture was stirred at room temperature for 3.5 h and poured into aqueous 1 M sodium hydroxide. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 50% EtOAc in hexane; silica gel, 10% MeOH in $CHCl_3$) to give a colorless oil. After 4 M hydrogen chloride in EtOAc (5 mL) was added to a solution of the above oil in EtOAc (1 mL), the mixture was stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with Et_2O, and dried under reduced pressure to give **1e** (39 mg, 6%) as a white solid: mp 237.0–240.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.38–1.84 (m, 8H), 1.89–2.00 (m, 1H), 2.88–3.05 (m, 5H), 4.19–4.30 (m, 3H), 7.07 (d, J = 7.9 Hz, 1H), 7.14 (t, J = 7.3 Hz, 1H), 7.44–7.52 (m, 2H), 7.55 (dd, J = 8.6, 1.8 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.43 (d, J = 7.9 Hz, 1H), 9.46 (br s, 1H), 9.75 (br s, 2H), 11.23 (br s, 1H); ESI MS m/z 538 [M(free)$^+$+1, 95%], 540 [M(free)$^+$+3, 100%]; Anal. Calcd for $C_{24}H_{27}BrF_3N_5O \cdot 2HCl \cdot 0.5H_2O$: C, 46.47; H, 4.87; N, 11.29; Br, 12.88; Cl, 11.43; F, 9.19. Found: C, 46.48; H, 4.68; N, 11.18; Br, 12.76; Cl, 11.58; F, 9.19.

5.15. N^4-Methyl-N^2-[*cis*-4-({[2-(trifluoromethoxy)benzyl]amino}methyl)cyclohexyl]quinazoline-2,4-diamine dihydrochloride (1f)

The title compound was prepared from **9B** and 2-(trifluoromethoxy)benzaldehyde according to the experimental procedure for **1e** to provide a white solid (33% yield): mp 160.0–164.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.41–2.19 (m, 9H), 2.92 (d, J = 4.8 Hz, 2H), 2.99 (d, J = 4.5 Hz, 3H), 4.18–4.38 (m, 3H), 6.95–7.16 (m, 2H), 7.24–7.51 (m, 4H), 8.13 (dd, J = 7.5, 1.8 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.43 (d, J = 8.4 Hz, 1H), 9.22–10.0 (m, 2H), 11.49 (br s, 1H); ESI MS m/z 460 [M(free)$^+$+1, 100%]; Anal. Calcd for $C_{24}H_{28}F_3N_5O \cdot 2HCl \cdot 1.2H_2O$: C, 52.03; H, 5.89; N, 12.64; Cl, 12.80; F, 10.29. Found: C, 51.94; H, 5.75; N, 12.60; Cl, 13.05; F, 10.30.

5.16. N^2-{*cis*-4-[({2-[4-Bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)methyl]cyclohexyl}-N^4-methylquinazoline-2,4-diamine dihydrochloride (1g)

The title compound was prepared from **9B** and **13** according to the experimental procedure for **1e** to provide a white solid (10% yield): mp 170–175.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.30–2.24 (m, 9H), 2.75–3.31 (m, 7H), 3.32–3.51 (m, 2H), 4.26 (br s, 1H), 6.96–7.58 (m, 6H), 8.05–8.50 (m, 2H), 9.22–9.72 (m, 2H), 11.51–11.72 (m, 1H); ESI HRMS m/z 552.1605 [M(free)$^+$+1], calcd for $C_{25}H_{30}BrF_3N_5O$ 552.1586.

5.17. N^4-Methyl-N^2-{*cis*-4-[({2-[2-(trifluoromethoxy)phenyl]ethyl}amino)methyl]cyclohexyl}quinazoline-2,4-diamine dihydrochloride (1h)

To a solution of **1g** (290 mg, 0.525 mmol) in EtOH (5.8 mL) was added 10% Pd–C (87 mg). The mixture was stirred at room temperature under a hydrogen atmosphere for 18.5 h. The reaction mixture was filtered through a pad of Celite, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 50% EtOAc in hexane to EtOAc) to give a colorless oil. After 4 M hydrogen chloride in EtOAc (230 μL) was added to a solution of the above oil in EtOAc (8 mL), the mixture was stirred at room temperature for 2 h and concentrated. A suspension of the above oil in Et_2O (10 mL) was stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with Et_2O, and dried under reduced pressure to give **1h** (156 mg, 55%) as a white solid: mp 192.0–195.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.37–2.34 (m, 9H), 2.83–3.60 (m, 9H), 4.26 (br s, 1H), 6.84–7.60 (m, 7H), 8.20–8.66 (m, 2H), 9.23–9.94 (m, 3H), 11.72 (br s, 1H); ESI MS *m*/*z* 474 [M(free)$^+$+1, 100%]; Anal. Calcd for $C_{25}H_{30}F_3N_5O \cdot 1.9HCl \cdot 1.5H_2O$: C, 52.69; H, 6.17; N, 12.29; Cl, 11.82; F, 10.00. Found: C, 52.97; H, 5.95; N, 11.93; Cl, 11.99; F, 10.36.

5.18. *tert*-Butyl (*cis*-4-{[4-(dimethylamino)quinazolin-2-yl]amino}cyclohexyl)carbamate (10A)

The title compound was prepared from **5A** and *tert*-butyl (*cis*-4-aminocyclohexyl)carbamate according to the experimental procedure for **9A** to provide a pale yellow foam (93% yield): ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.81 (d, J = 8.4 Hz, 1H), 7.25–7.50 (m, 2H), 6.98–7.05 (m, 1H), 4.96–5.04 (m, 1H), 4.50–4.65 (m, 1H), 4.05–4.16 (m, 1H), 3.55–3.72 (m, 1H), 3.26 (s, 6H), 1.48–1.88 (m, 8H), 1.45 (s, 9H); ESI MS *m*/*z* 386 (M$^+$+1, 30%), 408 (M$^+$+23, 100%).

5.19. *tert*-Butyl (*cis*-4-{[4-(methylamino)quinazolin-2-yl]amino}cyclohexyl)carbamate (10B)

The title compound was prepared from **5B** and *tert*-butyl (*cis*-4-aminocyclohexyl)carbamate according to the experimental procedure for **9A** to provide a white solid (65% yield): ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.46 (s, 9H), 1.50–1.94 (m, 8H), 3.12 (d, J = 4.8 Hz, 3H), 3.54–3.73 (m, 1H), 4.05–4.22 (m, 1H), 4.49–4.63 (m, 1H), 5.01–5.22 (m, 1H), 5.64–5.82 (m, 1H), 7.03–7.10 (m, 1H), 7.39–7.56 (m, 3H); ESI MS *m*/*z* 372 (M$^+$+1, 100%).

5.20. N^2-(*cis*-4-{[4-Bromo-2-(trifluoromethoxy)benzyl]amino}cyclohexyl)-N^4,N^4-dimethylquinazoline-2,4-diamine (2a)

To a solution of **10A** (16.1 g, 41.7 mmol) in EtOAc (160 mL) was added 4 M hydrogen chloride in EtOAc (145 mL). The mixture was stirred at room temperature for 70 min and concentrated. Aqueous 1 M sodium hydroxide was poured into the residue and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 1–9% MeOH in $CHCl_3$) to give N^2-(*cis*-4-aminocyclohexyl)-N^4,N^4-dimethylquinazoline-2,4-diamine (11.0 g, 92%) as a pale yellow solid. To a solution of the above solid (400 mg, 1.40 mmol) in CH_2Cl_2 (4 mL) were added **12** (377 mg, 1.40 mmol), AcOH (84 mg, 1.40 mmol), and $NaBH(OAc)_3$ (446 mg, 2.10 mmol), successively. The mixture was stirred at room temperature for 5 h and poured into aqueous saturated $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 50% EtOAc in hexane) to give **2a** (416 mg, 55%) as a white solid: mp 63.5–65.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.80 (dd, J = 7.9, 0.9 Hz, 1H), 7.36–7.51 (m, 5H), 7.01 (ddd, J = 8.3, 6.4, 1.9 Hz, 1H), 4.95–5.18 (m, 1H), 4.08–4.22 (m, 1H), 3.81 (s, 2H), 3.25 (s, 6H), 2.55–2.70 (m, 1H), 1.65–1.90 (m, 6H), 1.29–1.65 (m, 2H); ESI MS *m*/*z* 538 (M$^+$+1, 30%), 560 (M$^+$+23, 100%); Anal. Calcd for $C_{24}H_{27}BrF_3N_5O \cdot 0.2H_2O$; C, 53.18; H, 5.10; N, 12.92; Br, 14.74; F, 10.52. Found: C, 52.92; H, 5.02; N, 12.56; Br, 15.03; F, 10.83.

5.21. N^2-(cis-4-{[4-Bromo-2-(trifluoromethoxy)phenyl]amino}cyclohexyl)-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride (2b)

A mixture of 18-C-6 (647 mg, 2.45 mmol), 4-bromo-1-iodo-2-(trifluoromethoxy)benzene (770 mg, 2.10 mmol), and N^2-(*cis*-4-aminocyclohexyl)-N^4,N^4-dimethylquinazoline-2,4-diamine (500 mg, 1.75 mmol) obtained by the same method as compound **2a**, sodium *tert*-butoxide (235 mg, 2.45 mmol), tris(dibenzylideneacetone)dipalladium (160 mg, 0.175 mmol), and (*R*)-(+)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (109 mg, 0.175 mmol) in THF (3.5 mL) was stirred at reflux for 18.5 h. After cooling, Et_2O was added and the mixture was stirred at room temperature for 30 min, filtered through a pad of Celite, washed with $CHCl_3$, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 20–33% EtOAc in hexane; silica gel, 1–17% MeOH in $CHCl_3$) to give a colorless oil. After 4 M hydrogen chloride in EtOAc (300 μL) was added to a solution of the above oil in Et_2O (2 mL), the mixture was stirred at room temperature for 15 min. The precipitate was collected by filtration, washed with Et_2O, and dried under reduced pressure to give **2b** (189 mg, 18%) as a white solid: mp 254.0–258.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.64–2.08 (m, 8H), 3.30–3.78 (m, 8H), 4.20–4.38 (m, 1H), 6.65 (s, 1H), 6.79 (s, 1H), 6.94 (s, 1H), 7.22–7.31 (m, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.61–7.70 (m, 1H), 7.90 (d, J = 8.1 Hz, 1H), 8.85 (d, J = 7.9 Hz, 1H), 13.04 (s, 1H); ESI MS *m*/*z* 524 [M(free)$^+$+1, 95%], 526 [M(free)$^+$+3, 100%]; Anal. Calcd for $C_{23}H_{25}BrF_3N_5O \cdot 1.4HCl \cdot H_2O \cdot 0.3Et_2O$; C, 47.21; H, 5.14; N, 11.38; Br, 12.98; Cl, 8.06; F, 9.26. Found: C, 47.51; H, 4.78; N, 11.76; Br, 12.63; Cl, 7.92; F, 9.38.

5.22. N^2-[*cis*-4-({2-[4-Bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride (2c)

The title compound was prepared from **10A** and **13** according to the experimental procedure for **2a** to provide a white solid (26% yield): mp 170.0–174.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.62–1.96 (m, 2H), 2.00–2.48 (m, 6H), 3.20–3.64 (m, 11H), 4.31 (br s, 1H), 7.19–7.77 (m, 6H), 7.93 (d, J = 6.6 Hz, 1H), 8.72 (d, J = 7.5 Hz, 1H), 9.91 (br s, 2H), 12.66 (br s, 1H); ESI MS m/z 552 [M(free)$^+$+1, 95%], 554 [M(free)$^+$+3, 100%]; Anal. Calcd for $C_{25}H_{29}BrF_3N_5O \cdot 2HCl \cdot 1.5H_2O$; C, 46.03; H, 5.25; N, 10.74; Br, 12.25; Cl, 10.87; F, 8.74. Found: C, 46.06; H, 5.36; N, 10.58; Br, 12.05; Cl, 10.87; F, 8.74.

5.23. N^4,N^4-Dimethyl-N^2-[*cis*-4-({2-[2-(trifluoromethoxy)phenyl]ethyl}amino)cyclohexyl]quinazoline-2,4-diamine dihydrochloride (2d)

The title compound was prepared from **2c** according to the experimental procedure for **1h** to provide a white solid (48% yield): mp 169.5–173.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.62–1.89 (m, 2H), 1.93–2.53 (m, 6H), 3.15–3.71 (m, 11H), 4.33 (br s, 1H), 7.14–7.37 (m, 4H), 7.39–7.77 (m, 3H), 7.82–8.00 (m, 1H), 8.71 (br s, 1H), 9.78 (br s, 2H), 12.62 (br s, 1H); ESI MS m/z 474 [M(free)$^+$+1, 100%]; Anal. Calcd for $C_{25}H_{30}F_3N_5O \cdot 2HCl \cdot 1.5H_2O$: C, 52.36; H, 6.15; N, 12.21; Cl, 12.36; F, 9.94. Found: C, 52.36; H, 6.13; N, 12.21; Cl, 12.35; F, 9.73.

5.24. N^2-[*cis*-4-({3-[4-Bromo-2-(trifluoromethoxy)phenyl]propyl}amino)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride (2e)

The title compound was prepared from **10A** and **15** according to the experimental procedure for **2a** to provide a white solid (42% yield): mp 129.0–133.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.59–1.85 (m, 2H), 1.97–2.36 (m, 8H), 2.78 (t, J = 7.8 Hz, 2H), 3.02–3.14 (m, 2H), 3.19–3.35 (m, 1H), 3.52 (s, 6H), 4.31 (br s, 1H), 7.12–7.52 (m, 6H), 7.60–7.70 (m, 1H), 7.91 (d, J = 7.9 Hz, 1H), 8.81 (d, J = 7.2 Hz, 1H); ESI MS m/z 566 [M(free)$^+$+1, 95%], 568 [M(free)$^+$+3, 100%]; Anal. Calcd for $C_{26}H_{31}BrF_3N_5O \cdot 2HCl \cdot 1.6H_2O \cdot 0.3EtOAc$; C, 47.03; H, 5.60; N, 10.08; Br, 11.50; Cl, 10.21; F, 8.20. Found: C, 47.18; H, 5.51; N, 10.30; Br, 11.11; Cl, 10.10; F, 8.40.

5.25. N^2-[*cis*-4-({4-[4-Bromo-2-(trifluoromethoxy)phenyl]butyl}amino)cyclohexyl]-N^4,N^4-dimethylquinazoline-2,4-diamine dihydrochloride (2f)

To a solution of **10A** (16.1 g, 41.7 mmol) in EtOAc (160 mL) was added 4 M hydrogen chloride in EtOAc (145 mL). The mixture was stirred at room temperature for 70 min and concentrated. Aqueous 1 M sodium hydroxide was poured into the residue and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 1–9% MeOH in $CHCl_3$) to give N^2-(*cis*-4-aminocyclohexyl)-N^4,N^4-dimethylquinazoline-2,4-diamine (11.0 g, 92%) as a pale yellow solid. To a solution of the above solid (240 mg, 0.841 mmol) in MeOH (3 mL) were added **17** (262 mg, 0.842 mmol), AcOH (51 mg, 0.849 mmol), and $NaBH_3CN$ (79 mg, 1.26 mmol), successively. The mixture was stirred at room temperature for 8 h and poured into aqueous 1 M sodium hydroxide. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by medium-pressure liquid column chromatography (NH-silica gel, 50% EtOAc in hexane) to give a colorless oil. After 4 M hydrogen chloride in EtOAc (10 mL) was added to a solution of the above oil in EtOAc (2 mL), the mixture was stirred at room temperature for 1 h and concentrated. A suspension of the above oil in Et_2O (20 mL) was stirred at room temperature for 1 h. The precipitate was collected by filtration, washed with Et_2O, and dried under reduced pressure to give **2f** (220 mg, 40%) as a white solid: mp 121.5–125.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.58–2.59 (m, 12H), 2.60–2.78 (m, 2H), 2.92–3.42 (m, 3H), 3.52 (s, 6H), 4.20–4.42 (m, 1H), 7.12–7.40 (m, 3H), 7.48 (d, J = 7.7 Hz, 1H), 7.66 (t, J = 7.3 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 8.66–8.88 (m, 1H), 9.55 (br s, 2H), 12.73 (br s, 1H); ESI HRMS m/z 580.1911 [M(free)$^+$+1], calcd for $C_{27}H_{34}BrF_3N_5O$ 580.1899.

5.26. N^2-(*cis*-4-{[4-Bromo-2-(trifluoromethoxy)benzyl]amino}cyclohexyl)-N^4-methylquinazoline-2,4-diamine dihydrochloride (2g)

The title compound was prepared from **10B** and **12** according to the experimental procedure for **2a** to provide a white solid (20% yield): mp 197.0–202.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.41–1.94 (m, 8H), 2.57–2.72 (m, 1H), 3.12 (d, J = 4.8 Hz, 3H), 3.82 (s, 2H), 4.09–4.27 (m, 1H), 5.52 (br s, 1H), 7.05 (ddd, J = 8.2, 6.8, 1.4 Hz, 1H), 7.34–7.57 (m, 6H); ESI MS m/z 286 [M(free)$^+$−237, 100%], 522 [M(free)$^+$+1, 75%], 524 [M(free)$^+$+3, 75%]; Anal. Calcd for $C_{23}H_{25}BrF_3N_5O \cdot 2HCl \cdot H_2O$; C, 44.90; H, 4.75; N, 11.38; Br, 12.99; Cl, 11.52; F, 9.26. Found: C, 44.71; H, 4.77; N, 11.32; Br, 12.66; Cl, 11.42; F, 9.22.

5.27. N^2-[*cis*-4-({2-[4-Bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)cyclohexyl]-N^4-methylquinazoline-2,4-diamine dihydrochloride (2h)

The title compound was prepared from **10B** and **13** according to the experimental procedure for **2a** to provide a white solid (40% yield): mp 173.0–177.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.64–2.42 (m, 8H), 2.94 (s, 3H), 3.08–3.33 (m, 3H), 3.35–3.58 (m, 2H), 3.94–4.26 (m, 1H), 7.21–7.45 (m, 5H), 7.60 (t, J = 7.7 Hz, 1H), 7.71–7.94 (m, 1H), 8.62–8.82 (m, 1H), 9.93 (br s, 3H), 12.18 (br s, 1H); ESI MS m/z 538 [M(free)$^+$+1, 98%], 540 [M(free)$^+$+3, 100%]; Anal. Calcd for $C_{24}H_{27}BrF_3N_5O \cdot 1.9HCl \cdot 1.5H_2O$; C, 45.42; H, 5.07; N, 11.03; Br, 12.59; Cl, 10.61; F, 8.98. Found: C, 45.32; H, 4.93; N, 10.64; Br, 12.92; Cl, 10.68; F, 9.37.

5.28. N^2-[*cis*-4-({3-[4-Bromo-2-(trifluoromethoxy)phenyl]propyl}amino)cyclohexyl]-N^4-methylquinazoline-2,4-diamine dihydrochloride (2i)

The title compound was prepared from **10B** and **15** according to the experimental procedure for **2f** to provide a white solid (7% yield): mp 169.5–173.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.60–1.98 (m, 4H), 2.09–2.50 (m, 6H), 2.51–2.89 (m, 5H), 2.94–3.24 (m, 3H), 3.78–3.96 (m, 1H), 7.27–7.43 (m, 5H), 7.55–7.67 (m, 2H), 8.68 (d, J = 8.2 Hz, 1H), 9.59 (br s, 2H), 9.78 (br s, 1H), 12.37 (s, 1H); ESI MS *m*/*z* 552 [M(free)$^+$+1, 100%], 554 [M(free)$^+$+3, 98%]; Anal. Calcd for $C_{25}H_{29}BrF_3N_5O \cdot 2.1HCl \cdot 1.4H_2O \cdot 0.3Et_2O$; C, 46.52; H, 5.50; N, 10.35; Br, 11.81; Cl, 11.01; F, 8.43. Found: C, 46.88; H, 5.34; N, 10.71; Br, 11.49; Cl, 10.99; F, 8.67.

5.29. 4-Bromo-2-(trifluoromethoxy)benzaldehyde (12)

A solution of 4-bromo-1-iodo-2-(trifluoromethoxy)benzene (1.00 g, 2.72 mmol) in THF (15 mL) was cooled to −78 °C and 2.66 M *n*-BuLi in hexane (2.05 mL, 5.44 mmol) was added dropwise. After the mixture was stirred at −78 °C for 1.5 h, *N*-formylmorpholine (648 mg, 5.63 mmol) was added. The mixture was stirred at −78 °C for 15 min and at room temperature for 80 min. The reaction was quenched with aqueous 0.25 M citric acid (10 mL) and the aqueous layer was extracted with EtOAc three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 2–5% EtOAc in hexane) to give **12** (560 mg, 77%) as a pale brown solid: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 10.33 (s, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.50–7.67 (m, 2H); CI MS *m*/*z* 269 (M^++1, 95%), 271 (M^++3, 100%).

5.30. [4-Bromo-3-(trifluoromethoxy)phenyl]acetaldehyde (13)

To a suspension of (methoxymethyl)triphenylphosphonium chloride (31.5 g, 91.9 mmol) in Et_2O (300 mL) was added 1.8 M PhLi in cyclohexane–ether (7:3) (51.0 mL, 91.9 mmol) and the mixture was stirred at room temperature for 10 min. To the mixture was added **12** (23.8 g, 88.4 mmol) in Et_2O (100 mL) and the mixture was stirred at room temperature for 1 h, filtered, and concentrated. To the residue was added 10% H_2SO_4 (23.8 mL) in AcOH (214 mL) and the mixture was stirred at room temperature for 30 min and quenched with H_2O. The aqueous layer was extracted with Et_2O three times. The combined organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 9% EtOAc in hexane) to give **13** (16.7 g, 67%) as a pale brown oil: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.76 (s, 2H), 7.11–7.55 (m, 3H), 9.73 (s, 1H); ESI MS *m*/*z* 283 (M^++1, 100%), 285 (M^++3, 65%).

5.31. Ethyl (2*E*)-3-[4-bromo-3-(trifluoromethoxy)phenyl]acrylate (14)

To a solution of ethyl (diethoxyphosphoryl)acetate (3.45 g, 15.4 mmol) in THF (230 mL) was added 60% NaH dispersion in mineral oil (616 mg, 15.4 mmol) under a nitrogen atmosphere and the mixture was stirred at room temperature for 50 min. After cooling to 4 °C, a solution of **12** (3.00 g, 11.2 mmol) in THF (100 mL) was added and the mixture was stirred at room temperature for 11 h and poured into water. The aqueous layer was extracted with EtOAc three times. The combined organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 5% EtOAc in hexane) to give **14** (2.98 g, 79%) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.35 (t, J = 7.3 Hz, 3H), 4.29 (q, J = 7.0 Hz, 2H), 6.48 (d, J = 16.3 Hz, 1H), 7.43–7.58 (m, 3H), 7.83 (d, J = 15.8 Hz, 1H); CI MS *m*/*z* 339 (M^++1, 100%), 341 (M^++3, 98%).

5.32. 3-[4-Bromo-3-(trifluoromethoxy)phenyl]propan-1-ol (15–1)

To a suspension of LAH (834 mg, 22.0 mmol) in Et_2O (20 mL) cooled to 4 °C was added a solution of **14** (2.98 g, 8.79 mmol) in Et_2O (9 mL). The mixture was stirred at 0 °C for 1 h and at room temperature for 2.5 h and quenched with saturated aqueous ammonium chloride. The aqueous layer was extracted with EtOAc three times. The combined organic layer was washed with aqueous 1 M hydrochloric acid, dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography (silica gel, 25% EtOAc in hexane) to give **15–1** (1.14 g, 43%) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.58 (s, 1H), 1.75–1.96 (m, 2H), 2.65–2.82 (m, 2H), 3.60–3.76 (m, 2H), 7.11–7.44 (m, 3H); EI MS *m*/*z* 280 (M^+-18, 100%), 282 (M^+-16, 95%), 298 (M^+, 20%), 300 (M^++2, 20%).

5.33. 3-[4-Bromo-3-(trifluoromethoxy)phenyl]propanal (15)

To a suspension of **15–1** (1.03 g, 3.44 mmol) and Celite (1.40 g) in CH_2Cl_2 (47 mL) cooled to 4 °C was added PCC (1.11 g, 5.16 mmol). The mixture was stirred at room temperature for 6.5 h, filtered through a pad of Celite, washed with $CHCl_3$, concentrated, and purified by flash column chromatography (silica gel, 17% EtOAc in hexane) to give **15** (659 mg, 64%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.71–2.80 (m, 2H), 2.91–3.01 (m, 2H), 7.17 (d, J = 8.1 Hz, 1H), 7.33–7.42 (m, 2H), 9.80 (s, 1H); CI MS *m*/*z* 297 (M^++1, 100%), 299 (M^++3, 90%).

5.34. Ethyl (2*E*)-4-[4-bromo-3-(trifluoromethoxy)phenyl]but-2-enoate (16)

The title compound was prepared from **13** according to the experimental procedure for **14** to provide a colorless oil (26% yield): ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.24–1.33 (m, 3H), 3.28 (dd, J = 7.1, 1.5 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 6.28–6.46 (m, 2H), 6.65 (d, J = 16.2 Hz, 1H), 7.34–7.51 (m, 3H); ESI MS *m*/*z* 353 (M^++1, 100%).

5.35. 4-[4-Bromo-3-(trifluoromethoxy)phenyl]butanal (17)

The title compound was prepared from **16** according to the experimental procedure for **15** to provide a colorless

oil (35% yield): ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.83–2.05 (m, 2H), 2.42–2.56 (m, 2H), 2.60–2.78 (m, 2H), 7.07–7.49 (m, 3H), 9.79 (s, 1H); ESI MS *m*/*z* 311 (M^++1, 10%).

6. Assay

6.1. Membrane preparations

Membranes expressing human WT-MCH-R1 were purchased from Euroscreen and diluted in the assay buffer (25 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 μM phosphoramidon, 0.5 mM PMSF, and 0.2% BSA, pH7.4) for human WT-MCH-R1 binding assay.

6.2. Receptor binding assays for WT-MCH-R1

Binding assays were performed in 96-well plates. The receptor binding reaction was initiated by incubating 0.2 mL of membrane preparations with 0.1 nM [^{125}I](Phe^{13}, Tyr^{19})MCH (Amersham). Non-specific binding was determined using 10 μM T-226926. The reaction mixture was incubated for 2 h at room temperature. The binding reaction was terminated by rapid filtration through a GF/C glass filter plate presoaked with 0.3% polyethylenimine, followed by washing three times with 0.3 mL of wash buffer (PBS containing 0.5 M NaCl). The radioactivity retained in the filterplates was quantified with a Topcount™ scintillation counter (Packard). Specific binding was determined by subtracting non-specific binding from the total binding. In the competition binding assay, the concentration of the test compound that caused 50% inhibition of specific radioligand binding (IC_{50} values) was determined from each concentration–response curve. Data were analyzed by a non-linear least-squares curve-fitting procedure using Origin software (Microcal).

6.3. Metabolic stability studies in liver microsomes

Pooled human and rat liver microsomes were purchased from XenoTech LLC. (Kansas, KS, USA). $NADP^+$, G-6-P, G-6-P dehydrogenase (G-6-P DH), and $MgCl_2$ were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents and solvents used in this study were of the highest quality commercially available.

The reaction mixture in a final volume of 1 mL contained 1 μM compound and 0.5 mg of liver microsomes in 50 mM of a sodium-potassium phosphate buffer (pH 7.4). The reaction was started by adding 0.8 mM $NADP^+$, 8 mM G-6-P, 0.83 enzyme units of G-6-P DH, and 8 mM $MgCl_2$ after 5 min of preincubation at 37 °C. At zero minute and four time points ranging to 15 min, aliquots (100 μL) were removed and added to 400 μL acetonitrile. After the samples were centrifuged at 11,000 rpm (Microfuge R, Beckman Instruments, Inc., Fullerton, CA, USA) for 10 min, the supernatants were evaporated to dryness with nitrogen. The resulting residue was dissolved in methanol injected LC/MS/MS system, and substrate quantification was performed. LC/MS/MS analysis was performed in positive ion ESI mode on an API3000 triple quadrupole mass spectrometer (AB/MPS SCIEX, Thornhill, Ontario, Canada) equipped with an 1100 HPLC system (Agilent Technologies, Inc., Mississauga, ON, USA). The separation was performed with a Zorbax SB-C18 column (5 μm, 2.1 × 50 mm or 3.5 μm, 2.1 × 15 mm, Agilent) using linear gradient elution (0.1% aqueous acetic acid to 0.1% acetic acid containing acetonitrile) at a flow rate of 250 μL/min.

To determine the in vitro $T_{1/2}$, the slope of the linear regression from log concentration versus incubation time relationships ($-k$) was used in the conversion to in vitro $T_{1/2}$, values by in vitro $T_{1/2} = -0.693/k$.

References and notes

1. Kawauchi, H.; Kawazoe, I.; Tsubokawa, M.; Kishida, M.; Baker, B. I. *Nature* **1983**, *305*, 321–323.
2. Vaughan, J. M.; Fischer, W. H.; Hoeger, C.; Rivier, J.; Vale, W. *Endocrinology* **1989**, *125*, 1660–1665.
3. Kolakowski, L. F., Jr.; Jung, B. P.; Nguyen, T.; Johnson, M. P.; Lynch, K. R.; Cheng, R.; Heng, H. H. Q.; George, S. R.; O'Dowd, B. F. *FEBS Lett.* **1996**, *398*, 253–258.
4. Chambers, J.; Ames, R. S.; Bergsma, D.; Muir, A.; Fitzgerald, L. R.; Hervieu, G.; Dytko, G. M.; Foley, J. J.; Martin, J.; Liu, W. S.; Park, J.; Ellis, C.; Ganguly, S.; Konchar, S.; Cluderay, J.; Leslie, R.; Wilson, S.; Sarau, H. M. *Nature* **1999**, *400*, 261–265.
5. Shimomura, Y.; Mori, M.; Sugo, T.; Ishibashi, Y.; Abe, M.; Kurokawa, T.; Onda, H.; Nishimura, O.; Sumino, Y.; Fujino, M. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 622–626.
6. Bachner, D.; Kreienkamp, H. J.; Weise, C.; Buck, F.; Richter, D. *FEBS Lett.* **1999**, *457*, 522–524.
7. Saito, Y.; Nothacker, H. P.; Wang, Z.; Lin, S. H. S.; Leslie, F.; Civelli, O. *Nature* **1999**, *400*, 265–269.
8. Wang, S.; Behan, J.; O'Neill, K.; Weig, B.; Fried, S.; Laz, T.; Bayne, M.; Gustafson, E.; Hawes, B. E. *J. Biol. Chem.* **2001**, *276*, 34664–34670.
9. Hill, J.; Duckworth, M.; Murdock, P.; Rennie, G.; Sabido-David, C.; Ames, R. S.; Szekeres, P.; Wilson, S.; Bergsma, D. J.; Gloger, I. S.; Levy, D. S.; Chambers, J. K.; Muir, A. I. *J. Biol. Chem.* **2001**, *276*, 20125–20129.
10. Mori, M.; Harada, M.; Terao, Y.; Sugo, T.; Watanabe, T.; Shimomura, Y.; Abe, M.; Shintani, Y.; Onda, H.; Nishimura, Y.; Fujino, M. *Biochem. Biophys. Res. Commun.* **2001**, *283*, 1013–1018.
11. Sailer, A. W.; Sano, H.; Zeng, Z.; McDonald, T. P.; Pan, J.; Pong, S.; Feighner, S. D.; Tan, C. P.; Fukami, T.; Iwaasa, H.; Hreniuk, D. L.; Morin, N. R.; Sadowski, S. J.; Ito, M.; Ito, M.; Bansal, A.; Ky, B.; Figueroa, D. J.; Jiang, Q.; Austin, C. P.; MacNeil, D. J.; Ishihara, A.; Ihara, M.; Kanatani, A.; Van der Ploeg, L. H. T.; Howard, A. D.; Liu, Q. *Proc. Natl. Acad. Sci. U.S.A* **2001**, *98*, 7564–7569.
12. An, S.; Cutler, G.; Zhao, J. J.; Huang, S. G.; Tian, H.; Li, W.; Liang, L.; Rich, M.; Bakleh, A.; Du, J.; Chen, J. L.; Dai, K. *Proc. Natl. Acad. Sci. U.S.A* **2001**, *98*, 7576–7581.
13. Tan, C. P.; Sano, H.; Iwaasa, H.; Pan, J.; Sailer, A. W.; Hreniuk, D. L.; Feighner, S. D.; Palyha, O. C.; Pong, S.; Figueroa, D. J.; Austin, C. P.; Jiang, M. M.; Yu, H.; Ito, J.; Ito, M.; Ito, M.; Guan, X. M.; MacNeil, D. J.; Kanatani, A.; Van der Ploeg, L. H. T.; Howard, A. D. *Genomics* **2002**, *79*, 785–792.
14. Boutin, J. A.; Suply, T.; Andinot, V.; Rodriguez, M.; Beauverger, P.; Nicolas, J. P.; Galizzi, J. P.; Fauchere, J. L. *Can J. Physiol. Pharmacol.* **2002**, *80*, 388–395.

15. Qu, D.; Ludwig, D. S.; Grammeltoft, S.; Piper, M.; Pelleymounter, M. A.; Cullen, M. J.; Mathes, W. F.; Przypek, J.; Kanarek, R.; Maratos-Flier, E. *Nature* **1996**, *380*, 243–247.
16. Rossi, M.; Choi, S. J.; O'Shea, D.; Miyoshi, T.; Ghatei, M. A.; Bloom, S. R. *Endocrinology* **1997**, *138*, 351–355.
17. Shimada, M.; Tritos, N. A.; Lowell, B. B.; Flier, J. S.; Maratos-Flier, E. *Nature* **1998**, *396*, 670–674.
18. Chen, Y.; Hu, C.; Hsu, C. K.; Zhang, Q.; Bi, C.; Asnicar, M.; Hsiung, H. M.; Fox, N.; Slieker, L. J.; Yang, D. D.; Heiman, M. L.; Shi, Y. *Endocrinology* **2002**, *143*, 2469–2477.
19. Marsh, D. J.; Weingarth, D. T.; Novi, D. E.; Chen, H. Y.; Trumbauer, M. E.; Chen, A. S.; Guan, X. M.; Jiang, M. M.; Feng, Y.; Camacho, R. E.; Shen, Z.; Frazier, E. G.; Yu, H.; Metzger, J. M.; Kuca, S. J.; Shearman, L. P.; Gopal-Truter, S.; MacNeil, D. J.; Strack, A. M.; MacIntyre, D. E.; Van der Ploeg, L. H. T.; Qian, S. *Proc. Natl. Acad. Sci. U.S.A* **2002**, *99*, 3240–3245.
20. Shearman, L. P.; Camacho, R. E.; Stribling, D. S.; Zhou, D.; Bednarek, M. A.; Hreniuk, H. L.; Feighner, S. D.; Tan, C. P.; Howard, A. H.; Van der Ploeg, L. H. T.; MacIntyre, D. E.; Hickey, G. J.; Strack, A. M. *Eur. J. Pharmacology* **2003**, *475*, 37–47.
21. Borowsky, B.; Durkin, M. M.; Ogozalek, K.; Marzabadi, M. R.; Deleon, J.; Heurich, R.; Lichtblau, H.; Shaposhnik, Z.; Daniewska, I.; Blackburn, T. P.; Branchek, T. A.; Gerald, C.; Vaysse, P. J.; Forray, C. *Nat. Med.* **2002**, *8*, 825–830.
22. Gonzalez, M. I.; Vaziri, S.; Wilson, C. A. *Peptides* **1996**, *17*, 171–177.
23. Monzon, M. E.; De Barioglio, S. R. *Physiol. Behav.* **1999**, *67*, 813–817.
24. Takekawa, S.; Asami, A.; Ishihara, Y.; Terauchi, J.; Kato, K.; Shimomura, Y.; Mori, M.; Murakoshi, H.; Kato, K.; Suzuki, N.; Nishimura, O.; Fujino, M. *Eur. J. Pharmacol.* **2002**, *438*, 129–135.
25. Handlon, A. L.; Al-Barazanji, K. A.; Barvian, K. K.; Bigham, E. C.; Carlton, D. L.; Carpenter, A. J.; Cooper, J. P.; Daniels, A. J.; Garrison, D. T.; Goetz, A. S.; Green, G. M.; Grizzle, M. K.; Guo, Y. C.; Hertzog, D. L.; Hyman, C. E.; Ignar, D. M.; Peckham, G. E.; Speake, J. D.; Britt, C.; Swain, W. R. 228th National Meeting of the American Chemistry Society, Philadelphia, PA, 2004, MEDI193.
26. Receveur, J. M.; Bjurling, E.; Ulven, T.; Little, P. B.; Norregaard, P. K.; Hogberg, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5075–5080.
27. McBriar, M. D.; Guzik, H.; Xu, R.; Paruchova, J.; Li, S.; Palani, A.; Clader, J. W.; Greenlee, W. J.; Hawes, B. E.; Kowalski, T. J.; O'Neill, K.; Spar, B.; Weig, B. *J. Med. Chem.* **2005**, *48*, 2274–2277.
28. Kanuma, K.; Omodera, K.; Nishiguchi, M.; Funakoshi, T.; Chaki, S.; Semple, G.; Tran, T. A.; Kramer, B.; Hsu, D.; Casper, M.; Thomsen, B.; Beeley, N.; Sekiguchi, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2565–2569.
29. Kanuma, K.; Omodera, K.; Nishiguchi, M.; Funakoshi, T.; Chaki, S.; Semple, G.; Tran, T. A.; Kramer, B.; Hsu, D.; Casper, M.; Thomsen, B.; Beeley, N.; Sekiguchi, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3853–3856.
30. Chaki, S.; Funakoshi, T.; Hirota-Okuno, S.; Nishiguchi, M.; Shimazaki, T.; Iijima, M.; Grottick, A. J.; Kanuma, K.; Omodera, K.; Sekiguchi, Y.; Okuyama, S.; Tran, T. A.; Semple, G.; Thomsen, W. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 831–839.
31. Curd, F. H. S.; Landquist, J. K.; Rose, F. L. *J. Chem. Soc.* **1947**, 775–783.
32. Olah, G. A.; Ohannesian, L.; Arvanaghi, M. *J. Org. Chem.* **1984**, *49*, 3856–3857.