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Design and optimization of quinazoline derivatives as melanin concentrating hormone receptor 1 (MCHR1) antagonists ☆

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ABSTRACT

Melanin concentrating hormone (MCH) is an important mediator of energy homeostasis and plays a role in metabolic and CNS disorders. The modeling-supported design, synthesis and multi-parameter optimization (biological activity, solubility, metabolic stability, hERG) of novel quinazoline derivatives as MCHR1 antagonists are described. The in vivo proof of principle for weight loss with a lead compound from this series is exemplified. Clusters of refined hMCHR1 homology models derived from the X-ray structure of the β2-adrenergic receptor, including extracellular loops, were developed and used to guide the design.

Obesity, a chronic disorder associated with an imbalance between energy intake and expenditure,[1] is progressively developing into a global pandemic affecting the lives of more than a billion people worldwide.[2] It is emerging as a leading cause of morbidity and associated risk factors such as dyslipidemia, type 2 diabetes, stroke, cardiovascular disease, certain forms of cancer, osteoarthritis, and sleep apnea.[3] Many biological targets for treating obesity, including centrally modulated satiety and hunger regulating systems, have been evaluated.[4] Amongst centrally acting targets, melanin concentrating hormone (MCH) and its receptor have been studied extensively. The hormone MCH is a 19-amino acid cyclic neuropeptide expressed predominantly in the lateral hypothalamus and zona incerta.[5] MCH is an important mediator of energy homeostasis,[6] stimulating food intake in rats after injection in the central nervous system (CNS)[7] and increasing body weight.[8] Transgenic mice over expressing MCH gene are susceptible to insulin resistance and obesity.[9] The obese rodents, such as *ob/ob*, *db/db*, and Ay/a mice show over expression of MCH mRNA,[10] whereas mice lacking the gene encoding MCH are hypophagic, lean, and tend to maintain elevated metabolic rates.[11] Consistent with this phenotype, genetically altered mice that lack the gene encoding MCH receptor maintain elevated metabolic rates and remain lean despite hyperphagia on a normal diet.[12] The accumulated data supports the therapeutic utility of MCHR1 antagonists in treatment of obesity.

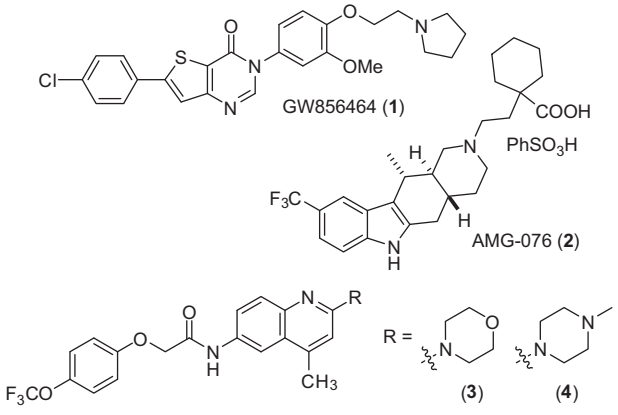

Figure 1. Potent MCHR1 antagonists.

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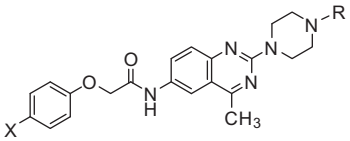
The pharmaceutical industries have spent significant efforts to identify and develop MCHR1 antagonists as anti-obesity agents.[13] So far three candidates GW856464[14] (**1**) (Fig. 1), AMG-076[15] (**2**), and NGD-4715 (structure undisclosed)[16] have entered phase I clinical trials. One of our laboratories[17] and Argenta[18] have previously reported acylamino-2-aminoquinolines as potent MCHR1 antagonists such as **3** and **4** (Fig. 1). Our SAR and modeling studies showed that the quinoline nitrogen was the primary interaction point with ^{123}Asp in the MCH1 receptor rather than the distal nitrogen.[17] Thus, compounds **3** and **4** are equipotent having an IC_{50} of ~50 nM in the SPA binding assay. Extensive SAR explorations of scaffold substituents also showed the 4-methyl group to be essential and optimal for activity.[17] We reasoned the quinoline site also to be invoked in the hERG interaction, that has been an issue for several chemotypes, and by modifying the basicity and introducing an additional nitrogen we could improve hERG liability as well as solubility by replacing the quinazoline with a quinazoline system. For example, the microscopic pKa for **4** is 6.6 for the quinoline nitrogen whereas the corresponding quinazoline nitrogen is calculated to have considerably lower basicity, that is, 4.5. As will become apparent we lost in affinity for the MCH1 receptor by this scaffold switch which called for additional SAR explorations of the eastern side chain that are described in this Letter leading up to a molecule with good efficacy in an obesity model.

The target compounds (Table 1–3) were synthesized as outlined in Schemes 1–4. For the synthesis of compounds **5–23** various piperazine analogs **II** were coupled with 2-chloro-4-methyl-6-nitroquinazoline (**I**)[19] to afford intermediate **III**. The nitro group was reduced to amine (**IV**) and coupled with phenoxyacetic acids **Va–b** to furnish the desired compounds (**5–23**) as outlined in Scheme 1.

Compounds **24–33** were prepared following Scheme 2. Cyclopropyl(piperidin-4-yl)methanone (**VI**) was synthesized from *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate[20] following four-steps protocols viz., oxidation of alcohol to aldehyde, Grignard reaction with cyclopropyl magnesium bromide, PCC oxidation followed by Boc-deprotection. Compound **VI** and other piperidines **VII** were coupled with **I** followed by reduction of the nitro group to afford amines **VIII** which after subsequent coupling with **V**

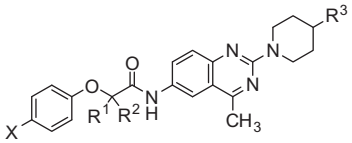
Table 1
Human MCHR1 binding and functional activity of piperazine derivatives



Compd	X	R	hMCHR1[a] (IC_{50})	
			SPA[b] (nM)	IP3[b] (nM)
5	Cl	Me	220	3800
6	Cl	Et	690	[c]
7	Cl		782	246
8	Cl	^{c}Pr	270	350
9	Cl		96	150
10	Cl		45	115
11	OCF_3		7	31
12	Cl	SO_2Me	>10000	>10000
13	Cl	CO^{i}Pr	225	91
14	OCF_3	CO^{i}Pr	36	33
15	Cl	$COCH_2{}^{i}$Pr	141	160
16	Cl	$COCH_2OMe$	215	295
17	Cl	CO^{c}Pr	251	146
18	OCF_3	CO^{c}Pr	49	30
19	Cl	CO^{c}Bu	325	158
20	OCF_3	CO^{c}Bu	84	45
21	Cl		305	350
22	Cl		116	1035
23	Cl		7300	>10000

[a] Values are mean of at least two experiments.
[b] See Refs.21,22 for the protocol.
[c] Not tested.

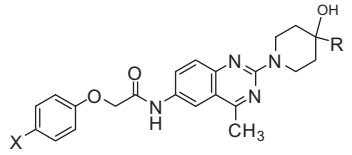
Table 2
Human MCHR1 binding and functional activity of piperidine derivatives



Compd	X	R^1	R^2	R^3	hMCHR1[a] (IC_{50})	
					SPA[b] (nM)	IP3[b] (nM)
24	Cl	H	H	H	180	305
25	Cl	H	H	OH	150	163
26	Cl	H	H	OMe	213	181
27	Cl	Me	Me	OH	>10000	>10000
28	Cl	H	H	CONH^{i}Pr	15	25
29	OCF_3	H	H	CONH^{i}Pr	17	9
30	OCF_3	H	H	$CONMe_2$	47	18
31	OCF_3	H	H	CONH^{c}Pr	9	3
32	OCF_3	H	H	$CONH_2$	23	32
33	OCF_3	H	H	CO^{c}Pr	10	14
34	Cl	H	H	NH_2	150	610
35	OCF_3	H	H	NH_2	22	80
36	Cl	H	H	NHCO^{i}Pr	15	54
37	Cl	H	H	NHCONH^{i}Pr	7	4
38	Cl	H	H	Pyrrolidine	20	81
39	Cl	Me	H	Pyrrolidine	258	401
40	Cl	Me	Me	Pyrrolidine	>10000	>10000
41	OCF_3	H	H	Pyrrolidine	31	120
42	OCF_3	H	H	Morpholine	6	16
43	OCF_3	H	H	2-Pyrrolidinone	19	4

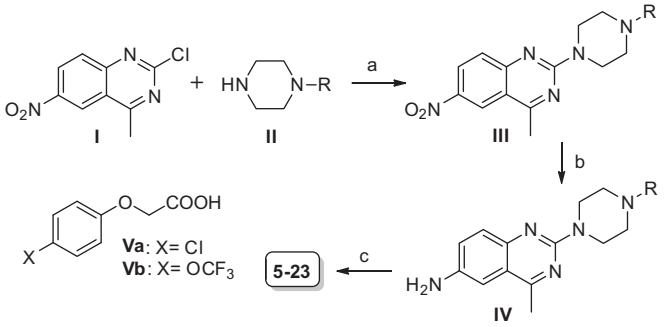
[a] Values are mean of at least two experiments.
[b] See Refs.21,22 for the protocol.

Table 3
Human MCHR1 binding and functional activity of of 4-hydroxypiperidine derivatives



Compd	X	R	hMCHR1[a] (IC_{50})	
			SPA[b] (nM)	IP3[b] (nM)
44	Cl	Me	237	827
45	Cl	Et	208	384
46	OCF_3	Et	116	59
47	OCF_3	*c*-Pr	101	33
48	OCF_3	*c*-Bu	79	27
49	OCF_3	*c*-Hexyl	327	156
50	OCF_3	$CONH_2$	636	[c]
51	OCF_3	Ph	354	67
52	OCF_3	*p*-F-phenyl	150	55
53	OCF_3	*m*-F-phenyl	635	100
54	OCF_3	5-F-2-pyridyl	213	59

[a] Values are mean of at least two experiments.
[b] See Refs.21,22 for the protocol.
[c] 72% response at 10 μM.



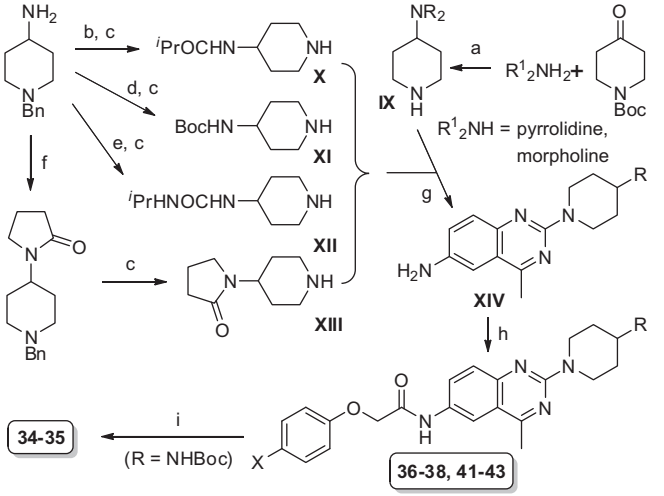
Scheme 1. Reagents and conditions: (a) Et_3N, MeOH, reflux, 2 h, 60–100%; (b) 10% Pd/C, H_2, EtOH, 2 h, 97%; (c) **V**, HOBt, EDCI, Et_3N, CH_2Cl_2, rt, 6 h, 25–60%.

produced compounds **24**–**26** and **32**–**33**. Hydrolysis of the ester group on the piperidine ring followed by amide coupling furnished the desired target structures **28**–**31**. Compound **27** was prepared analogous to the procedure for **25** by coupling with 2-(4-chlorophenoxy)-2-methylpropanoic acid.

Syntheses of compounds **34**–**43** are outlined in Scheme 3. Piperidines **IX** were prepared from *tert*-butyl 4-oxopiperidine-1-carboxylate by reductive amination and Boc-deprotection. Piperidines **X** and **XI** were synthesized by N-acylation/carbamoylation of 1-benzylpiperidin-4-amine followed by debenzylation. The reaction of isopropylisocyanate with 1-benzylpiperidin-4-amine followed by debenzylation gave piperidine **XII**. Alkylation of 1-benzylpiperidin-4-amine with ethyl 4-bromobutanoate followed by hydrolysis and EDC mediated intramolecular cyclization afforded the intermediate 1-(1-benzylpiperidin-4-yl)pyrrolidin-2-one which after debenzylation furnished piperidine **XIII**. All these piperidines **IX–XIII** were coupled with **I** and subsequently converted to target compounds **36**–**38** and **41**–**43**. Deprotection of the Boc group provided amino compounds **34**–**35**. Compounds **39** and **40** were prepared analogous to the procedure for **39** by coupling with 2-(4-chlorophenoxy)propanoic acid and 2-(4-chlorophenoxy)-2-methylpropanoic acid, respectively.

The synthesis of compounds **44**–**54** is illustrated in Scheme 4. Piperidines **XV** were prepared by various Grignard reactions on 1-benzylpiperidin-4-one followed by debenzylation. The cyanohydrine intermediate **XVI** was converted to amide **XVII** by standard protocols. The coupling of **I** with **XV** and **XVII** and subsequent manipulations afforded the desired compounds.

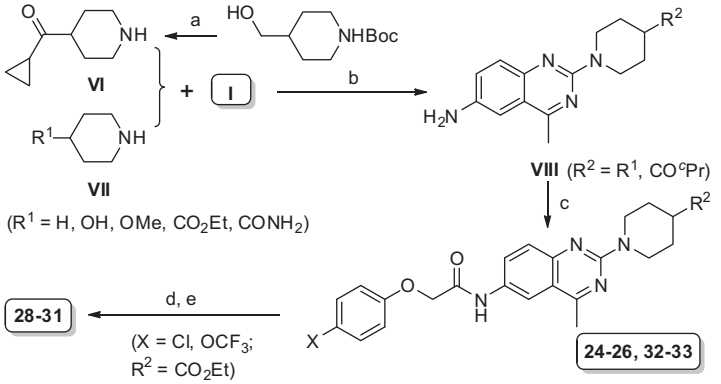
The binding affinity for the MCHR1 of the target compounds was assessed in the SPA based [^{125}I]MCH binding screen[21] using



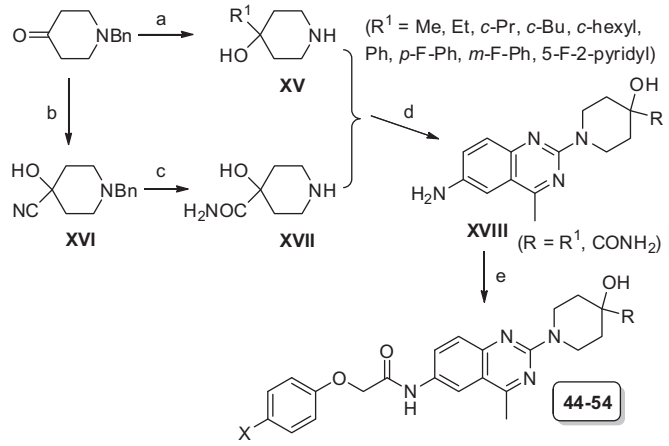
Scheme 3. Reagents and conditions: (a) (i) Amine, 10% Pd/C, H_2, MeOH, 53–65%; (ii) TFA, CH_2Cl_2, rt, 2 h, 70–74%; (b) *i*-PrCOCl, Et_3N, CH_2Cl_2, rt, 1 h, 60%; (c) 10% Pd/C, H_2, EtOH, 2–4 h, 72–90%; (d) Boc_2O, $NaHCO_3$, CH_2Cl_2, rt, 4 h, 60%; (e) *i*-PrNCO, DIPEA, CH_2Cl_2, rt, 8 h, 72%; (f) ethyl 4-bromobutanoate, K_2CO_3, DMF, rt, 2 h, 42%; (g) (i) **I**, Et_3N, MeOH, reflux, 2 h, 72–90%; (ii) 10% Pd/C, H_2, EtOH, 2 h, 83–93%; (h) **V**, HOBt, EDCI, Et_3N, CH_2Cl_2, rt, 6 h, 25–60%; (i) TFA, CH_2Cl_2, rt, 2 h, 75–78%.

Chinese hamster ovary (CHO-K1) cell membranes expressing human recombinant MCHR1 receptors. The functional antagonism was measured in an IP3 SPA-YSI assay.[22] Selected compounds were chosen for solubility[23] and microsomal stability[24] studies.

The Table 1 shows a summary of results obtained from the *N*-alkyl, amide and sulfonamide variations of eastern piperazine derivatives. The quinazolines **5** and **6** displayed considerably lower binding affinity than the corresponding quinolines having 58 and 22 nM, respectively, in the SPA assay.[17] This trend turned out to be consistent throughout the series which called for the extended SAR exploration described herein. Increasing the bulk of alkyl moieties such as **7** and **8** did not have any significant impact whereas morpholine was beneficial (**10**). The potency could be further improved with a *para*-trifluoromethoxy substituent (**11**) having IC_{50}s of 7 and 31 nM in binding and functional assays, respectively. As we knew that the basicity of the distal nitrogen was not crucial for activity in the quinoline series[17] we explored other more polar amide motives and found some striking differences. Surprisingly the sulfonamide **12** is completely detrimental to MCHR1 potency whereas the amides **13–20** are tolerated. The trend in slight potency improvement with 4-OCF_3 compounds compared to their



Scheme 2. Reagents and conditions: (a) (i) PCC, CH_2Cl_2, rt, 6 h, 90%; (ii) *c*-PrMgCl, THF, −78 °C to rt, 2 h, 44%; (iii) PCC, CH_2Cl_2, rt, 6 h, 88%; (iv) TFA, CH_2Cl_2, rt, 30 min, 99%; (b) (i) Et_3N, MeOH, reflux, 2 h, 75–93%; (ii) 10% Pd/C, H_2, EtOH, 2 h, 85–97%; (c) **V**, HOBt, EDCI, Et_3N, CH_2Cl_2, rt, 6 h, 25–60%; (d) LiOH, THF, EtOH, H_2O, rt, 3 h, 75–82%; (e) amine, HOBt, EDCI, Et_3N, DMF, 52–73%.



Scheme 4. Reagents and conditions: (a) (i) R^1MgBr, THF, 0 °C to rt, 43–56%; (ii) 10% Pd/C, H_2, EtOH, 2 h, 85–92%; (b) NaCN, $NaHCO_3$, THF, H_2O, rt, 6 h, 78% (c) (i) H_2SO_4, rt, 16 h, 52%; (ii) 10% Pd/C, H_2, EtOH, rt, 3 h, 43%; (d) (i) **I**, Et_3N, MeOH, reflux, 2 h, 72–90%; (ii) 10% Pd/C, H_2, EtOH, 2 h, 83–93%; (e) **V**, HOBt, EDCI, Et_3N, CH_2Cl_2, rt, 6 h, 51–65%.

4-chloro analogs was also observed in this series. Increasing the lipophilicity and bulk of amides from cyclopropyl to cyclohexyl led to gradual loss in MCHR1 potency. The benzamide derivative **23** is practically devoid of activity.

The effects of replacing piperazines to piperidines are shown in Tables 2 and 3. The unsubstituted piperidine **24** has a moderate potency and we explored small substituents at the 4-position like OH, OMe and NH_2 (**25**–**26** and **34**) that also could be beneficial with respect to hERG binding, without improvements. The OCF_3 analog once again showed improved activity (**34** vs **35**). However, most of the amides **28**–**32** and the *c*-propyl ketone **33** are very potent MCHR1 antagonists in the functional assay. The reverse amide **36** displays an activity comparable to the amide analog **28**. The replacement of *c*-propyl amide with *c*-propyl ketone (**31** vs **33**) did not show much difference in terms of MCHR1 binding and functional profile. The urea derivative **37** was very potent displaying MCHR1 IC_{50}s of 7 and 4 nM in binding and functional assay but the poor solubility of this compound hindered its further profiling. A pyrrolidine substitution at the 4-position was tolerated though no difference in replacing Cl with OCF_3 group at the western end was observed (**38** vs **41**). The replacement of pyrrolidine with morpholine as in **42** showed significant improvement in MCHR1 potency displaying binding affinity of 6 nM and functional antagonism of 16 nM. As the other amides and retroamides, the cyclic 2-pyrrolidinone derivative **43** showed to be very potent with binding affinity of 19 nM and functional antagonism of 4 nM.

Docking experiments were used to guide the SAR explorations. We have previously described a homology model based on the rhodopsin structure.[17a] Herein we use the recently published X-ray structure of the β2-adrenergic receptor also including the extracellular loops.[25] Figure 2 describes the receptor ensemble used to emulate the induced fit during the docking (see Supplementary section for details).

To account for receptor variability and ligand induced fit, compound **42**, as an illustration, was subjected to full flexible ligand docking to each of the 50 receptor models according to a Monte Carlo routine under softened van der Waals conditions (details are given in the Supplementary data). Several key hydrogen bond interactions were identified for the linker Gln_{196} III:12 (3.36), Gln_{281} V:08 (5.42), Gln_{345} VI:20 (6.55).[26] One of the quinazoline nitrogen atoms is engaged in an ionic interaction with Asp_{192} III:08 (3.32), whereas the other is forming a hydrogen bond to Thr272 in extracellular loop (ECL) 2b (Fig. 3). This additional binding to the extracellular loop could explain why we do not see a larger potency drop in the less basic quinalzolines compared to the quinazolines only interacting with Asp_{192} III:08. The proposed docking mode also supported the rather accommodating pocket allowing substantial variations in conjunction to the quinazoline part. We were not able to convincingly explain the lack of activity of the sulfonamide **12** albeit it displayed an energetically less favored skewed piperazine conformation of the docked structure compared to the other compounds having pronounced chair conformations. The docking pose also accounts for the limited space available in the 4-position of the phenoxy moiety in the opposite part of the molecules. A van der Waals interaction between the 4-methyl in the quinazoline and Leu_{274} on ECL2b is identified (cf Supplementary data) and the tryptophan on helix 4 limit the space for accommodating groups larger than a methyl in accordance with the SAR of the corresponding quinolines.[17]

The introduction of a methyl group in the oxymethylene linker to improve the linker stability has shown to decrease MCHR1 potency whereas the gem-dimethyl group is completely detrimental (compare **25** vs **27**; **38** vs **39** and **40**) which lends support from modeling studies as well.

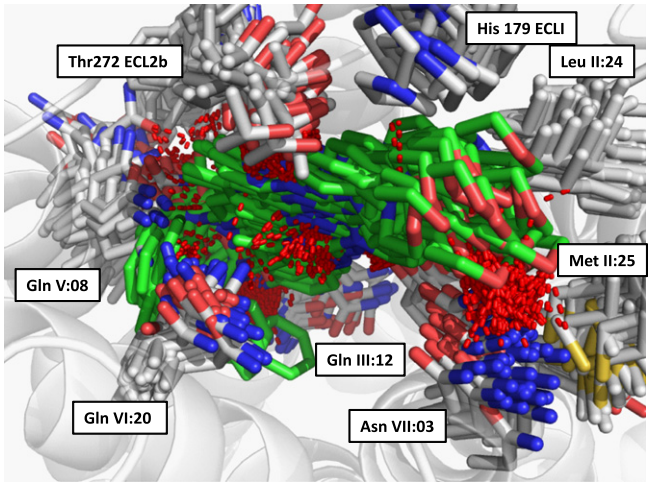


Figure 2. Cluster of 50 hMCHR1 models in complex with **42** shown in green. Constructed, docked and refined from the pair-wise sequence alignment to the β2-X-ray structure.[25] The annotations are in accordance with the Schwartz numbering system.[26] ECL refers to extracellular loop.

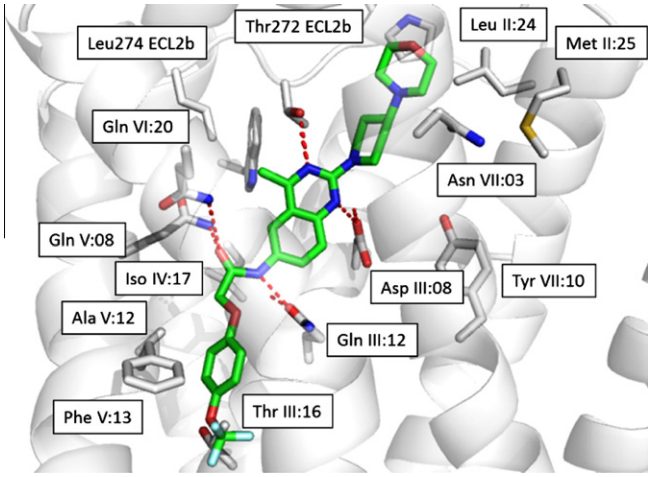


Figure 3. Docking pose of **42** in homology model of hMCHR1 depicting crucial interactions including residues on the extracellular loop 2b. Annotations as in Figure 2.

Table 4
Solubility and stability of selected MCHR1 antagonists

Compd	MCHR1_IC_{50} IP3 (nM)	Sol (μM) [a]	MLM Stab.[b]	HLM Stab.[c]	M-blood Stab.[d]
10	115	15	1	39	[e]
18	30	8	43	59	[e]
20	45	4	69	52	45
28	25	132	11	70	49
36	54	205	9	23	[e]
39	401	272	34	81	67
42	16	12	45	59	29

[a] Determined as precipitation from 5% DMSO and 95% PBS solution.
[b] Mice liver microsomal stability, % remaining after 30 min.
[c] Pooled human liver microsomal stability, % remaining after 30 min.
[d] Mice blood stability, % remaining after 30 min.
[e] Not tested.

Table 5
hERG data of selected MCHR1 antagonists

Compd	MCHR1_IC_{50} IP3 (nM)	% Inhibition at 1 μM or IC_{50} (μM)
33	14	32%
38	81	0.45
39	401	76%
42	16	78%
43	4	3.7
48	27	31%

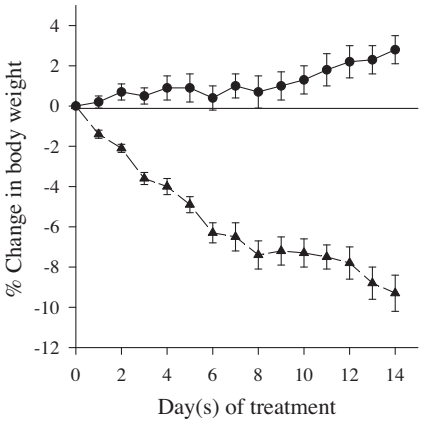


Figure 4. Effect on the body weight of DIO C57BL/6J mice after subchronic oral administration of **42** (▲30 mg/kg, b.i.d.) for 14 days versus vehicle control animals (●) (n = 12).

4-Hydroxy piperidine derivatives have been useful motifs for many GPCR receptors (internal chemometrics analysis)[27] and we explored a small set to see if the activity of **25** could be improved (Table 3). Amongst various alkyl substitutions, *c*-Pr and *c*-Bu provided some activity enhancement. The carboxamide group showed decreased binding affinity to MCHR1. The phenyl analogs **51** and **52** showed useful MCHR1 functional potency but with moderate binding affinity. The introduction of an additional nitrogen as in **54** compared to **52** had significant impact in solubility but without sufficient MCHR1 potency.

The compounds were profiled in parallel with respect to some physicochemical and ADMET properties (Table 4 and 5). The favorable piperazinyl-morpholine derivatives (e.g., **10**) showed poor metabolic stability, especially in mouse liver microsomes. The piperazinyl-amides, such as **18** and **20**, had poor solubility but good microsomal and blood stability. The piperidinyl amides (**28**) displayed much better solubility but showed a pronounced difference in mouse and human microsomal stability. The retroamides (e.g., **36**) also showed good solubility but poor metabolic stability. The introduction of a methyl substituent in the oxymethylene linker (**39**) led to good solubility and improved plasma stability but at the expense of reduced potency. The morpholine substituted piperazines like **42** had good metabolic stability and reasonable stability in blood but low solubility. With respect to hERG the *c*-propyl ketone **33**, the 2-pyrrolidinone **43** and 4-hydroxypiperidine **48** were better than the pyrrolidines **38** and **39** and morpholine **42**.

Clearly we have not yet arrived at a fully optimized compound of this chemotype, but we wanted to verify their potential also in a subchronic in vivo model. Compound **42**, showing good oral PK profile,[28] was chosen as a prototype molecule to investigate the effect in a diet induced obesity (DIO) mouse model using C57BL/6J mice. On oral administration **42** (30 mg/kg, b.i.d.),[29] showed steady loss of body weight culminating in a statistically significant weight loss of 12% on day 14 as shown in Figure 4.[30]

In summary, we have disclosed the SAR of a quinazoline-derived novel series of potent MCHR1 antagonists. Systematic structural activity studies helped identify key modifications required for optimal potency of this inherently less potent scaffold. The representative molecule **42** showed significant anti-obesity effect in a DIO mice model but further optimizations are needed to improve stability in plasma related to the oxymethylene linker, which are disclosed in the subsequent paper.

Acknowledgment

We thank the scientists of the analytical department of Discovery Research for spectral data of all compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.050.

References and notes

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21. [^{125}I]-MCH binding was performed by incubating membranes from CHO-K1 cells stably expressing hMCHR1 with SPA beads and tracer in presence of various test compounds for 2 h. Non specific binding was measured by conducting the reaction in presence of 1 μM of cold MCH peptide. Readings were taken in the top count scintillation counter. The extent of antagonism was expressed as % displacement. The IC_{50} for the compound was calculated by Assay Explorer from MDL software using the average CPM values.
22. Functional response of compounds was assessed using the IP3-SPA-YSI method. Briefly, CHO-K1 cells stably expressing hMCHR1 were incubated overnight with 0.5 μCi/well of 3*H*-myo-inositol to generate a pool of 3*H*-PIP2. After aspiration of labeling medium, the cells were incubated with test compounds followed by stimulation with 80 nM of agonist (MCH peptide, corresponds to EC_{80}). The resulting pool of 3*H*-IPs was extracted with formic acid and the amount of 3*H*-IPs generated in the cells was detected using the non derivatized yttrium silicate SPA beads.
23. *Protocol for solubility measurements*: A solution of 25 μL of compound dissolved in DMSO (10 mM) is added to a PBS solution to a final solution of 500 μM in a 96-well PP Greiner deep-well plate. This mixture is allowed to precipitate overnight at RT with the plate located on a plate-shaker. Next day solution (and precipitate) is centrifuged and absorbance (260, 280 and 620 nm) of supernatant is measured. Also a reference (100 and 500 μM compound) dissolved in acetonitrile is measured and solubility (i.e., remaining compound in PBS solution) is determined by dividing the absorbance measured in PBS versus acetonitrile. A blind DMSO is also measured for all wave lengths as reference and the absorbance of 620 nm is used to exclude possible contaminations (i.e., visible) in samples.
24. *Protocol for microsomal stability studies*: Metabolic stability studies were conducted by incubating the compounds at 10 μM with 1 mg/ml of mouse/human liver microsomes diluted in phosphate buffer (0.1 M, pH 7.4) .The reaction was initiated by the addition of NADPH and the samples were incubated for 30 min at 37 °C. The reaction was terminated by the addition of an organic solvent containing an appropriate internal standard. The samples were vortexed, centrifuged and the clear supernatant was analysed via HPLC.
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26. Generic numbering of amino acids in TM bundle according to Schwartz, T.W. (e.g., Gln_{196} III:12), *Curr. Opin. Biotechnol.* **1994**, *5*, 434. A comparison with the Ballesteros–Weinstein nomenclature (e.g., Gln_{196} 3.36) is detailed in Frimurer, T.M.; Högberg, T. *Curr. Top. Med. Chem.* **2011**, *11*, 1882.
27. A recent substructure search for compounds containing 4-hydroxypiperidines of the ChEMBL (former Starlight) database containing more than 500000 bioactive ligands (predominantly small molecules) identified a total of 803 small molecules containing 4-hydroxypiperidine motifs (many more if additional substituents in the piperidine ring are included). The compounds distributed on 153 different chemotype clusters based on Daylight 2D fingerprints and a Tannimoto similarity threshold of 0.7. The ligands were associated with various different targets including several GPCR families.
28. Oral PK profile of compound **42** in Swiss Albino Mice (30 mg/kg); Plasma: $AUC_{(0-8)}$ 1.02 μM.h, C_{max} 0.43 μM, T_{max} 1 h, $t_{1/2}$ 3.0 h; Brain: $AUC_{(0-8)}$ 6.21 μM h, C_{max} 2.66 μM, T_{max} 1 h, $t_{1/2}$ 1.7 h.
29. b.i.d. stands for Latin word *bis in die* meaning twice a day.
30. C57BL/6J mice were fed a high fat diet (60% calories from fat, D12492 feed) for nearly 3 months until they reached an average body weight of approximately 45 g. Animals were grouped based on initial body weight. Compounds to be tested were then administered per orally twice daily for a period of 14 days. Body weight and food intake of the animals was recorded on a daily basis during the experimental duration.