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Discovery of 4-(dimethylamino)quinazolines as potent and selective antagonists for the melanin-concentrating hormone receptor 1

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Abstract—A series of 4-(dimethylamino)quinazoline based antagonists of the melanin-concentrating hormone receptor 1 (MCH-R1) is described. This series was derived from a lead compound, AR129330, identified by HTS of a GPCR-directed library using a functional assay with a constitutively activated (CART) form of the receptor. The preliminary optimization resulted in the identification of compounds **20**, **21**, and **23**.

Melanin-concentrating hormone (MCH) is a cyclic 19 amino acid peptide expressed in the lateral hypothalamic area (LHA) and the rostromedial zona incerta.[1,2] There has been significant interest in the pharmacology of MCH since the discovery of the MCH-R1.[3] Based on the localization of MCH-containing neurons, a role for MCH in feeding behavior and energy expenditure has been suggested. Indeed, the intracerebroventricular (icv) injection of MCH in rats results in increased food intake.[4,5] Moreover, MCH mRNA was reported to be upregulated by fasting in both normal and ob/ob mice and to be increased in ob/ob mice relative to wild-type animals.[4] In contrast, prepro-MCH-knockout mice were found to be hypophagic and have reduced body weight and increased leanness relative to their wild-type counterparts.[6] It is suggested that MCH-R1, the only form of the receptor shown to be present in rodents, may mediate the physiological effects of MCH in those species. From these data it can be inferred that a centrally acting MCH-R1 antagonist may reduce food intake and bodyweight, and hence may be effective in treating obesity. In addition, MCH was reported to induce an anxiogenic effect when injected into the medical preoptic area in rats,[7] while icv injection of MCH increased the number of entries into the open arms in the elevated plus-maze test,[8] suggesting that MCH may also be implicated in the modulation of emotional states. This hypothesis is supported by the recent finding that SNAP-7941, a selective antagonist for the MCH-R1, exhibited anxiolytic- and antidepressant-like effects in rodent models.[9]

MCH-R1 antagonists reported in the literature to date encompass a wide range of structural types (Fig. 1). The first non-peptide MCH-R1 antagonist T-226296 has a tetralin substructure and has been found to have low nanomolar affinity for the MCH-R1.[10] One of the most potent MCH-R1 antagonists hitherto described is compound SNAP-7941, with a reported IC_{50} value of 0.04 nM in an MCH-R1 binding assay.[11]

Our initial hit identification however came not from modification of such known structures, but from high-throughput screening of our in-house GPCR-directed library. The library contains a series of collections based on a number of privileged fragments, some of which

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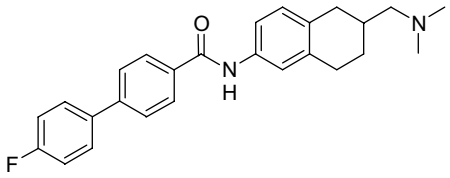
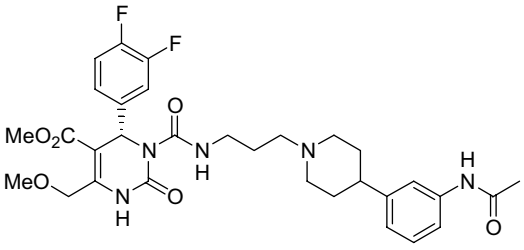
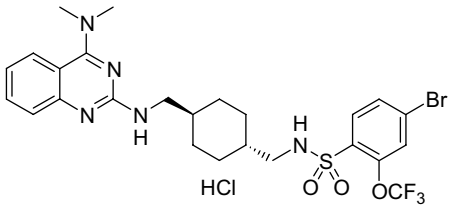


T-226296



SNAP-7941

Figure 1. Representative non-peptide MCH-R1 antagonists.



AR129330

MCH IC_{50} = 160 nM

Y5 IC_{50} = 2.7 nM

α_{2A} IC_{50} = 7.7 nM

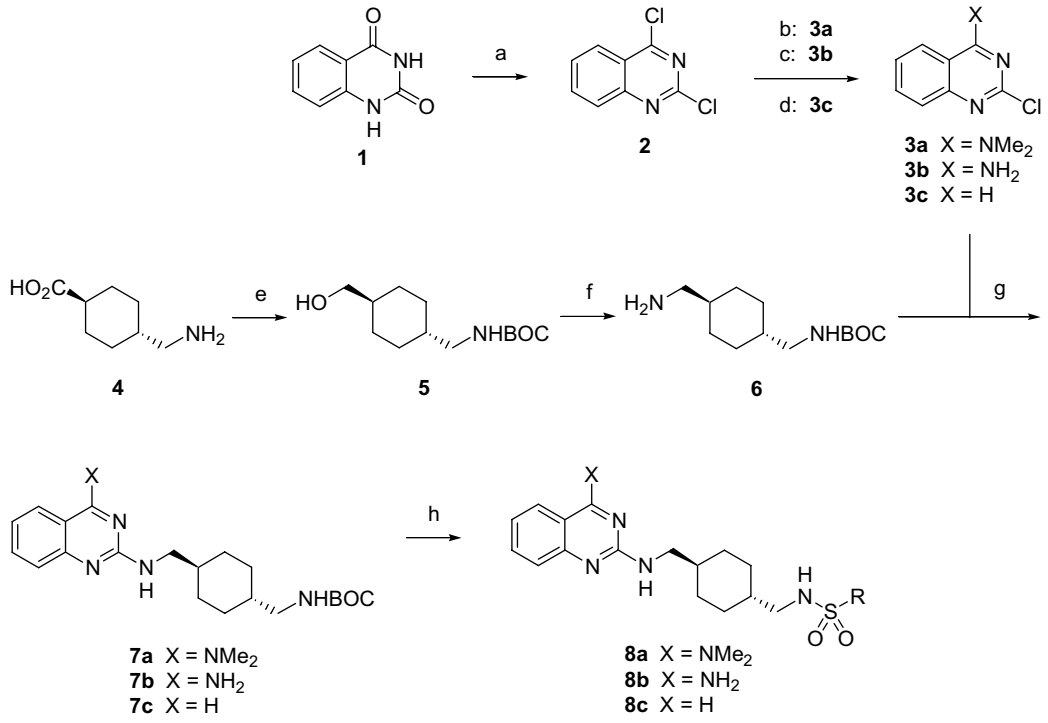
Figure 2. Quinazoline lead structure from HTS.

have been previously well documented and others which have been identified by proprietary pharmacophore mapping algorithms. In addition, we have designed and synthesized libraries based on specific core structures selected from a broad range of known ligands for GPCRs, with the focus on class 1 GPCRs. One such sub-library was built around the known NPY Y5 antagonist series of quinazolines, as exemplified by CGP-71683A. Using a fluorescence-based assay to measure inhibition of MCH-induced calcium flux in HEK-293 cells stably expressing a constitutively activated mutant on the human MCH-R1 (hMCH-R1), we identified a series of 4-(dimethylamino)quinazolines and 4-(methylamino)quinazolines from this Y5-like sub-library as functional MCH-R1 antagonists. Our most potent initial screening hit was 4-bromo-*N*-{[*trans*-4-({[4-(dimethylamino)quinazolin-2-yl]amino}methyl)cyclohexyl]methyl}-2-(trifluoromethoxy)benzenesulfonamide hydrochloride (AR129330), a resynthesized and purified sample demonstrating an IC_{50} value of 160 nM in our assay. This is by no means the first example of using families of GPCR antagonist ligands to hop from activity at one GPCR to another, and this initial success strengthens the case for screening of such target class directed libraries as an approach to accelerate the hit detection phase of drug discovery. Although AR129330 lacked selectivity against a panel of other GPCRs, in particular α_{2A} adrenergic receptor and, unsurprisingly, Y5, we used AR129330 as our initial chemical starting point for our discovery program targeting MCH-R1 antagonists (Fig. 2). In addition to the poor selectivity, the in vitro metabolic stability of most members of the initial hit series was found to be moderate at best and these two properties were identified as key areas in need of improvement before we would consider this a lead series. Our initial efforts to develop structure–activity relationships (SAR) at the MCH-R1 based on the 4-(dimethylamino)quinazoline series and to improve selectivity are described in this communication.

A representative scheme for the preparation of quinazoline derivatives described herein is shown in Scheme 1.

The quinazoline cores **3a**–**c** were synthesized in two steps from commercially available 1*H*,3*H*-quinazoline-2,4-dione **1**. The starting material was reacted with phosphorous oxychloride ($POCl_3$) under reflux in the presence of *N*,*N*-dimethylaniline to provide 2,4-dichloro-quinazoline **2**. Selective substitution of the chlorine at the 4-position with 50% aqueous dimethylamine or 28% aqueous ammonia gave the corresponding 4-amino-2-chloro-quinazoline **3a** or **3b**. Alternatively, the chlorine at the 4-position could be selectively reduced by activated zinc to yield 2-chloroquinazoline **3c**.[12] The mono-protected *trans*-1,4-cyclohexylmethyldiamine **6** was prepared in six steps from commercially available tranexamic acid **4**. The carboxylic acid was reduced to alcohol **5** by $NaBH_4$ via the mixed acid anhydride after the amine was protected as its *tert*-butyl (BOC) carbamate. Tosylation of the alcohol followed by azidation gave an intermediate azide, which was further converted into the amine **6** by reduction with lithium aluminum hydride. Coupling of **3a**–**c** with **6** was accomplished upon reflux in isopropanol to afford coupling products **7a**–**c**, respectively. Deprotection of the BOC group was achieved with hydrogen chloride to provide amines as precursors for target quinazoline derivatives **8a**–**c**. The amines were coupled to an appropriate sulfonyl chloride to afford the desired quinazoline sulfonamides **8a**–**c**.

Throughout our hit-to-lead and lead optimization program, we employed a combination of two assays to assess potency of new analogues at the hMCH-R1. In each case, we used a constitutively activated (CART) form of the receptor mutated at the third intracellular loop of the WT-MCH-R1 and measured competitive inhibition of binding of [^{125}I](Phe^{13}, Tyr^{19})MCH or transient intracellular calcium-mobilization evoked by the MCH agonist in HEK293 cells stably expressing the CART-MCH-R1.[13] We used the former to compare binding to MCH-R1 with binding data at other receptors to enable us to determine selectivity, whereas the latter we used to measure functional antagonist potency. We found that the CART form of the receptor provided better signal-to-noise ratios in these assays than the wild-type hMCH-R1 transfected into the same cell line. However, to verify that the mutation would not significantly impact the activity of compounds at the WT-MCH-R1, we compared data from a number of our quinazoline analogues across two assay platforms with both



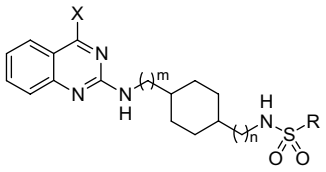
Scheme 1. Representative synthetic route of quinazolines. Reagents and conditions: (a) $POCl_3$, *N*,*N*-dimethylaniline, reflux, 7 h, 86%; (b) aq 50% Me_2NH, THF, room temp., 80 min, 94%; (c) aq 28% NH_3, room temp., 28.5 h, 96%; (d) activated Zn, aq 9% NH_3 saturated with NaCl, CH_2Cl_2, reflux, 3 h, 70%; (e) (i) $(BOC)_2O$, aq 1.3 M NaOH, *tert*-BuOH, room temp, 18 h, 67%, (ii) $ClCO_2Et$, Et_3N, CH_2Cl_2, 0 °C, 50 min, (iii) $NaBH_4$, THF, MeOH, 4 °C, 3 h, 84% in two steps; (f) (i) TsCl, pyridine, 4 °C to room temp, 15 h, (ii) NaN_3, DMF, 50 °C, 4 h, 91% in two steps, (iii) LAH, THF, room temp., 6 h, 91%; (g) isopropanol, reflux; (h) (i) 4 M HCl in EtOAc, EtOAc, room temp, 1.5 h, (ii) RSO_2Cl, *iso*Pr_2NEt, CH_2Cl_2, 4 °C or room temp.

the CART-MCH-R1 and WT-MCH-R1. The IC_{50} values of the lead compound AR129330 in the receptor binding assay and intracellular calcium-mobilization assay utilizing the CART-MCH-R1 were 160 ± 9.0 nM ($n = 8$) and 140 ± 10 nM ($n = 6$), respectively, whereas the IC_{50} in the receptor binding assay using the WT-MCH-R1 was 200 ± 38 nM ($n = 3$). From these data, we were satisfied that measuring binding at the CART-MCH-R1 would provide a meaningful SAR-driving assay for our medicinal chemistry approaches and downstream spot checks, where we never observed statistically significant differences in IC_{50} values in the CART- and WT-MCH-R1 assays, further validated this assumption.

We initially examined the effect of changes in the dimethylamino group at the 4-position of the quinazoline as shown in Table 1. With some SAR information already in hand from screening of our directed library, we knew that substituents larger than dimethylamino in the quinazoline 4-position were very poorly tolerated so we were able to focus our efforts around very conservative changes in this region of the molecule. Compound **9**, in which the dimethylamino group was replaced by an amino group, was approximately 12-fold less potent at the MCH-R1 than AR129330, and still showed very high affinity for the Y5 (IC_{50} for Y5 = 1.6 nM). Compound **10** with no substituent at the 4-position was devoid of activity for the MCH-R1 at a concentration of 10 μM. Simultaneously, we attempted to replace the terminal phenyl ring with linear and branched alkyl groups. Replacement with either methyl substituent **11** or isopropyl **12** was found to be completely lacking for MCH-R1 activity whilst retaining significant affinity for the Y5 and the α_{2A} receptors. Likewise, butyl substituent **13** and octyl substituent **14** markedly decreased MCH-R1 activity compared to AR129330. These early results indicated that both 4-amino-substitution of the quinazoline and the terminal phenyl group are critical for MCH-R1 activity and form two key parts of the MCH-R1 pharmacophore with the *trans*-1,4-cyclohexane–dimethyleneamine serving to arrange the two pharmacophoric groups into three-dimensional space. We expected that chemical modification of the length or conformation of the spacer between the quinazoline ring and the sulfonamide linker could be used to optimize activity at the receptor by more closely defining the required spatial arrangement. This hypothesis then, led us to focus our next series of investigations around this area of our hit series.

Next we examined the effects of altering the spacer portion upon MCH-R1 activity and the selectivity over the Y5 and the α_{2A} receptors. Shortening the length of the *trans*-1,4-cyclohexane spacer by removing one or both methylene groups significantly reduced MCH-R1 affinity (e.g., **15**: $m = 1$, $n = 0$; **16**: $m = 0$, $n = 1$; **17**: $m = 0$, $n = 0$). Changing the relative orientation of the substituents

Table 1. In vitro data of quinazoline derivatives



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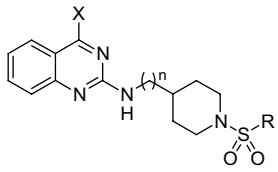

II

Compound	Structure	Conformation	*m*	*n*	X	R	IC_{50}[a] (nM)		
							MCH-R1	Y5	α_{2A}
CGP71683A	I	*trans*	1	1	NH_2	1-Naphthyl	3500	1.3	97
AR129330	I	*trans*	1	1	NMe_2	4-Br-2-OCF_3-Ph	160	2.7	7.7
9	I	*trans*	1	1	NH_2	4-Br-2-OCF_3-Ph	1900	1.6	100
10	I	*trans*	1	1	H	4-Br-2-OCF_3-Ph	> 10000	78	140
11	I	*trans*	1	1	NMe_2	CH_3	> 10000	41	86
12	I	*trans*	1	1	NMe_2	*iso*C_3H_7	> 10000	NE[b]	NE
13	I	*trans*	1	1	NMe_2	C_4H_9	2900	4.9	58
14	I	*trans*	1	1	NMe_2	C_8H_{17}	6700	NE	NE
15	I	*trans*	1	0	NMe_2	4-Br-2-OCF_3-Ph	810	2.5	21
16	I	*trans*	0	1	NMe_2	4-Br-2-OCF_3-Ph	1500	150	110
17	I	*trans*	0	0	NMe_2	4-Br-2-OCF_3-Ph	2200	31	47
18	I	*cis*	1	1	NMe_2	4-Br-2-OCF_3-Ph	2900	NE	NE
19	I	*cis*	1	0	NMe_2	4-Br-2-OCF_3-Ph	2700	NE	NE
20	I	*cis*	0	1	NMe_2	4-Br-2-OCF_3-Ph	140	120	130
21	I	*cis*	0	0	NMe_2	4-Br-2-OCF_3-Ph	83	880	57
22	II	—	—	1	NMe_2	4-Br-2-OCF_3-Ph	6000	3000	5.6
23	II	—	—	0	NMe_2	4-Br-2-OCF_3-Ph	250	8900	170

[a] Compounds were evaluated for their ability to compete with [^{125}I](Phe^{13}, Tyr^{19})MCH, [^{125}I]PYY, or [^{3}H]MK912 at a human CA-MCH-R1 stably expressed in HEK293 cells or a human Y5 or α_{2A} transiently expressed in COS-1 cells. Data represent the mean of 1–3 separate experiments performed from 5 concentrations and in duplicate.

[b] Not evaluated.

across the cyclohexyl ring of AR129330 from *trans* to *cis* provided compound **18**, which resulted in an 18-fold decrease in activity. Similarly 4-aminomethyl-*cis*-cyclohexylamine derivative **19** showed significantly reduced affinity for the MCH-R1. Compound **20**, in which the spacer used in **19** was reversed, exhibited comparable affinity to AR129330 but still lacked selectivity over the other receptors. Use of the shorter *cis*-1,4-cyclohexyldiamine spacer, as in **21** however, resulted in a 2-fold increase in activity relative to AR129330 and more importantly provided our first hint of selectivity over the Y5 receptor. Further shortening of the linker by replacement with piperidine rings led to divergent selectivities. Compound **22** showed very potent affinity for the α_{2A} receptor (IC_{50} for α_{2A} = 5.6 nM) and was significantly less active at both the MCH-R1 and the Y5 receptor. In contrast, compound **23** maintained MCH-R1 activity and showed remarkably decreased Y5 activity compared to AR129330. These data emphasize the importance of the nature, ring conformation, and length of the spacer to the MCH-R1 activity.

In summary, high-throughput screening of our in-house GPCR-directed library detected AR129330 as a potential chemical starting point for the discovery of MCH-R1 antagonists. Through the preliminary SAR investigation we demonstrated that both 4-amino-substitution of the quinazoline and the terminal phenyl group were critical for MCH-R1 activity. Subsequently, further optimization of the spacer portion between the quinazoline ring and the sulfonamide linker resulted in the identification of compounds **20**, **21**, and **23**, which in next investigation led us to the finding of other series involving oral active ATC0175.

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