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Identification of 2-aminobenzimidazoles as potent melanin-concentrating hormone 1-receptor (MCH1R) antagonists

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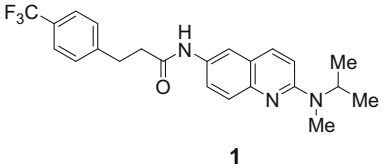
ABSTRACT

A series of 2-aminobenzimidazole-based MCH1R antagonists was identified by core replacement of the aminoquinoline lead **1**. Subsequent modification of the 2- and 5-positions led to improvement in potency and intrinsic clearance. Compound **25** exhibited good plasma and brain exposure, and attenuated MCH induced food intake at 30 mg/kg PO in rats.

Melanin-concentrating hormone (MCH) is a cyclic nonadecapeptide with a disulfide bond that is expressed predominantly in the lateral hypothalamus. MCH was originally isolated from salmon pituitaries as a factor of skin color alteration.[1] Thus far, several lines of evidence have shown that MCH is an important mediator of energy homeostasis. Up-regulation of prepro-MCH mRNA has been reported in several obese animals.[2–4] Chronic intracerebroventricular infusion of MCH causes obesity with hyperphagia.[5,6] Targeted disruption of the prepro-MCH gene reduces food intake and increases the metabolic rate, generating a lean phenotype.[7] In contrast, overexpression of the prepro-MCH gene causes moderate obesity in mice.[8] MCH binds to and activates two types of G-protein-coupled receptors, MCH1R[9,10] and MCH2R.[11] MCH1R is expressed in rodents and higher mammals, whereas MCH2R is expressed in ferrets, dogs, rhesus monkeys, and humans, but not in rodents. Recently, we showed that chronic administration of a peptidic MCH1R antagonist suppresses food intake and body weight gain in diet-induced obesity (DIO) rats and mice.[12,13] Thus, the MCH1R antagonist could be a potential therapeutic agent for the treatment of obesity.[14]

Discovery of 2-aminoquinoline-6-carboxamide MCH1R antagonists, exemplified by **1**, was reported first by DeVita et al. (Fig. 1).[15] Identification of the related compounds series, using a homology model of MCH1R based on the crystal structure of rhodopsin[16] and virtual screening techniques,[17] was subsequently published by other laboratories. This 2-aminoqunoline series exhibits excellent binding activity to MCH1R; however, no orally available compound has been reported in this class. We speculated that the lipophilic quinoline core might be detrimental to metabolic stability and solubility, resulting poor bioavailability in this series. In addition, we conjectured that the nitrogen atom at the 1-position in the quinoline ring may be involved in a key interaction with the receptor, and that the basicity coming from the nitrogen atom in the quinoline moiety is critical for potency. These hypotheses led us to attempt to create potent and orally available MCH1R antagonists by replacing the quinoline core with hydrophilic bioisostere, which possesses a basic site capable of interacting with the receptor. A bioisosteric 2-aminobenzimidazole template was found to show potent MCH1R binding activity, and subsequent structure–activity relationship (SAR) studies led to the identification of potent and in vivo active compounds exemplified by **25**.[18]



1

Figure 1. Aminoquinoline MCH1R antagonist.

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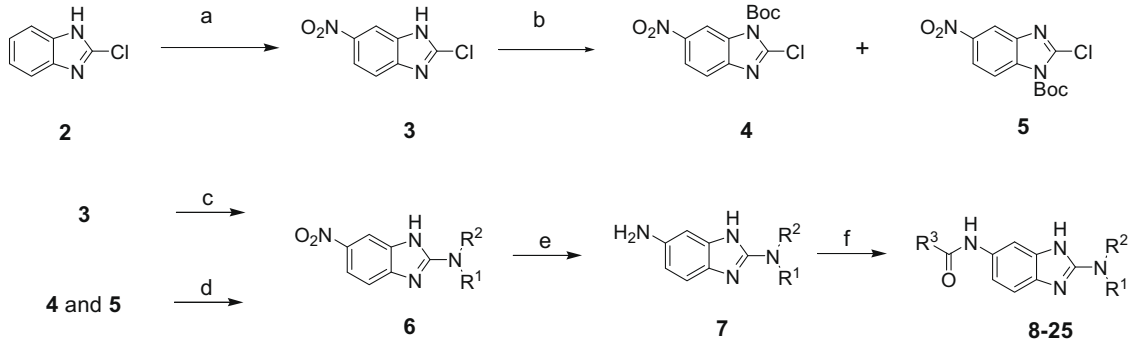
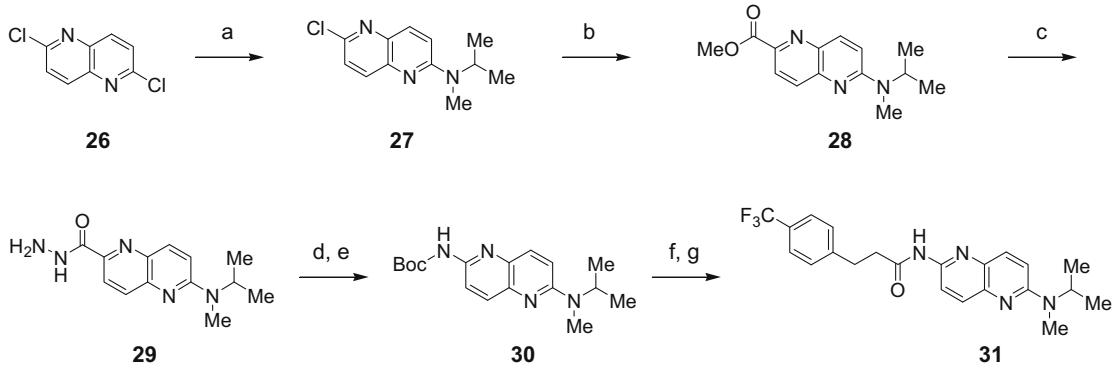


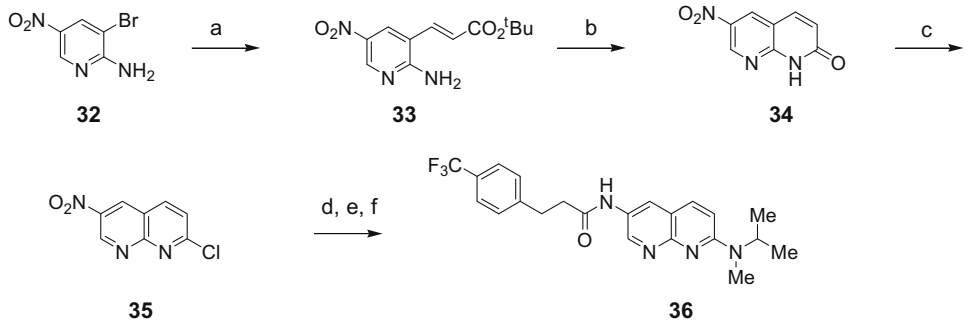
Scheme 1. Reagents and conditions: (a) 70% HNO_3, H_2SO_4; (b) Boc_2O, DMAP, THF; (c) R^1R^2NH (8–50 equiv), neat or in dioxane, sealed tube, 130 °C; (d) R^1R^2NH or HCl salt (2.2–3.3 equiv), DIEA, dioxane or DMSO, 80–100 °C; (e) H_2, $Pd(OH)_2$–C, MeOH; (f) R_3COOH, HATU, DIEA, CH_2Cl_2 or R_3COCl, Et_3N, CH_2Cl_2.

In this Letter, we report the development of a series of 2-aminobenzimidazole-5-carboxamide MCH1R antagonists.

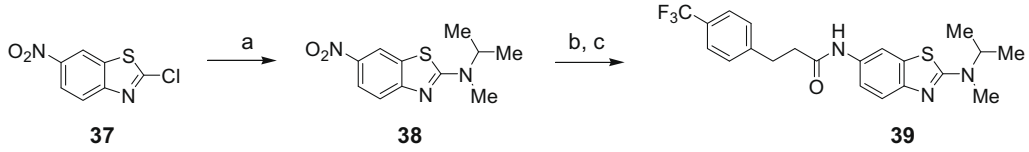
The synthesis of the 2-aminobenzimidazole-5-carboxamide derivatives is described in Scheme 1. Commercially available 2-chlorobenzimidazole (**2**) was converted to 5-nitro derivative **3** by treatment with 70% HNO_3 and H_2SO_4. Displacement of the chloro substituent with desired amino groups was accomplished by reaction of **3** with a large excess amount of the corresponding amines in a sealed tube at 130 °C, providing 2-aminobenzimidazole **6**. Alternatively *N*-Boc-protected 2-chloro-5-nitrobenzimidazole can be used as a mixture of regioisomers (**4** and **5**) instead of **3**, In this case, the substitution reaction proceeded under milder conditions,



Scheme 2. Reagents and conditions: (a) *N*-methylisopropylamine (10 equiv), dioxane, sealed tube, 120 °C; (b) CO (140 psi), $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, $NaHCO_3$, MeOH/dioxane, 100 °C; (c) $NH_2NH_2{\cdot}H_2O$, MeOH; (d) $NaNO_2$, 1 N aq HCl, 0 °C; (e) *tert*-BuOH/dioxane, reflux; (f) CF_3COOH; (g) RCOCl, pyridine, $CHCl_3$.

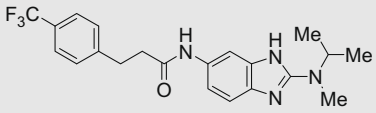
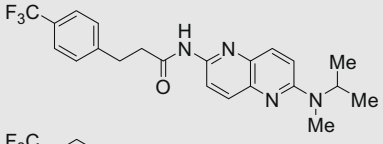
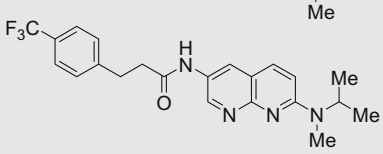
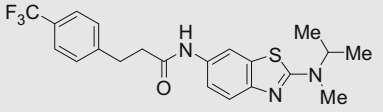


Scheme 3. Reagents and conditions: (a) *tert*-butyl acrylate, $Pd(OAc)_2$, (*o*-tol)$_3$P, Et_3N/DMF, 130 °C; (b) 5 N aq HCl, sealed tube, 130 °C; (c) $POCl_3$, reflux; (d) *N*-methylisopropylamine, K_2CO_3, DMF, 80 °C; (e) H_2, Pd–C, MeOH; (f) RCOCl, Et_3N, CH_2Cl_2.



Scheme 4. Reagents and conditions: (a) *N*-methylisopropylamine (3 equiv), K_2CO_3, DMF, 80 °C; (b) H_2, Pd–C, MeOH/THF; (c) RCOOH, 2-chloro-1,3-dimethylimidazolidinium chloride, pyridine, $CHCl_3$.

Table 1
MCH1R binding and calculated pK_a data for bioisosteric derivatives

Compound	Structure	Binding assay IC_{50}[a] (nM)	Calculated pK_a[b]
8		5.9	7.8
31		375	5.1
36		365	5.3
39		>750	3.9

[a] The data represent means of at least two experiments.
[b] pK_a values were calculated by the SPARC on-line calculator.[21]

namely **4** and **5** reacted with 2.2–3.3 equiv of the desired amine at a lower temperature of 80–100 °C. Under the present reaction conditions, the Boc group at the resulting 2-amino-benzimidazole was removed to afford **6**. Hydrogenation of the 6-nitro group in **6** followed by a coupling reaction with carboxylic acid in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) or acyl chloride afforded the target compounds **8**–**25**.

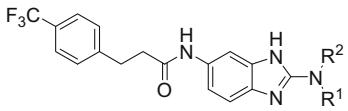
Scheme 2 shows the synthesis of 1,5-naphthyridine derivative **31**. Mono-amination of commercially available 2,6-dichloronaphthyridine (**26**) followed by palladium catalyzed methoxy carbonylation provided methyl ester **28**. Compound **28** was condensed with hydrazine to give hydrazide **29**, which was then subjected to diazotization and subsequent Curtius rearrangement in the presence of *tert*-butanol to give compound **30**. Treatment of **30** with trifluoroacetic acid followed by acylation afforded compound **31**.

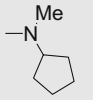
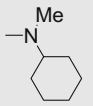
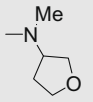
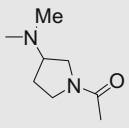
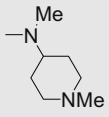
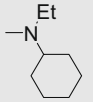
Synthesis of 1,8-naphthyridine derivative **36** was performed as shown in Scheme 3. Commercially available 2-amino-3-bromo-5-nitropyridine (**32**) was converted to naphthyridinone **34** by Heck reaction employing *tert*-butyl acrylate, subsequent cyclization under acidic conditions. Treatment of **34** with $POCl_3$ provided 2-chloro-2-nitronaphthyridine (**35**), which was converted to compound **36** through a similar fashion (amination, reduction, and then acylation) to the synthesis for benzimidazoles **8**–**25**.

Benzothiazole derivative **39** was prepared from commercially available 2-chloro-6-nitrobenzothiazlole (**37**) through a similar fashion (amination, reduction, and then acylation) to the synthesis for benzimidazoles **8**–**25** as shown in Scheme 4.

Compounds were evaluated for their binding affinity to the membranes of CHO-K1 cells expressing human MCH1R (hMCH1R) in a competition binding assay with [^{125}I]-MCH as the radioligand.[19] Antagonistic activity was estimated by the inhibitory effect of compounds on intracellular calcium mobilization induced by MCH using a FLIPR in CHO-K1 cells expressing human MCH1R.[20]

Table 2
MCH1R binding and function data for 2-aminobenzimidazole-5-carboxamides

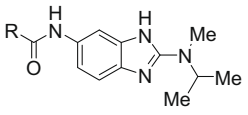


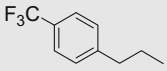
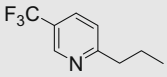
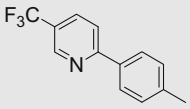
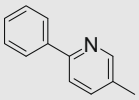
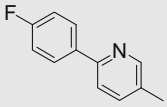
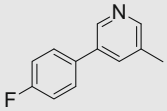
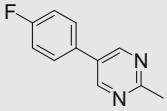
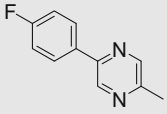
Compound	NR^1R^2	Binding assay IC_{50}[a] (nM)	Functional assay IC_{50}[a] (nM)
8		5.9	21
9		12	nd
10		19	nd
11		24	nd
12		4	22
13		6.7	27
14		27	nd
15		30	nd
16		23	nd
17		135	nd
18		428	nd

[a] The data represent means of at least two experiments (nd = not determined).

Related compounds possessing the bioisostere for quinoline were prepared and evaluated for MCH1R binding activity (benzimidazole **8**, 1,5-naphthyridine **31**, 1,8-naphthyridine **36**, and benzothiazole **39**). Compounds **31**, **36**, and **39** exhibited modest binding affinity. Interestingly, benzimidazole **8** showed excellent binding affinity (IC_{50}: 5.9 nM) and functional activity (IC_{50}: 21 nM). The calculated pK_a values of compounds **31**, **36**, and **39** are less than 5.3.[21] In contrast, the calculated pK_a value of compound **8** is 7.8,[21] supporting our hypothesis that appropriate basicity at the 1-position is critical for potency in this series (Table 1). Discovery of benzimidazole **8** with excellent MCH1R binding activity encouraged us to explore further SAR studies of this series.

Table 3
MCH1R binding and function data for 2-aminobenzimidazole-5-carboxamides



Compound	R	Binding assay IC_{50}[a] (nM)	Rat hepatic clearance CL[b] (mL/min/kg)
8		5.9	32
19		41	nd
20		3.1	16
21		8.8	12
22		5.5	8
23		900	nd
24		15	9
25		2.7	10

[b] Intrinsic clearance measured in rat hepatocytes.[22]
[a] The data represent means of at least two experiments (nd = not determined).

The hMCH1R binding affinities and functional inhibitory potencies of compounds with a variety of amino substituents at the 2-position are shown in Table 2. Replacement of the *iso*-propyl group on the amino group in **8** with (cyclo) lower alkyl groups such as methyl, ethyl, and cyclopropyl groups led to a decrease in binding affinity (**9**, **10**, and **11**). Introduction of larger cycloalkyl groups such as cyclopentyl and cyclohexyl groups retained the binding affinity (**12** and **13**), although introduction of oxygen- or nitrogen-containing cycloalkyl groups was deleterious to binding activity as seen in **14**, **15**, and **16**. Replacing the methyl group in **13** with a larger alkyl group, an ethyl group, produced a 20-fold less active compound **17** (IC_{50}: 135 nM). The combination of a methyl group and a bulky group on the amine provided potent MCH1R antagonists. Interestingly, morpholine **18** (IC_{50}: 428 nM) showed a significant loss in binding activity.

Compound **8** was then evaluated for its metabolic stability using rat hepatocytes.[22] Compound **8** was found to show modest clearance (32 mL/min/kg). We turned our attention to expand SAR to the carboxamide moiety to improve binding affinity and intrinsic clearance value (Table 3). Replacement of the terminal phenyl ring with a 2-pyridyl ring **19** (IC_{50}: 41 nM) showed a sevenfold decrease in binding activity compared to the parent **8**. The ethylene linkage in the dihydrocinnamyl moiety, a potentially metabolically labile moiety, was replaced with aromatic rings to give *p*-biaryl compound **20** (IC_{50}: 3.1 nM, clearance: 16 mL/min/kg) with significant improvement in binding affinity (compound **19** vs **20**) and clearance (compound **8** vs. **20**). Therefore, we investigated a series of biaryl analogs. Azine rings such as pyridine, pyrimidine, and pyrazine rings were well tolerated. Incorporation of a fluorine atom to the phenyl group in **21** provides a positive impact on the binding activity and clearance as seen in **22**. The meta-

Table 4
Pharmacokinetic data of **25** in rats

	25
F[a] (%)	35
CL[a] (mL/min/kg)	11
Vd[a] (L/kg)	1.2
$T_{1/2}$[a] (h)	2.3
Plasma level[b,c] (μM)	3.9
Brain level[b,c] (nmol/g)	1.4

[a] The rats were dosed at 1 mg/kg IV and 3 mg/kg PO (mean values, $n = 3$).
[b] The rats were dosed at 30 mg/kg PO (mean values, $n = 3$).
[c] Plasma and brain levels were determined at 2 h post-dose.

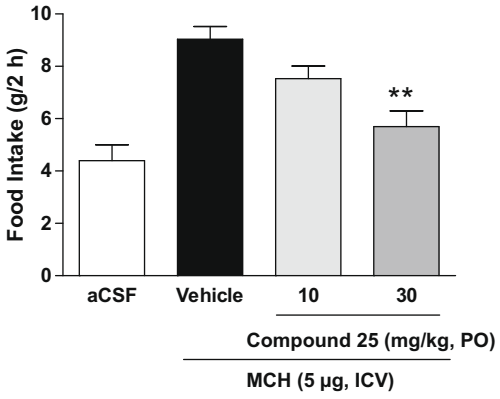


Figure 2. Effect of compound **25** on MCH-induced feeding response in SD rats. Values are the mean ± SEM of 15–20 rats per group. $^{**}P$ <0.01 versus vehicle/MCH group.

orientated biaryl analog **23** (IC_{50}: 900 nM) displayed a significant decrease in affinity. The pyrazinecarboxamide derivative **25** (IC_{50}: 2.7 nM, clearance: 10 mL/min/kg) showed the best balance of potency and clearance.

We evaluated the pharmacokinetic properties of compound **25** in rats as shown in Table 4. Compound **25** demonstrated a low clearance (11 mL/min/kg) and good oral bioavailability (35%). Furthermore, its levels in plasma and brain at 2 h were 3.9 μM and 1.4 nmol/g, respectively (brain/plasma ratio: 0.36) at an oral dose of 30 mg/kg. We also examined the effect of compound **25** on the MCH-induced feeding response in SD rats as shown in Figure 2. Oral dosing at 10 and 30 mpk dose-dependently suppressed food intake by 32% and 72% for 2 h, respectively, compared to the vehicle treatment group.[23]

In summary, we discovered that 2-aminobenzimidazoles were potent MCH1R antagonists with bioisosteric replacement of the quinoline core. Subsequent optimization of 2-amino and 6-carboxamide positions led to identification of compound **25** that has demonstrated excellent in vitro activity and good brain penetrability. Compound **25** also significantly suppressed MCH induced food intake after oral dosing in rats.

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19. Membranes from CHO-K1 cells stably expressing human MCH1R were incubated with [^{125}I]-MCH (PerkinElmer, Waltham, MA, USA) and test compounds in binding buffer (50 mM Tris, 10 mM $MgCl_2$, 2 mM EDTA, 50 μg/mL bacitracin, 0.2% BSA, pH 7.4) at room temperature for 2 h. The membranes were filtrated onto GF/C filter plates and dried. The radioactivity was counted using a microplate scintillation counter (TopCount, PerkinElmer). Non-specific binding was determined by including 1 μM unlabeled MCH in the binding reaction.
20. CHO-K1 cells stably expressing human MCH1R were seeded into 96-well culture plates at 3.0×10^4 cells per well and cultured in culture medium (Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsberg, CA, USA), 10% Hepes, 1 mg/mL G418, 100 U/ml penicillin, and 100 g/ml streptomycin) for 24 h. The cells were loaded with Fluo-4 AM in DMEM containing 10% FBS and 2.5 mM probenecid at 37 °C for 1 h. Then the cells were washed with assay buffer (Hanks' balanced salt solution containing 20 mM Hepes, 0.5% BSA, and 2.5 mM probenecid, pH 7.4) and analyzed using a FLIPR system (Molecular Devices, Sunnyvale, CA) to measure the mobilization of intracellular Ca^{2+} in response to MCH. The cells were treated with the test compounds for 5 min before addition of MCH.
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23. A 26-gauge guide cannula was stereotaxically implanted into the third ventricle of male SD rats as described previously (Endocrinology 146:3080-3086, 2005). After at least 1 week recovery, rats were fed a high fat diet for ~3 h and compound **25** (10 or 30 mg/kg) or vehicle was orally administered. One hour after the drug administration, MCH (5 μg/μL/head, dissolved in artificial cerebrospinal fluid) was injected ICV and food intake for 2 h was measured. MCH injection was performed 1 h before the onset of the dark cycle.