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Bioorganic & Medicinal Chemistry Letters 16 (2006) 5445–5450
Bioorganic & Medicinal Chemistry Letters

Identification of substituted 4-aminopiperidines and 3-aminopyrrolidines as potent MCH-R1 antagonists for the treatment of obesity

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Received 15 June 2006; revised 14 July 2006; accepted 17 July 2006
Available online 1 August 2006

Abstract—A substituted 4-aminopiperidine was identified as showing activity in an MCH assay from an HTS effort. Subsequent structural modification of the scaffold led to the identification of a number of active MCH antagonists. 3,5-Dimethoxy-*N*-(1-(naphthalen-2-ylmethyl)piperidin-4-yl)benzamide (**5c**) was among those with the highest binding affinity to the MCH receptor (K_i = 27 nM), when variations were made at benzoyl and naphthylmethyl substitution sites from the initial HTS hit. Further optimization via piperidine ring contraction resulted in enhanced MCH activity in a 3-aminopyrrolidine series, where (*R*)-3,5-dimethoxy-*N*-(1-(naphthalen-2-ylmethyl)-pyrrolidin-3-yl)benzamide (**10i**) was found to be an excellent MCH antagonist (K_i = 7 nM).

Obesity and overweight account for greater than 50% of the US population and western countries.[1] The epidemic is also increasing in children and adolescents. The complications of obesity and overweight include diabetes, cardiac diseases, and certain forms of cancer.[2] While there is a significant effort at identifying effective pharmacotherapies for obesity, there is currently no good obesity drug on the market and the currently available therapies are limited due to variable efficacies and undesirable side effects.[3] Over the past decade, multiple pharmaceutical companies have focused on central nervous system (CNS) targets, including the melanocortin,[4] endocannabinoid,[5] and melanin concentrating hormone receptors.[6]

Melanin concentrating hormone (MCH) is a cyclic 19 amino acid CNS neuropeptide that is highly conserved in vertebrates.[7] MCH is implicated in diverse physiological processes and, importantly, in feeding behavior and energy balance.[8] The MCH producing neurons in the CNS are located in the lateral hypothalamus and zona incerta with neuronal connections to widespread regions in the brain.[9] When injected intracerebroventricularly in rats, MCH stimulates food intake.[10] MCH levels are found to increase during fasting and in leptin-deficient obese mice.[11] Transgenic MCH over-expression in the brain leads to overweight mice with hyperphagia. In contrast, MCH-deficient mice have reduced body weight and leanness due to reduced feeding and an increased metabolic rate.[12] Finally, MCH-R1 deficient mice are lean and hyperactive. Based on this evidence in mice, there is a wide interest in modulating this ligand receptor system as a mechanism to induce weight loss or as a long-term weight maintenance therapy in treating the obesity epidemic.

Structure **1** was identified as one of the hits from our in-house high-throughput screening effort[13] and showed good MCH binding activity (K_i 125 nM).[14] This scaffold possessed a variety of attractive attributes like a potent in vitro activity, low molecular weight, a number of H-bonding acceptors/donors, and various sites for structural modifications for the optimization. Our strategy included a three-component approach as delineated in Figure 1: benzoyl (Section A), 4-aminopiperidine (Section B), and naphthalene (Section C) moieties. In this

Keywords: MCH-R1; Antagonist; Obesity; Aminopiperidine; Aminopyrrolidine.
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doi:10.1016/j.bmcl.2006.07.053

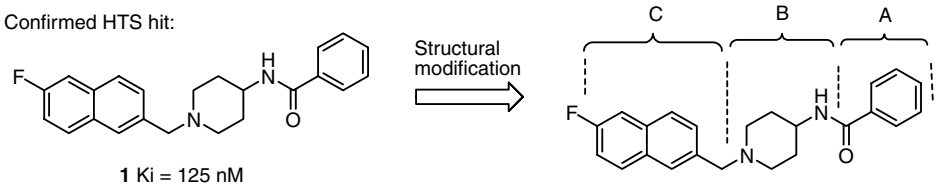


Figure 1.

context, we wish to report our SAR results from the structural modification of the 4-aminopiperidine series, as well as the identification of the more potent 3-aminopyrrolidine series.

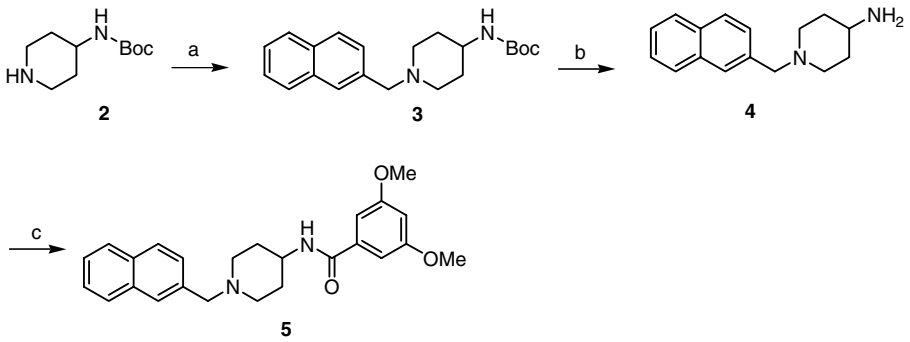
Our initial interest was to modify the benzoyl moiety to examine the substitution impact on MCH in vitro activity by introducing various groups to the aryl ring (Section A in Fig. 1). The chemistry for the preparation of **5a**–**n** is outlined in Scheme 1. Starting from *tert*-butyl piperidin-4-ylcarbamate, alkylation with 2-(bromomethyl)naphthalene and deprotection of the Boc group followed by coupling gave the final products. The robust chemistry procedure allowed us to quickly generate a pool of analogs for the in vitro MCH screening, and the results are tabulated in Table 1.

Structural modification at the benzoyl moiety in the 4-aminopiperidine scaffold had shown significant substituent effects impacting the MCH-R1-binding affinity. It was found that an electron-rich alkoxy group at meta positions resulted in an increase of binding activity in **5b**–**d**, compared to the non-substituted parent **5a**. Interestingly, para substitution of methoxy in **5e**–**f** was detrimental to the activity. In addition, incorporation of fluorine atom(s) to the phenyl proved to be beneficial as seen in **5g**–**j**. In this case, the substitution position does not seem to influence binding results. Since both methoxy and fluoro groups were contributing to in vitro activity, the combination of 3-methoxy and 4-fluoro in **5k** exhibited more pronounced binding activity relative to its mono-substituted counterparts (**5b** and **5g**) as expected. The great potency of the fluorinated analogs coupled with potential metabolic stability warrants further investigation of these compounds in in vivo testing. In contrast, other halogen substitution such as 4-chloro in **5l** showed a decrease in the binding affinity. Similar results were observed in 3-hydroxy and 3-dimethylamino analogs (**5m**–**n**), in which an H-bond donor or acceptor group negatively impacted the MCH activity.

The 3,5-dimethoxybenzoyl moiety in the 4-aminopiperidine was found to be among the most promising substituents based on the SAR profile for the modification in Section A. Therefore, this moiety was chosen during the course of our SAR investigation in Section C of the 4-aminopiperidine scaffold. The chemistry for preparing these analogs in Scheme 2 includes a 3-step sequence starting from commercially available 1-benzyl-4-aminopiperidine **6**. Coupling with substituted 3,5-dimethoxybenzoyl acid followed by hydrogenation afforded a key intermediate **8**. The alkylation with naphthylmethylbromide finished the synthesis of compound **9**.

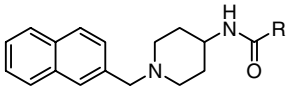
A part of our interest in developing SAR was to explore an alternative group for the naphthalyl moiety to improve the solubility property of analogs in the series. Substitution of the naphthalene with fluoro (**9b**) showed basically no effect in binding affinity compared to the parent **5c**, whereas methoxy analog (**9c**) was about 3-fold less active. Interestingly, α-substitution of the naphthalenylmethyl in **9d** (a racemate) retained the in vitro activity. This implies that a space tolerance at this particular site is present in the MCH-R1-binding pocket. Although heteroaryl substitution was, in general, less favored, an indole analog (**9f**) was found to be about 4-fold more active than a quinoline counterpart (**9e**). Partial saturation of the naphthalene in analog **9g** (a racemate) resulted in a slight loss of the in vitro activity.

These compelling data suggest the outer benzene ring is likely more important for the MCH binding activity. This was further illustrated in cinnamyl analogs **9h** exhibiting excellent binding affinity.[15] In contrast, **9i**

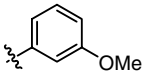
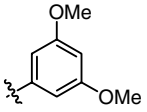
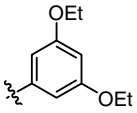
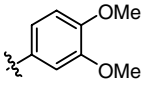
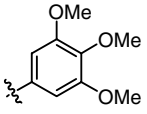
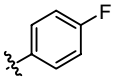
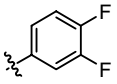
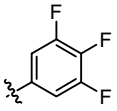
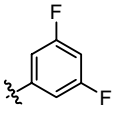
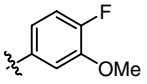
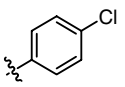
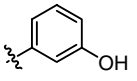
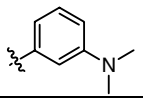


Scheme 1. Reagents: (a) 2-(bromomethyl)naphthalene, TEA, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) HOBT, EDCI, NMM, THF.

Table 1. MCH-R1-binding for 4-aminopiperidines with various benzoyl moieties[a]



5a-r

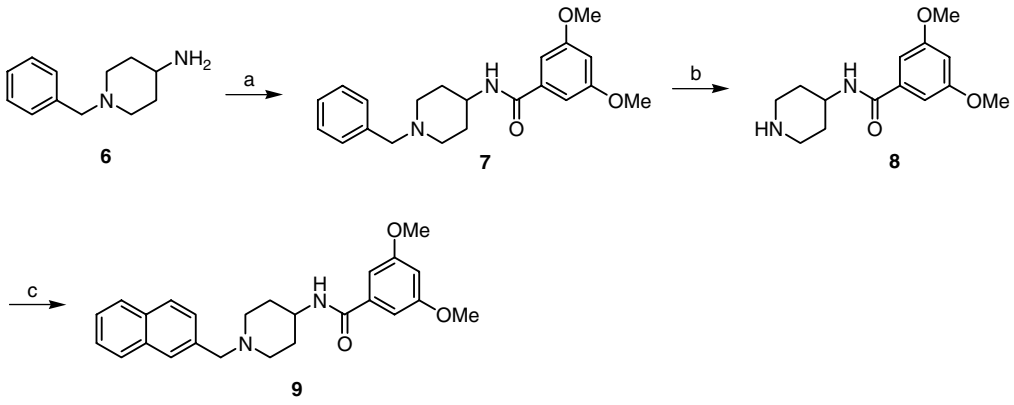
Compound	Moiety (R)	K_i (nM)
5a		347
5b		29
5c		27
5d		56
5e		1000
5f		286
5g		36
5h		18
5i		9
5j		65
5k		13
5l		232
5m		570
5n		125

[a] The data represent means of at least two experiments, unless otherwise indicated.

showed little activity in the MCH binding assay due to lack of conformational rigidity. However, adding a hydroxyl group α to the benzene in **9l** regained considerable in vitro activity, which may be rationalized by re-positioning of the phenyl group through a directing effect from the hydroxyl group in the binding pocket. Moreover, substitution with only the inner benzene ring also produced some in vitro activity as shown in analog **9k–l**, however these compounds were still 7-fold less active compared to the parent **5c** and ~20-fold less to the cinnamyl analog **9h**.

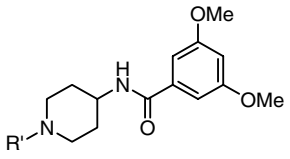
With the encouraging SAR results from the modification in the Sections A and C, we turned our attention to expand SAR to the aminopiperidine core portion (Section B) to further improve the in vitro activity. Initially, we substituted the 4-aminopiperidine with alkyl functionality at different positions to see how these changes might impact the MCH in vitro activity. *N*-Methyl-4-aminopiperidine **10a** showed about a 7-fold decrease in binding activity compared to the unsubstituted **5c**, whereas 4-methyl-4-aminopiperidine **10b** showed a 3-fold loss in the activity. Interestingly, stereochemical bias did not seem to be a factor governing thein vitro activity as shown in *cis* and *trans* 3-methyl isomers **10c** and **10d**. It was also found that a heavily substituted piperidine near the ring nitrogen in **10e** resulted in a significant loss of the in vitro activity, which suggests the basic nitrogen may play an important role for the binding affinity. In addition, 3-amido benzoyl piperidine analog **10f** exhibited essentially no MCH activity, presumably due largely to the positional distortion of the benzoyl moiety in the MCH-R1-binding pocket. We then focused our attention on the modification of the ring size and opened ring system to evaluate SAR outcomes. Intriguingly, fused bicyclic analogs showed a great stereochemical preference, excellent MCH activity in *trans* isomer **10g** over its counterpart **10h**. We hypothesized the *trans* isomer adapts the requisite structural conformation accommodated in the receptor for tight binding. The most promising finding in the structural modification in Section B was the identification of pyrrolidine **10i** showing single digit nano-molar activity.[16] We suspect this compelling result reveals a key conformational requirement needed to allow MCH antagonists to fit into the receptor pocket tightly. The fine tuning of the conformational orientation of the benzamide group defined the activity in the following order of activity: 3-aminopyrrolidine (**10i**, K_i 7 nM) > 4-aminopiperidine (**5c**, K_i 27 nM) > 3-aminopiperidine (**10f**, K_i > 10,000 nM). Finally, an open ring form in **10j** was found to be completely inactive because of missing ring rigidity.

As a follow-up to the modification of the 4-aminopiperidine series, the 3-aminopyrrolidine was identified to be the most active MCH antagonist series. Therefore, we focused on additional iteration of analogs for the 3-aminopyrrolidine to further optimize MCH activity. Using the SAR information from Tables 1 and 2, a number of aminopyrrolidine analogs were assembled and screened for in vitro data (Tables 3 and 4). The 3-aminopyrrolidine analogs clearly demonstrated a conformational preference, in which the (*R*)-isomer **10i**

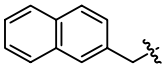
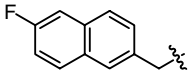
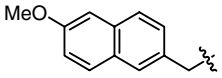
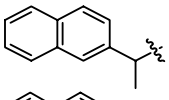
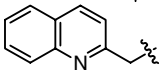
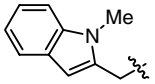
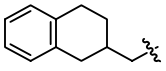
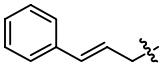
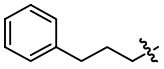
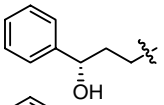
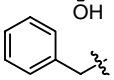
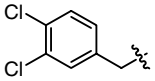


Scheme 2. Reagents: (a) HOBT, EDCI, NMM, THF; (b) Pd/C, H_2, MeOH; (c) 2-(bromomethyl)naphthylene, TEA, CH_2Cl_2.

Table 2. MCH-R1-binding for 4-(3,5-dimethoxybenzamido)peridines containing *N*-alkyl moieties[a]

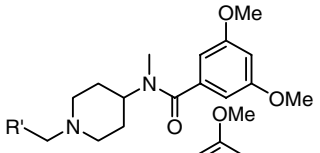
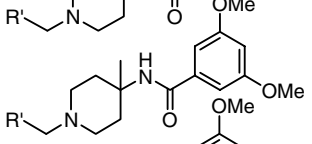
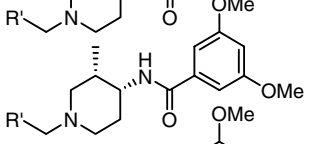
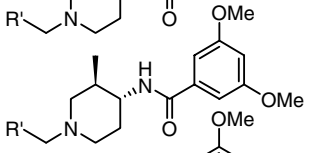
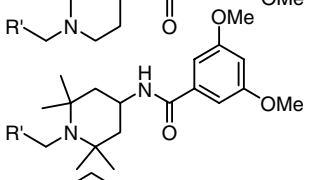
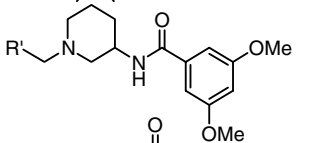
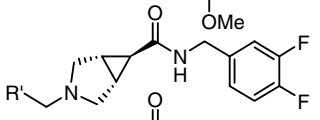
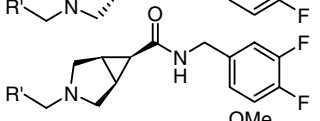
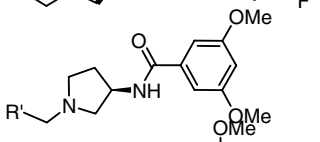
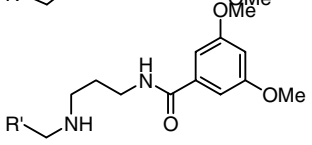


9a-9r

Compound	Moiety (R′)	K_i (nM)
5c		27
9b		32
9c		99
9d		25
9e		625
9f		142
9g		136
9h		12
9i		$>10^5$
9j		505
9k		302
9l		205

[a] The data represent means of at least two experiments, unless otherwise indicated.

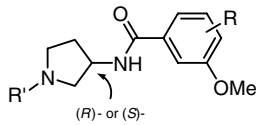
Table 3. MCH-R1-binding for aminopiperidine, aminopyrrolidine and aminoazitidine analogs[a,b]

Compound	Moiety (R′ = 2-naphthyl)	K_i (nM)
10a		169
10b		86
10c		170
10d		133
10e		$>10^5$
10f		$>10^5$
10g		40
10h		$>10^5$
10i		7
10j		$>10^5$

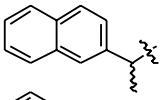
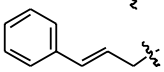
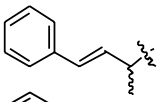
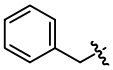
[a] The data represent means of at least two experiments, unless otherwise indicated.

[b] The procedures described in Scheme 1 were used to prepare analogs **10a**–**j**.

Table 4. MCH-R1-binding for 3-aminopyrrolidine analogs[a]



10j and 11a-d

Compound	Moiety (R′ = 2-naphthyl)	Config.	K_i (nM)
10i	5-MeO	(*R*)-	7
11a	5-MeO	(*S*)-	484
11b	H	(*R*)-	67
11c	4-F	(*R*)-	62
11d		(*R*)-	12
11e		(*R*)-	17
11f		(*R*)-	23
11g		(*R*)-	8070

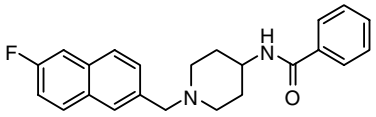
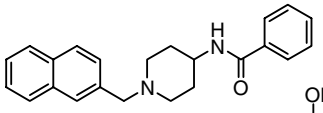
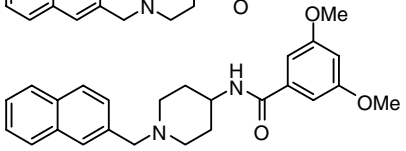
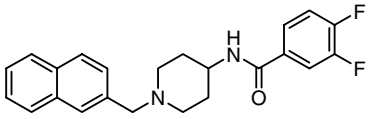
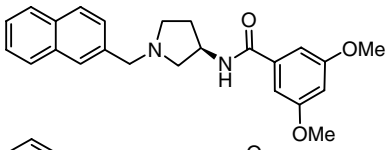
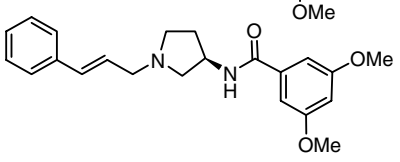
[a] The data represent means of at least two experiments, unless otherwise indicated.

was significantly more active (~70-fold) than the (*S*)-isomer **11a**. Therefore, SAR was focused in the (*R*)-series in this subclass. The mono-substituted 3-methoxybenzamide (**11b**) showed about 10-fold diminution in binding activity. In contrast to **5k** in the 4-aminopiperidine series, mixed substitution of 4-fluoro-3-methoxybenzamide in **11c** was not preferred in the 3-aminopyrrolidine series. In this case, the SAR trend did not correlate well when we duplicated the substitution patterns from the 4-aminopiperidine series to the 3-aminopyrrolidine series. Moreover, replacement of a cinnamyl group for the naphthalene in **11e** resulted in improved MCH activity consistent with that in the 4-aminopiperidine series. α-Methyl substitution in analogs **11d** and **11f** also retained the MCH in vitro activity. Again, a smaller aryl group in **11g** for the naphthalene showed a significant decrease of the binding affinity.

We used MCH-R1-induced Ca^{2+} release from CHO cells transfected with human MCH-R1 to measure the functional antagonism.[17] To confirm our binding affinity relative to functional antagonism at MCH-R1, a group of selected compounds are tabulated in Table 5. Most of compounds tested in our in vitro assays showed good correlation between binding activity and function potency. The discrepancy within a 3-fold range between the assays indicates the compounds bind to the MCH receptor as well as display functional antagonism.

In summary, our HTS finding led us to explore SAR in a 4-aminopiperidine scaffold, which resulted in the identification of a number of highly potent MCH antagonists. The combination of a 3,5-dimethoxybenzamide or a 3,4-difluorobenzamide with a 2-naphthylmethyl moiety proved to be the optimal substituents for MCH binding

Table 5. MCH-R1-binding and function data for 4-aminopiperidine and (3*R*)-aminopyrrolidine analogs[a]

Compound	Structure	MCH binding K_i (nM)	MCH function IC_{50} (nM)
1		125	109
5a		347	948
5c		27	62
5h		18	52
10i		7	18
11e		17	42

[a] The data represent means of at least two experiments, unless otherwise indicated.

activity. In addition, modification on the piperidine ring enabled us to discover the 3-aminopyrrolidine series, leading to further enhancement in the MCH in vitro activity (to nano-molar values). Further optimization and in vivo activity of these compounds will be reported in due course.

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14. Compounds were assayed for binding using an HEK-293 cell line that overexpresses the MCH-R1 (SLC-1). Competitive binding assays were performed using europium labeled MCH at a concentration of 25 nM. Varying concentrations of compounds were incubated with the MCH-R1 cells in the presence of 25 nM europium labeled MCH. Subsequently, cells were washed free of excess europium labeled MCH and residual bound MCH was quantified. Non-specific binding was determined by incubating cells with 10 μM unlabeled MCH.
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17. Functional MCH activity was assayed in the same HEK-293 cell line that overexpresses the MCH-R1 (SLC-1). The cells stably expressed a reporter construct containing the serum response element regulating the expression of the firefly luciferase gene. Functional MCH activity was detected by assaying luciferase activity. Prior to initiating the assay, cells were washed free of serum-containing media and incubated overnight in serum-free media. For functional antagonist assays, cells were incubated with varying concentrations of the test compound and 25 nM MCH for 4 h. Cells were then processed for luciferase activity as a measure of receptor activation.