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Synthesis and evaluation of 2-amino-8-alkoxy quinolines as MCHr1 antagonists. Part 1

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Abstract—A high-throughput screen was performed in order to identify chemotypes that are bound by the melanin concentrating hormone receptor-1 (MCHr1). A novel 2-amino-8-alkoxyquinoline compound (**1**) was identified and subsequently optimized using a parallel and automated procedure for the rapid production of multiple analogs. The structure–activity relationships that emerged from this effort are described, along with selected pharmacokinetic parameters of compound (**d**)-**61** when dosed orally in diet-induced obese mice.

Melanin-concentrating hormone (MCH) is a cyclic, 19-amino acid peptide that is synthesized in cell bodies in the lateral hypothalamus and zona incerta of the central nervous system (CNS). While it derives its name from its ability to promote aggregation of pigment-containing granules in the skin cells of fish,[1] the MCH-peptide is now understood to play a major role in body weight regulation in rodents.[2,3] A single injection of MCH into the CNS stimulates food intake in rats,[4] and chronic administration leads to increased body weight.[5] Similarly, transgenic mice altered to increase expression of the MCH gene are susceptible to insulin resistance and obesity.[6] In contrast, mice lacking the gene encoding MCH are hypophagic, lean, and maintain elevated metabolic rates.[7] Consistent with this phenotype, genetically altered animals that lack the gene encoding the MCH receptor maintain elevated metabolic rates and thus remain lean, even though they are hyperphagic on a normal diet.[8,9] The consistency of the beneficial phenotypes in animals lacking MCH or its single relevant receptor MCHr1 in rodents indicates that successful antagonism of this system could lead to weight loss. Further validation of the mechanism as a novel target for antiobesity pharmacotherapy comes from the observation that chronic administration of small-molecule antagonists leads to the reduction of food intake and body weight in animal models.[10,11] MCHr1 is widely recognized as an important target across the pharmaceutical industry, and several classes of small-molecule MCHr1 antagonists have recently been disclosed.[12,13]

As part of our effort to identify and assess the effects of MCHr1 antagonists, a high-throughput screen was performed from which several structurally diverse compounds with affinity for the MCHr1 receptor were identified. This report describes the generation of structure–activity relationships (SAR) of one of these hits via the use of pre-organized building blocks and automated chemical transformations aimed at rapid lead optimization.

The 2-amino-8-alkoxyquinoline **1** (Fig. 1) had an IC_{50} of 0.091 μM in an assay designed to measure the ability of test compounds to displace MCH from MCHr1 in IMR-32 cells.[14] Further characterization in an assay designed to measure functional antagonism of MCH-mediated Ca^{2+} release using a fluorometric imaging plate reader (FLIPR™)[15] showed a nearly 20-fold decrease in potency.[16] Despite this, the structural simplicity of this unique G-protein coupled receptor (GPCR) pharmacophore[17,18] prompted further investigation.

Keywords: Melanin concentrating hormone; Obesity.
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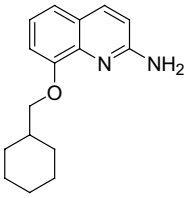


1 (A-224940)

MCHr1 IC_{50} = 0.091 μM

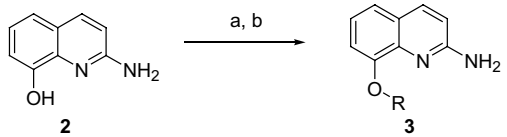
MCHr1 FLIPR IC_{50} = 1.68 μM

Figure 1. MCHr1 ligand.

Since the available SAR around this chemotype were limited and no structural information regarding the receptor was available, we utilized a technique routinely performed in our laboratory that employs a standardized automated chemical transformation performed with an optimized monomer set to generate a set of analogs and the associated SAR in a single experiment. A typical monomer set consists of a homologous series of linear, branched, cyclic, and fused cyclic building blocks that are compatible with the chosen transformation and automation platform. The set is designed to encompass typical medicinal chemistry experiments such as homologations and ring expansions while being filtered of reactive functionalities and high-molecular weight monomers. Following the structure of lead compound **1**, we chose an aliphatic set of alcohols and the corresponding automated Mitsunobu protocol[19] in order to produce a set of aryl–alkyl ethers containing substitution patterns typically employed to probe nonaromatic hydrophobic interactions.[20]

The set of aryl–alkyl ethers (**4**–**50**) was prepared starting from 2-amino-8-hydroxyquinoline (**2**) using a DBAD/polymer supported triphenylphosphine reagent system (Scheme 1). The products were separated from their corresponding reaction mixtures via sequestration onto PS-TsOH[21] resin and then purified by reverse-phase HPLC.

Screening this comprehensive set of analogs (Fig. 2) for their MCHr1 binding affinity provided invaluable SAR trends. Nearly all the products containing α-branched sidechains had sub-micromolar IC_{50} values with the notable exception of cyclobutyl and cyclopentyl ethers **5** and **7**, which were inactive. Interestingly, the presence of a 3-methyl substituent on the cyclopentane ring of compound **13** increased the potency relative to **7**. The potency-enhancing effect of α-branching is best exemplified by comparing analogs **6**, **8**, and **9** to the corresponding linear derivatives **24**, **26**, and **27**, respectively, which are roughly 10-fold less potent. Additionally, analogs



Scheme 1. Reagents and conditions: (a) ROH, DBAD, PS-PPh_3, THF; (b) PS-TsOH, DMA, then NH_3/MeOH.

with terminal branching (**19**, **44**, **47**) were generally more active than their corresponding non-branched derivatives. The trend is most clearly observed when comparing analogs **26**, **37**, and **44**, which increase in potency with the increasing number of terminal methyl groups. Analog **19**, which combines α-branching and terminal branching, has an IC_{50} value of 41 nM. In contrast, the presence of oxygen or sulfur generally confers a deleterious effect on potency. This is observed when comparing the branched and linear carbon-chain analogs **8** and **26** to their considerably less active oxygenated and thiolated analogs **11** and **39**, respectively, although the 3-tetrahydrofuran substituted **42** is just over 2-fold less active than its carbocyclic derivative **40**. Finally, all unsaturated analogs tested were inactive (IC_{50} > 2 μM) in the binding assay.

The 10 most potent compounds in the binding assay were evaluated for functional antagonism in the FLIPR™ assay (Fig. 2). The IC_{50} values were generally 5- to 20-fold lower than the corresponding binding values. Analogs **8**, **19**, and **20** showed the least dramatic decreases in functional antagonism, while the other α-branched analogs tested (**14**, **18**) dropped as much as 40-fold. Significant decreases in functional activity were also observed with non-α-branched compounds such as **40** and **47**, as well as oxygenated analog **42**. Compound **19**, which is 2-fold more potent than lead compound **1** in the binding assay but over 8-fold more potent in antagonism of MCH-mediated Ca^{2+} release, demonstrated the best combination of binding and functional potency.

We next explored the effect of substitution at the 2-amine position, and chose analog **19** as a focal point for exploration. To this end, compound **19** was reductively aminated with a diverse set of aldehydes to afford the corresponding secondary 2-aminoquinolines (Scheme 2). To access the tertiary amine products, the des-amino analog **53** was first prepared via Mitsunobu reaction with 8-hydroxyquinoline and 4,4-dimethyl-2-pentanol. *N*-oxidation with *m*-CPBA and rearrangement with refluxing $POCl_3$ to afford the 2-chloro intermediate was then followed by S_NAr displacement with various amines under microwave heating conditions to furnish the corresponding tertiary 2-aminoquinoline derivatives **54** (Scheme 3).

The des-amino analog **53** was inactive in both assays (Table 1). Secondary amines represented by **55** and **56** exhibited significantly reduced binding and functional potencies. Analog **57**, which was comparable to parent compound **19** in binding potency, was the lone exception. Tertiary amines exemplified by compounds **58**–**60** were also inactive. Other modifications to the 2-amino substituent, including acylation and urea formation, resulted in inactive compounds (data not shown).

Because of the deleterious effects of *N*-substitution, the unsubstituted 2-amino-8-alkoxyquinoline **19** was chosen for further investigation. In order to probe for preferential receptor binding of one of the enantiomers of **19**, the racemate was separated into two enantiomers (**d**)-**61** and (**l**)-**61** via chiral HPLC chromatography.[23] Interestingly,

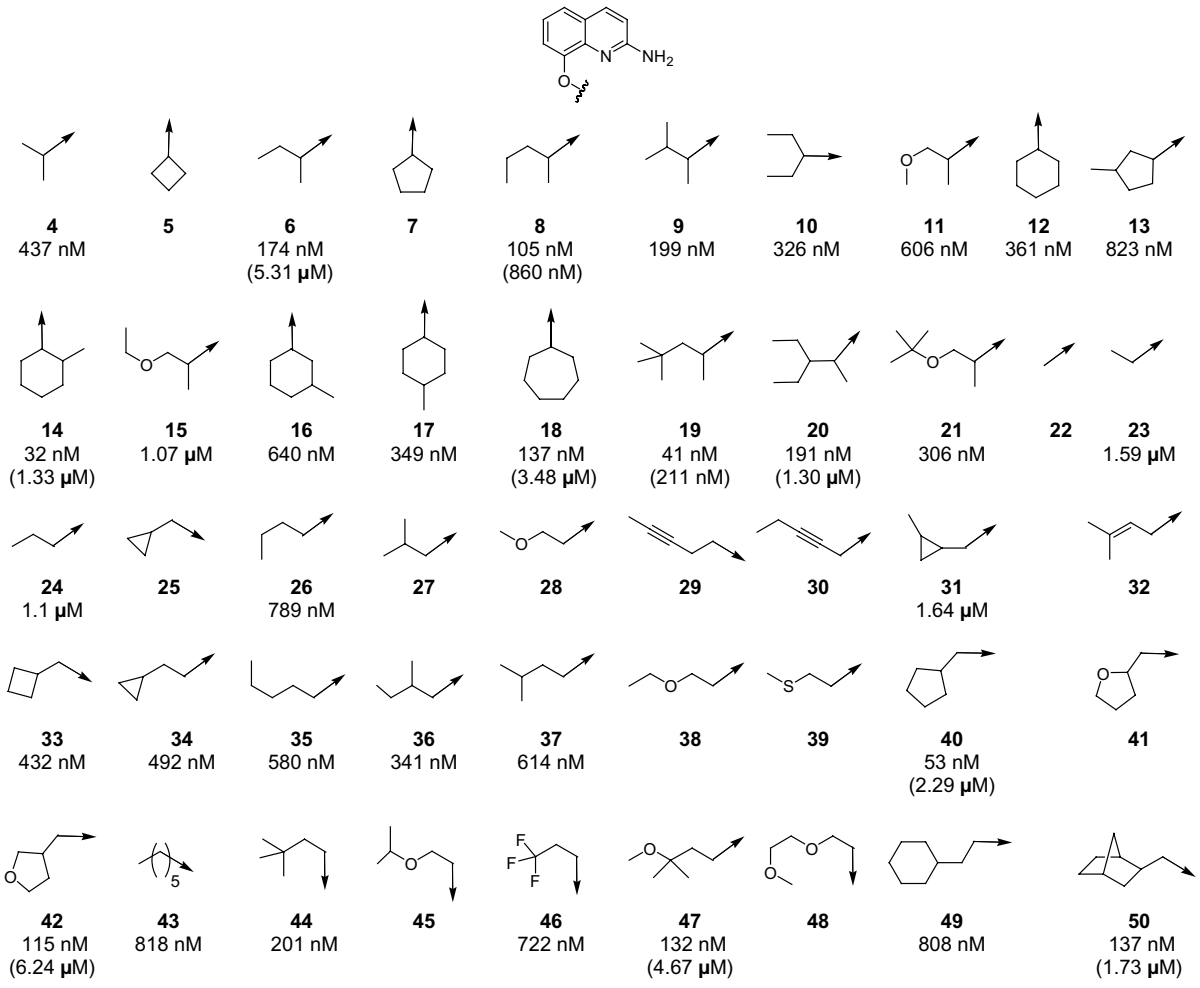
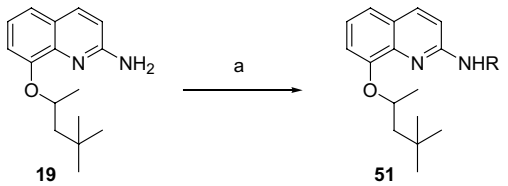


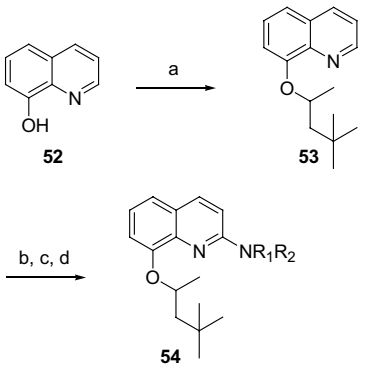
Figure 2. MCHr1 binding and functional potency (in parentheses) of a set of aryl–alkyl ethers. (Values are means of three experiments.) MCHr1 binding IC_{50} is greater than 2 μM if value not shown.



Scheme 2. Reagents and conditions: (a) RCHO, 1:1 DCE/MeOH (1% AcOH), MP-$CNBH_3$,[22] 55 °C.

MCHr1 preferentially binds enantiomer **(d)-61** (Table 2).

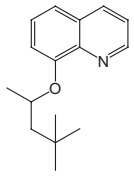
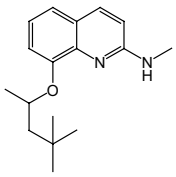
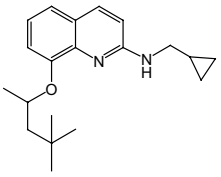
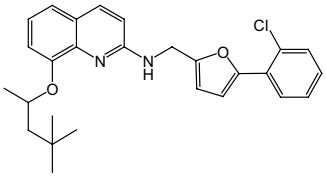
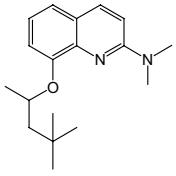
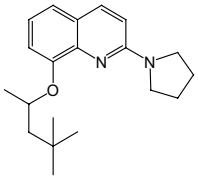
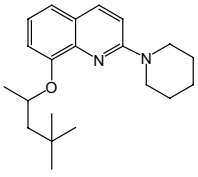
Since the largest concentration of MCHr1 is located in the lateral hypothalamus, it is likely that potential drug candidates acting through this receptor will be required to penetrate the blood–brain barrier. In order to assess brain penetration and plasma exposure of **(d)-61**, this compound was dosed orally at 10 mg/kg in diet-induced obese (DIO) mice.[24] As shown in Table 3, the ratio of brain to plasma exposure of **(d)-61** is greater than 4, indicating a favorable partitioning of this compound into the CNS.



Scheme 3. Reagents and conditions: (a) 4,4-dimethyl-2-pentanol, DBAD, PS-PPh_3, THF; (b) *m*-CPBA, CH_2Cl_2; (c) $POCl_3$, reflux; (d) R_1R_2NH, NMP, 220 °C.

In summary, preliminary optimization of a series of 2-aminoquinoline-based MCHr1 antagonists is described. The use of pre-organized building blocks with a standardized automated platform allowed for the rapid synthesis of multiple alkoxy ethers and the generation of valuable SAR in a single experiment. This culminated

Table 1. Binding and functional potency of MCHr1 antagonists

Entry	Compound	IMR32 binding IC_{50}[a] (μM)	IMR32 FLIPR™ IC_{50}[a] (μM)
53		>2.00	>10.0
55		0.318	6.09
56		>2.00	>10.0
57		0.069	0.536
58		>2.00	>10.0
59		>2.00	>10.0
60		>2.00	>10.0

[a] Values are means of three experiments.

Table 2. Binding and functional potency of the enantiomers of **19** (absolute stereochemistry undetermined)

Enantiomer	IMR32 binding IC_{50}[a] (μM)	IMR32 FLIPR™ IC_{50}[a] (μM)
(d)-61	0.02	0.098
(l)-61	0.55	4.51

[a] Values are means of three experiments.

in the identification of potent compound **(d)-61**, which antagonizes MCHr1 binding and MCH-mediated Ca^{2+} release with IC_{50} values of 20 and 98 nM, respectively.

Table 3. Selected PK parameters of **(d)-61** in DIO mice (10 mg/kg po)

	Cmax (ng/mL or ng/g)	$T_{1/2}$ (h)	AUC (ng · h/ml or ng · h/g)
Plasma	127	1.9	247
Brain	595	2.3	1026

In addition, **(d)-61** shows efficient CNS penetration when dosed orally at 10 mg/kg in DIO mice, and represents a novel, low molecular weight, MCHr1 antagonist.

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14. MCHR1 binding assay using IMR-32 membrane preparations: Inhibition of binding of MCH to MCHr1 was determined using cell membranes prepared from I3.4.2 cells. In 96-well plates, I3.4.2 membranes (6 μg/well) were incubated in the presence of test compound in binding buffer (25 mM HEPES pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.5% BSA) and with 0.05 nM ^{125}I-MCH (NEN; 2200 Ci/mmol) per well for 60 min at rt. Nonspecific binding controls consisted of I3.4.2 membranes, 0.05 nM human ^{125}I-MCH, and 300 nM human MCH. Total

binding controls of I3.4.2 membranes and 0.05 nM ^{125}I-MCH were also included on each plate. The plates were centrifuged for 5 min at 1380*g* in a Beckman GS-6R desktop centrifuge. The reaction buffer was carefully aspirated from each well without disturbing the pellet. Wash buffer (25 mM HEPES pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.5 M NaCl) was added to each well and then the pellets were transferred to a 0.5% polyethylenimine treated GF/B filtration plate (Packard) using a plate Filtermate Harvester (Packard). The filter plate was washed three times with wash buffer, Microscint 20 (Packard) was added to each well, and the plate was read using a Topcount microplate scintillation counter (Packard).

15. Assay for release of intracellular calcium in IMR-32 cells: Activation of MCHR by MCH induces the release of Ca^{2+} from intracellular stores, which is measured using a fluorometric imaging plate reader (FLIPR™, Molecular Devices Corp.) in conjunction with the Ca^{2+}-sensitive dye reagent (Calcium Assay Reagent, Molecular Devices). IMR-32 I3.4.2 cells were plated at 100,000 cells/well in 96-well plates. After two days, cells were loaded with the Calcium Assay Reagent for 1 h at rt. Test compounds were prepared at 60 μM in 6% DMSO. The cell plate was placed in the FLIPR™, 50 μL/well of test compound was delivered, and the calcium signal was followed for 3 min to assess potential agonist activity. Then 50 μL/well of 6 μM human MCH (in D-PBS containing 0.1% BSA) was added and the ligand-induced calcium signal was followed for an additional 3 min. Antagonist activity is reported as concentration of test compound required to inhibit 50% of MCH-induced Ca^{2+} flux.
16. Discrepancies between the binding and functional activities of MCHr1 antagonists have been observed for some compounds. While the conditions used for the binding and functional assays are significantly different, the actual cause of the observed discrepancy is unknown and currently under investigation.
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22. Macroporous cyanoborohydride (MP-$CNBH_3$) was purchased from Argonaut Technologies. www.argotech.com.
23. HPLC column and conditions: Chiralcell OJ™ column, Hex/EtOH/MeOH = 50/25/25, flow rate = 0.8 mL/min, column temp. = 25 °C.
24. Generation of diet-induced obese (DIO) mice: Male C57Bl/6J mice were placed on a 60% kcal lard diet for 3–4 months, during which time they became obese (45 g vs 30 g for lean controls on normal chow). Compounds were dosed in DIO-mice orally @ 10 mpk in a vehicle containing 1% Tween-80 and water. Whole brains and plasma were harvested at 0.5, 1, 2, 4, 6, 8, 12, and 24 h after dosing, and drug concentrations were determined by mass spectroscopy analysis in comparison with a standard curve.