Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 832–835
Bioorganic & Medicinal Chemistry Letters

MCH-R1 antagonists based on an arginine scaffold: SAR studies on the amino-terminus

José Méndez-Andino,* Anny-Odile Colson, Daniel Denton, Maria C. Mitchell, Doreen Cross-Doersen and X. Eric Hu†

Procter & Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, OH 45039, USA

Received 11 September 2006; revised 13 October 2006; accepted 23 October 2006
Available online 25 October 2006

Abstract—We have identified a novel series of potent MCH-R1 antagonists based on L-arginine. As predicted by computational methods, there was an activity dependence on the π-electronic character of the aromatic systems corresponding to the amino-terminus of these molecules. These results have enhanced our understanding of the MCH-R1 receptor and the potential for a predictive homology model.

Estimates suggest that over 30% of the US adult population is overweight and that obesity-related health care cost is in the range of $100 billion per year.[1] Obesity has become a major health concern worldwide since many countries are also experiencing increases in obese population. These trends have led to an increased number of drug discovery programs directed to find anti-obesity therapies.[2,3]

The G-protein-coupled melanin-concentrating hormone receptor 1 (MCH-R1) has received significant attention in recent years as a potential target for effective anti-obesity therapies.[3] It has been suggested that CNS-located MCH-R1 is involved in biological processes related to mammal feeding behaviors and energy expenditure.[4] Small molecule MCH-R1 antagonists are being heavily pursued by many laboratories trying to find an effective drug-molecule for the treatment of obesity.[5]

Aided by molecular modeling studies of various truncated analogs of the natural ligand peptide for human MCH-R1, we identified arginine-based peptidomimetics **1** and **2** as potent MCH-R1 antagonists in the early stages of our research efforts.[6] Subsequently, SAR studies focused on variations at the amino-terminus, carboxy terminus, and the guanidine group were performed to identify the key pharmacophore contact points leading to MCH-R1 antagonist activity. The work presented herein focuses on the optimization of the amino-terminus of these arginine-based MCH-R1 antagonists (Fig. 1).[7]

Optimization of the amino-terminus requires an understanding of its role in MCH-R1 binding and function.[7]

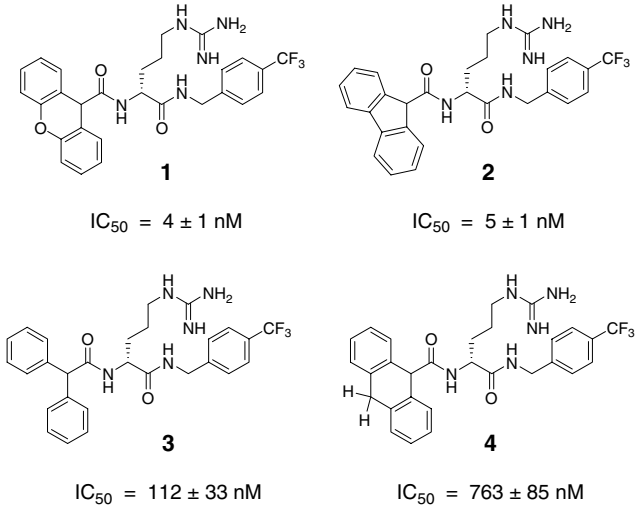


Figure 1. MCH-R1 antagonists based on an arginine scaffold.

Keywords: Melanin-concentrating hormone; MCH-R1 antagonists; Obesity; Cation–π interactions.
* Corresponding author. Tel.: +1 513 622 0304; fax: +1 513 622 1433; e-mail: mendezandino.jl@pg.com
† Present address: Genome Research Institute, University of Cincinnati, 2180 E. Galbraith Road, Cincinnati, OH 45237, USA.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.056

Simple structural changes around this region, as exemplified in compounds **3** and **4**, led to a significant loss in biological activity. We initially hypothesized that the interaction was hydrophobic in nature, but our results did not support that assumption and demanded an alternative explanation.

To help generate new hypotheses, further docking experiments were performed in our homology model of the MCH-R1 receptor. The model of the receptor was built using the known human MCH-R1 receptor amino acid sequence (SWISS_PROT Primary Accession No. Q96S47) mapped onto the crystal structure of rhodopsin.[8] Details of the construction are presented elsewhere.[6b,9] The model was subsequently refined using inhouse and published site-directed mutagenesis studies,[10] as well as SAR data generated in our laboratories. Individual ligands were then docked manually and energy minimized (SYBYL Version 6.7. Tripos: St. Louis, MO, USA) within the putative binding site of the receptor located between transmembrane helices (TMH) 3, 5, 6, and 7. At the bottom of this site lies Asp 123 (TMH3) which has been shown to be essential for cationic agonist and antagonist binding at homologous GPCRs.[11] For the antagonist–receptor complex, the charge-reinforced H-bond between the protonated nitrogen atom of the ligand and the carboxyl of Asp 123 was a required interaction for docked poses to be considered further. Upon docking of the arginine-based compound **1** (Fig. 2), our model suggests that two arginine residues, Arg197 (extra-cellular loop 2) and Arg284 (TMH6), are key components of the binding site where the aromatic groups putatively interact.

It has been suggested in the literature that the guanidine unit of an arginine can engage in strong cation–π interactions with electron-rich aromatic functionalities in aqueous environments.[12] It is worth pointing out that our MCH-R1 model predicts that this interaction occurs on the extra-cellular side of the receptor. This information, along with careful analysis of our SAR data, led us to hypothesize that the delocalized π-electron-rich xanthene and fluorene groups in **1** and **2** were key for MCH-R1 antagonist activity and that they were participating in cation–π interaction with arginine residues of the receptor. Based on our model, the cation–π interaction only occurs over one of the phenyl rings of the symmetric xanthene unit while the additional phenyl ring is only necessary to achieve the required spatial arrangement and electronic properties of the aromatic ring system.

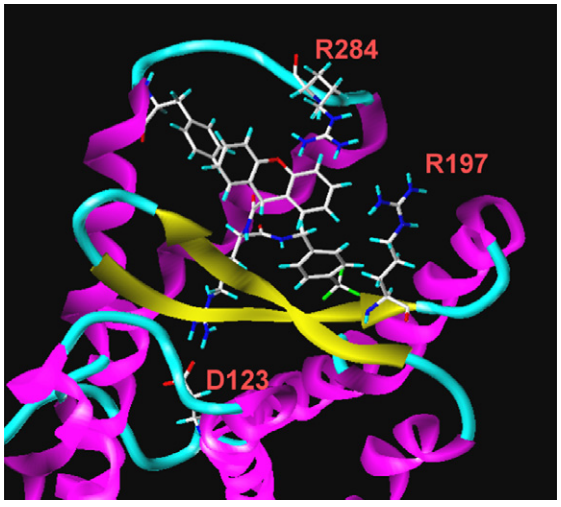


Figure 2. Bound conformation of compound **1** in the h-MCH-R1 model.

In order to test this hypothesis, we focused our efforts on finding replacements for the xanthene and fluorene groups based on cation–π interaction theory. Furthermore, we aimed at producing effective MCH-R1 antagonists with lower molecular weight and/or solubility properties suitable for in vivo dosing. We therefore envisioned that a heterocyclic electron-rich naphthalene analog could mimic the electronic properties of the xanthene and fluorene rings while also reducing the molecular weight and potentially improving aqueous solubility.

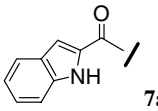
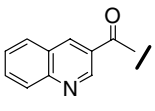
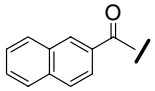
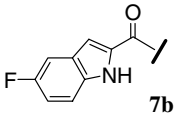
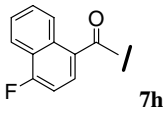
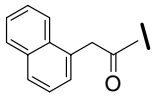
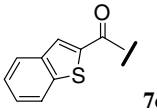
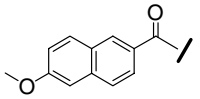
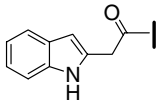
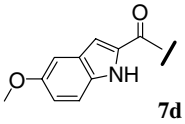
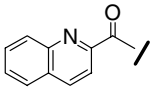
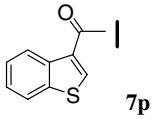
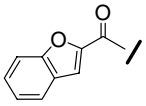
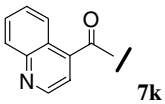
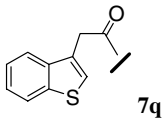
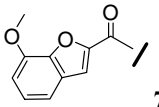
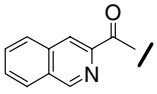
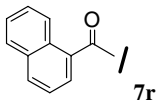
The magnitude of the cation–π interaction depends on distance, orientation, and is directly proportional to the delocalization of π electrons (aromaticity).[13] The order of aromaticity for heteroatomic naphthalene analogs on the basis of the electronegativity of heteroatoms is as follows: benzothiophene > indole > benzofuran.[14] However, it is well known that indoles display great cation–π-stacking capabilities in combination with the ability of the NH bond to engage in additional H-bonding interactions.[13c,d]

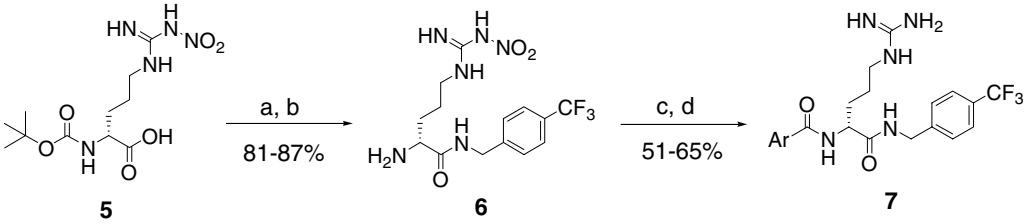
In order to understand, the activity dependence on the π-electronic character of the aromatic systems, we identified a series of π-excessive and π-deficient naphthalene analogs. Isoquinolines and quinolines are a common example of π-deficient naphthalene analogs, while benzofurans, benzothiophenes, and indoles represent π-aexcessive naphthalene analogs.[14] Spatial arrangements based on substitution patterns were part of our survey as well. Based on these criteria, a series of compounds containing naphthalene-analog units were prepared (Table 1).

The compounds needed for our study were easily accessible by a four-step synthetic route from readily available starting materials (Scheme 1).[15] The synthesis initiated with the coupling of the L-arginine derivative **5** with 4-trifluoromethylbenzylamine followed by cleavage of the *t*-butylcarbamate protecting group. The resulting primary amine was coupled to the corresponding naphthoic acid analog followed by the deprotection of the guanidine functionality to yield the desired compounds **7**.

As predicted, the π electron-excessive series produced the more potent compounds, whereas, the π-deficient series primarily led to less potent antagonists (Table 1).[16] The indole analog **7a** was one of the most active compounds. It was suggested by our model (Fig. 3) to form π–cation interactions with both arginines in addition to forming a

Table 1. SAR studies:[16] examples of naphthalene analogs and electronic properties

π Electron-excessive systems		π Electron-deficient systems		Substitution patterns	
Structure	IC_{50} (nM)	Structure	IC_{50} (nM)	Structure	IC_{50} (nM)
 **7a**	20 ± 10	 **7g**	96 ± 8	 **7m**	212 ± 76
 **7b**	25 ± 17	 **7h**	311 ± 75	 **7n**	243 ± 30
 **7c**	45 ± 4	 **7i**	443 ± 118	 **7o**	512 ± 104
 **7d**	78 ± 6	 **7j**	904 ± 52	 **7p**	952 ± 80
 **7e**	313 ± 184	 **7k**	1034 ± 330	 **7q**	1008 ± 189
 **7f**	622 ± 344	 **7l**	1814 ± 387	 **7r**	1797 ± 594



Scheme 1. Reagents and conditions: (a) 4-trifluoromethylbenzylamine, EDCI, HOBT, NMM, DMF; (b) TFA/CH_2Cl_2 (1:1); (c) ArCOOH, EDCI, HOBT, NMM, DMF; (d) 5% Pd/$BaSO_4$, H_2, AcOH/MeOH (1:4).

hydrophobic interaction with F289. It is worth noting that arginines are known to participate in π–cation interaction in two limiting geometries: T-shaped and parallel.[13c] Both of these geometries are illustrated with the docked poses of compounds **1** and **7a** (Fig. 3). The indole compound **7a** not only offers a good biological activity, but it also has a lower molecular weight than the benchmarks **2** and **3**. Furthermore, its significantly improved aqueous solubility (433 μg/mL) over both prior analogs (7–14 μg/mL) made it readily formulable for subsequent in vivo studies.

It is interesting to note that upon docking of compound **7r**, the naphthyl ring positioned itself off-the-plane of the limiting geometries to form optimal π–cation interactions with the arginines, hence suggesting poor mimics of either system **1** or **7a**. The addition of a methylene linker (as in **7n)** satisfactorily positioned the ring system to mimic compound **1**. Synthesis and subsequent testing of these compounds showed a 10-fold increase in functional activity for **7n** compared to **7r**. Although there was no improvement over compound **1**, the correct prediction of the relative functional activity of these two compounds infers a certain degree of reliability of our model.

We report here the design, modeling, and synthesis of novel, effective MCH-R1 antagonists based on an arginine scaffold. In these systems, the indole unit (**7a**) proved to be an effective replacement for the xanthene and fluorene groups displaying similar biological activity, but improved aqueous solubility over **1** and **2**. This work, which was led by π–cation interaction theory aided by docking experiments within a putative receptor binding model, suggests that rational design employing in silico approaches contributes to efficient optimization

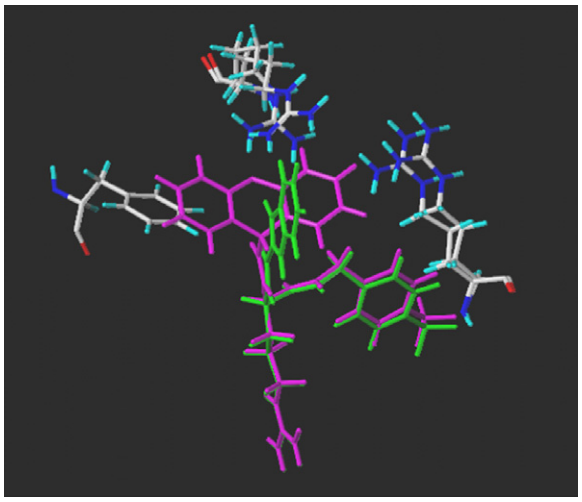


Figure 3. Bound conformation of **1** (magenta) and **7a** (green) in the MCH-R1 model.

of in vitro active series. Further optimization will be pursued, including the addition of new contact residues within the pharmacophore, the modeling of explicit solvent on the extra-cellular side of the receptor and the exploration of alternative binding orientation.

Acknowledgments

The authors extend their gratitude to Dr. Frank H. Ebetino and Dr. John A. Wos for the invaluable technical discussions and assistance.

References and notes

1. *The Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity 2001, Public Health Service*: Office of the Surgeon General, US Department of Health and Human Sevices: Rockville, MD, 2001; www.surgeongeneral.gov/topics/obesity.
2. (a) Powell, D. R. *Obesity Rev.* **2006**, *7*, 89; (b) Fong, T. M. *Expert Opin. Invest. Drugs* **2005**, *14*, 243.
3. Dyke, H. J.; Ray, N. C. *Expert Opin. Ther. Patents* **2005**, *15*, 1303.
4. (a) Kowalski, T. J.; McBriar, M. D. *Expert Opin. Invest. Drugs* **2004**, *13*, 1113; (b) Receveur, J. M.; Bjurling, E.; Ulven, T.; Little, P. B.; Norregaard, P. K.; Hogberg, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5075.
5. (a) Kanuma, K.; Omodera, K.; Nishiguchi, M.; Funakoshi, T.; Chaki, S.; Semple, G.; Tran, T.-A.; Kramer, B.; Hsu, D.; Casper, M.; Thomsen, B.; Sekiguchi, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3853; (b) Ulven, T.; Frimurer, T. M.; Receveur, J.-M.; Little, P. B.; Rist, Ø.; Nørregaard, P. K.; Högberg, T. *J. Med. Chem.* **2005**, *48*, 5684.
6. (a) Chen, Z.; Kende, A.; Colson, A.-O.; Mendezandino, J.; Ebetino, F.; Bush, R.; Hu, X. *Synth. Commun.* **2006**, *36*, 473; (b) Mieling, G. E.; Mieling, K. K.; Bush, R. D.; Colson, A.-O. US 2005170433 A1, 2005.
7. For examples of π-rings in molecular recognition, see: Sulpizi, M.; Carloni, P. *J. Phys. Chem. B* **2000**, *104*, 10087, and references therein.
8. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. *Science* **2000**, *289*, 739.
9. Colson, A.-O.; Perlman, J. H.; Smolyar, A.; Gershengorn, M. C.; Osman, R. *Biophys. J.* **1998**, *74*, 1087.
10. Macdonald, D.; Murgolo, N.; Zhang, R.; Durkin, J. P.; Yao, X.; Strader, C. D.; Graziano, M. P. *Mol. Pharmacol.* **2000**, *58*, 217.
11. (a) Gether, U. *Endocr. Rev.* **2000**, *21*, 90; (b) Cavalli, A.; Fanelli, F.; Taddei, C.; De Benedetti, P. G.; Cotecchia, S. *FEBS Lett.* **1996**, *399*, 9.
12. (a) Tsou, L. K.; Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 14917; (b) Flocco, M. M.; Mowbray, Sl. *J. Mol. Biol.* **1994**, *235*, 709.
13. (a) Gallivan, J. P.; Dougherty, D. A. *J. Am. Chem. Soc.* **2000**, *122*, 870; (b) Minoux, H.; Chipot, C. *J. Am. Chem. Soc.* **1999**, *121*, 10366; (c) Gallivan, J. P.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9459; (d) Dougherty, D. A. *Science* **1996**, *271*, 163.
14. Gupta, R. R.; Kumar, M.; Gupta, V. *Heterocyclic Chemistry I*; Spinger-Verlag: Germany, 1998.
15. (a) For compound **6** see Ryan, J. W.; Chung, A. *Eur. Pat. Appl.*, 78703, 11 May **1983**; (b) **7a**–**r**: a mixture of the corresponding nitro-guanidine compound (0.25 mmol) and 5% Pd/$BaSO_4$ (15 mg) in 20% AcOH/MeOH (3 mL) was stirred for 8–12 h under H_2 atmosphere. The mixture diluted with MeOH (5 mL) and filtered through a syringe-filter (0.45 μm). The filtrates purified by preparative HPLC (Polaris C18-A 10μ, 250 × 500 R, 1% TFA-water/acetonitrile as eluent) to yield the desired products as TFA salts.
16. MCH functional antagonist was detected using HEK 293 cells that expressed the MCH-R1 (SLC-1) receptor in a firefly luciferase reporter assay. Cells were incubated for 4 h in the presence of 25 nM MCH and varying concentrations of drug of interest. Receptor activation was measured by luminescence.