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Novel pyrazolopiperazinone- and pyrrolopiperazinone-based MCH-R1 antagonists

Kenneth M. Meyers,[†] José Méndez-Andino,[*] Anny-Odile Colson, X. Eric Hu,[‡] John A. Wos, Maria C. Mitchell, Karen Hodge, Jeremy Howard, Jennifer L. Paris, Martin E. Dowty, Cindy M. Obringer and Ofer Reizes[§]

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

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Abstract—The synthesis and biological testing of novel classes of potent melanin-concentrating hormone (MCH-R1) antagonists based on pyrazolopiperazinone and pyrrolopiperazinone scaffolds are described.

The World Health Organization considers obesity a major health concern for many countries, especially the United States. Estimates indicate that more than 30% of the US adult population is overweight, and that obesity-related health care costs are in the range of $100 billion per year.[1] These trends have promoted increased research activity in the pharmaceutical industry directed to find anti-obesity therapies.[2]

The G-protein-coupled receptor MCH-R1 has received significant attention in recent years as a potential target for effective anti-obesity therapy.[3] It has been suggested that CNS-located MCH-R1 is involved in biological processes related to mammalian feeding behaviors and energy expenditure.[4] Identification of a small molecule MCH-R1 antagonist is being heavily pursued by many laboratories trying to find an effective drug-molecule that may be effective for the treatment of obesity.[5]

Recently, we have disclosed phenethyl ketopiperazine compound **1** as a potent MCH-R1 antagonist in vitro ($K_i = 11 \pm 7$ nM).[6] But despite its high in vitro MCH antagonist activity, the compound did not promote any weight loss in a mouse Diet Induced Obesity study (mDIO). We hypothesized that compound **1** was not active in vivo likely due to insufficient brain exposure. In addition, we hypothesized that the hydrophilic nature of **1** (water solubility >45 mg/mL at pH 7.4) could have contributed to its inability to effectively cross the blood–brain barrier. This led us to consider making compounds with increased lipophilicity to address potential

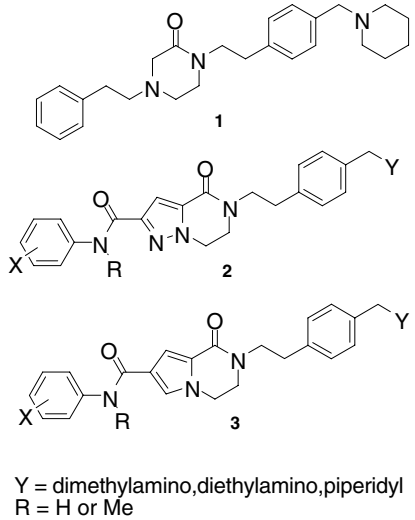

Figure 1. MCH-R1 antagonist **1**, and alternatives based on pyrazolopiperazinone **2** and pyrrolopiperazinone **3** scaffolds.

Keywords: Melanin-concentrating hormone; MCH-R1 antagonists; 5HT-2C receptor; Obesity; Pyrazolopiperazinone; Pyrrolopiperazinone.
* Corresponding author. Tel.: +1 513 622 0304; fax: +1 513 622 1433; e-mail: mendezandino.jl@pg.com
† Present address: Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Road, Ridgefield, CT 06877, USA.
‡ Present address: Genome Research Institute, University of Cincinnati, 2180 E. Galbraith Road, Cincinnati, OH 45237, USA.
§ Present address: Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue-NC10, Cleveland, OH 44195, USA.

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blood–brain barrier penetration issues associated with compound **1**.

We have identified pyrazolopiperazinone **2** and pyrrolopiperazinone **3** scaffolds as alternatives to **1** (Fig. 1). These scaffolds were derived from **1** by fusing a pyrazole or pyrrole ring with the ketopiperazine moiety. As a result, **2** and **3** should have decreased solubility compared to **1** due to increased lipophilic character and decreased pKa of the ketopiperazine nitrogen. We predicted that scaffold **2** would lead to potent MCH-R1 binders because key interactions observed between the dimethylamine analog of **1** and the MCH-R1 homology model[7] were conserved with **2** (Fig. 2).

Pyrazolopiperazinone analogs **2a**–**g** were synthesized according to Scheme 1. Coupling of 4-(2-Boc-aminoethyl)benzoic acid **4** and secondary amines with EDCI and HOBT gave amide compounds **5**. Reduction of the amide with $BH_3 \cdot THF$ followed by Boc-deprotection with trifluoroacetic acid gave diamine compounds **6**. Condensation with **7**[8] under basic conditions gave pyrazolopiperazinone compounds **8**. Ester saponification followed by amide formation yielded final compounds **2a**–**g**.

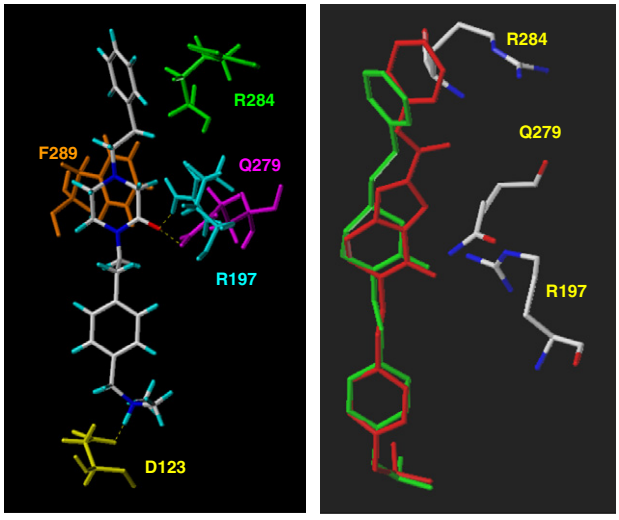
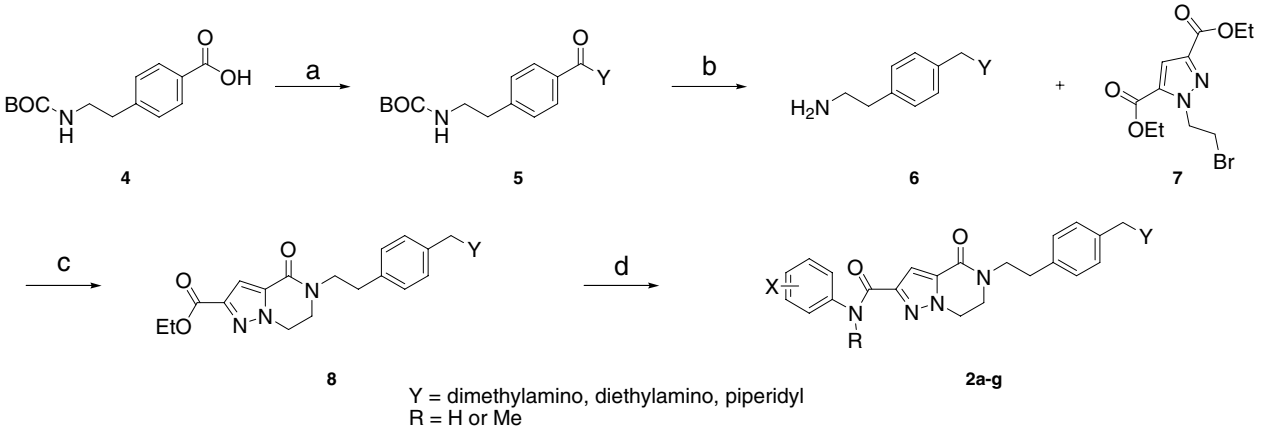


Figure 2. Key interactions observed between the dimethylamine analog of **1** and MCH-R1 homology model (left). Analog of **1** (green) and **2c** (red) in MCH-R1 homology model (right).

In vitro MCH-R1 and $5HT_{2C}$ binding data for compounds **2a**–**g** are summarized in Table 1.[9,10] We desired selectivity over $5HT_{2C}$ due to the relatively high homology of the receptor with MCH-R1 and its suggested role in the modulation of feeding behavior that could interfere with effects related to inhibition of MCH-R1. Simple variations at the tertiary alkyl amine position did not significantly affect the MCH-R1 activity. These results were consistent with our findings in the earlier ketopiperazine (**1**) series.[6] Fluorine substituted aniline compounds **2a**–**c** were more potent MCH-R1 binders than trifluoromethyl substituted aniline compounds **2d**–**e**. Interestingly, in both the fluoro-aniline and trifluoromethyl-aniline analog pairs, the meta-substituted compound displayed higher in vitro activity than the corresponding para-substituted analog. The substitution of a methyl group for hydrogen at the amide position decreased MCH in vitro activity for reasons unclear to us.

Compounds **2a**–**b** and **2f**–**g** were selective for MCH-R1 over $5HT_{2C}$ (Table 1). Compound **2b** displayed good MCH-R1 activity, high selectivity over $5HT_{2C}$ and decreased water solubility (26.1 mg/mL at pH 7.4) compared to **1**. However, this compound was not efficacious in our 4-day mDIO weight loss model likely due to limited blood–brain barrier penetration. This hypothesis was supported by Caco-2 cell assay[11] data identifying **2b** as an efflux substrate ($A - B/B - A = 4.1/21$).[12]

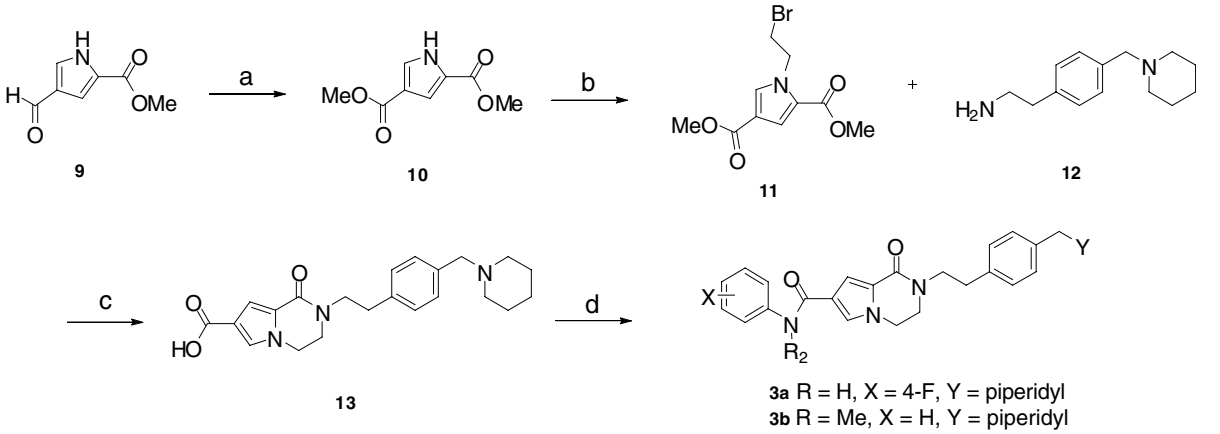
The analogous pyrrolopiperazinones were prepared according to Scheme 2. Aldehyde **9**[13] was oxidized to the acid with $KMnO_4$ and converted to methyl ester **10**. Alkylation with 1,2-dibromoethane gave pyrrole compound **11**. Unlike the pyrazolopiperazinone synthe-



Scheme 1. Reagents and conditions: (a) secondary amine, EDCI, HOBT, NMM, DMF, rt, 81–89%; (b) i—$BH_3 \cdot THF$, 65 °C, 63–81%; ii—trifluoroacetic acid, CH_2Cl_2, rt, 91–99%; (c) K_2CO_3, CH_3CN, 80 °C, 68–82%; (d) i—LiOH, THF, H_2O, rt, 90–98%; ii—corresponding aniline, PyBop, *N*,*N*-diisopropylethylamine, DMF, rt, 58–76%.

Table 1. MCH-R1 in vitro activity for compounds **2a**–**f**[9,10]

Compound	R	X	Y	MCH-R1 Binding K_i (nM)[10]	$5HT_{2C}$ K_i (μM)
2a	H	3-F	Piperidyl	22	>100
2b	H	4-F	Diethylamino	78	>100
2c	H	4-F	Dimethylamino	82	—
2d	H	3-CF_3	Diethylamino	497	—
2e	H	4-CF_3	Diethylamino	2482	—
2f	Me	4-F	Piperidyl	5294	>100
2g	Me	H	Piperidyl	NA	>100



Scheme 2. Reagents and conditions: (a) i—$KMnO_4$, acetone, H_2O, rt, 88%; ii—TMSCl, MeOH, rt, 94%; (b) 1,2-dibromoethane, NaH, DMF, 65 °C, 70%; (c) NaH, CH_3CN, 80 °C; then H_2O, 37–46%; (d) aniline, PyBop, *N*,*N*-diisopropylethylamine, DMF, rt, 61–73%.

sis, condensation of **11** and **12** required the stronger base sodium hydride. Upon aqueous workup, acid **13** was isolated. Amide formation with PyBoP yielded the final compounds **3a**–**b**.

Compound **3a** had a MCH K_i of 620 nM and **3b** was inactive in our in vitro binding assay. Similar to the pyrazolopiperazinone series, *N*-methyl amide **3b** had decreased activity compared to secondary amide **3a**. Comparison across scaffolds revealed that substitution of the pyrazole moiety by a pyrrole resulted in decreased activity, therefore suggesting that the second nitrogen of the pyrazole moiety interacts favorably with the receptor.

In an attempt to improve the in vitro efficacy of the pyrrolopiperazinone series, we examined the role of the amide linker between the pyrrole and the halogenated aromatic ring. Based on structure **1** and its high potency, we hypothesized that the amide linker was not essential for biological activity. Modifications to the amide linker were performed in order to test this hypothesis (Fig. 3).

The synthesis of **14** and **15** is outlined in Scheme 3. Friedel–Crafts acylation of ethyl-pyrrole-2-carboxylate **18** with 4-fluorobenzoyl chloride followed by N-alkylation with 1,2-dibromoethane gave **19**. Condensation with **12** yielded ketone linked **14**. Ketone reduction with triethylsilane in trifluoroacetic acid[14] gave methylene linked variant **15**.

The synthesis of **16** is outlined in Scheme 4. Wittig olefination of **9** with 4-fluorobenzyl triphenylphosphonium chloride **20** gave a mixture of alkene isomers that was reduced with hydrogen in the presence of Pd/C to yield **21**. Alkylation of the pyrrole nitrogen with 1,2-dibromoethane followed by condensation with **12** in the presence of sodium hydride gave **16**.

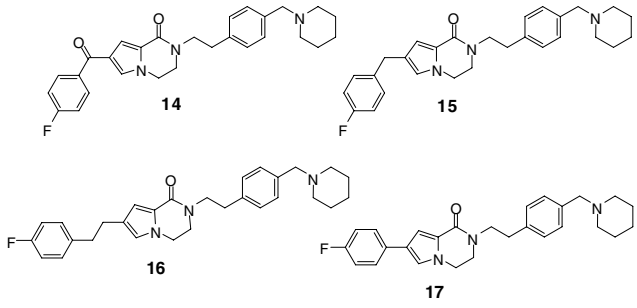
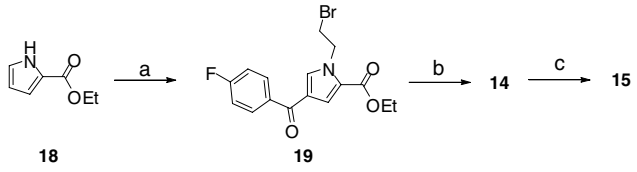
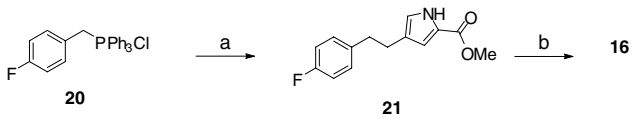


Figure 3. Amide linker replacements in the pyrrolopiperazinone series.



Scheme 3. Reagents and conditions: (a) i—4-fluorobenzoyl chloride, $AlCl_3$, 1,2-dichloroethane −20 °C, 71%; ii—1,2-dibromoethane, NaH, DMF, 65 °C, 58%; (b) **12**, NaH, CH_3CN, 80 °C, 51%; (c) triethylsilane, trifluoroacetic acid, rt, 82%.

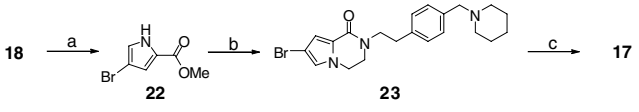


Scheme 4. Reagents and conditions: (a) i—KHMDS, THF, 0 °C; **9**, rt; ii—H_2, Pd/C, MeOH, rt, 73% two steps; (b) i—1,2-dibromoethane, NaH, DMF, 65 °C, 67%; ii—**12**, NaH, CH_3CN, 80 °C, 41–47%.

The synthesis of **17** is outlined in Scheme 5. Bromination of ethyl-pyrrole-2-carboxylate **18** gave a mixture of 4-bromo, 5-bromo, and 4,5-dibromo products, which was easily separable employing reverse-phase preparative HLPC. 4-Bromo product **22** was N-alkylated with 1,2-dibromoethane and condensed with **12** in the presence of sodium hydride to give compound **23**. Suzuki coupling of **23** and 4-fluorophenyl boronic acid yielded final compound **17**.

The MCH-R1 in vitro activity of **3a**–**b** and **14**–**17** is shown in Table 2. Through linker modifications in the pyrrolopiperazinone series, MCH-R1 binding activity comparable to the pyrazolopiperazinones was achieved. This suggests that the detrimental effect of switching the pyrazole to pyrrole may be circumvented by modifications to the linker between the 4-fluorophenyl and pyrrolopiperazinone rings. Reduction of ketone **14** to methylene **15** did not significantly affect MCH activity. Chain-length changes resulted in the most potent MCH binders of the pyrrolopiperazinone series (**16** and **17**). These results suggest that the overall orientation plays a more significant role for binding than the polar character of the linker. In addition, these results are in agreement with our hypothesis that the amide of **3a** is not required for high-affinity binding with MCH-R1. However, removal of the amide functionality between the pyrrolopiperazinone and 4-fluorophenyl rings resulted in complete loss of MCH-R1 selectivity over $5HT_{2C}$ (compounds **14**–**17**).

In summary, we have identified pyrazolopiperazinone and pyrrolopiperazinone scaffolds as potent MCH-R1 antagonists in vitro. Despite an attractive in vitro profile, the lead pyrazolopiperazinone compound **2b** was not active in vivo likely due to limited blood–brain barrier penetration. Substitution of the pyrazole moiety with pyrrole led to decreased MCH-R1 activity. Through modification of the amide linker between the 4-fluorophenyl and the pyrrole rings in the pyrrolopiperazinone series, potency similar to that of pyrazolopiperazinones was achieved. Unfortunately, these changes led to significant losses in MCH-R1 selectivity over $5HT_{2C}$.



Scheme 5. Reagents and conditions: (a) i—Br_2, CCl_4, 0 °C, 53%; (b) i—1,2-dibromoethane, NaH, DMF, 65 °C, 71%; ii—**12**, NaH, CH_3CN, 80 °C, 48–53%; (c) 4-fluorophenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, DMF, 100 °C, 80–83%.

Table 2. MCH-R1 in vitro activity for compounds **3a**–**b** and**14**–**17**

Compound	MCH-R1 K_i (nM)	$5HT_{2C}$ K_i (μM)
3a	625	>100
3b	NA	—
14	148	5.3
15	228	0.7
16	15	2.5
17	23	0.1

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9. MCH binding using a Flashplate radioligand binding assay was performed by Perkin-Elmer Biosignal (Toronto, Ontario). Full competition curves were generated with compound concentration varied from 100 μM to 11 fM. Potency (K_i) and maximal efficacy were determined and used to define structure–activity relationship.
10. $5HT_{2C}$ assay was performed using a membrane preparation from cells that over-express the receptor. Membranes were purchased from Euroscreen (ES-318-M) and used according to established protocols. Briefly, membranes were incubated in a reaction mixture containing varying concentrations of the compound and [^{3}H] mesulergine (final concentration 0.33 nM; Amersham), a compound known to bind to the $5HT_{2C}$ receptor. The binding buffer contained 50 mM Tris–HCl, 0.1% Ascorbic Acid, 5 mM $CaCl_2$, and 10 μg/ml Saponin. Nonspecific binding was assessed by incubating the membranes with [^{3}H] mesulergine and excess 10 μM mianserin (ICN). After incubating the mixture at room temperature for 60 min, bound mesulergine was separated from unbound mesulergine by filtration through glass fiber B filter (GF/B) plates (Millipore). Bound radioactive mesulergine was detected using a scintillation counter.
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12. (a) Within our MCH program, we observed that compounds predicted to transport passively across the blood–brain barrier in the Caco-2 assay usually promoted statistically significant weight loss in our 4-day mDIO model, and displayed [brain]/[serum] > 2. Compounds predicted efflux substrates did not show in vivo activity and displayed [brain]/[serum] < 1; (b) Please refer to: Meyers, K. M.; Méndez-Andino, J. L.; Colson, A. O.; Warshakoon, N. C.; Wos, J. A.; Mitchell, M. C.; Hodge, K. M.; Howard, J. M.; Ackley, D. C; Holbert, J. K.; Mittelstadt, S. W.; Dowty, M. E.; Obringer, C. M.; Ofer Reizes, O.; Hu, X. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, in press.
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