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Discovery of novel spiro-piperidine derivatives as highly potent and selective melanin-concentrating hormone 1 receptor antagonists

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ABSTRACT

Optimization of high-throughput screening hit **1a** led to the identification of a novel spiro-piperidine class of melanin-concentrating hormone 1 receptor (MCH-1R) antagonists. Compound **3c** was identified as a highly potent and selective MCH-1R antagonist, which has an IC₅₀ value of 0.09 nM at hMCH-1R. The synthesis and structure–activity relationships of the novel spiro-piperidine MCH-1R antagonists are described.

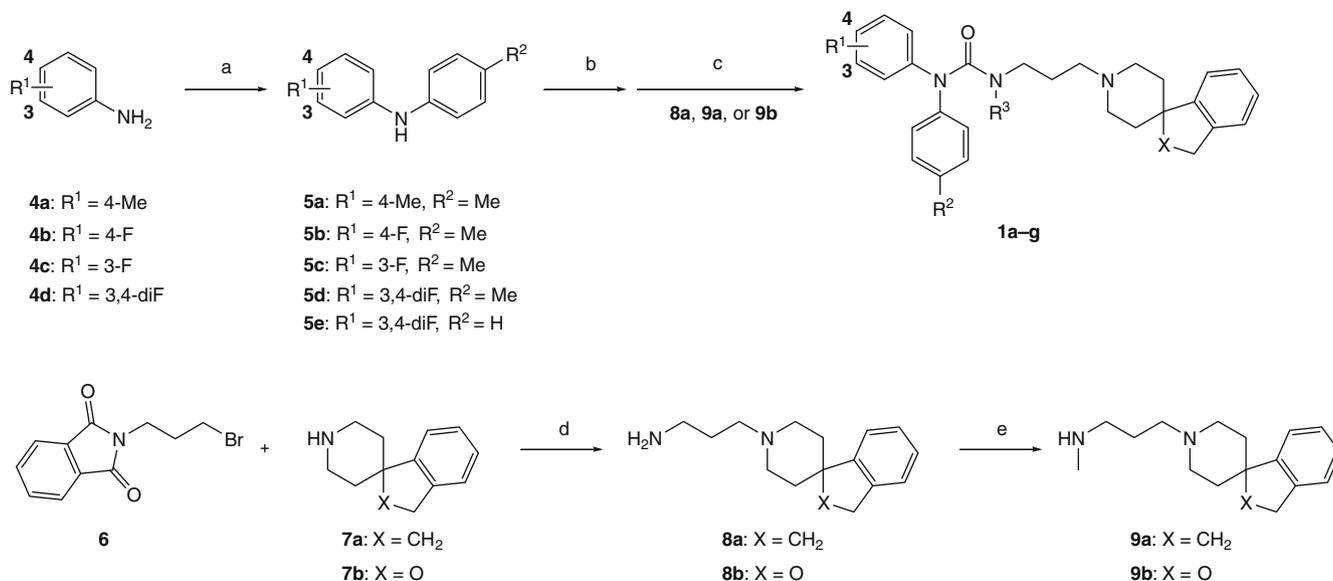
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Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid polypeptide that is expressed predominantly in the lateral hypothalamus (LH). The LH is a region of the brain involved in the regulation of feeding, the neuroendocrine axis, and thermogenesis. Several lines of investigation suggest that MCH is an important mediator of energy homeostasis. Mice lacking prepro-MCH are lean, hypophagic, and have an elevated metabolic rate.¹ Conversely, prepro-MCH overexpression in mice results in a greater susceptibility to obesity.² Furthermore, overexpression of MCH mRNA has been found in obese rodents, such as ob/ob, db/db, and Ay/a mice.^{3–5} Exogenous administration of MCH stimulates food intake,^{3,6} and chronic ICV infusion of the MCH^{7,8} or a related MCH-1R agonist⁹ produces obesity with hyperphagia. Even when pair-feeding is employed to prevent hyperphagia, ICV infusion of MCH still produces anabolic changes.¹⁰ The effects of MCH are mediated through G protein-coupled receptors located in the CNS, and thus far two receptor subtypes, MCH-1R and MCH-2R, have been identified.^{11–14} Since rodents possess only MCH-1R, all pharmacological effects of MCH in rodents are likely mediated via MCH-1R.¹⁵ Recently, peptidic and non-peptidic MCH-1R antagonists have been developed; both antagonists produced anti-obese effects in diet-induced obese rats.^{9,16,17} Collectively, these data indicate that MCH-1R is an important regulator of energy homeo-

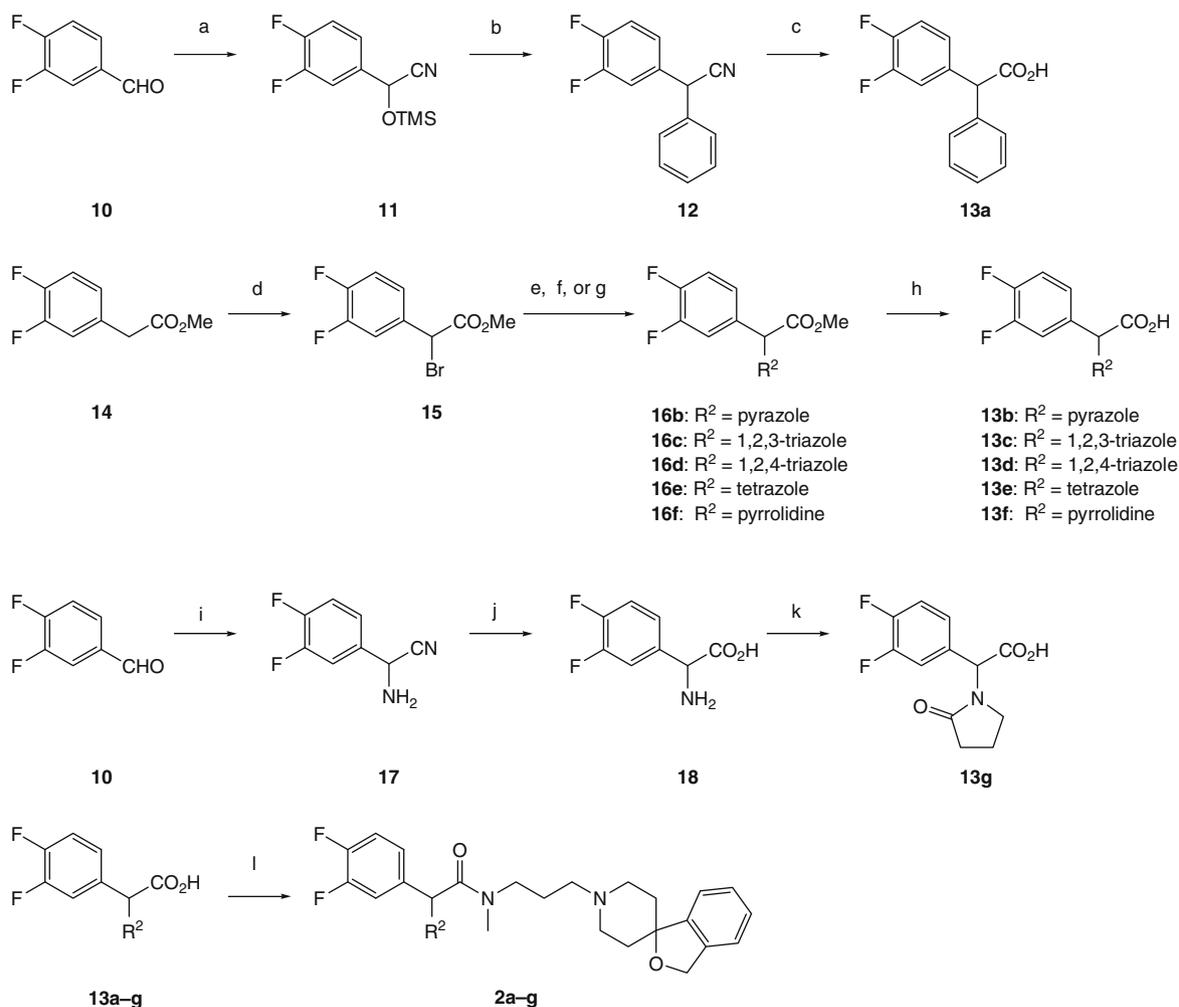
stasis, and suggest that it may play an important role in the development of obesity. Hence, MCH antagonists could be effective therapeutic agents for the treatment of obesity. Screening of Merck sample collections against human MCH-1R (hMCH-1R) resulted in the identification of a spiro-piperidine class of lead **1a**, which has an IC₅₀ value of 42 nM. Subsequent optimization efforts were centered on improvement of the MCH-1R activity and reduction of human α_{1A} -adrenoceptor (h α_{1A}) activity to identify the potent and selective derivative **3c**. The synthesis and structure–activity relationships (SAR) of this novel spiro-piperidine class of MCH-1R antagonists are described.

The synthesis of compounds described herein is outlined in Schemes 1–3. Compounds **1a–g** were prepared as shown in Scheme 1. Substituted anilines **4** were coupled with 4-methylphenylboronic acid or phenylboronic acid in the presence of copper acetate (II) and triethylamine to give diaryl amines **5a–e**, which were reacted with triphosgene followed by amines **8a**, **9a**, or **9b** to give compounds **1a–g**. Amines **8a**, **9a**, and **9b** were derived from *N*-(3-bromopropyl)-phthalimide **6** using standard procedures as depicted in Scheme 1.^{18,19} Compounds **2a–g** were synthesized as outlined in Scheme 2. 3,4-Difluorobenzaldehyde (**10**) was converted to the corresponding cyanohydrin trimethylsilyl ether **11**. The trimethylsilyloxy group of **11** was displaced by a phenyl group followed by hydrolysis of the cyano group to give **13a**. Ester **14** was brominated to give **15**. The bromo group of **15** was displaced by the desired azoles and pyrrolidine followed by hydrolysis of the ester

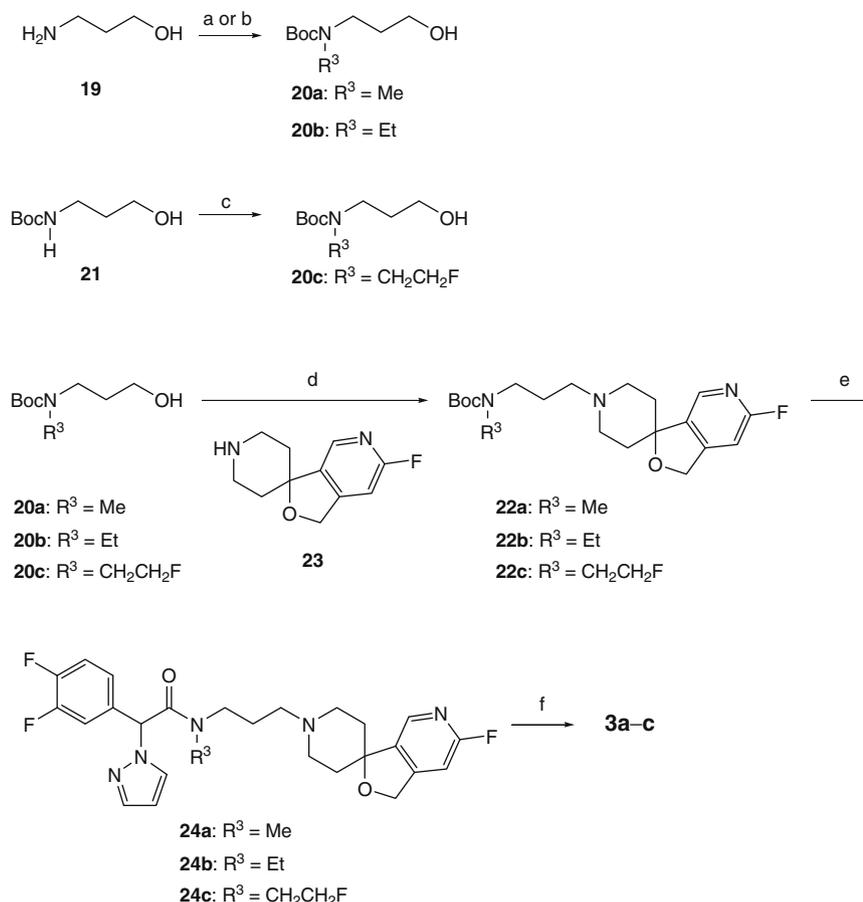
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Scheme 1. Preparation of urea derivatives **1a–g**. Reagents and conditions: (a) Cu(OAc)₂, Et₃N, 4-methylphenylboronic acid or phenylboronic acid, CH₂Cl₂, rt, 17 h, 48–90%; (b) triphosgene, Et₃N, CHCl₃, 0 °C → rt, 2–17 h, 62–99%; (c) Et₃N, ClCH₂CH₂Cl, 60–100 °C, 2–3 h, 50–85%; (d) (i) K₂CO₃, KI, DMF, 80 °C, 17 h, 46–95%, (ii) H₂NNH₂·H₂O, EtOH, reflux, 3 h, 99%; (e) (i) ClCO₂Et, Et₃N, CHCl₃, rt, 17 h, 99%, (ii) LiAlH₄, 1,4-dioxane, reflux, 17 h, 98–99%.



Scheme 2. Synthesis of compounds **2a–g**. Reagents and conditions: (a) TMSCN, ZnCl₂, CH₂Cl₂, rt, 4 h, 93%; (b) benzene, conc. H₂SO₄, 0 °C, 1.5 h, 15%; (c) conc. H₂SO₄, H₂O, 100 °C, 17 h, 66%; (d) NBS, HBr, CCl₄, reflux, 17 h, 98%; (e) pyrazole, *n*-BuLi, cat. 2,2'-bipyridyl, THF, –30 °C → rt, 20 h, 60%; (f) 1,2,3-triazole or 1,2,4-triazole, NaH, THF, 0 °C → rt, 17 h, 34–46%; (g) tetrazole or pyrrolidine, K₂CO₃, DMF–THF, rt, 3 h, 40–78%; (h) 4 N NaOH aq, MeOH, rt → 50 °C, 2–17 h, 60–97%; (i) NH₃ aq, MeOH, 0 °C, 3 h, and then TMSCN, rt, 17 h, 94% over two steps; (j) 40% H₂SO₄ aq, 90 °C, 17 h, 99%; (k) (i) 4-chlorobutanoyl chloride, Et₃N, 1,4-dioxane–H₂O, 0 °C, 30 min, 60%, (ii) *tert*-BuOK, THF, 0 °C, 2 h, 85%; (l) amine **9b**, EDCI·HCl, HOBT·H₂O, NaHCO₃, DMF, rt, 17 h, 17–98%.



Scheme 3. Preparation of spiro-fluoropyridine derivatives **3a–c**. Reagents and conditions: (a) (i) HCO_2Et , THF, rt, 17 h, (ii) LiAlH_4 , $0 \rightarrow 60^\circ\text{C}$, 3 h, (iii) Boc_2O , 1 N NaOH aq, THF, rt, 2 h, 67% over three steps; (b) (i) Ac_2O , THF, rt, 17 h, (ii) LiAlH_4 , $0 \rightarrow 70^\circ\text{C}$, 2 h, (iii) Boc_2O , 1 N NaOH aq, THF, rt, 2 h, 85% over three steps; (c) (i) TBDMSCl, imidazole, DMF, rt, 2.5 h, 99%, (ii) 2-fluoroethyltosylate, NaH, DMF, rt, 60 h, 67%, (iii) TBAF, THF, $0^\circ\text{C} \rightarrow \text{rt}$, 1 h, 92%; (d) (i) MsCl, *N,N*-diisopropylethylamine, EtOAc, 0°C , 30 min, 99%, (ii) **23**, K_2CO_3 , KI, DMF, 80°C , 17 h, 43–81%; (e) (i) 4 N HCl–EtOAc, 0°C , 30 min, 99%, (ii) **13b**, EDCl–HCl, HOBT– H_2O , NaHCO_3 , DMF, rt, 17 h, 28–76%; (f) chiral resolution by HPLC (CHIRALCEL OD, hexanes/EtOH = 8/2).

group to afford carboxylic acids **13b–f**. Treatment of aldehyde **10** with aqueous ammonia followed by trimethylsilylcarbonitrile furnished **17**, which was subjected to acidic conditions for hydrolysis of the nitrile group to give carboxylic acid **18**. The amino group of **18** was coupled with 4-chlorobutanoyl chloride followed by treatment with potassium *tert*-butoxide to effect intramolecular cyclization to afford **13g**. The α -substituted phenyl acetic acids **13a–g** were coupled with amine **9b** to give the target compounds **2a–g**. The preparation of compounds **3a–c** is outlined in Scheme 3. The 3-alkylaminopropanols **20a–c** protected by a *tert*-butoxycarbonyl (Boc) group were prepared by standard procedures as shown in Scheme 3.²⁰ The hydroxyl group of **20a–c** were mesylated and displaced by the piperidine analogue **23**²¹ to give **22a–c**. Com-

pounds **24a–c** were obtained by deprotection of the Boc group of **22a–c** followed by coupling of the resulting amino group with the carboxylic acid **13b**. The racemates **24a**, **24b**, and **24c** were resolved by HPLC to give the corresponding active enantiomers **3a**, **3b**, and **3c**, respectively.²²

High-throughput screening of Merck sample collections against hMCH-1R led to the identification of **1a**, which has an IC_{50} value of 42 nM. Compound **1a** was found to have moderate activity for $\text{h}\alpha 1\text{A}$ ($\text{IC}_{50} = 540$ nM) (Fig. 1). Modification of **1a** was initiated to increase MCH-1R activity and reduce $\text{h}\alpha 1\text{A}$ activity (Table 1). The 4- and 3-fluorophenyl derivatives **1b** and **1c** were equipotent to **1a**. The 3,4-difluorophenyl derivative **1d** was found to be more potent than **1a**. The potency was further improved by *N*-methylation as in **1e** ($\text{IC}_{50} = 2.1$ nM), a 20-fold improvement over **1a**. The introduction of an oxygen atom to the spiro-indane portion, as in **1f**, resulted in reduced $\text{h}\alpha 1\text{A}$ activity while retaining MCH-1R activity. Removal of a 4-methyl group on the distal *N*-phenyl group (**1g**) was found to be detrimental for MCH-1R activity ($\text{IC}_{50} = 19$ nM). Conversion of the urea linkage of **1g** to the amide led to the identification of the potent amide derivative **2a**, which shows an IC_{50} value of 1.0 nM at hMCH-1R (Table 2). The R^2 substituent of **2a** was further explored. The pyrazole and triazole derivatives **2b–d** displayed good activities for hMCH-1R and decreased $\text{h}\alpha 1\text{A}$ activities. The tetrazole derivative **2e** exhibited decreased activity. The pyrrolidine derivative **2f** showed a significant loss of potency; however, the corresponding oxo-derivative **2g** was surprisingly potent, with an IC_{50} value of 2.7 nM at hMCH-1R. Permeability-glyco-

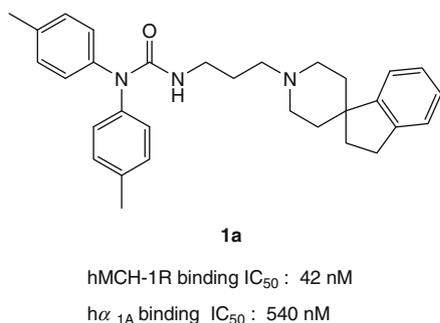
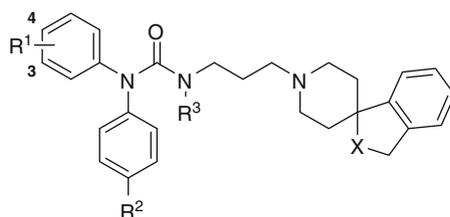


Figure 1. Structure of the lead compound **1a**.

Table 1
Human MCH-1R and α_{1A} binding activity of urea derivatives **1a–g**^a



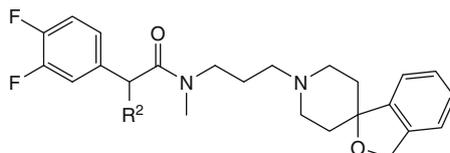
Compound	R ¹	R ²	R ³	X	hMCH-1R ^b (IC ₅₀ , nM)	h α _{1A} ^c (IC ₅₀ , nM)
1a	4-Methyl	Me	H	CH ₂	42	540
1b	4-Fluoro	Me	H	CH ₂	54	340
1c	3-Fluoro	Me	H	CH ₂	42	490
1d	3,4-Difluoro	Me	H	CH ₂	9.6	350
1e	3,4-Difluoro	Me	Me	CH ₂	2.1	230
1f	3,4-Difluoro	Me	Me	O	2.1	470
1g	3,4-Difluoro	H	Me	O	19	920

^a The values are the means of two experiments.

^b Inhibition of [¹²⁵I]MCH binding to hMCH-1R in CHO cells.

^c Inhibition of [³H]prazosin binding to human α_{1A} -adrenoceptor in LMtk⁻ cells.

Table 2
Human MCH-1R and α_{1A} binding activity and P-gp susceptibility of compounds **2a–g**^a



Compound	R ²	hMCH-1R ^{b,c} (IC ₅₀ , nM)	h α _{1A} ^{b,d} (IC ₅₀ , nM)	P-gp Susceptibility ^{e,f} transcellular transport ratio (B-to-A)/(A-to-B)	
				MDR1	mdr1a
2a		1.0	600	1.4	3.7
2b		1.7	3600	1.8	4.9
2c		1.9	1920	4.1	4.9
2d		2.9	1910	9.2	3.4
2e		9.9	1910	NT	NT
2f		125	1160	NT	NT
2g		2.7	1000	24.8	32.9

^a Compounds **2a–g** were tested as racemates.

^b The values are the means of two experiments.

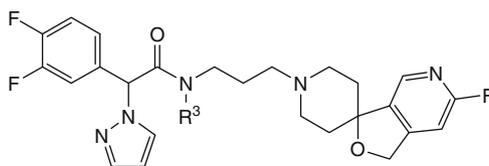
^c Inhibition of [¹²⁵I]MCH binding to hMCH-1R in CHO cells.

^d Inhibition of [³H]prazosin binding to human α_{1A} -adrenoceptor in LMtk⁻ cells.

^e Transcellular transport ratio ((B-to-A)/(A-to-B)) in human MDR1- and mouse mdr1a-transfected LLC-PK1 cell line.

^f NT, not tested.

Table 3
Human MCH-1R and α_{1A} binding activity and P-gp susceptibility of compounds **24a** and **3a–c**



Compound	R ³	hMCH-1R ^{a,b} (IC ₅₀ , nM)	h α 1A ^{a,c} (IC ₅₀ , nM)	P-gp Susceptibility ^d transcellular transport ratio (B-to-A)/(A-to-B)	
				MDR1	mdr1a
24a (racemate)		1.3	>10,000	3.3	6.8
3a (active isomer of 24a) ^e		0.46	>10,000	5.5	16.9
3b (active isomer of 24b) ^e		0.15	>10,000	2.2	1.5
3c (active isomer of 24c) ^e		0.09	>10,000	2.7	3.7

^a The values are the means of two experiments.

^b Inhibition of [¹²⁵I]MCH binding to hMCH-1R in CHO.

^c Inhibition of [³H]prazosin binding to human α_{1A} -adrenoceptor in LMtk⁻ cells.

^d Transcellular transport ratio ((B-to-A)/(A-to-B)) in human *MDR1*- and mouse *mdr1a*-transfected LLC-PK1 cell line.

^e Single isomer. The absolute configuration was not determined.

protein (P-gp) susceptibility of potent derivatives was evaluated by transcellular transport ratios obtained from human *MDR1*- and mouse *mdr1a*-transfected porcine renal epithelial (LLC-PK1) cell monolayers.²³ P-gp is expressed in the blood-brain barrier and excludes its substrates from the brain. In this P-gp transport assay, a compound with a B-to-A/A-to-B ratio above 3 is considered to be a P-gp substrate. All test compounds were human and mouse P-gp substrates except for **2a** and **2b** in human P-gp; compounds **2a** and **2b** were found not to be a human P-gp substrate (Table 2). In addition, **2b** showed the best selectivity against h α 1A among the derivatives **2a–g**.

We subsequently realized that the replacement of the spiroisobenzofurane structure of **2b** with the spiro-fluorofuropyridine structure as in **24a** is very effective to reduce the h α 1A activity (Table 3) while retaining MCH-1R activity. The racemate **24a** was resolved, and the active isomer **3a**²⁴ was found to have a remarkably potent MCH-1R activity (IC₅₀ = 0.46 nM). Further potency enhancement was achieved by modification of the *N*-alkyl group (R³). The ethyl derivative **3b** (active isomer)²⁴ displayed an IC₅₀ value of 0.15 nM. Compound **3b** was further substituted with fluorine as in **3c**,^{24,25} which has an IC₅₀ value of 0.09 nM at the MCH-1R. This highly potent compound **3c** showed good selectivity over MCH-2R (IC₅₀ > 1 μ M) and a panel of 171 diverse unrelated binding sites (IC₅₀ > 1 μ M for all the binding sites tested) and not a human P-gp substrate.

In summary, the highly potent and selective MCH-1R antagonist **3c** was identified by structural optimization of high-throughput screening hit **1a**. Evaluation of **3c** to assess its potential for clinical development is ongoing. Compound **3c** is also an attractive compound as a PET tracer due to its excellent potency, hydrophilicity (log *D*_{7.4} = 2.3), and possible labeling with ¹⁸F.

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22. Compounds **24a–c** were resolved by CHIRALCEL OD, eluting with hexanes/EtOH = 8/2. Compound **3a** was obtained as the second-eluted enantiomer by the HPLC resolution of **24a**. Compounds **3b** and **3c** were obtained as the first-eluted enantiomers by the HPLC resolution of **24b** and **24c**, respectively.
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24. The IC₅₀ values for the corresponding enantiomers of **3a**, **3b** and **3c** are 9.1, 1.1, and 22 nM, respectively.
25. Data of **3c**: ¹H NMR (400 MHz, CDCl₃) δ 1.65–2.12 (m, 6H), 2.34–2.52 (m, 4H), 2.72–2.97 (m, 2H), 3.32–3.42 (m, 1H), 3.45–3.67 (m, 2H), 3.70–3.93 (m, 1H), 4.46–4.78 (m, 2H), 5.04 (s, 2H), 6.30 (t, 3/4H, J = 2.0 Hz), 6.32 (t, 1/4H, J = 2.0 Hz), 6.56 (s, 1/4H), 6.77 (s, 1H), 6.90 (s, 3/4H), 7.02–7.12 (m, 1H), 7.13–7.24 (m, 2H), 7.45 (d, 3/4H, J = 2.4 Hz), 7.50 (d, 1/4H, J = 2.4 Hz), 7.54 (d, 3/4H, J = 2.0 Hz), 7.56 (d, 1/4H, J = 2.0 Hz), 7.96 (s, 1/4H), 7.98 (s, 3/4H); MS (ESI) m/z 532.3 [M+H]⁺; HPLC purity (99.6%).