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Optimization of 2-piperidin-4-yl-acetamides as melanin-concentrating hormone receptor 1 (MCH-R1) antagonists: Designing out hERG inhibition

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ABSTRACT

Herein, we disclose the discovery and optimization of 2-piperidin-4-yl-acetamide derivatives as MCH-R1 antagonists. Structural investigation of piperidin-4-yl-amide and piperidin-4-yl-ureas identified 2-piperidin-4-yl-acetamide-based MCH-R1 antagonists with outstanding in vivo efficacy but flawed with high affinity towards the hERG potassium channel. While existing hERG SAR information was employed to discover highly potent MCH-R1 antagonists with minimized hERG inhibition, additional hurdles prevented their subsequent clinical exploration.

Obesity, once regarded as a cosmetic consideration, is now progressively developing into a global pandemic affecting the lives of about 1.1 billion people worldwide and exerting an increasing financial burden on health systems and patients.[1] Pharmacological intervention would therefore be highly advantageous to address the needs of an ever-increasing patient population. Consequently, pharmaceutical companies have been very proactive in the obesity arena. Here, melanin-concentrating hormone receptor 1 (MCH-R1) antagonists have recently attracted widespread attention as a potentially viable treatment.[2–6] A significant amount of experimental results in rodent models supports such an hypothesis. For instance, the MCH system was shown to be critically involved in the fine regulation of food intake and in balancing energy homeostasis in mice.[7–10] Additionally, a number of MCH-R1 antagonists with antiobesity effects in animals have been presented and recently reviewed.[11]

We previously identified piperidin-4-yl-amides as potent MCH-R1 antagonists[12] and reported their subsequent optimization to piperidin-4-yl-ureas in the accompanying Letter (Fig. 1).[13] Unfortunately, both scaffolds demonstrated inhibition of the hERG potassium channel which is involved in the ventricular repolarization of the heart. Several cardiovascular liabilities have been associated with hERG blockade: these include death, proarrhythmia and QT interval prolongation.[14–16] Building on the hERG–MCH-R1 SAR reported in the preceding Letter,[13] we focused on enhancing MCH-R1–hERG selectivity in the present series.

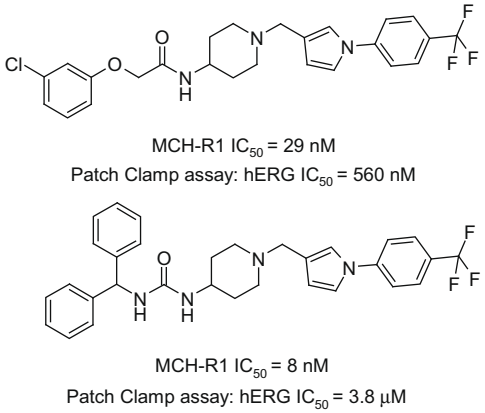

Figure 1. Piperidin-4-yl urea and piperidin-4-yl amide containing MCH-R1 antagonists.

When dealing with the optimization of off-target activities, one normal approach is to set a selectivity goal based on in vitro poten-

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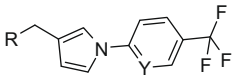
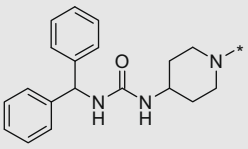
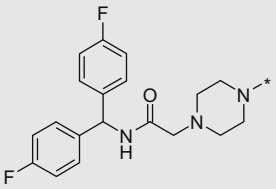
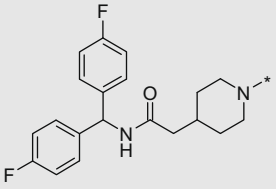
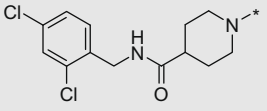
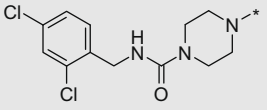
cies. As an example, the ratio between hERG and MCH-R1 IC_{50}s would, ideally, describe the margin between toxic (hERG induced arrhythmia) and pharmacological (MCH-R1 mediated obesity reduction) effect. Instead of setting an arbitrary value for such a parameter based on predicted free human concentrations required for a potential anti-obesity effect, we worked towards complete removal of hERG inhibition in the series, as a more prudent risk-mitigation strategy, and our findings are herein presented.

hERG inhibitors containing a positively ionized moiety are thought to bind to the potassium channel through a cation–π interaction involving one of the tyrosine residues lining in the channel pore.[17,18] Following this hypothesis, reducing the pK_a of the cation group was reported as a common and effective strategy to affect hERG inhibition.[19,20] Scaffold exploration was therefore aimed at replacing the piperidine core with less basic piperazine alternatives, as detailed in Table 1. The synthetic pathway leading to **1**–**5** has been reported elsewhere.[21]

Table 1
In vitro MCH-R1 and hERG binding data for **1**–**5**



No.	R	Y	pK_a[a]	MCH-R1 IC_{50}[b,c] (μM)	hERG IC50[b] (μM)
1		C	9.2	0.039	3.8[d]
2		N	6.5	0.055	0.8[d]
3		N	9.1	0.013 0.017[f]	0.8[d]
4		N	8.3	0.444	3.5[e]
5		C	6.2	0.008	1.6[e]

[a] pK_a measured using pressure-assisted capillary electrophoresis.[22]
[b] Values are mean of at least two experiments. Experimental errors within 15% of value.
[c] Compounds competed with ^{125}I-MCH for binding at the human MCH1 receptor (h-MCH-R1) expressed in the HEK293 cell line.
[d] Patch clamp assay using IONWORKS™ technology in hERG-expressing CHO cells.
[e] Rubidium (Rb+) efflux assay. Compounds were tested for their ability to inhibit activation of the hERG potassium channel expressed in CHO cells.
[f] Functional assay measuring [^{35}S]GTPγS accumulation.

We initially walked the nitrogen atom adjacent to the piperidine ring of **1** to produce the corresponding piperazine compound **2**. Although MCH-R1 potency was somehow retained, this transformation did not affect hERG affinity, as presented in Table 1. Direct comparison of **2** and the additional piperidine analog **3** indicated that, despite the structural changes influencing basicity, hERG inhibition was maintained (Table 1). Unfortunately, pK_a modulation did not ameliorate hERG affinity, as further indicated by the **4**–**5** molecular pair (Table 1).

hERG potency notwithstanding, **3** was a very promising MCH-R1 antagonist with extremely high efficacy in our diet induced obesity (DIO) mouse model.[23] Following oral administration of a 50 μmol/kg dose of **3** (once daily), a 18.2% (StdDev: 2.9%) weight reduction was achieved after 21 days, as summarized in Figure 2. Encouraged by these promising in vivo results, we set out to remove hERG inhibition. Building on our previous hERG experience in the urea series,[13] we investigated the effects of structural modifications in the present 2-piperidin-4-yl-acetamide derivatives. Specifically, introduction of hydrophilic groups was evaluated in an effort to increase polarity, a common strategy employed in hERG optimization.[19,20] The results are presented in Table 2. Schemes 1 and 2 detail the synthetic procedures employed to prepare the piperidin-4-yl acetamide and piperidin-4-yl carboxyamide derivatives, respectively, as previously described.[13]

Flexible alcohol chains (**6**–**10**) still afforded potent hERG inhibitors in the low micromolar range (Table 2). Here, **10** yielded the best MCH-R1/hERG separation (MCH-R1 IC_{50} = 40 nM, hERG IC_{50} = 11.7 μM). Disappointingly, inclusion of more polarized (**11**–**12**) and weakly acidic (**13**) phenol groups did not reduce hERG affinity, as shown in Table 2. While our first attempt at reducing hydrophobicity in the present series increased the hERG/MCH-R1 margin compared to the original starting points, it did not completely abolish hERG inhibition, as we originally intended. A new series of side chains, bearing an amide function as polar moiety, was thus designed to further investigate the effects of polarity on hERG inhibition, as detailed in Table 3.

Pyridine and *N*-methyl-pyrrole side chains (**14**–**15**) offered varying degrees of hERG inhibition but markedly reduced MCH-R1 potency (Table 3). Introduction of a second amide function in the molecule resulted in a significant improvement in hERG selectivity, with IC_{50} values as high as 31 μM (**16**–**21**, Table 3). Here, **18** coupled acceptable MCH-R1 potency (IC_{50} = 71 nM) with minimized hERG inhibition potential (IC_{50} = 31 μM). Interestingly, simple isosteric substitution of piperidine (**18**) to morpholine (**20**) improved hERG selectivity but deteriorated MCH-R1 affinity (IC_{50}s: 71 and 173 nM, respectively).

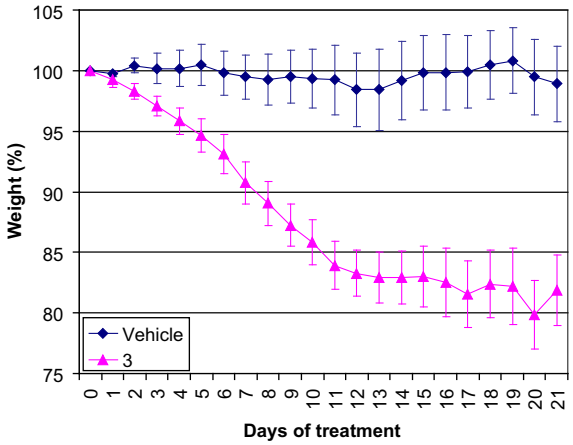
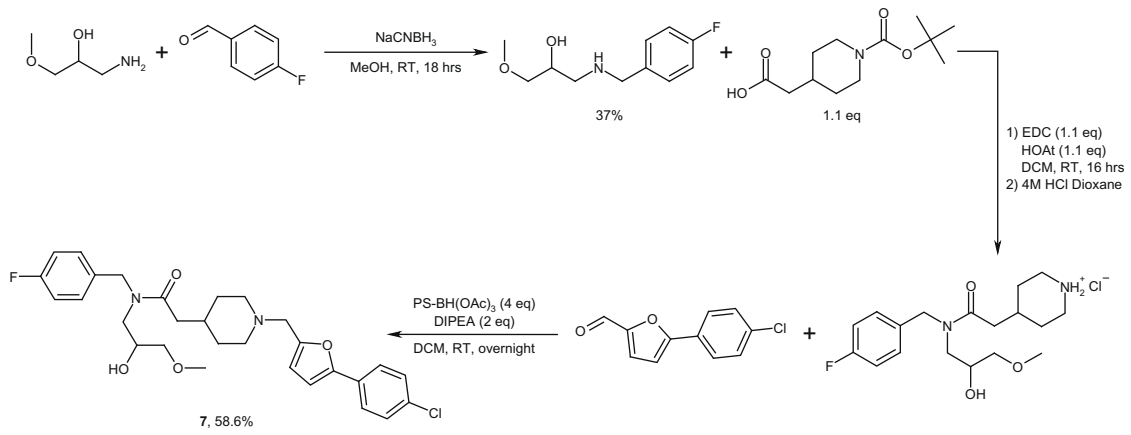
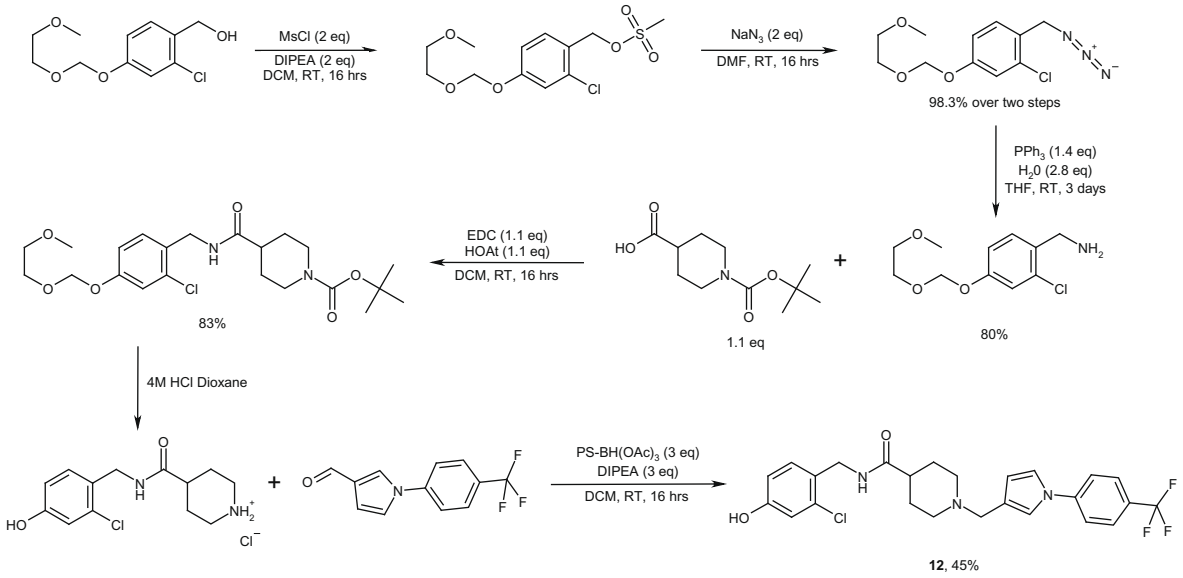


Figure 2. In vivo antiobesity effect of **3** in a 21 days DIO mouse study.[23]

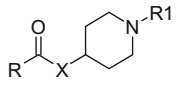


Scheme 1.



Scheme 2.

Table 2
In vitro MCH-R1 and hERG binding data for **6**–**13**



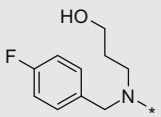
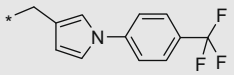
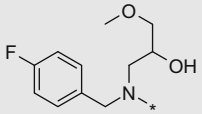
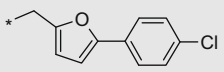
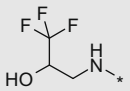
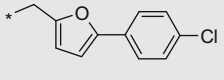
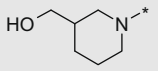
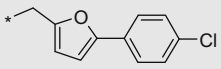
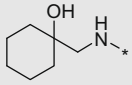
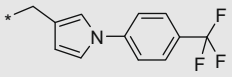
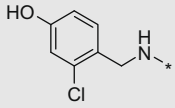
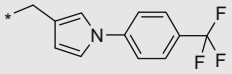
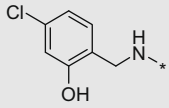
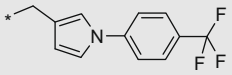
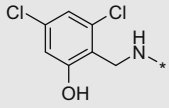
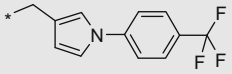
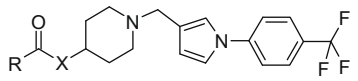
No.	R	X	R1	MCH-R1 IC_{50}[a,b] (μM)	hERG IC_{50}[a,c] (μM)
6		CH_2		0.004	0.63
7		CH_2		0.15	1.09
8		CH_2		0.892 (0.815)	1.02

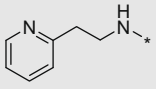
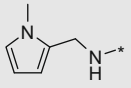
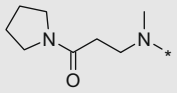
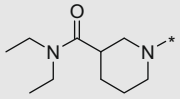
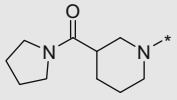
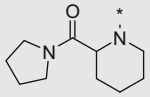
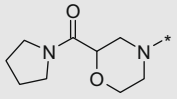
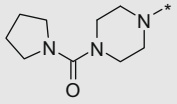
Table 2 (*continued*)

No.	R	X	R1	MCH-R1 IC_{50}[a,b] (μM)	hERG IC_{50}[a,c] (μM)
9		CH_2		0.597 (0.655)	2.34
10		CH_2		0.040	11.7
11		–		0.030	1.55
12		–		0.022	1.55
13		–		0.144	4.2

[a] Values are mean of at least two experiments. Experimental errors within 15% of value.
[b] Compounds competed with ^{125}I-MCH for binding at the human MCH1 receptor (h-MCH-R1) expressed in the HEK293 cell line. Second measurements from duplicates are shown in parentheses.
[c] Patch clamp assay using IONWORKS™ technology in hERG-expressing CHO cells.

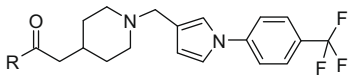
Table 3
In vitro MCH-R1 and hERG binding data for **14**–**21**

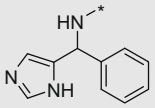
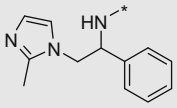
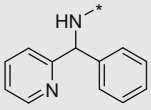
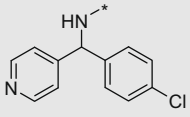
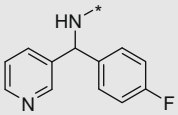
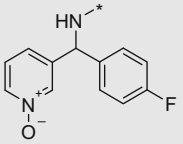
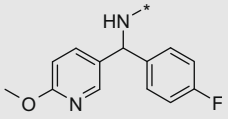


No.	R	X	MCH-R1 IC_{50}[a,b] (μM)	hERG IC_{50}[a,c] (μM)
14		–	0.339	10
15		CH_2	0.313	2.4
16		CH_2	0.142	28
17		CH_2	0.037	16
18		CH_2	0.071	31
19		CH_2	0.066	10
20		CH_2	0.173	>31.6[d]
21		CH_2	0.417	14

[a] Values are mean of at least two experiments. Experimental errors within 15% of value.
[b] Compounds competed with ^{125}I-MCH for binding at the human MCH1 receptor (h-MCH-R1) expressed in the HEK293 cell line.
[c] Patch clamp assay using IONWORKS™ technology in hERG-expressing CHO cells.
[d] No hERG inhibition at the highest measured concentration (31.6 μM).

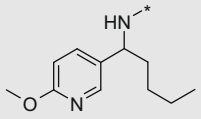
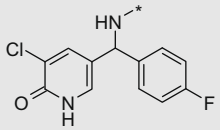
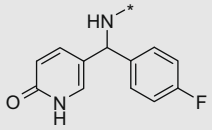
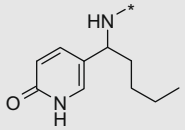
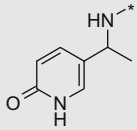
Table 4
In vitro MCH-R1 and hERG binding data for **22**–**33**



No.	R	MCH-R1 IC_{50}[a,b] (μM)	hERG IC_{50}[a] (μM)
22		0.017	11.4[c]
23		0.065	26.6[c]
24		0.041	2.3[d]
25		0.017	1.7[c]
26		0.028	2.17[c]
27		0.021	>31.6[c,e]
28		0.002	1.75[c]

(*continued on next page*)

Table 4 (*continued*)

No.	R	MCH-R1 IC_{50}[a,b] (μM)	hERG IC_{50}[a] (μM)
29		0.005	1[c]
30		0.006 0.021[e]	>31.6[c,f]
31		0.002 0.025[e]	>31.6[c,f]
32		0.001 0.019[e]	>31.6[c,f]
33		0.067	>31.6[c,f]

[a] Values are mean of at least two experiments. Experimental errors within 15% of value.
[b] Compounds competed with ^{125}I-MCH for binding at the human MCH1 receptor (h-MCH-R1) expressed in the HEK293 cell line.
[c] Patch clamp assay using IONWORKS™ technology in hERG-expressing CHO cells.
[d] Rubidium (Rb+) efflux assay. Compounds were tested for their ability to inhibit activation of the hERG potassium channel expressed in CHO cells.
[e] Functional assay measuring [^{35}S]GTPγS accumulation.
[f] No hERG inhibition at the highest measured concentration (31.6 μM).

A substantial amount of MCH-R1 and hERG data were generated for the current series. The original starting points were lipophilic MCH-R1 antagonists with high affinity to the hERG channel. Introduction of polarity in the molecule improved hERG/MCH-R1 separation but also reduced MCH-R1 binding. We therefore reasoned that a lipophilic element might be a prerequisite for MCH-R1 antagonism while a polar moiety could be required for minimized hERG inhibition. Accordingly, combination of hydrophobic and hydrophilic groups into a single ligand could afford MCH-R1 antagonists devoid of hERG inhibition.

A number of amide derivatives were then prepared to validate this assumption, as reported in Table 4. Synthetic methods leading to **22**–**33** follow the procedure presented in Scheme 1.[13] The full synthetic route to **32** is outlined in Scheme 3.

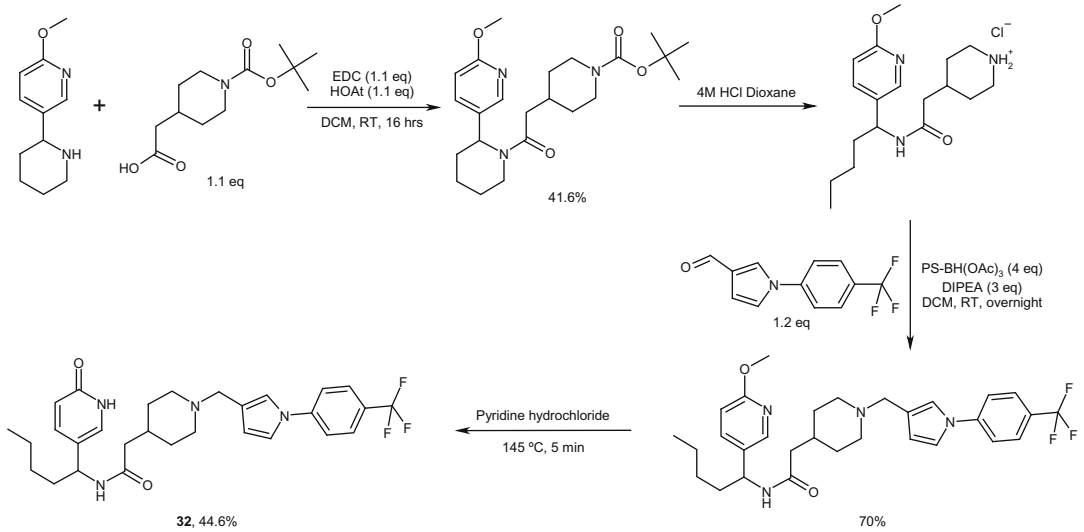
Combination of polar imidazole and lipophilic phenyl rings afforded potent MCH-R1 antagonists with improved hERG selectivity (**22**–**23**, Table 4). When pyridines were used as polar features (**24**–**29**) very good MCH-R1 potency was achieved but hERG inhibition was normally high, as presented in Table 4. Due to its marked polar character, the N-oxide derivative **27** was a notable exception, as shown in Table 4. Introduction of 1*H*-pyridin-2-ones (**30**–**33**) yielded the desired enhancement. **30**–**32** did not show any detectable hERG inhibition in the patch clamp assay while competing for binding to MCH-R1 at single digit nanomolar concentrations (Table 4). Compound **33** is a hERG-free, weaker MCH-R1 antagonist due to the lack of a hydrophobic moiety, as displayed in Table 4. These results support our previous hypothesis requiring a hydrophobe and a sufficiently polar feature for maximal MCH-R1/hERG selectivity in the current series. Considering the promising MCH-R1/hERG profile of the pyridone derivatives, additional in vitro tests were performed to further characterize the compounds, as exemplified in Table 5 for **30**.

Despite being metabolically stable and offering excellent selectivity at both the voltage-gated V1.5 Na^+ channel and the MCH-R2, the compounds showed minimal cellular permeability, as pre-

Table 5
Selectivity, stability and permeability data for **30**

Parameter[a]	Value
Na^+V1.5 IC_{50} (μM)[b]	>31.6[c]
MCH-R2 IC_{50} (μM)[d]	4.6
HLM Cl_{int} (mL/min/mg)	<15
A-B P_{app} (1E-6 cm/s)	0.16
B-A P_{app} (1E-6 cm/s)	77.5

[a] Values are mean of at least two experiments.
[b] Patch clamp assay using IONWORKS™ technology in Na^+V1.5-expressing CHO cells.
[c] No Na^+V1.5 inhibition at the highest measured concentration (31.6 μM).
[d] Compounds competed with ^{125}I-MCH for binding at the human MCH2 receptor (h-MCH-R2) expressed in the CHO cell line.



Scheme 3.

sented in Table 5 for **30**. Subsequent investigations identified P-glycoprotein (P-gp) as the transporter responsible for their efflux. Reduction of hydrophobicity offered outstanding hERG selectivity in the present series but triggered efflux mechanisms at a cellular level. On the contrary, increasing hydrophobicity afforded good cellular permeability and optimal MCH-R1 potency but also provided very effective hERG inhibitors. Although the pharmacodynamic and selectivity profile of these compounds was successfully optimized, it was not possible to combine it with acceptable pharmacokinetic properties.

In summary, preliminary scaffold hopping from piperidin-4-yl-ureas to 2-piperidin-4-yl-acetamides resulted in the identification of **3**, a potent and in vivo effective MCH-R1 antagonist with unacceptable hERG inhibition. Subsequent structural optimization of **3** balanced polarity and lipophilicity in the molecule to enhance MCH-R1/hERG selectivity. As a result, **30**–**32** were discovered as novel, potent MCH-R1 antagonists without hERG-associated liabilities. Regrettably, the limited cellular permeability observed due to P-gp activity prevented further development of these compounds.

Acknowledgments

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References and notes

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23. *Measurement of body weight and food intake.* Mice used for weight-gain studies were housed three to four per cage and given free access to standard mouse chow containing 4% of total energy as fat (R-34; Lactamin AB, Stockholm, Sweden) or to cafeteria diet, an ad libitum choice of standard mouse chow, full-fat cheese (38% fat), almond paste, milk chocolate, and pastry (Delicatobollar; Delicato, Solna, Sweden) based on vegetarian butter, sugar, rolled oats, cocoa, and coconut shreds). From 4 wk of age, mice were weighed weekly to the nearest 0.1 g on an electronic scale. Treatment of mice began after 10 weeks on diet. Mice were dosed with compound or vehicle *per os*, once daily, 90–60 min before the onset of dark.