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4-Aminophthalazin-1(2*H*)-one Derivatives as Melanin Concentrating Hormone Receptor 1 (MCH-R1) Antagonists

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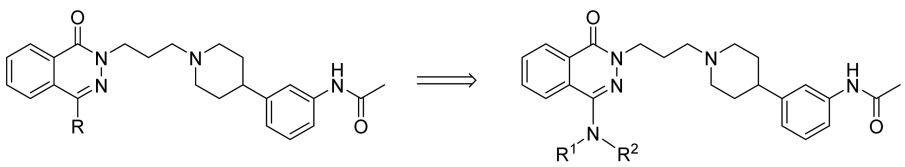
Obesity, characterized by the accumulation of excess fat on the body, has become a global epidemic.[1] Moreover, obesity is a major risk factor associated with a number of severe comorbidities including type 2 diabetes, dyslipidemia, coronary heart disease, stroke and certain cancers.[2] Unfortunately, few efficacious and safe anti-obesity drugs have been developed thus far.[3]

MCH, an orexigenic cyclic 19-amino acid polypeptide predominantly expressed in the lateral hypothalamus of the brain, plays a physiological role in both the regulation of feeding and energy homeostasis.[4] This peptide exerts its effects through interactions with two types of G protein-coupled receptors called MCH receptor 1 and 2 (MCH-R1 and -R2).[5] While the exact biological functions of MCH-R2 are still unknown, the role of MCH-R1 in regulating food intake and body weight has been demonstrated in previous genetic and pharmacological studies.[6] Therefore, antagonism of MCH-R1 is considered to be one of the most attractive strategies for the design of potential anti-obesity therapeutic agents. Indeed, numerous MCH-R1 antagonists have been shown to display anti-obesity efficacy in diet-induced obesity (DIO) animal models.[7] Although numerous efforts in pharmaceutical companies and academic laboratories have been devoted to the identification various types of pharmacophore derivatives of MCH-R1 antagonists, only a few potential drug candidates have advanced to the phase 1 clinical stage owing to their unsuitable pharmacokinetic (PK) profiles and safety issues.[8]

In previous studies, we demonstrated that selected substances, having structures in which the phthalazin-1(2*H*)-one ring system is linked to a piperidinylphenylacetamide group, are potent MCH-R1 antagonists.[9] An earlier systematic structure-activity relationships (SAR) study, focusing mainly on members of this family that possess variously C-4 substituted phthalazin-1(2*H*)-one moieties, led to the identification of the 3,4-difluorophenyl (**1**) and 5-chlorothiophenyl (**2**) derivatives as highly potent MCH-R1 antagonists, which have respective IC_{50} values of 1 and 10 nM (Figure 1). In a continuation of efforts aimed at the development of potent MCH-R1 antagonists as anti-obesity agents,[10] we explored the effect on MCH-R1 binding affinity of replacement of the aryl or heteroaryl groups at the C-4 position of the phthalazin-1(2*H*)-one skeleton by various amine groups.[11] Below, we describe the results of this effort, which focused on the synthesis, biological evaluation, and SAR study of a variety of 4-aminophthalazin-1(2*H*)-one derivatives.

The general synthetic route employed for the preparation of 4-aminophthalazin-1(2*H*)-one derivatives **3** is outlined in Scheme 1. For each target, the sequence was initiated by aromatic nucleophilic substitution reaction of commercially available 1,4-dichlorophthalazine with a selected amine, which produced the corresponding 1-chloro-4-aminophthalazine **4**. The 4-aminophthalazin-1(2*H*)-one intermediate **5,** generated by acetic acid promoted hydrolysis of **4**,[12] was then treated with 1-chloro-3-iodopropane, using sodium hydride as base to produce 2-(3-chloropropyl)-4-aminophthalazin-1(2*H*)-one **6**. Finally, coupling reaction of **6** with the known *N*-[3-(4-piperidinyl)phenyl]acetamide[13] in the presence of sodium carbonate gave the target 4-aminophthalazin-1(2*H*)-one derivative **3**.

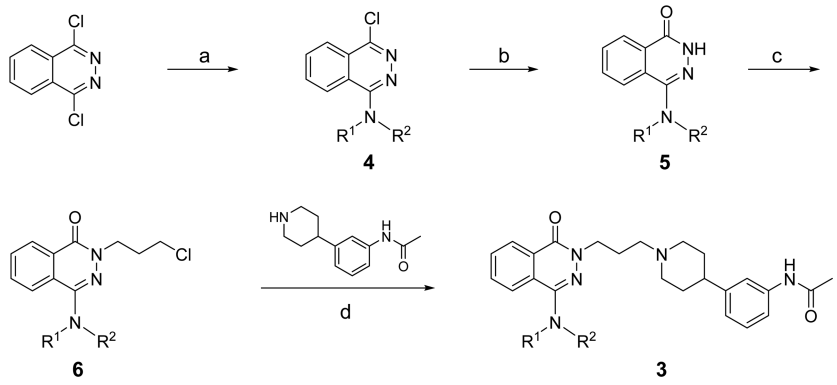
Binding affinities of the 4-aminophthalazin-1(2*H*)-one derivatives **3** to the membranes of CHO cells expressing



1 R = 3,4-di-fluorophenyl
2 R = 5-chlorothiophen-2-yl

3

Figure 1. Structural modification of phthalazin-1(2*H*)-one based MCH-R1 antagonists.



Scheme 1. (a) HNR^1R^2, K_2CO_3, DMF, 80 °C, 4 h; (b) acetic acid, reflux, 2 h; (c) NaH, 1-chloro-3-iodopropane, DMF, rt, 1 h; (d) Na_2CO_3, NaI (cat), DMF, 100 °C, 3 h.

human MCH-R1 were determined by using a competitive binding assay with Eu-labeled MCH and a time-resolved fluorometric (TRF) assay.[14] The results show that the *N*,*N*-diethylamino substituted derivative **3a** displays a moderate binding affinity to MCH-R1 and that increases in the steric bulk of the amine substituent, as represented by the respective *N*,*N*-dipropyl and *N*,*N*-diisobutyl derivatives **3b** and **3c**, cause a small decrease in binding ability. Incorporation of a pyrrolidine group at the C-4 position of phthalazin-1(2*H*)-one as in **3d**, led to an improved binding affinity (IC_{50} = 90 nM) compared with that of the *N*,*N*-diethyl analog **3a**. Moreover, the 4-piperidine substituted phthalazin-1(2*H*)-one **3e** displayed a 3-fold greater binding affinity (IC_{50} = 30 nM) as compared to that of **3d**. The results of an exploration of the effect of substituents at the 4-position of piperidine ring in **3e** demonstrate that methyl (**3f**), chloro (**3g**), and ketone (**3h**) substituted analogs all have comparatively lower binding affinities than **3e**.

Additionally, the effects on binding affinity caused by replacing the piperidine moiety in **3e** by other 6-membered heterocyclic ring systems were evaluated. The observations show that while substitution by a morpholine group (**3i**) leads to a reduced binding affinity, substitution by a thiomorpholine ring (**3j**) promotes a dramatic increase in MCH-R1 binding (IC_{50} = 5 nM). Interestingly, incorporation of unsubstituted piperazine (**3k**) and *N*-methylpiperazine (**3l**) moieties at the 4-psition of the phthalazin-1(2*H*)-one skeleton caused a complete loss of binding affinity. Lastly, although the respective cyclohexylamino and 4-chlorophenylamino phthalazin-1(2*H*)-ones **3m** and **3o** had high binding affinities, the substance containing the *N*-methylaniline group (**3n**) exhibited very low binding to MCH-R1.

Further studies were carried out with the 4-thiomorpholino analog **3j**, the phthalazin-1(2*H*)-one that was observed to have the most potent MCH-R1 binding ability. This substance was found to display good metabolic stability in human and rat liver microsomes (100% and 100% for 30 min, respectively). In addition, **3j** did not inhibit the cytochrome P450 enzymes 2D6 and 3A4 (< 10% at 10 μM) and it has a low hERG binding activity (IC_{50} = 52 μM). Furthermore, in an iv pharmacokinetic study (10 mg/kg), **3j** was found to exhibit an acceptable plasma level (AUC = 0.85 μg h/mL), but high values were observed for its volume of distribution (V_{ss} = 15 L/kg) and clearance (Cl = 196 mL/min/kg).

In summary, the studies described above have led to the discovery that substances, in which the 4-aminophthalazin-1(2*H*)-one moieties are linked to piperidinylphenylacetamide group, serve as potent MCH-R1 antagonists. The results of a systematic SAR exploration led to the identification of the thiomorpholino analog **3j** as a highly potent MCH-R1 antagonist. This phthalazin-1(2*H*)-one derivative also displayed good metabolic stability, no inhibition of CYP450 enzymes, and low hERG binding activity. However, it possessed unfavorable pharmacokinetic properties. Further studies focusing on improvement of pharmacokinetic properties of substances in this series are now in progress.

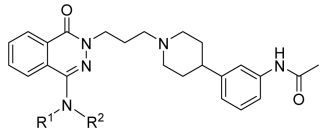
Experimental Section

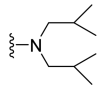
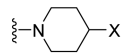
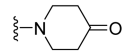
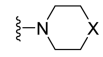
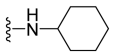
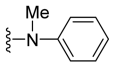
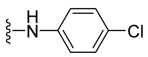
Synthesis of 4-(4-Chlorophthalazin-1-yl)thiomorpholine (4j). To a solution of 1,4-dichlorophthalazine (500 mg, 2.51 mmol) in DMF (10 mL) were added thiomorpholine (388 mg, 3.76 mmol) and K_2CO_3 (1.04 g, 7.53 mmol). The mixture was stirred at 80 °C for 4 h, cooled, diluted with 50 mL of water, and extracted with ethyl acetate (50 mL). The organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure, giving a residue that was subjected to silica gel column chromatography (ethyl acetate/*n*-hexane, 1/3) to give **4j** (310 mg, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20-8.26 (m, 1H), 7.97-8.03 (m, 1H), 7.86-7.94 (m, 2H), 3.77-3.81 (m, 4H), 2.90-2.94 (m, 4H).

Synthesis of 4-Thiomorpholinophthalazin-1(2*H*)-one (5j). A solution of **4j** (310 mg, 1.16 mmol) in acetic acid (10 mL) was stirred at reflux for 2 h. The mixture was cooled and diluted with 10 mL of water. The resulting precipitate was separated by filtration, washed with ethyl acetate, and dried *in vacuo* to give **5j** (250 mg, 87%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.13 (s, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 7.86-7.91 (m, 2H), 7.79-7.84 (m, 1H), 3.28-3.38 (m, 4H), 2.80-2.90 (m, 4H).

Synthesis of 2-(3-Chloropropyl)-4-thiomorpholinophthalazin-1(2*H*)-one (6j). To a solution of **5j** (250 mg, 1.01

Table 1. MCH-R1 binding affinities of C-4 amino substituted phthalazin-1(2*H*)-one derivatives



Compound	NR^1R^2	MCH-R1 IC_{50}[a,b] (nM)
3a		190
3b		250
3c		490
3d		90
		
3e	X = H	30
3f	X = Me	620
3g	X = Cl	190
3h		
		
3i	X = O	110
3j	X = S	5
3k	X = NH	1,000
3l	X = NMe	1,000
3m		30
3n		760
3o		10

[a]MCH-R1 binding affinities were determined by using a competitive binding with Eu-MCH and a TRF assay. [b]Values are means of at least two measurements

mmol) in DMF (10 mL) were added sodium hydride (48 mg, 1.21 mmol) and 3-iodo-1-chloropropane (0.17 mL, 1.52 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h, diluted with water (50 mL), and extracted with ethyl acetate (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (ethyl acetate/*n*-hexane, 1/3) to afford **6j** (309 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42-8.45 (m, 1H), 7.72-7.84 (m, 3H), 4.32 (t, *J* = 6.7 Hz, 2H), 3.63 (t, *J* = 6.7 Hz, 2H), 3.45-3.48 (m, 4H), 2.87-2.90 (m, 4H), 2.28-2.37 (m, 2H).

Synthesis of 2-{3-[4-(3-Acetamidophenyl)piperidin-1-yl]propyl}-4-thiomorpholinophthalazin-1(2*H*)-one (3j). To a solution of **6j** (57 mg, 0.18 mmol) in DMF (1 mL) were added *N*-[3-(piperidin-4-yl)phenyl]acetamide (47 mg, 0.22 mmol), Na_2CO_3 (57 mg, 0.54 mmol), and catalytic amount of NaI. The mixture was stirred at 100 °C for 3 h, cooled, diluted with 50 mL of water, and extracted with ethyl acetate (50 mL). The organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure, giving a residue that was subjected to silica gel column chromatography (10% MeOH/CH_2Cl_2) to give **3j** (22 mg, 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42-8.45 (m, 1H), 7.71-7.84 (m, 3H), 7.31-7.41 (m, 3H), 7.23 (d, *J* = 7.9 Hz, 1H), 6.93 (d, *J* = 7.9 Hz, 1H), 4.23 (t, *J* = 7.0 Hz, 2H), 3.45-3.48 (m, 4H), 3.14 (d, *J* = 11.3 Hz, 2H), 2.86-2.90 (m, 4H), 2.57-2.61 (m, 2H), 2.46-2.53 (m, 1H), 2.17 (s, 3H), 2.13-2.15 (m, 4H), 1.83-1.90 (m, 4H).

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Supporting Information. Experimental procedures and spectral data of compounds **3a-3o**. This material can be found in the online version.

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