http://dx.doi.org/10.5012/bkcs.2012.33.7.2389

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

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Received March 15, 2012, Accepted March 26, 2012

Key Words : Melanin concentrating hormone (MCH), MCH-R1 antagonist, 4-Heteroaryl phthalazin-1(2*H*)-one, Obesity

Obesity is a chronic disorder in which there is an imbalance between energy intake and expenditure. According to a recent World Health Organization (WHO) report, more than one billion of adults in world population are overweight, and 200 million of these individuals are clinically obese.[1] Moreover, obesity has emerged as a major risk factor for several associated diseases including type 2 diabetes, dyslipidemia, coronary heart disease, stroke and certain cancers.[2] Therefore, a significant need exists for the development of efficacious and safe anti-obesity therapeutic agents.

Among many centrally-acting neuropeptides, the melanin concentrating hormone (MCH), a cyclic 19-amino acid polypeptide, has attracted considerable recent attention as a target for the treatment of obesity. MCH, which is expressed predominantly in the lateral hypothalamus of the brain, is known to be involved in both regulation of feeding and energy homeostasis.[3] The effects of this peptide are mediated by two types of G protein-coupled receptors called MCH receptor 1 and 2 (MCH-R1 and MCH-R2).[4] The results of previous genetic and pharmacological studies have demonstrated that MCH-R1 plays an important role in the control of food intake and body-weight.[5] These findings suggest that this receptor is one of the most promising targets for the treatment of obesity.[6] Indeed, numerous MCH-R1 antagonists have been found to display anti-obesity efficacy in diet-induced obesity (DIO) animal models.[7] Although numerous pharmaceutical companies have devoted considerable efforts to develop pharmacophore derivatives of MCH-R1 antagonists as potential anti-obesity agents, few candidates have advanced to the phase 1 clinical stage owing to their unsuitable pharmacokinetic (PK) profiles and safety concerns.[8]

Previously, we demonstrated that the 4-aryl phthalazin-1(2*H*)-one derivatives linked to 4-aryl piperidine moiety are potent MCH-R1 antagonists.[9] An extensive structure-activity relationship (SAR) investigation, probing the effects of substituents on the C-4 phenyl group of phthalazin-1(2*H*)-one, led to the identification of the highly potent 3,4-difluorophenyl derivative **1** (Figure 1), which was found to exhibit a high binding affinity to MCH-R1 (IC_{50} = 1 nM). In a continuing effort aimed at the development of potent MCH-R1 antagonists as anti-obesity agents,[10] we have explored substances in which the C-4 aryl group of the phthalazin-1(2*H*)-one is replaced by heteroaryl groups (Figure 1). We envisioned that this modification might not only lead to an improved pharmacokinetic profile but also to enhance physicochemical properties while maintaining MCH-R1 binding activity. Below, we describe the results of this study which have led to the synthesis, biological evaluation, and evaluation of the structure-activity relationships of a variety of 4-heteroaryl phthalazin-1(2*H*)-one derivatives.

The general synthetic route employed for the preparation of 4-heteroaryl phthalazin-1(2*H*)-one derivatives **2** is outlined in Scheme 1. The target compounds **2** were prepared starting from 2-bromobenzoic acid in four-step sequences by utilizing simple synthetic manipulations. Specifically, lithium-halogen exchange reaction of 2-bromobenzoic acid using excess *n*-butyllithium, followed by acylation with appropriate heteroaroyl esters, produced the corresponding 2-heteroaroyl substituted benzoic acids **3**.[11] The key 4-heteroaryl phthalazin-1(2*H*)-ones intermediates **4** were then generated by condensation of the keto acids **3** with hydrazine.[12] *N*-

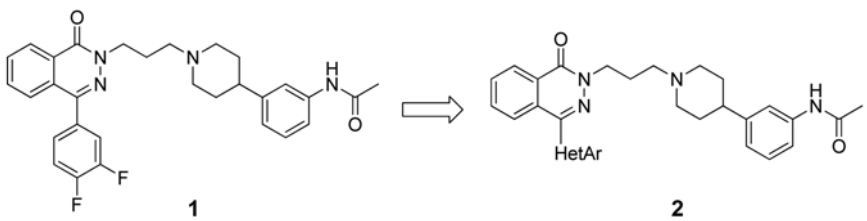
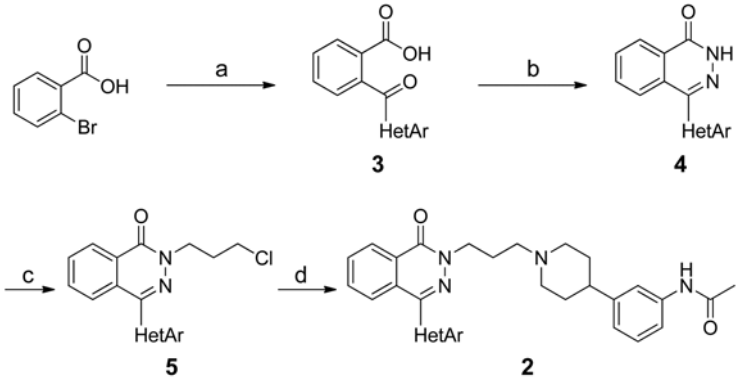
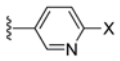
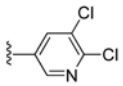
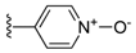
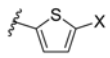
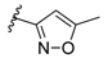


Figure 1. Structural modification of phthalazin-1(2*H*)-one-based MCH-R1 antagonists.



Scheme 1. Reagents and conditions: (a) *n*-BuLi; heteroaryl carboxylic ester, THF; (b) hydrazine, EtOH, reflux; (c) NaH, 1-chloro-3-iodopropane, DMF, rt; (d) *N*-[3-(4-piperidinyl)phenyl]acetamide, Na_2CO_3, NaI (cat), DMF, 100 °C.

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

Compound	HetAr	MCH-R1 IC_{50}[a,b](nM)
2a		90
2b	 X=H	230
2c	X=Cl	40
2d		20
2e		30
2f		130
2g		20
2h		840
2i		130
2j	 X= H	132
2k	X= Me	50
2l	X=Cl	10
2m		50
2n		170
2o		50

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

Alkylation reactions of **4** with 1-chloro-3-iodopropane, using sodium hydride as base, afforded 2-(3-chloropropyl)-4-heteroaryl phthalazin-1(2*H*)-ones **5**, which were transformed to the target compounds **2** through coupling reactions with the known *N*-[3-(4-piperidinyl)phenyl]acetamide[13] in the presence of sodium carbonate.

Binding affinities of the 4-heteroaryl phthalazin-1(2*H*)-ones **2** to the membranes of CHO cells expressing human MCH-R1 were evaluated (Table 1) by using a competitive binding assay with Eu-labeled MCH and a time-resolved fluorometric (TRF) assay.[14] The unsubstituted pyridyl derivatives **2a**, **2b** and **2f** were observed to exhibit moderate binding affinities. Interestingly, incorporation of chloro substituents on the pyridyl ring resulted in a significant increase in binding affinity. For example, the 6-chloro-3-pyridyl derivative **2c** (IC_{50} = 40 nM) has a 5.7-fold more potent binding affinity in comparison to the unsubstituted 3-pyridyl analog **2b** (IC_{50} = 230 nM). Moreover, repositioning the chloro substituent from the 6- to the 5-position (**2d**) resulted in an additional 2-fold enhancement in binding affinity in contrast to that of **2c**, and 5,6-dichloro analog **2e** also possessed a good binding activity. Similarly, the 6-chloro-4-pyridyl analog **2g** displayed a nearly 6.5-fold improvement in binding affinity compared to the unsubstituted 4-pyridyl analog **2f**. Conversion of the pyridine ring to the corresponding pyridine *N*-oxide (**2h**) proved detrimental to MCH-R1 binding.

The effects of replacement of the pyridyl ring by a 5-membered heteroaryl group at the C-4 of the phthalazin-1(2*H*)-one to MCH-R1 binding affinity were evaluated. The presence of a 2-furyl group (**2i**) at this location led to a binding IC_{50} value of 130 nM. In addition, the 2-thiophenyl derivative **2j** had a binding affinity that equaled that of **2i** while introduction of methyl group (**2k**) at C-5 of the 2-thiophenyl ring further improved the binding affinity. As in the case of pyridyl derivatives, introduction of a 5-chloro group in the thiophene ring gave a substance (**2l**) that has the highest binding affinity (IC_{50} = 10 nM) in the series studied. Finally, phthalazin-1(2*H*)-ones containing *N*-methyl-2-pyrrole (**2m**), 2-thiazole (**2n**), and 5-methyl-3-isoxazole (**2o**) groups at C-4 displayed comparatively lower binding affinities.

The 4-(5-chlorothiophen-2-yl) substituted phthalazin-1(2*H*)-one derivative **2l** with the most potent MCH-R1 binding ability was subjected to further evaluation. The results

showed that **2l** displays good metabolic stability in human and rat liver microsomes (63% and 67% for 30 min, respectively).[15] In addition, this compound is not an inhibitior of the cytochrome P450 enzyme 3A4 (< 10% at 10 μM) and it has low hERG binding activity (IC_{50} = 64 μM).[16] Furthermore, the pharmacokinetic profile of **2l** in an iv/po pharmacokinetic study (10 mg/kg) displayed an acceptable clearance (Cl = 1.8 mL/min/kg), plasma level (AUC = 1.0 μg h/mL) and half-life ($t_{1/2}$ = 7.5 h).[17] However, **2l** showed low oral bioavailability (F = 16%).

In the investigation described above, we observed that phthalazin-1(2*H*)-one derivatives, possessing heteroaryl group at C-4 position, are potent MCH-R1 antagonists. An extensive SAR study carried out with substances in this family that contain C-4 pyridyl and 5-membered heteroaryl groups led to the identification of the 5-chlorothiophen-2-yl analog **2l** as a highly potent MCH-R1 antagonist. This substance displayed good metabolic stability, no inhibition of CYP450 enzymes, and low hERG binding activity. Further optimization studies based on **2l** as a lead compound, focusing on improving oral bioavailability, are in progress.

Experimental Section

Synthesis of 2-(5-Chlorothiophene-2-carbonyl)benzoic acid (3l). To a solution of 2-bromobenzoic acid (1.0 g, 5.0 mmol) in THF (10 mL) was added *n*-butyllithium (5.5 mL, 2 M in THF) at −78 °C. The mixture was stirred for 30 min before adding methyl 5-chlorothiophene-2-carboxylate (0.97 g, 5.5 mmol). After stirring for 1 h at −78 °C, the mixture was extracted with ethyl acetate (50 mL). The combined organic layer was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (10% MeOH/CH_2Cl_2) to obtain **3l** (300 mg, 23%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.04 (br s, 1H), 7.93-7.98 (m, 1H), 7.58-7.74 (m, 2H), 7.48-7.52 (m, 1H), 7.21 (d, *J* = 4.1 Hz, 1H), 7.10 (d, *J* = 4.1 Hz, 1H).

Synthesis of 4-(5-Chlorothiophen-2-yl)phthalazin-1(2*H*)-one (4l). A solution of **3l** (300 mg, 1.12 mmol) and hydrazine hydrate (0.17 mL, 5.60 mmol) in ethanol (10 mL) was stirred at reflux for 8 h. The resulting precipitate was filtered, washed with ethyl acetate, and dried *in vacuo*. Recrystallization of the solid from ethanol gave **4l** (200 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 7.89-8.00 (m, 2H), 7.53 (d, *J* = 3.9 Hz, 1H), 7.28 (d, *J* = 3.9 Hz, 1H).

Synthesis of 2-(3-Chloropropyl)-4-(5-chlorothiophen-2-yl)phthalazin-1(2*H*)-one (5l). To a solution of **4l** (190 mg, 0.72 mmol) in DMF (10 mL) were added sodium hydride (35 mg, 0.86 mmol) and 3-iodo-1-chloropropane (0.12 mL, 1.08 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 **5l** (194 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51-8.54 (m, 1H), 8.09-8.13 (m, 1H), 7.79-7.88 (m, 2H), 7.26 (d, *J* = 3.9 Hz, 1H), 7.02 (d, *J* = 3.9 Hz, 1H), 4.44 (t, *J* = 6.8 Hz, 2H), 3.66 (t, *J* = 6.8 Hz, 2H), 2.33-2.42 (m, 2H).

Synthesis of 4-(5-Chlorothiophen-2-yl)-2-[3-[4-[3-(acetylamino)phenyl]piperidine-1-yl]propyl]phthalazin-1(2*H*)-one (2l). To a solution of **5l** (61 mg, 0.18 mmol) in DMF (1 mL) were added *N*-[3-(piperidin-4-yl)phenyl]acetamide (46 mg, 0.21 mmol), Na_2CO_3 (57 mg, 0.54 mmol), and catalytic amount of NaI. The mixture was stirred at 100 °C for 2 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 **2l** (40 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51-8.54 (m, 1H), 8.10-8.13 (m, 1H), 7.80-7.86 (m, 2H), 7.42-7.44 (m, 2H), 7.25-7.27 (m, 2H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 4.0 Hz, 1H), 6.89 (d, *J* = 7.6 Hz, 1H), 4.36 (t, *J* = 6.8 Hz, 2H), 3.16-3.19 (m, 2H), 2.66 (t, *J* = 6.8 Hz, 2H), 2.46-2.52 (m, 1H), 2.18 (s, 3H), 2.14-2.26 (m, 4H), 1.82-1.86 (m, 4H).

Acknowledgments. This research was supported by a grant from the Ministry of Education, Science and Technology (2011-0019397) and the R&D program of MKE/KEIT (10038744), Korea.

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