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4-Arylphthalazin-1(2*H*)-one derivatives as potent antagonists of the melanin concentrating hormone receptor 1 (MCH-R1)

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ABSTRACT

A novel series of 4-arylphthalazin-1(2*H*)-one linked to arylpiperidines were synthesized and evaluated as MCH-R1 antagonists. The results of an extensive SAR study probing the effects of substituents on the 4-arylphthalazin-1(2*H*)-one C-4 aryl group led to the identification of the 4-(3,4-difluorophenyl) derivative as a highly potent MCH-R1 inhibitor with an IC_{50} = 1 nM. However, further investigations showed that this substance has unacceptable pharmacokinetic properties including a high clearance and volume of distribution.

Obesity has become a global epidemic and a major risk factor for many serious diseases including type 2 diabetes, dyslipidemia, coronary heart disease, stroke and certain cancers.[1] 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 obesity treatment. MCH is expressed predominantly in the lateral hypothalamus of the brain and is known to be involved in both regulation of feeding and energy homeostasis.[2] The effects of MCH are mediated by two types of G protein-coupled receptors, MCH receptors-1 and -2 (MCH-R1 and -R2).[3] The results of previous genetic and pharmacological studies demonstrated that MCH-R1 plays an important role in the control food intake and body-weight and suggested that this receptor is one of the most promising targets for the obesity treatment.[4] Indeed, numerous MCH-R1 antagonists have been found to have antiobesity efficacy in diet-induced obesity (DIO) animal models.[5] Despite the large efforts of a number of pharmaceutical companies to develop a variety of pharmacophore derivatives of MCH-R1 antagonists as potential antiobesity therapeutic agents, few have led to candidates that have advanced to the phase 1 clinical stage owing to their unsuitable PK profiles and safety concerns.[6]

In a previous investigation, we discovered that a series of 2-aryl substituted benzimidazoles that are linked to arylpiperidines serve as MCH-R1 antagonists.[7] Among these substances, the *p*-chlorophenyl derivative **1** was found to exhibit high affinity to MCH-R1 (IC_{50} = 1 nM) (Fig. 1). As part of a continuing drug discovery program aimed at the development of potent MCH-R1 antagonists, we carried out a SAR study of newly prepared pyridazin-3(2*H*)-ones based on the lead structure **2**, which has an IC_{50} value of 120 nM. An initial effort focused on substances containing a modified pyridazin-3(2*H*)-one core and explored the effects of fusing rings to the pyridazin-3(2*H*)-one core. As the data in Table 1 show, introduction of methyl groups at the 5- and 6-positions of pyridazin-3(2*H*)-one ring gave a compound **3** that has a threefold improved affinity for MCH-R1 compared with unsubstituted analog **2**, whereas the cyclohexane fused analog **4** was found to possess a slightly lower binding affinity. Interestingly, thiophene ring fused (**5**) and phthalazin-1(2*H*)-one (**6**) analogs were observed to exhibit sixfold more potent binding than **2**.

Based on the results described above, the phthalazin-1(2*H*)-one heterocyclic system was proposed as a potentially interesting scaffold for the development of new MCH-R1 antagonists. The activities of 4-arylphthalazin-1(2*H*)-one derivatives, in which a variety

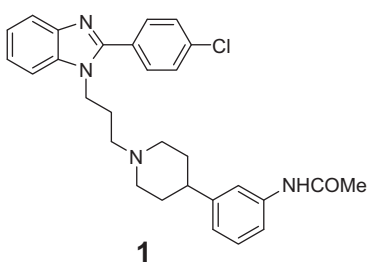

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Figure 1. Benzimidazole based MCH-R1 antagonist.

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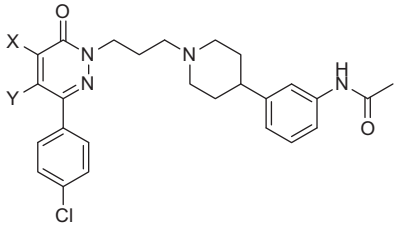
Table 1
Effects of substituents on the 5- and 6-positions of pyridazin-3(2*H*)-one derivatives on MCH-R1 binding affinity



Compound	X	Y	MCH-R1 IC_{50}[a,b] (nM)
2	H	H	120
3	Me	Me	40
4			170
5			20
6			20

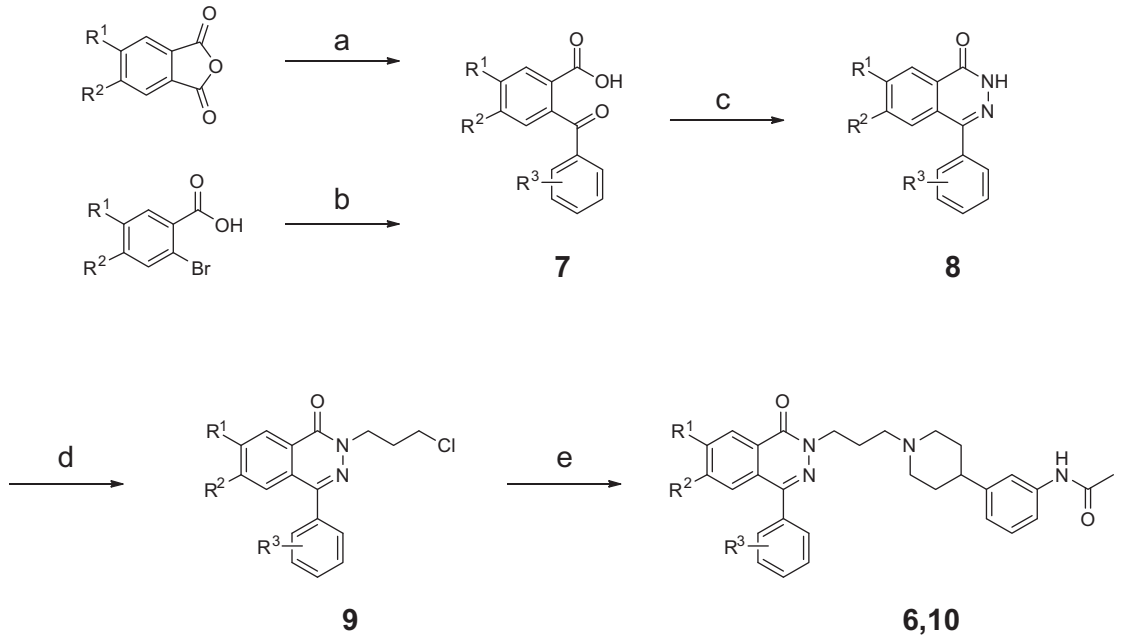
[a] Binding affinities of compounds for MCH-R1 were determined by using a competitive binding with Eu-MCH and a TRF assay.
[b] Values are means of at least two measurements.

of substituents were introduced on the phthalazin-1(2*H*)-one C-4 aryl moiety, were examined.[8] Below, the results of this effort involving the synthesis, biological evaluation, and structure–activity relationships of variously substituted 4-arylphthalazin-1(2*H*)-one derivatives are described.

The general synthetic routes employed for the preparation of **6** and **10**, containing 4-arylphthalazin-1(2*H*)-one rings linked to arylpiperidine through their 2-position, are outlined in Scheme 1. The key intermediates, 4-arylphthalazin-1(2*H*)-one **8**, are either commercially available or were readily prepared by condensation of 2-benzoyl substituted benzoic acids **7** with hydrazine.[9] The keto acids **7** were generated by reaction of the corresponding aryl Grignard reagents with phthalic anhydride. Alternatively, lithiation reactions of 2-bromobenzoic acids using *n*-butyllithium followed by acylation with aroyl esters were used to produce **7**.[10] Simple alkylation reactions of **8** with 1-chloro-3-iodopropane, utilizing sodium hydride as a base, gave the desired 2-(3-chloropropyl)-4-arylphthalazin-1(2*H*)-ones **9**. The targets **6** and **10** were then formed by using coupling reactions of **9** with the known *N*-[3-(4-piperidinyl)phenyl]acetamide[11] in the presence of sodium carbonate.

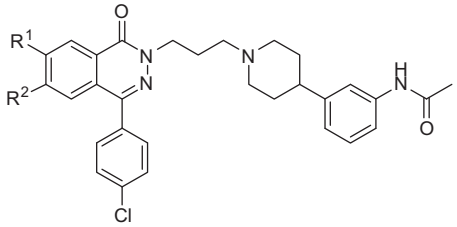
The binding affinities of the 4-arylphthalazin-1(2*H*)-one derivatives **6** and **10** to membranes of CHO cells expressing human MCH-R1 were evaluated. The measurements were performed by using a competitive binding assay with Eu-labeled MCH and a time-resolved fluorometric (TRF) assay.[12,13] The results show that, in a manner that is similar to those of benzimidazole derivatives,[7] a three carbon linker between phthalazin-1(2*H*)-one and arylpiperidine moieties is preferred over two, four, and five carbon linkers (data not shown). The effects of substituents at 6- and 7-positions of the phthalazin-1(2*H*)-one were examined utilizing 4-(*p*-chlorophenyl) substituted phthalazin-1(2*H*)-one. The results summarized in Table 2 demonstrate that, in general, substituents (R^1) at the 7-position of the phthalazin-1(2*H*)-one moiety, such as F-(**6a**), Cl (**6b**), and OMe (**6c**), improved binding affinity slightly. In addition, the 6-chloro (**6e**, R^2 = Cl) and 6-methyl (**6f**, R^2 = CH_3) analogs were equipotent to the unsubstituted compound **6** while 6-fluoro derivative **6d** (R^2 = F) was slightly less potent. Finally, the dichloro analog **6g** (R^1 = R^2 = Cl) also exhibited a good binding activity. The combined results indicate that non-sterically bulky substituents at the 6- and 7-positions of the phthalazin-1(2*H*)-one group do not greatly affect MCH-R1 binding affinity.

Attention was next given to the effects of substituents on the aryl ring positioned at C-4 of the phthalazin-1(2*H*)-one containing hydrogens at C-6 and C-7, and the results are summarized in Table 3. Removal (**10a**, R^3 = H) or repositioning the chloro



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

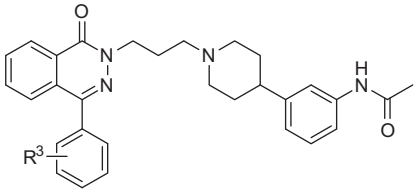
Table 2
Effects of substituents (R^1 and R^2) of phthalazin-1(2*H*)-one derivatives on MCH-R1 antagonist activity



Compound	R^1	R^2	MCH-R1 IC_{50}[a,b] (nM)
6	H	H	20
6a	F	H	14
6b	Cl	H	14
6c	OMe	H	15
6d	H	F	30
6e	H	Cl	20
6f	H	Me	20
6g	Cl	Cl	20

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

Table 3
Effects of substituents on the 4-aryl group of phthalazin-1(2*H*)-one derivatives on MCH-R1 antagonist activity



Compound	R^3	MCH-R1 IC_{50}[a,b] (nM)
6	4-Cl	20
10a	H	30
10b	2-Cl	96
10c	3-Cl	26
10d	4-F	5
10e	4-Br	50
10f	4-CF_3	136
10g	4-CN	34
10h	4-OMe	179
10i	4-SMe	141
10j	4-NMe_2	423
10k	4-Me	91
10l	4-*i*-Pr	380
10m	2,4-Di-F	7
10n	3,4-Di-F	1
10o	2,4-Di-Cl	27
10p	3,4-Di-Cl	12
10q	2-F-4-Cl	50
10r	3-F-4-Cl	6
10s	2,3,5-F	27
10t	2,3,4,5,6-F	30

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

substituent from the *para*-(**6**) to the *ortho* (**10b**, R^3 = *o*-Cl) or *meta* (**10c**, R^3 = *m*-Cl) positions led to a slight reduction in binding ability. A further exploration of the effects of substituents at the *para*-position of phenyl group revealed that fluoro substituted derivative **10d** (R^3 = *p*-F) has a fourfold higher binding affinity (IC_{50} = 5 nM) compared to the *p*-chloro analog **6**. However, substrates possessing *p*-bromo (**10e**), *p*-trifluoromethyl (**10f**), *p*-cyano (**10g**), *p*-methoxy (**10h**), *p*-methylthio (**10i**), *p*-dimethylamino (**10j**), *p*-methyl (**10k**), and *p*-*i*-propyl (**10l**) substituents all displayed reduced binding activities.

Table 4
Pharmacokinetic profile of **10n** in rats

Parameter[a]	Value
iv $t_{1/2}$ (h)	5.2
Oral AUC (μg h/mL)	1.0
iv CL (mL/kg min)	50
V_{dss} (L/kg)	19
%F	25

[a] Determined in rats by administration of 10 mg/kg, iv and po (n = 3).

The effects of disubstitution on the 4-phenyl ring were also investigated. The 3,4-difluoro derivative **10n** exhibited the most potent binding (IC_{50} = 1 nM) to this receptor among all of the compounds studied. Moreover, 2,4-difluoro (**10m**) and 3-fluoro-4-chloro (**10r**) analogs retained high binding affinities as demonstrated by their respective low IC_{50} values of 7 and 6 nM. Other disubstituted substrates, such as the 2,4-dichloro (**10o**), 3,4-dichloro (**10p**), 2-fluoro-4-chloro (**10q**), 2,3,5-trifluoro (**10s**), and pentafluoro (**10t**) derivatives, displayed comparatively lower binding affinities.

The 4-(3,4-difluorophenyl) substituted phthalazin-1(2*H*)-one **10n** that has the most potent MCH-R1 binding ability was subjected to further studies. The results show that this compound does not inhibit the cytochrome P450 enzymes 2D6 and 3A4 (<10% at 10 μM) and has a low hERG binding activity (IC_{50} = 16 μM). In addition, compound **10n** displays good metabolic stability in human and rat liver microsomes (62% and 86% for 30 min, respectively). Furthermore, in an in vivo rat iv/po pharmacokinetic study (10 mg/kg), **10n** displays modest oral bioavailability (*F* = 25%) with an acceptable plasma level (AUC = 1.0 μg h/mL) and half-life ($t_{1/2}$ = 5.2 h) as shown in Table 4. However, compound **10n** exhibits a high clearance (Cl = 50 mL/kg min) and a high volume of distribution (V_d = 19 L/kg).

In summary, optimization of the initial pyridazin-3(2*H*)-one lead compound **2** led to the discovery of the 4-arylphthalazin-1(2*H*)-one derivatives as potent MCH-R1 antagonists. Further extensive SAR studies probing substituents on the phenyl group at the 4-position of the phthalazin-1(2*H*)-one resulted in the identification of the difluoro derivative **10n** as a highly potent MCH-R1 antagonist. This compound also displayed good metabolic stability, no significant inhibition of CYP450 enzymes, and acceptable hERG binding activity. Further investigations of 4-arylphthalazin-1(2*H*)-one derivatives, concentrating on the improvement of pharmacokinetic properties such as clearance and volume of distribution, are now in progress.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.111.

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13. Receptor binding assays with europium-labeled MCH (Eu-MCH) were performed in 96-well AcroWell™ plates. The MCH labeled with europium at N1 position was supported from Wallac labeling service (PerkinElmer Oy). The human recombinant MCH-1 receptor membrane preparation (MCH-1/SLC1 membrane) was from Euroscreen S.A. (PerkinElmer Oy). The assay buffer contained 25 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% bovine serum albumin pH 7.4. Non-specific Eu-MCH binding was determined experimentally by the presence of 0.5 μM unlabeled MCH (human). After incubation at room temperature for 90 min, the incubation mixtures were filtered in the automatic vacuum filtration system for filter plates and rapidly washed three times with 300 μl of ice-cold 25 mM HEPES buffer (pH 7.4). The europium was dissociated from the bound ligand by the addition of 150 μl of DELFIA enhancement solution (PerkinElmer Oy) and incubated for 10 min with shaking. Dissociated europium created highly fluorescent complexes, which were measured in a multilabel counter with a time-resolved fluorescence (TRF) option (Victor II, PerkinElmer Oy). The counter setting was 340 nm excitation, 400 μs delay, and emission collection for 400 μs at 615 nm. The extent of antagonism was expressed as% displacement. The IC_{50} value was characterized in an 8-dose response study to generate the compound concentration required to yield 50% displacement.