Bioorganic & Medicinal Chemistry Letters 18 (2008) 4997–5001

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Syntheses and structure–activity relationships of novel, potent, and selective *trans*-2-[3-oxospiro[isobenzofuran-1(3*H*),1′-cyclohexan]-4′-yl]benzimidazole NPY Y5 receptor antagonists

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ARTICLE INFO

Article history:
Received 28 February 2008
Revised 31 July 2008
Accepted 8 August 2008
Available online 13 August 2008

Keywords:
Neuropeptide Y
Y5 receptor
Antagonist
Anti-obesity
Benzimidazole

ABSTRACT

Syntheses and structure–activity relationships of a novel class of 2-[3-oxospiro[isobenzofuran-1(3*H*),1′-cyclohexan]-4′-yl]benzimidazole NPY Y5 receptor antagonists are described. Optimization of the lead compound **2a** by incorporating substituents into the 5-position or into both the 5- and 6-positions of the benzimidazole core part led to the identification of 5-(5-methyl-1,2,4-oxadiazol-2-yl)benzimidazole (**2r**: IC_{50} = 3.3 nM) and 5-(2-methyltetrazol-5-yl)benzimidazole (**2u**: IC_{50} = 5.9 nM), both of which are potent, selective, and orally bioavailable Y5 receptor antagonists.

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Neuropeptide Y (NPY) is a highly conserved 36-amino acid peptide with potent, centrally mediated orexigenic effects.[1] There have been five distinct types of G protein-coupled NPY receptors, Y1, Y2, Y4, Y5, and y6 receptor subtypes so far.[2] Among these, the Y5 receptor subtype is thought to play a role in the regulation of food intake.[3] Recently, we disclosed that the Y5 receptor subtype is involved in the regulation and development of diet-induced obesity in rodents, suggesting the utility of potent Y5 receptor antagonists in the treatment of obesity.[4] To date, a number of potent Y5 receptor antagonists with distinct structure classes such as sulfonamide, pyrrolo[3,2-*d*]pyrimidine, carbazole, imidazolylpyridine, and imidazolone classes have been reported.[5]

In our previous paper, we reported the structure–activity relationships (SARs) of 2-[3-oxospiro[isobenzofuran-1(3*H*),4′-piperidin]-1′-yl]benzimidazole derivatives (**1**).[6] In parallel, we have continued our research that is examining the lead potential of 2-[3-oxospiro[isobenzofuran-1(3*H*),1′-cyclohexan]-4′-yl]benzimidazole structure (**2a**) as a novel Y5 antagonist lead. In this letter, we describe the syntheses and SARs for these types of benzimidazoles, along with the biological profiles of selected compounds (Fig. 1).

General synthetic methods for the benzimidazole derivatives (**2a**–**u** and **14**–**16**) are shown in Scheme 1 through 3. Addition reaction of the dianion, generated from **3** and *n*-BuLi, to cyclohexane-1,4-dione *mono*-ethyleneketal and the subsequent acidic treatment of the reaction mixture afforded cyclohexanone (**4**) in a 48% yield. Stereoselective reduction of the ketone moiety of **4** ($NaBH_4$) gave a mixture of diastereomers (*cis*:*trans* = ~7:1), which was purified by recrystallization to give **5** exclusively. Mesylation of the resulting secondary alcohol and the subsequent substitution reaction with *n*-tetrabutylammonium cyanide provided cyanocyclohexane (**6**). Acidic hydrolysis of the cyanide moiety produced the carboxylic acid (**7**) in 96% yield.[7]

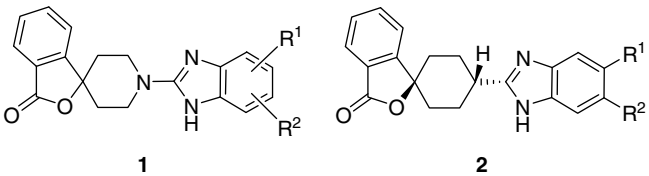

Figure 1. Structures of benzimidazole Y5 receptor antagonists.

Syntheses of some representative phenylenediamines (**10f**, **10g**, **10i**, **10j**, and **10t**) are described in Scheme 2. Suzuki coupling of commercially available 4-bromo-2-nitroaniline **8** with arylboronic acids gave 4-aryl-2-nitroanilines **9**, which were hydrogenated in the presence of 10% Pd–C to produce the phenylenediamines.

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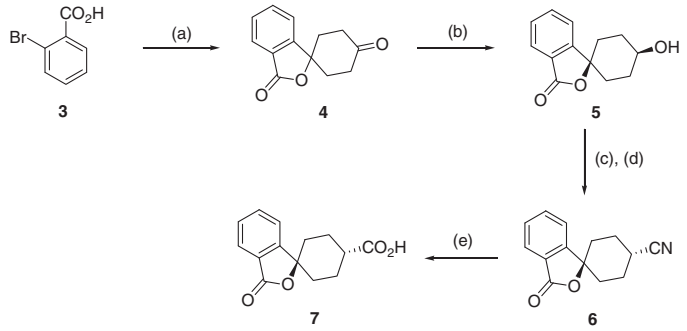
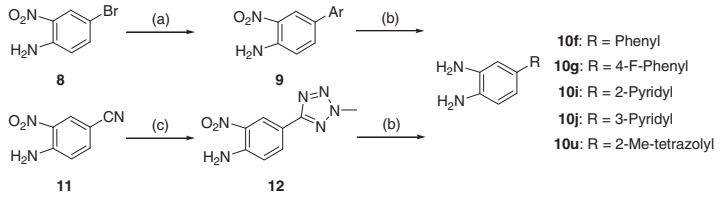
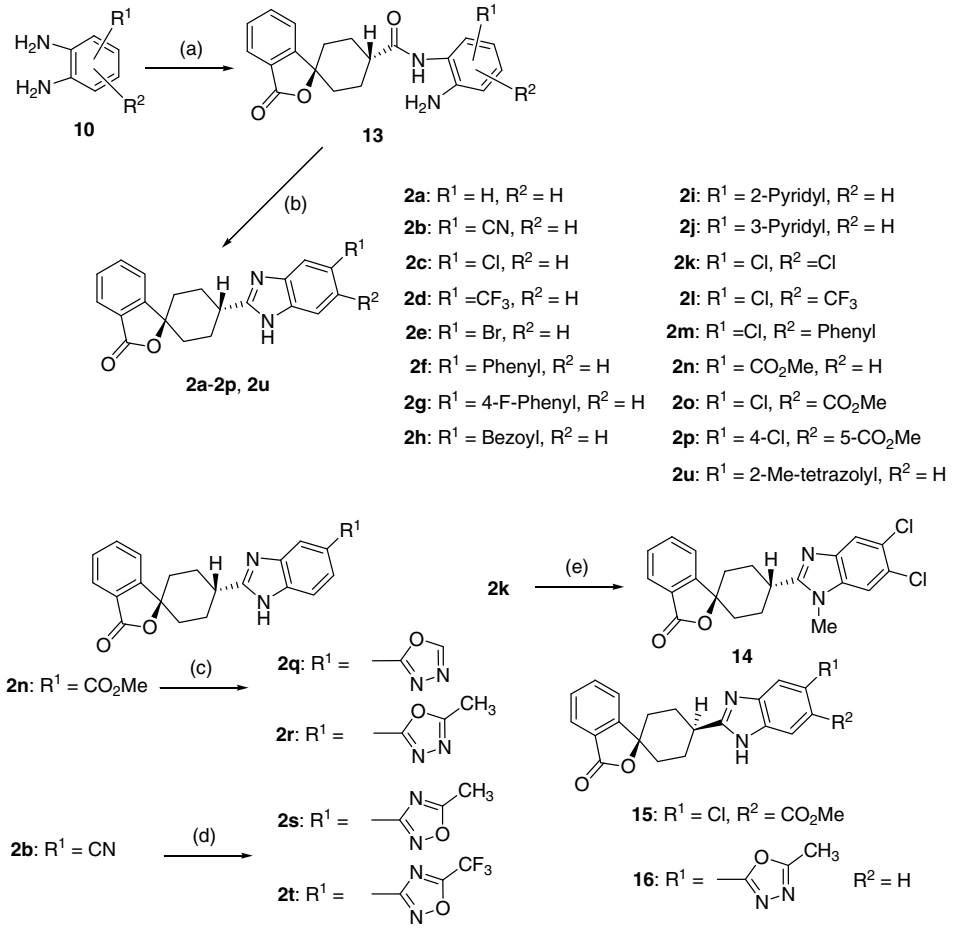
doi:10.1016/j.bmcl.2008.08.021



Scheme 1. Syntheses of the core part (**7**) of benzimidazole derivatives. Reagents and conditions: (a) 1—*n*-BuLi, 1,4-cyclohexanedione mono-ethyleneketal, THF, −70 °C; 2—concd HCl, H_2O–acetone, 80 °C, 3 h, 48%; (b) $NaBH_4$, THF–H_2O, 0 °C, 71%; (c) MsCl, NEt_3, 0 °C, 86%; (d) NEt_4CN, DMF, 100 °C, 76%; (e) concd H_2SO_4, dioxane–H_2O, 115 °C, 96%.



Scheme 2. Syntheses of some of the representative phenylenediamines (**10f**, **10g**, **10i**, **10j**, and **10u**). Reagents and conditions: (a) $ArB(OH)_2$, cat. $Pd(PPh_3)_4$, 2 N Na_2CO_3, DME, 80 °C; (b) H_2, 10% Pd–C, THF–MeOH, rt; (c) NaN_3, $MgCl_2$, 120 °C, DMF; (d) MeI, NEt_3, acetone, rt.



Scheme 3. Syntheses of benzimidazole derivatives (**2a**–**u** and **14**–**16**). Reagents and conditions: (a) EDCI, **7**, Py, rt; (b) $POCl_3$, 100 °C; (c) 1) NH_2NH_2, EtOH; (c) $CH(OEt)_3$, or $CH_3CH(OEt)_3$, DMF; (d) 1—NH_2OH–HCl, NEt_3, EtOH, 60 °C; 2—Ac_2O, 100 °C, or (CF3CO)2O, 100 °C; (e) 60% NaH, MeI, DMF, rt.

Coupling of phenylenediamines **10** with the carboxylic acid **7** by a conventional method (EDCI, Py, rt) produced amides **13**. Treatment of **13** with $POCl_3$ at 100 °C for 2 h followed by separation of the mixture of the *trans*- and *cis*-benzimidazoles (2:1∼>10:1) by silica-gel column chromatography afforded *trans*-benzimidazoles (**2a**–**p** and **2u**) as the major products in 30–70% yields.

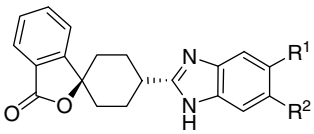
Other benzimidazole derivatives (**2q**–**t**) bearing a heteroaryl group at the 5-position were prepared from 5-methoxycarbonyl- (**2n**) or 5-cyanobenzimidazole (**2b**) by conventional synthetic methods (Scheme 3).[8] During preparation of **2o** and **2r**, the corresponding *cis*-benzimidazoles (**15** and **16**) were isolated. The 1-methyl-5,6-dichlorobenzimidazole (**14**) was prepared by methylation of **2k** (MeI, 60% NaH, DMF).

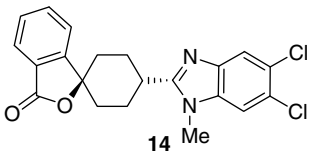
Compounds were tested in an initial screen for the binding affinity against human NPY Y5 receptor subtype (displacement of [^{125}I]PYY binding) in transfected LMtk$^-$ cells.[3b] Subsequently, selected compounds were examined for their subtype selectivity over the other NPY receptors (Y1, Y2, and Y4) in the binding assays, as well as being compared for their Y5 antagonist activity (inhibition of NPY induced Ca^{2+} flux in LMtk$^-$ cells expressing the human Y5 receptor).[3b]

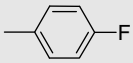
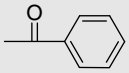
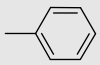
Initial SAR study was conducted by incorporating a substituent into the 5-position of the benzimidazole core part of **2a**, based on the SARs of the 2-[3-oxospiro[isobenzofuran-1(3*H*),4′-piperidin]-1′-yl]benzimidazole derivatives we previously developed.[6] The results revealed a strong preference for sterically bulky substitution at the 5-position (Table 1). When a chlorine atom (**2c**) or trifluoromethyl group (**2d**) was introduced into this position, there was improvement of the Y5 binding affinity to some extent. However, the binding affinities of these compounds were still moderate (double-digit nanomolar affinity). With a more sterically bulky substituent such as a phenyl, 4-fluorophenyl, or benzoyl group, the compounds (**2f**–**h**) attained low nanomolar binding affinities to the Y5 receptor (IC_{50}: ∼2 nM). The potent Y5 binding affinity was also retained, when the phenyl moiety in **2f** is replaced with a pyridin-2-yl (**2i**) or pyridin-3-yl (**2j**) group. Comparison of the Y5 affinity of 5,6-dichloro- (**2k**), 5-chloro-6-trifluoromethyl- (**2l**), 5-trifluoromethyl- (**2c**), 5-chloro- (**2d**), or 5-phenylbenzimidazole (**2f**) suggested that the 5,6-substitution was more favorable than the 5-substitution.

To clarify the effect of the benzimidazole N–H group on the Y5 affinity, 1-methyl-5,6-dichlorobenzimidazole (**14**) was

Table 1
Binding affinity of benzimidazole derivatives (**2a**–**m** and **14**) to the Y5 receptor

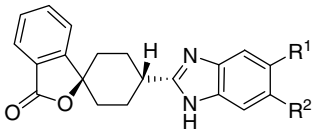

2a-2m

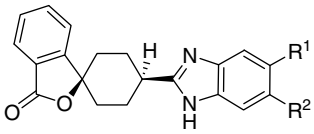

14

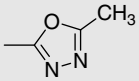
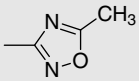
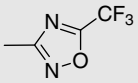
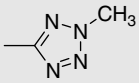
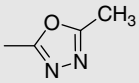
Compound	R^1	R^2	Y5 (IC_{50}, nM)[a]	clog$D_{7.4}$[b]
2a	H	H	91	4.0
2b	CN	H	20	3.8
2c	Cl	H	39	4.7
2d	CF_3	H	47	5.1
2e	Br	H	45	4.8
2f		H	2.3	5.9
2g		H	1.7	6.4
2h		H	2.4	5.6
2i		H	2.4	5.0
2j		H	3.1	4.8
2k	Cl	Cl	8.0	5.2
21	Cl	CF_3	2.6	5.6
2m	Cl		2.2	6.6
14			>1000	5.6

[a] Binding affinities were determined by displacement of [^{125}I]PYY binding on the recombinant human Y5 receptor transfected in LMtk" cells. IC_{50} values are the mean of two or more independent assays (Ref. 9).
[b] clog$D_{7.4}$ values were calculated based on Ref. 10.

Table 2
Binding affinity of benzimidazole derivatives (**2n**–**u**, **15**, and **16**) to the Y5 receptor


2n-2u


15, 16

Compound	R^1	R^2	Y5 (IC_{50}, nM)[a]	clogD_{74}[b]
2n	CO_2Me	H	7.7	4.1
2o	Cl	CO_2Me	2.2	4.8
2p	4-Cl[c]	5-CO_2Me[d]	4.5	4.8
2q		H	15	1.4
2r		H	3.3	3.3
2s		H	5.1	3.7
2t		H	2.1	4.8
2u		H	5.9	3.6
15	Cl	CO_2Me	>1000	4.8
16		H	>1000	3.3

[a] Binding affinities were determined by displacement of [^{125}I]PYY binding on the recombinant human Y5 receptor transfected in LMtk" celis. IC_{50} values are the mean of two or more independent assays (Ref. 9).
[b] clog*D* values were calculated based on Ref. 10.
[c] Chlorine atom in **2p** was attached to the 4-position.
[d] Methoxycarbonyl group in **2p** was attached to the 5-position.

Table 3
Brain and plasma levels of the compound (**2n**)

Compound	Plasma[a] (μM)	Brain[a] (nmol/g)	B/P ratio[b]
2n	3.1	1.7	0.55

[a] Compound **2n** (2.7 mg/kg) was intraveniously administered to rats ($n = 3$), and the plasma and brain levels were measured at 10 min. Values are the means of the tests.
[b] B/P means the ratio of the brain level (nmol/g) to plasma level (μM) of the compound.

Table 4
Biological properties of the representative compounds (**2r**, **2s**, and **2u**)

Compound	IC_{50}[a] (nM)					Rat plasma (μM) levels[c]		B/P ratio[d]
	Y5	Y1	Y2	Y4	Y5[b]	2 h	4 h	
2r	3.3	>10,000	>10,000	>10,000	5.5	1.4	0.85	0.05
2s	5.1	>10,000	>10,000	>10,000	7.0	0.4	n.d.[e]	–
2u	5.9	>10,000	>10,000	>10,000	7.2	3.2	0.99	0.12

[a] IC_{50} values are the mean of two or more independent assays.
[b] Antagonist activity was determined by the inhibition of NPY-induced [Ca^{2+}] increase in LMtk$^-$ cells expressing the human Y5 receptor.
[c] Compounds (10 mg/kg) were orally administered to rats ($n = 3$), and the plasma levels were measured at 2 and 4 h.
[d] Compounds (10 mg/kg) were orally administered to rats ($n = 3$), and the plasma and brain levels were measured at 2 h. B/P means the ratio of the brain level (nmol/g) to plasma level (μM) of the compounds.
[e] Not detected.

prepared and its Y5 affinity was compared with that of **2k**. The 1-methylbenzimidazole derivative **14** almost completely lost its potency, suggesting that the N–H group plays a key role in the binding interaction between **14** and the Y5 receptor.

The above SAR study demonstrated that steric factors in the substituents at the 5-position or at both the 5- and 6-positions of the benzimidazole core ring were important for high Y5 binding affinity. However, if there is a high lipophilicity associated with compounds (clog$D_{7.4}$: >5),[10] this may worsen the aqueous solubility of compounds and influence their ADME aspects, which can result in problems during the drug development phase.[11] Therefore, we focused further modification on reducing the lipophilicity of compounds, while the compounds are retaining high Y5 potency (Table 2).

Incorporation of a methoxycarbonyl group (**2n**) into the benzimidazole core ring was found to be well tolerated (IC_{50}: 7.7 nM), and this group clearly contributed to reducing the lipophilicity (clog$D_{7.4}$: **2n** = 4.1). In addition, as shown in Table 3, it is noteworthy that an intravenous administration of **2n** to rats revealed a fairly good brain/plasma ratio (B/P = 0.55). This result prompted us to further replace the methyl ester with ester bioisosters such as 1,3,4-oxadiazole and 1,2,4-oxadiazole moieties, because ester bioisosters are thought to be metabolically more stable than the ester moiety.[10] In addition, theoretically, incorporation of these heteroaryl groups should not only reduce the lipophilicity but also increase the aqueous solubility. As expected, compounds bearing a 5-methyl-1,3,4-oxadiazole (**2r**), 5-methyl-1,2,4-oxadiazole (**2s**), or 2-methyltetrazole (**2u**) retained the potent Y5 binding affinity, and had better metabolic stability than **2n** in the rat hepatocyte assay.[12] In addition, these compounds were more likely to be hydrophilic than **2n** (clog$D_{7.4}$: **2r** = 3.3, **2s** = 3.7, **2u** = 3.6, and **2n** = 4.1). Comparison of the aqueous solubility of the compounds (**2r** and **2u**) with that of the representative lipophilic compounds (**2l** and **2n**) in 0.2 M phosphate buffer (pH 6.8) indicated that the heteroaryl groups contributed to the aqueous solubility of these compounds (**2r**: 0.94 μg/mL, **2u**: 5.9 μg/mL, **2l**: <0.01 μg/mL, and **2n**: 0.60 μg/mL).

To determine the importance of the *trans*-configuration of this class of compounds in terms of the Y5 binding affinity, the *cis*-isomers (**15** and **16**) of the potent compounds (**2o** and **2r**) were evaluated as representatives of this class. As expected, these had far less Y5 binding affinity, which provides some conformational insight into the binding interaction of this class of compounds and the Y5 receptor.

As seen in Table 4, further characterization of the representative benzimidazoles (**2r**, **2s**, and **2u**) was carried out. In the binding assay, these compounds showed greater than 1000-fold subtype selectivity over the other NPY receptors (Y1, Y2, and Y4). Moreover, these exhibited potent antagonist activity by inhibiting NPY-induced [Ca^{2+}] increase in LKtk$^-$ cells that expressed the human Y5 receptor. These results suggested that compounds (**2r**, **2s**, and **2u**) are potent and subtype-selective Y5 receptor antagonists. To examine the oral absorbability and brain penetrability, these compounds were orally administered to rats at 10 mg/kg dosage. Plasma levels were then measured at 2 and 4 h after dosing, and the brain penetrability (B/P) was also examined at 2 h after oral administration. Even at 4 h after oral administration, compounds (**2r** and **2u**) showed moderate plasma exposures (~1 μM), suggesting that these were indeed orally bioavailable Y5 antagonists in rats. With regard to the brain penetrability, these compounds showed marginal brain penetrability unlike that seen in the case of **2n** (B/P = 0.55). The marginal brain exposures of the compounds (**2r** and **2u**) suggested that these would not show *in vivo* efficacy in rats (10 mg/kg po).[13]

In conclusion, structure–activity relationship studies were performed in a class of novel benzimidazoles with the aim of identifying potent, selective, and orally bioavailable NPY Y5 receptor antagonists, leading to the discovery of the two potent compounds (**2r** and **2u**). These results were achieved by incorporating a heteroaromatic ring into the 6-position of the benzimidazole core part. At the present time, further modifications of this class that focus on improving brain penetrability are underway to identify in vivo potent compounds.

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