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Identification of a novel and orally available benzimidazole derivative as an NPY Y5 receptor antagonist with in vivo efficacy

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ABSTRACT

Optimization of lead compound **2** is described, mainly focusing on modification at the C-2 position of the benzimidazole core. Replacement of the phenyl linker of **2** with saturated rings resulted in identification of compound **8b** which combines high Y5 receptor binding affinity with a good ADME profile leading to in vivo efficacy.

Obesity is associated with several comorbidities, including hypertension, type 2 diabetes (DM), osteoarthritis, dyslipidemia, obstructive sleep apnea, and some cancers. Many of these diseases can be prevented or ameliorated by a reduction in body weight. However, diet and exercise strategies alone, although successful in the short term, are difficult to maintain in the long term for the majority of patients. Given these limitations in achieving weight control, medications and alternative treatment options have been sought.[1]

Neuropeptide Y (NPY) is a 36-amino acid peptide[2] which is widely distributed in the central[3–5] and peripheral nervous systems.[6–8] The biological effects of NPY are mediated through its interaction with five G-protein coupled receptors (Y1, Y2, Y4, Y5 and Y6).[9] Among them, the Y5 receptor is thought to play a key role in the central regulation of food intake and energy balance.[10–13] Recently, two Y5 antagonists, MK-0577 and Velneperit in Figure 1, have advanced to clinical trials. While clinical data of MK-0577 showed modest efficacy,[14] Velneperit was proved to be an agent that have a statistically significant effect on weight loss.[15] Therefore, antagonism of the Y5 receptor represents an attractive target for potential therapeutic application against obesity.

Recently, we reported the optimization of HTS hit **1** and the discovery of novel NPY Y5 receptor antagonists **3a**–**c** bearing the pyridone moiety as their linkers and having high binding affinity and attractive in vitro profiles with respect to CYP450 inhibition, solubility and metabolic stabilities (Fig. 1).[16] Despite these attractive properties, pyridone analogues exhibited only little absorption after oral administration.[17] We thought that replacing the aromatic linker with corresponding saturated rings, such as pyrrolidine, piperidine, piperazine, morpholine, tetrahydropyran and lactam, might lead us to structurally novel series of compounds without impairing their high binding affinity and attractive in vitro ADME profiles (Fig. 1). Herein we report our efforts to identify a novel and orally available Y5 antagonist endowed with in vivo efficacy.

Representative synthetic routes for derivatives **4**–**8** are shown in Scheme 1. 2-Bromobenzimidazole **12** and phenylenediamine **14** were prepared according to the preceding report.[16] 2-Bromobenzimidazole **12** was coupled with various secondary amines **13** to afford derivatives **4**–**8a**. To evaluate the Structure–Activity Relationship (SAR) focused on R^1 and R^2 groups of **8**, phenylenediamines **14** and **16** were treated with CDI, followed by chlorination in $POCl_3$ to give 2-chlorobenzimidazoles **15** and **17**, respectively. Next, **15** and **17** were coupled with **13** (Y = O) to afford derivatives **8b**, **8f** and **8i**–**l**. To explore the other R^1 groups, 2-chlorobenzimidazole **18** was coupled with **13** (Y = O), then various alkylthio (R^1S) groups were introduced at the C-5 position of the benzimidazole core using a coupling reaction,[18] followed by oxidation to the corresponding alkylsulfonyl (R^1SO_2) groups. As shown in Scheme 2, the synthesis of **9a** commenced from the Prins reaction.[19] The

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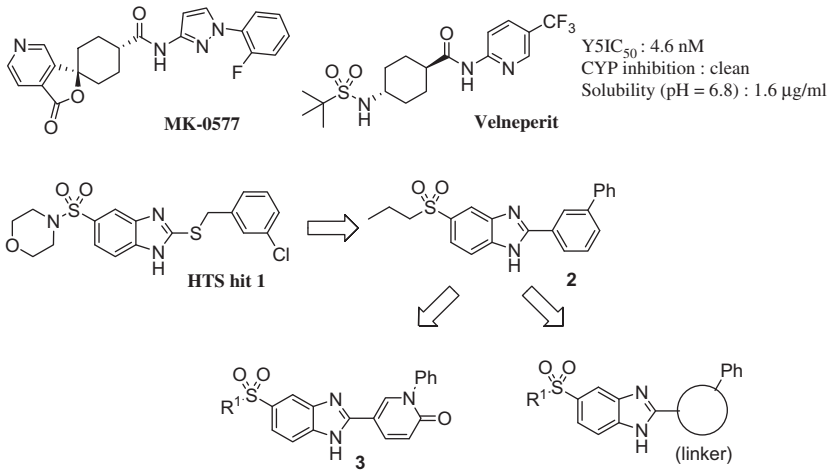
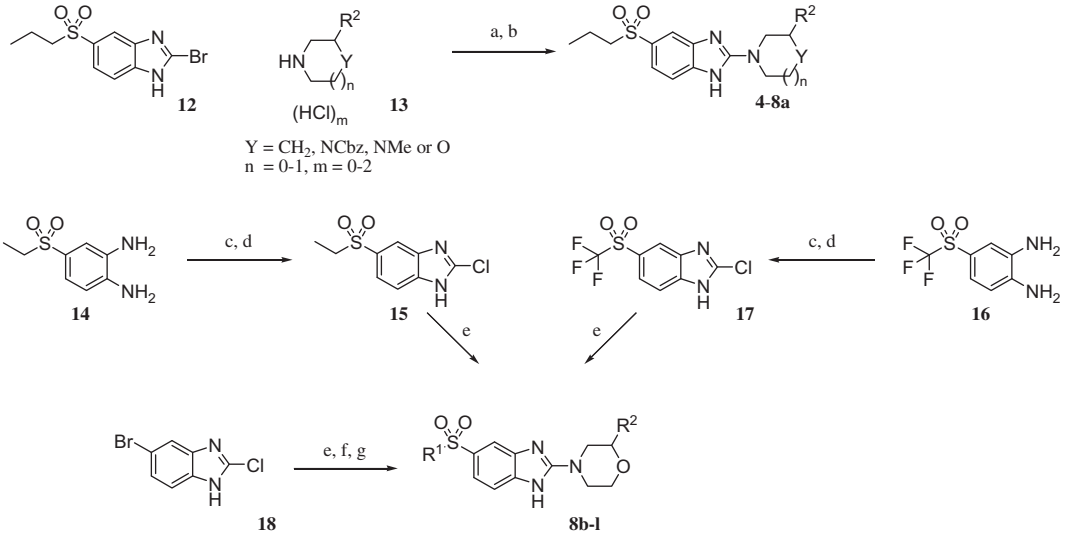


compds	Y5 IC_{50} (nM)[a]	CYP450 inhibition (μM) 1A2 / 2C19 / 2C9 / 2D6 / 3A4	Solubility (μM)[b]	hMs / rMs (%)[c]
1	19.8	>20 / 0.4 / 1.5 / 11 / 1.9	8.9	6.7 / 0.19
2	0.43	2.7 / 19 / 9.5 / >20 / 6.0	0.50	68.6 / 57.1
3a (R^1=*n*-Pr)	1.93	All > 20	43.3	88.5 / 78.6
3b (R^1=*t*-Bu)	7.50	All > 20	9.4	94.2 / >99.9
3c (R^1=CF_3CH_2)	0.29	All > 20	7.7	94.7 / 93.6

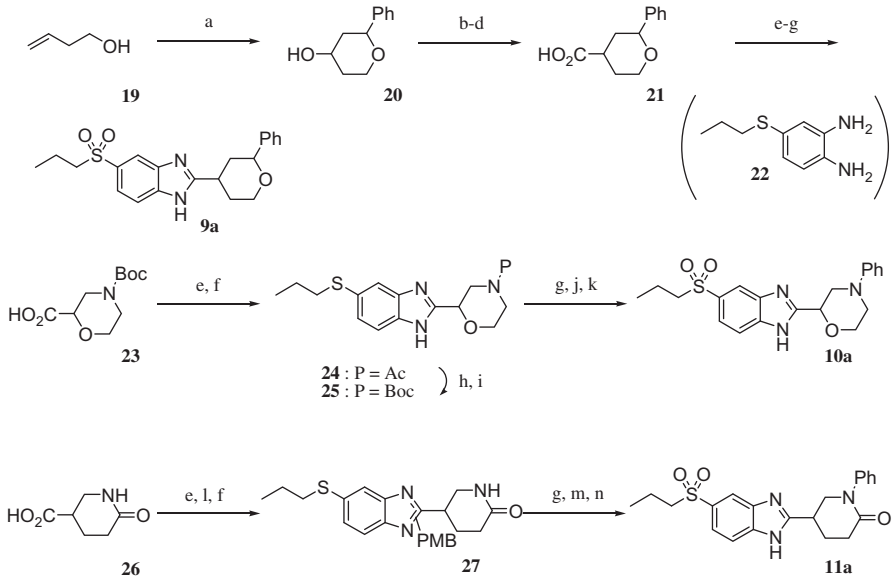
[a] Concentration of the compound that inhibited 50% of total specific binding of ^{125}I-PYY as a ligand to mouse NPY Y5 receptors; obtained from the mean value of two or more independent assays.
[b] Solubility was measured as kinetic solubility using 1% DMSO solution at pH 6.8.
[c] Metabolic stability in human or rat liver microsomes was measured as the percentage of the compound remaining after 30 min incubation.

Figure 1. Structures of MK-0577, Velneperit and benzimidazole derivatives.



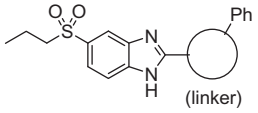
Scheme 1. Representative synthetic routes for compounds **4**–**8**. Reagents and conditions: (a) K_2CO_3, CH_3CN, 120 °C (microwave); (b) Y = NCbz: Pd/C, H_2, MeOH, rt; (c) CDI, THF, rt; (d) $POCl_3$, 100 °C; (e) **13**, K_2CO_3, CH_3CN, 120 °C (microwave); (f) R^1SH, Hunig's base, $Pd_2(dba)_3$, xantphos, dioxane, 130 °C (microwave); (g) *m*-CPBA, CH_2Cl_2, 0 °C to rt.

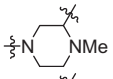
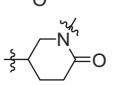
resulting alcohol **20** was mesylated and subsequently substituted with cyanide, followed by hydrolysis of the resulting cyano group under basic condition to afford **21**. Carboxylic acid **21** was treated with phenylenediamine **22** in the presence of HATU and Et_3N to produce a mixture of regioisomeric amides. The amides were subsequently cyclized in acetic acid to construct the benzimidazole core, followed by oxidation of the *n*-propylthio group with *m*-CPBA to provide **9a**. As for the synthesis of **10a**, a similar route to **9a** resulted in *N*-acetyl morpholine **24**. Hydrolysis of the acetyl group and Boc-protection of the resulting amine were conducted to ob-



Scheme 2. Representative synthetic routes for compounds **9**–**11**. Reagents and conditions: (a) PhCHO, conc.H_2SO_4, H_2O, 0 °C to rt; (b) MsCl, Et_3N, THF, 0 °C; (c) NaCN, TBNI, DMF, 100 °C; (d) 28% NaOMe in MeOH, 100 °C then H_2O; (e) **22**, HATU, Et_3N, DMF, 0 °C to rt; (f) AcOH, 80 °C to 150 °C (microwave); (g) *m*-CPBA, CH_2Cl_2, 0 °C to rt; (h) 28% NaOMe in MeOH, 100 °C; (i) Boc_2O, CH_2Cl_2, rt; (j) TFA, CH_2Cl_2, rt; (k) PhI, $Pd_2(dba)_2$, RuPhos, *t*-BuOK, dioxane, 100 °C; (l) *p*-Methoxybenzaldehyde, $NaBH(OAc)_3$, AcOH, rt; (m) PhI, CuI, *N*,*N*-dimethylglycine, K_2CO_3, DMA, 170 °C (microwave); (n) TFA, 110 °C (microwave).

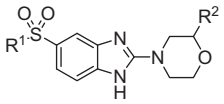
Table 1
IC_{50} values, CYP450 inhibition profiles, solubilities and metabolic stabilities of compounds **2** and **4**–**11a**



compds	linker	Y5 IC50[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)	hMs/rMs[c] (%)
2		0.43	2.7/19/9.5/>20/6.0	0.50	68.6/57.1
4a		1.01	>20/>20/17/>20/14	15.4	74.9/19.2
5a		0.29	>20/12/9.4/13/6.9	19.1	45.2/2.2
6a		6.82	All >20	>50	64.4/44.0
7a		4.99	All >20	>50	21.9/21.1
8a		0.6	>20/19/>20/>20/>20	>50	77.9/45.9
9a[d]		17.2	>20/20/>20/>20/>20	>50	65.0/74.0
10a		15.9	All >20	>50	66.1/7.5
11a		193	All >20	>50	81.1/62.0

[a] Concentration of the compound that inhibited 50% of total specific binding of ^{125}I-PYY as a ligand to mouse NPY Y5 receptors; obtained from the mean value of two or more independent assays.
[b] Solubility was measured as kinetic solubility using 1% DMSO solution at pH 6.8.
[c] Metabolic stability in human or rat liver microsomes was measured as the percentage of the compound remaining after 30 min incubation.
[d] *Cis*/*trans* mixture.

Table 2
IC_{50} values, CYP450 inhibition profiles, solubility and metabolic stabilities of compounds **8a–l**



compds	R1	R2	Y5 IC50[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)	hMs/rMs[c] (%)
8a	*n*-Pr	Ph	0.60	>20/19/>20/>20/>20	>50	77.9/45.9
8b	Et	Ph	0.43	All >20	>50	89.0/76.1
8c	*i*-Pr	Ph	1.33	15/19/>20/>20/16	>50	87.0/49.5
8d	*t*-Bu	Ph	4.27	>20/>20/>20/>20/14	10.7	78.5/56.4
8e	*i*-Bu	Ph	0.28	>20/>20/17/>20/9.3	43.2	55.1/2.4
8f	CF_3	Ph	0.73	17/1.7/4.5/5.5/2.3	3.7	81.7/67.9
8g	CH_2CF_3	Ph	0.21	>20/>20/>20/>20/15	>50	85.0/63.5
8h	$C(CH_3)_2CF_3$	Ph	1.43	18/17/11/18/8.2	11.6	60.2/37.8
8i	Et	4-F-Ph	1.05	All >20	>50	92.4/87.1
8j	Et	4-Cl-Ph	0.85	9.2/9.1/>20/18/13	38.1	94.6/87.2
8k	Et	4-Me-Ph	0.80	>20/17/>20/>20/>20	21.6	87.8/34.0
8l	Et	4-OMe-Ph	3.03	All >20	>50	81.0/48.0

[a, b, c] See Table 1.

tain **25**. Oxidation of the *n*-propylthio group of **25** with *m*-CPBA, followed by acidic deprotection of the Boc-group and introduction of an outer phenyl group using the Buchwald–Hartwig reaction provided **10a**. The synthesis of **11a** commenced from condensation of **26** with **22**, followed by protection with a *p*-methoxybenzyl (PMB) group, and cyclization to produce a mixture of 1- and 3-PMB protected benzimidazoles **27**. Oxidation of the *n*-propylthio group with *m*-CPBA, followed by introduction of an outer phenyl group using the Ullmann reaction[20] and then removal of the PMB group provided **11a**.

In the course of our program, the hit to lead SAR study generated biphenyl derivative **2** as a lead compound which showed attractive Y5 receptor binding affinity, but possessed CYP450 inhibition profiles and low solubility. While our previous efforts to improve the issues related to **2** led us to identify the attractive pyridone analogues **3**, the oral absorption of **3** was insufficient. Although the reason was unclear, we tried to find a second-generation derivative with in vitro profiles comparable to **3** but having improved oral absorption. Our strategy was to replace the aromatic linker of **2** with several saturated rings to reduce structural planarity and change the ADME profiles (Fig. 1).[21–23]

As shown in Table 1, almost all of saturated derivatives showed moderate to appreciable improvement in the CYP450 inhibition profiles and the solubility. Piperidine **5a** exhibited sub-nanomolar binding affinity, which was equipotent to **2**. Pyrrolidine **4a** was threefold less potent than **5a**. These results suggest that the right-hand part, which is similar in shape to the biphenyl moiety of **2**, is important for the binding affinity. While **5a** afforded the best potency, its metabolic stabilities in human and rat liver microsomes were unacceptable. The metabolic instablility of **5a** may arise from the aliphatic carbon around the benzylic position. This hypothesis prompted us to incorporate heteroatoms into the piperidine moiety, which provided piperazine **6–7a** and morpholine **8a**. Among them, derivatives **6a** and **8a** showed improvement in metabolic stabilities. While piperazine **6a** exhibited a 20-fold decrease in the binding affinity, morpholine **8a** was equipotent to piperidine **5a**. On the other hand, replacement of the central phenyl linker of **2** by tetrahydropyran, regioisomeric morpholine or a lactam moiety resulted in a decrease of the Y5 receptor binding affinity (**9–11a**). These results combined with the SAR study of pyridone analogues **3** suggest that the sp^3 hybridized carbon atoms, which directly bonded with the C-2 position of benzimidazole core, have a negative influence on the binding affinity.

To further explore the potential of morpholine **8a**, we investigated some other compounds (Table 2). Initial investigations were focused on the left-hand part of **8a**, namely alkyl substitutions on the sulfonyl group (R^1SO_2). The replacement of *n*-$PrSO_2$ with $EtSO_2$ led to equipotent compound **8b** with further improved metabolic stabilities. The binding affinity decreased as the bulkiness of the α-position of the alkylsulfonyl group become larger (**8b–d**). In contrast, the bulkiness of the β-position of the alkylsulfonyl group showed a positive effect on the binding affinity (**8e** and **8g**). While **8g** showed high Y5 binding affinity with appreciable CYP450 profiles and high solubility, the $CF_3CH_2SO_2$ moiety was found to be easily hydrolyzed under basic condition due to the strong acidity of the α-proton (Fig. 2).[24] An attempt to improve the physicochemical stability of **8g** by hindering the α-position of $CF_3CH_2SO_2$ group worsened the CYP450 inhibition profiles and the solubility (**8h**). The influence of various substituents on the right-hand phenyl ring of **8b** was also investigated (Table 2). While all substituents, such as fluoro, chloro, methyl and methoxy, on the outer phenyl ring led to retaining of Y5 binding affinities, **8j** showed CYP450 inhibition potential and **8k–l** were metabolically unstable in rat liver microsomes.

On the basis of these results, morpholine **8b** was selected for further investigation. An in vivo cassette study in rats for **8b** (0.5 mg/kg iv, 1.0 mg/kg po) was conducted to examine the oral absorption[25] and showed acceptable plasma exposure with moderate blood clearance (C_{max} = 64.2 ng/ml, CL_{tot} = 9.9 ml/min/kg), indicating some effect of the saturated linker on the PK profiles. To

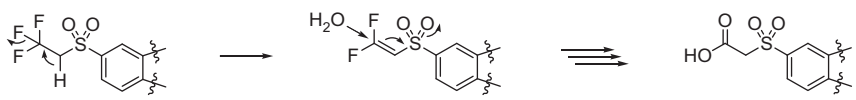


Figure 2. Mechanism for decomposition of **8g**.

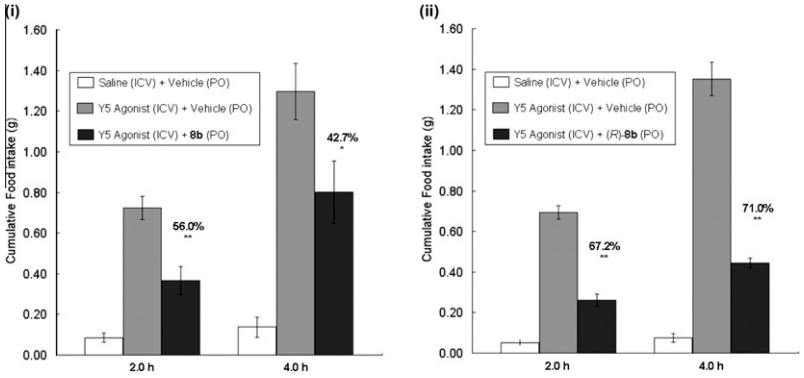
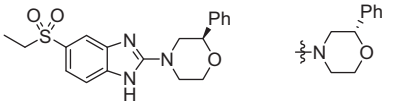


Figure 3. (i) Effect of **8b** (12.5 mg/kg) on Y5 agonist-stimulated food intake in diet-induced obese mice. (ii) Effect of (*R*)-**8b** (12.5 mg/kg) on Y5 agonist-stimulated food intake in diet-induced obese mice. $^{**}p$ >0.01 versus Y5 agonist and vehicle treated group. $n = 2$.



(*R*)-**8b** : 0.31 nM (*S*)-**8b** : 4.76 nM

Figure 4. Effect of chirality.

evaluate in vivo efficacy, compound **8b** (12.5 mg/kg) was orally administered 1 h before the mice were treated with Y5 selective agonist[26] (0.1 nmol icv) and cumulative food intake was measured for the following 4 h. As shown in Figure 3, compound **8b** blocked the increase in food intake in this feeding model. This result prompted us to verify the effect of the stereochemistry at the C-2 position of morpholine for in vivo efficacy. Thus, (*R*)-**8b** and (*S*)-**8b** were prepared separately[27] and their Y5 receptor binding affinities were investigated. Figure 4 reveals a strong preference for (*R*)-**8b**, which was 10-fold more potent than the corresponding (*S*)-isomer. Although not directly compared, the in vivo efficacy of (*R*)-**8b** was also tested and resulted in enhanced efficacy, correlating with its high binding affinity (Fig. 3 and 4).

In summary, replacement of the phenyl linker of lead compound **2** with corresponding saturated linkers resulted in several potent derivatives. Among them, morpholine **8b** showed in vivo efficacy in the agonist-induced food intake model. Extensive SAR exploration revealed a preference for the (*R*)-isomer. At present, further investigation of this compound is underway.

Acknowledgments

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17. To further characterize the pyridone analogues **3a**–**c**, in vivo cassette studies (0.5 mg/kg iv, 1.0 mg/kg po) were conducted. Unfortunately, these compounds exhibited low plasma levels with high clearance in spite of their acceptable solubility and high metabolic stability in rat liver microsomes. The poor correlation between the metabolic stability in liver microsomes and the in vivo clearance suggest that extrahepatic clearance routes may have been operating.
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25. The rats ($n = 2$) were dosed at 0.5 mg/kg iv and 1.0 mg/kg po.
26. [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]-human pancreatic polypeptide.
27. Zindell, R.; Riether, D.; Bosanac, T.; Berry, A.; Gemkow, M. J.; Ebneth, A.; Löbbe, S.; Raymond, E. L.; Thome, D.; Shih, D. T.; Thomson, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1604.