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Identification of a novel benzimidazole derivative as a highly potent NPY Y5 receptor antagonist with an anti-obesity profile

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ABSTRACT

Optimization of HTS hit **1** for NPY Y5 receptor binding affinity, CYP450 inhibition, solubility and metabolic stability led to the identification of some orally available oxygen-linker derivatives for in vivo study. Among them, derivative **4i** inhibited food intake induced by the NPY Y5 selective agonist, and chronic oral administration of **4i** in DIO mice caused a dose-dependent reduction of body weight gain.

Neuropeptide Y (NPY) is a 36-amino acid peptide[1] which is widely distributed in the central[2–4] and peripheral nervous systems.[5–7] The biological effects of NPY are mediated through its interaction with five G-protein coupled receptors (Y1, Y2, Y4, Y5 and Y6).[8] Among them, the Y5 receptor is thought to play a key role in the central regulation of food intake and energy balance,[9–12] suggesting the possibility of NPY Y5 antagonists being effective as anti-obesity drugs. Recently, two NPY Y5 antagonists, MK-0577 and Velneperit in Figure 1, showed modest but significant suppression of body weight gain in human clinical trials.[13,14]

Previously, we carried out optimization of HTS hit **1**, mainly focused on modification at the C-2 position of the benzimidazole core. Elimination of the flexible and metabolically liable –S–CH_2– part and utilization of pyridone and morpholine rings led to identification of novel NPY Y5 receptor antagonists **2b** and **2c**, respectively.[15] Although pyridone **2b** exhibited high affinity at the NPY Y5 receptor with attractive in vitro ADME profiles, it suffered from poor bioavailability. While morpholine **2c** was orally active for suppression of food intake induced by a NPY Y5 selective agonist, its chronic oral administration (25 mg/kg bid) to diet-induced obese (DIO) mice did not cause reduction of body weight gain. With regard to brain penetration, morpholine **2c** showed marginal brain exposure presumably due to the presence of a guanidine-like substructure, which would have a negative effect on in vivo efficacy.[16] Lipophilicity is known to be an important parameter governing brain penetration and a guanidine-like substructure show the lowest *C*Log*P* value (Fig. 2).[17] Therefore, we sought to eliminate this liability by replacing the nitrogen-linker with a sulfur-, oxygen- or carbon-linker. In this letter, we describe the design and identification of a novel and orally available NPY Y5 receptor antagonist, which has significant anti-obesity effects in DIO mice

The syntheses of benzimidazole derivatives **3a–d** and **4a–k** bearing a sulfur- or oxygen-linker at the C-2 position are shown in Scheme 1 together with their intermediates. These derivatives were synthesized through the coupling of thiol or alcohols with 2-chlorobenzimidazole **10**, followed by removal of SEM-protection. To prepare a mixture of 1- and 3-SEM-protected 2-chlorobenzimidazole **10**, phenylenediamine **9** was treated with CDI, followed by chlorination using $POCl_3$ and SEM-protection. To obtain alcohol **13**, ester **12** was reduced using $NaBH_4$. The synthetic routes of derivative **5**–**8a** are shown in Scheme 2. Carboxylic acid **14** was made to react with **9** in the presence of HATU and Et_3N to produce a mixture of regioisomeric amides. The resulting amides were subsequently cyclized in acetic acid to obtain **5a**. The benzyl position of **5a** was oxidized with SeO_2[18] to afford **6a**, which was followed by deoxofluorination using deoxofluor to give **7a**. As for the synthesis of **8a**, carboxylic acid **16** was prepared through phase

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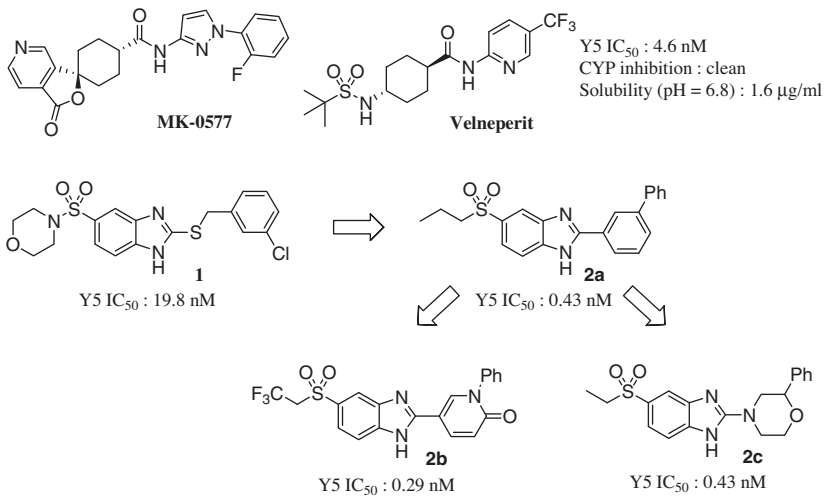


Figure 1. Structures of velneperit, MK-0577, HTS hit **1** and compound **2a–c**.

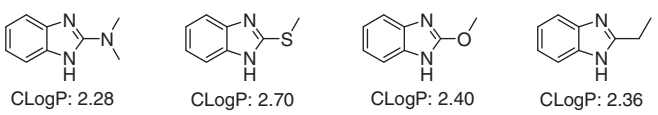
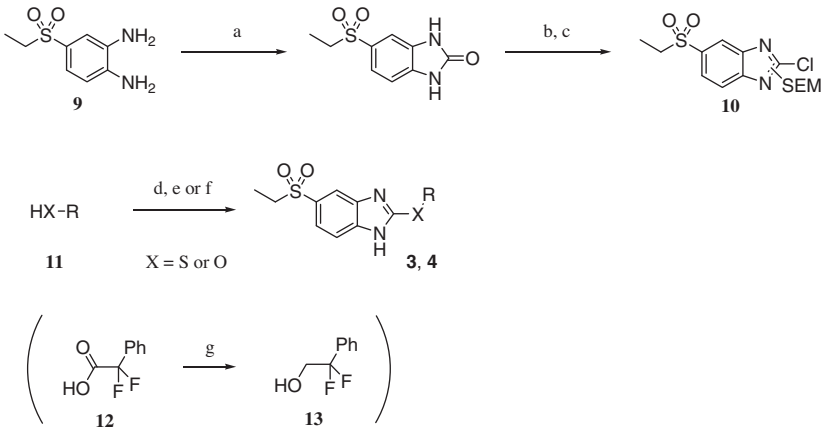


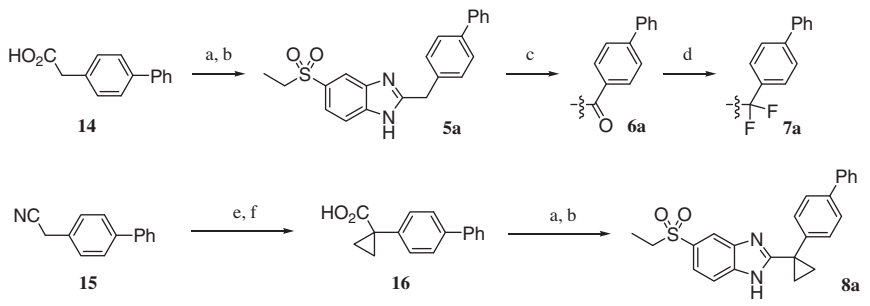
Figure 2. Lipophilicity.

transfer cycloalkylation with 1,2-dibromoethane[19] and hydrolysis of the nitrile group. Derivative **8a** was prepared in a similar manner to **5a**.

On the basis of findings from our previous work,[15] the initial structure–activity relationship (SAR) study was conducted by replacement of the sulfonamide moiety of HTS hit **1** with an

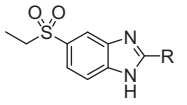


Scheme 1. Syntheses of **3** and **4**. Reagents and conditions: (a) CDI, DMF, rt; (b) $POCl_3$, 120 °C; (c) SEMCl, NaH, DMF, rt; (d) **10**, Cs_2CO_3 or NaH, DMF, 0–50 °C; (e) TBAF, THF, 70 °C; (f) TFA, CH_2Cl_2, rt, then MeOH; (g) $NaBH_4$, THF, rt.

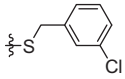
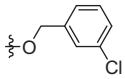


Scheme 2. Syntheses of benzimidazole derivatives **5–8a**.Reagents and conditions: (a) **9**, HATU, Et_3N, DMF, 0 °C to rt; (b) AcOH, reflux; (c) SeO_2, dioxane, 80 °C; (d) deoxofluor, THF, 60 °C; (e) 1,2-dibromoetane, $BnNEt_3Cl$, 12 M NaOH aq, THF, rt; (f) concd HCl, AcOH, 100 °C.

Table 1
IC_{50} values, CYP450 inhibition profiles, solubility and metabolic stabilities of compounds **1** and **3a–d**

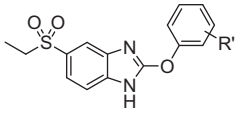


3

Compds	R	Y5 IC_{50}[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)	hMs/rMs[c] (%)
1	—	19.8	>20/0.4/1.5/11/1.9	8.9	6.7/0.19
3a		12.1	8.9/9.4/12/18/8.8	27.5	63.7/0.13
3b		80.4	8.9/>20/>20/>20/16	49.9	88.5/37.9
3c		25.0	14/>20/>20/>20/>20	>50.0	88.1/37.6
3d		4.39	14/17/>20/>20/>20	>50.0	97.9/83.8

[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 compound remaining after 30 min incubation.

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

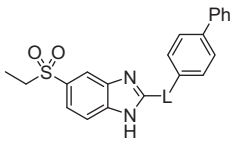


4

Compds	R′	Y5 IC_{50}[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)	hMs/rMs[c] (%)
4a	*ortho*-CF_3	3190	>20/>20/>20/0.4/>20	>50	94.1/>99.9
4b	*meta*-CF_3	41.2	>20/>20/>20/0.4/> 20	>50	>99.9/88.4
4c	*para*-CF_3	98.1	All >20	>50	95.2/86.9
4d	*meta*-OCF_3	7.11	All >20	>50	97.1/86.2
4e	*para*-OCF_3	22.4	>20/>20/>20/>20/17	>50	92.8/91.6
4f	*meta*-Ph	9.55	8.3/12/8.4/15/13	4.2	29.1/77.3
4g	*para*-Ph	2.82	11/15/6.9/>20/7.7	2.5	>99.9/>99.9

[a,b,c] See Table 1.

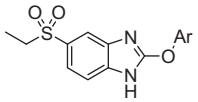
Table 3
IC_{50} values, CYP450 inhibition profiles, solubility and metabolic stabilities of compounds **4g** and **5–8a**



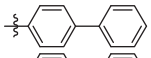
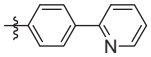
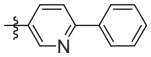
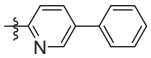
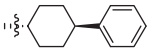
Compds	L	Y5 IC_{50}[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)	hMs/rMs[c] (%)
4g		2.82	11/15/6.9/>20/7.7	2.5	>99.9/>99.9
5a		112	>20/10/14/3.5/10	3.0	87.6/74.3
6a		2.61	All >20	0.3	>99.9/>99.9
7a		48.6	10/8.4/2.7/4.1/10	0.3	0.10/78.3
8a		608	11/9.4/3.2/1.2/5.4	0.5	88.5/81.3

[a,b,c] See Table 1.

Table 4
IC_{50} values, CYP450 inhibition profiles, solubility and metabolic stabilities of compounds **4g–k**



4

Compds	Ar	Y5 IC_{50}[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)	hMs/rMs[c] (%)
4g		2.82	11/15/6.9/>20/7.7	2.5	>99.9/>99.9
4h		4.07	>20/>20/17/>20/>20	>50	99.1/99.3
4i		2.92	17/>20/15/>20/11	39.7	98.6/>99.9
4j		96.4	15/>20/>20/>20/11	45.2	89.2/>99.9
4k		3.75	>20/17/7.3/>20/2.7	6.1	>99.9/93.8

[a,b,c] See Table 1.

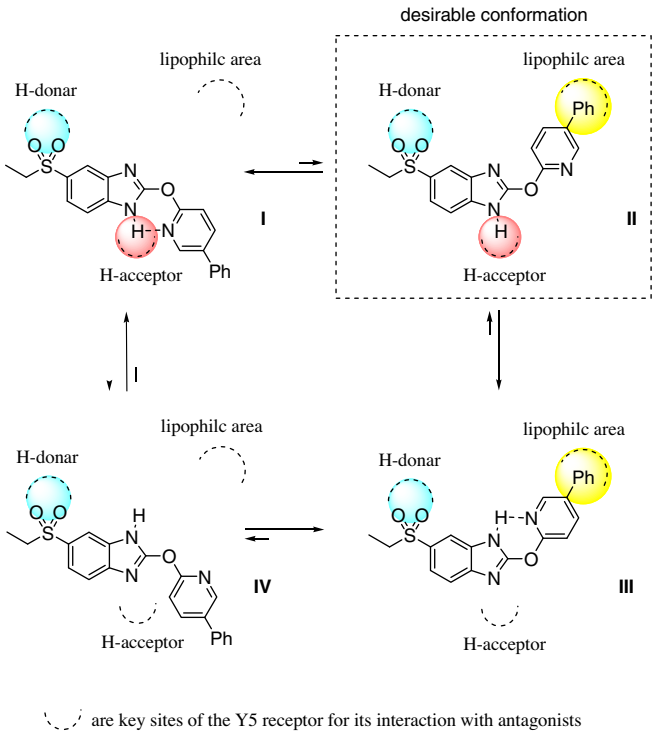


Figure 3. Conformational preference for **4j**.

ethylsulfonyl group. As shown in Table 1, derivative **3a** exhibited improved solubility and metabolic stability in human liver microsomes, however, its metabolic stability in rat liver microsomes was still low. Introduction of an –O–CH_2– linker in place of the –S–CH_2– moiety of **3a** led to improvement of metabolic stabilities in both human and rat liver microsomes to some extent but resulted in reduction of the Y5 receptor binding affinity. To enhance the binding affinity, phenethyl ether was utilized in order to place a terminal phenyl group in a similar position to the highly potent derivative **2c**. Although derivative **3c** had improved binding affinity relative to **3b**, the Y5 IC_{50} value was moderate. We hypothesized that the moderate binding affinity of **3c** could arise from the flexible nature of the –O–CH_2–CH_2– linker. To fix the conformation of

Table 5
Rat PK profile of **3d**, **4d**, **4h** and **4i** (0.5 mg/kg iv, 1.0 mg/kg po)

Compds	CL_{tot} (ml/min/kg)	AUC (ng hr/ml)	C_{max} (ng/ml)	BA[a] (%)	B/P ratio[b]
3d	5.29	2160	166	68.3	0.58
4d	30.7	74.8	17.9	13.5	0.98
4h	2.04	2880	287	33.9	0.04
4i	2.13	3630	320	46.5	0.14

[a] Bioavailability.
[b] Brain/plasma ratio.

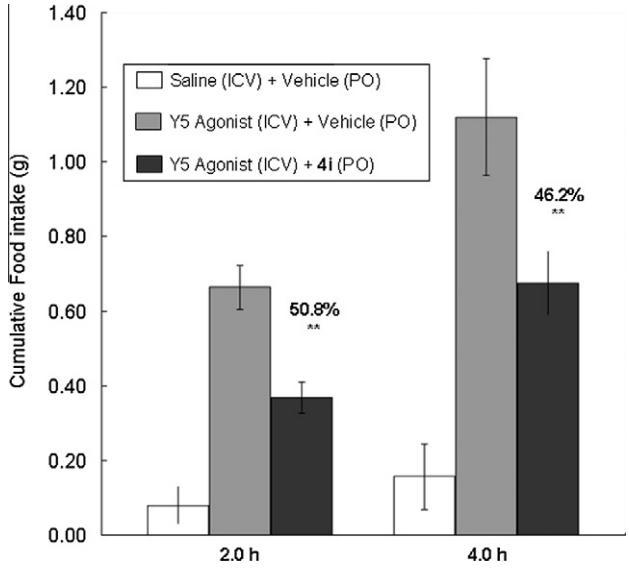


Figure 4. Effect of **4i** (12.5 mg/kg) on Y5 agonist-stimulated food intake in diet-induced obese mice. $^{**}p > 0.01$ versus Y5 agonist and vehicle treated group. $n = 4–7$.

the flexible linker, a gauche effect by introduction of fluorine atoms[20] on the benzylic position was considered, which was also expected to overcome the metabolic liability. As expected, derivative **3d** showed enhanced binding affinity with improved metabolic stabilities.

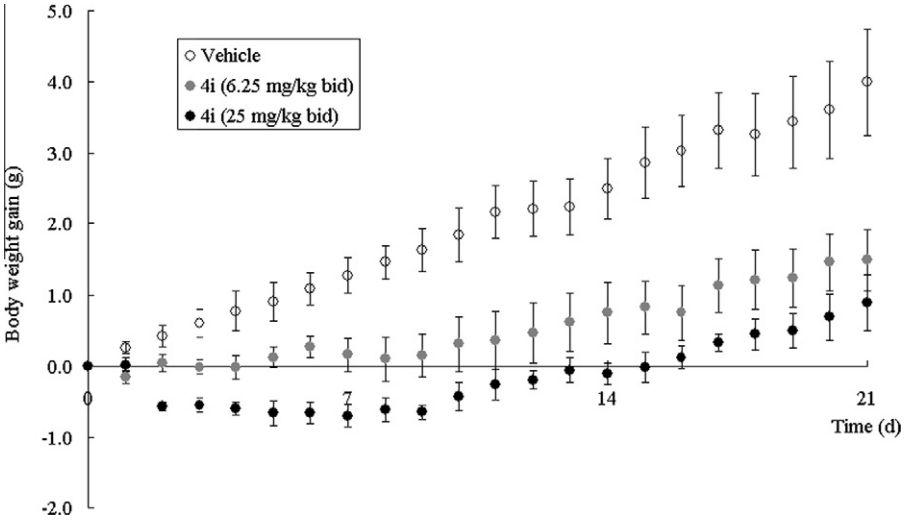


Figure 5. Effect of chronic oral administration of **4i** on body weight gain in diet-induced obese mice. $n = 7$.

As shown in Table 2, the next SAR study was conducted with phenoxy derivatives that seem to be a moderately flexible and useful linker. Positional scanning with the CF_3 group was investigated and *meta*- and *para*-CF_3 derivatives had modest binding affinities. On the basis of this result, additional *meta*- and *para*-substituted derivatives were explored. The most favorable substitution was *para*-phenyl, followed by *meta*-OCF_3. Interestingly, while *para*-phenyl was three-fold more potent than the *meta*- phenyl, *para*-OCF_3 was less potent than *meta*- OCF_3. To further explore the highest potent biphenyl derivative **4g**, the oxygen linker was replaced with a $-CH_2-$, $-CO-$, $-CF_2-$ or cyclopropylene substructure (Table 3). While $-CH_2-$, $-CF_2-$ and cyclopropylene derivatives resulted in significant decreases in the Y5 receptor binding affinity, the carbonyl derivative **6a** retained high binding affinity with improved CYP450 inhibition profiles but suffered from decreased solubility, probably due to its rigid structural nature.

Derivative **4g** was a highly potent Y5 antagonist with appreciable metabolic stabilities, but several issues were identified; it had potent CYP450 inhibition and low solubility which may have been a consequence of its high lipophilicity or rigid nature. This hypothesis prompted us to incorporate a nitrogen atom into the biphenyl region of **4g** to reduce its lipophilicity. As shown in Table 4, all pyridine derivatives exhibited improved CYP450 inhibition profiles and high solubility. While derivative **4h** and **4i** retained Y5 receptor binding affinity, derivative **4j** was not tolerated in the target affinity, probably due to undesirable conformational preference via intramolecular hydrogen bonding between benzimidazole N–H and pyridine nitrogen (**I** and **III** in Fig. 3). Next, we replaced the inner phenyl ring of **4g** with a cyclohexyl substructure to reduce structural planarity and change ADME profiles.[21] Cyclohexyl derivative **4k** maintained high binding affinity and metabolic stabilities, but did not show acceptable improvement in the CYP450 inhibition profiles and solubility.

Through our efforts, some derivatives presented an in vitro profile suitable for progression to in vivo studies (Y5 $IC_{50} < 10$ nM, CYP450 inhibition >10 μM, solubility >10 μM, hMs/rMs > 80%/>80%). In vivo cassette studies in rat for **3d**, **4d**, **4h** and **4i** were conducted and their pharmacokinetic (PK) parameters are shown in Table 5.[22] While derivative **4d** exhibited high brain/plasma (B/P) ratio, the plasma level was low probably due to high clearance. Derivatives **3d**, **4h** and **4i** had acceptable plasma levels with low clearance. Additionally, derivatives **3d** and **4i** had moderate to good B/P ratios.

Derivatives **3d** and **4i** were thus selected for evaluation of in vivo efficacy and tested in a Y5 selective agonist-induced food intake model.[23] While derivative **4i** (12.5 mg/kg po) blocked the increase in food intake in this feeding model (Fig. 4), derivative **3d** (12.5 mg/kg po) was not efficacious in spite of its desirable PK profile. To determine what led to the difference between **3d** and **4i**, the cerebrospinal fluid (CSF) concentrations of these compounds were measured. At 30 min after administration of **3d** (0.5 mg/kg iv) and **4i** (0.5 mg/kg iv), the CSF concentrations were 1.7 ng/ml and 2.9 ng/ml, respectively. This suggested that the CSF concentration has a stronger correlation with in vivo efficacy than the B/P ratio. In addition to the in vivo efficacy stated above, oral administration of **4i** to DIO mice for 21 days caused a dose-dependent reduction that was significantly different from the control group without any abnormal behavior (Fig. 5).

In summary, a series of novel and potent NPY Y5 receptor antagonists were identified by modification of HTS hit **1**. Among them, derivative **4i** exhibited an acceptable PK profile and inhibited food intake induced by the NPY Y5 selective agonist, which resulted in reduction of body weight gain in DIO mice. Further characterization of this compound will be reported in due course.

Acknowledgments

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22. Rats (n = 2) were dosed at 0.5 and 1.0 mg/kg iv and po, respectively.
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