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Design, synthesis and identification of novel benzimidazole derivatives as highly potent NPY Y5 receptor antagonists with attractive in vitro ADME profiles

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ABSTRACT

Optimization of our HTS hit **1**, mainly focused on modification at the C-2 position of the benzimidazole core, is described. Elimination of the flexible and metabolically labile –S-CH_2– part and utilization of less lipophilic pyridone substructure led to identification of novel NPY Y5 receptor antagonists **6**, which have low to sub-nanomolar Y5 receptor binding affinity with improved CYP450 inhibition profiles, good solubilities and high metabolic stabilities.

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 receptor antagonists being effective as anti-obesity drugs. Indeed, two Y5 antagonists, MK-0577 and Velneperit, have advanced to human clinical trials. While the clinical data of MK-0577 showed modest efficacy,[13] Velneperit induced statistically significant weight loss in obese subjects.[14] To explore for novel Y5 antagonists, we conducted an HTS campaign of our compound library and selected structurally diverse compounds.[15] Among them, benzimidazole **1** (Fig. 1) was one of the attractive hit compounds. In our previous SAR studies that led to the identification of Velneperit, we found that the pharmacophore of the Y5 antagonist consisted of two distinctive substructures, the SO_2 moiety and amidic N–H, as shown in Figure 1.[16] Comparison of the structural features of Velneperit and HTS hit **1** indicated that compound **1** would possess a pharmacophore similar to Velneperit and that the terminal lipophilic groups are also important for Y5 receptor binding affinity (Fig. 1). However, while **1** exhibited Y5 antagonistic activity, its profile regarding CYP450 inhibition and metabolic stability needed to be improved. We hypothesized that these issues could arise from oxidatively reactive and flexible –S-CH_2– linker of **1**. On the basis of this hypothesis, we focused on the introduction of metabolically stable and rigid aromatic linkers in place of the –S-CH_2– moiety of **1**. Here we report our efforts to identify novel Y5 antagonists endowed with improved CYP450 inhibition profiles, good solubility and high metabolic stabilities.

Schemes 1–3 show general synthetic methods for the benzimidazole derivatives and their intermediates. Derivatives **2** were synthesized from commercially available Albendazole (**9**), which has a benzimidazole core. The synthesis of derivatives **4**–**8** commenced from amidations of carboxylic acids with phenylenediamines and subsequent cyclocondensation to construct the benzimidazole core. As shown in Scheme 1, Albendazole (**9**) was first treated with *m*-CPBA to oxidize the *n*-propylthio group and then subjected to hydrolysis of the methyl carbamate group, followed by conversion of the resulting amino group to a bromo group using the Sandmeyer reaction, to give 2-bromobenzimidazole **10**. Bromide **10** and boronic acids **11** were coupled using Suzuki cross-coupling to synthesize derivatives **2.** Scheme 2 shows the preparation of the phenylenediamines. To prepare **13**, nucleophilic aromatic substitution of chloride **12** with $EtSO_2Na$[17] was carried out,[18] followed by hydrogenation of the nitro group in the presence of Pd/C. To obtain **15**, the nitro group of **14** was reduced using $Na_2S_2O_4$. Phenylenediamines **16** and **17** were commercially available. As shown in Scheme 3, the synthesis of derivatives **4** commenced from **18**, which were phenylated using Suzuki cross-coupling[19] to give **19**. Carboxylic acids **19** were made to react with phenylenediamine

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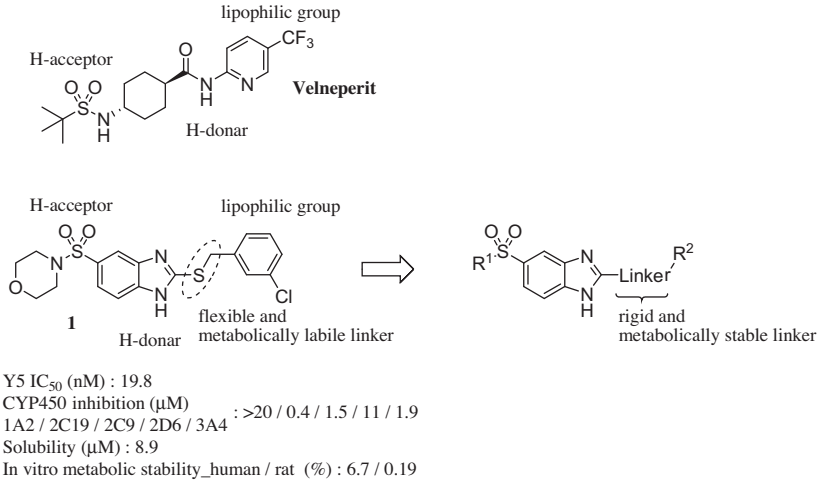
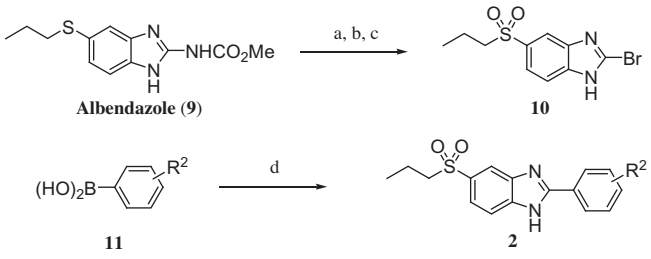


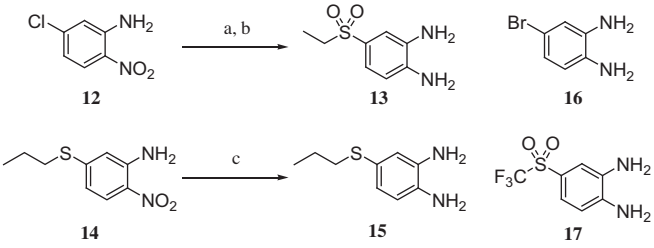
Figure 1. Structures of Velneperit and HTS hit **1** and design of novel derivatives.

13 in the presence of HATU and Et_3N to produce a mixture of regioisomeric amides. The amides were subsequently cyclized in acetic acid to provide derivatives **4**. Pyridine analogues **5** were prepared in a similar manner to **4**. As for syntheses of pyridone analogues **6** and **7**, carboxylic acids **20** were coupled with phenylenediamines (Scheme 1) and then subjected to cyclocondensation in acetic acid, followed by MOM-protection of the resulting benzimidazole N–H, to afford **21**. Next, the chloro group at the C-2 position of the pyridine moiety of **21** was converted into a benzyloxy group. Alkylthio (R^1S) groups at the C-5 position of the benzimidazole core, introduced using cross-coupling reaction,[20] were converted into alkylsulfonyl (R^1SO_2) groups by oxidation with *m*-CPBA. Hydrogenolysis of the benzyl ether was followed by introduction of an additional phenyl group using the Ullmann reaction.[21] Finally, removal of the MOM protecting group under acidic condition provided pyridone analogues **6** and **7**. In the case of analogue **8**, the corresponding carboxylic acid **24** was prepared and a method similar to the above was adopted.

The first approach to resolving the issues of HTS hit **1** was performed by conversion of the –S-CH_2– part to a phenyl linker and introduction of an additional phenyl ring as the R^2 group (Fig. 1). Considering the availability of starting material for the derivative synthesis, Albendazole (**9**) was utilized (Scheme 1), in which the sulfonyl group could be easily furnished at the C-5 position during synthesis and would be equivalent to the sulfonyl moiety of **1**. As shown in Table 1, positional scanning with the phenyl ring was investigated and showed a preference for the *meta*-position. While the *ortho*-phenyl **2b** lost Y5 receptor binding affinity, the *meta*-phenyl **2c** had very high binding affinity, which was 1000-fold more potent than unsubstituted **2a**. The *para*-phenyl **2d** showed single digit nanomolar binding affinity, which was slightly less potent than **2c**. On the basis of these observations, additional *meta*-substituted derivatives were explored and we found that the most favorable substitution was phenyl (**2c**), followed by trifluoromethoxy (**2f**) and trifluoromethyl (**2e**). These results prompted us to verify the pharmacophore of the highly potent derivative **2c** and to investigate the corresponding indole analogues **3a** and **3b** for their Y5 receptor binding affinity (Fig. 2). Interestingly, compound **3a** was approximately 60-fold more potent than **3b**, although both indole analogues had decreased binding affinity relative to **2c**. The moderate potency of **3a** and the loss of potency with **3b** suggest that form **I** would be the preferred binding mode of the possible tautomer, whose N–H is attached at the *para*-position to the sulfonyl group. As for in vitro metabolic stabilities**,** derivative **2c** exhibited improved metabolic stabilities in liver microsomes (human



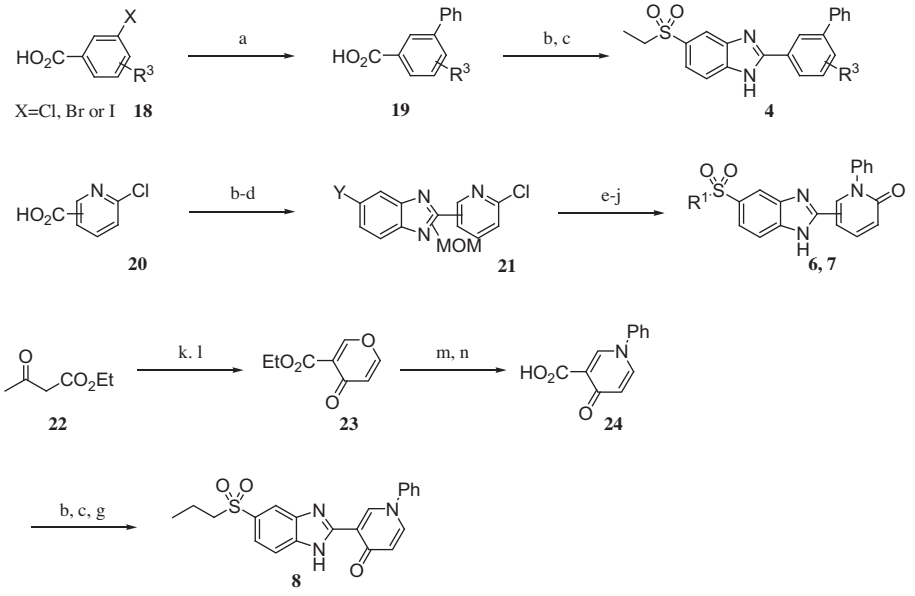
Scheme 1. Representative synthetic routes of analogues **2**. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, 0 °C to rt; (b) 2 N NaOH aq, 85 °C; (c) conc. HCl, $NaNO_2$, CuBr, 60 °C; (d) **10**, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane, H_2O, MW(180 °C).



Scheme 2. Preparation of phenylenediamines **12**–**17**. Reagents and conditions: (a) $EtSO_2Na$, DMSO, 100 °C; (b) Pd/C, H_2, MeOH, rt; (c) $Na_2S_2O_4$, EtOH, H_2O, 60 °C.

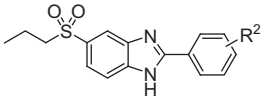
68.6%, rat 57.1%) relative to HTS hit **1** (human 6.7%, rat 0.19%).[22] In this way, derivative **2c** was a highly potent Y5 antagonist with improved metabolic stabilities, however, there was room for improvement in the CYP450 inhibition profiles and the solubility.

Initial efforts to improve the CYP450 inhibition profiles and the solubility of the key compound **2c** led to the introduction of a less lipophilic ethylsulfonyl group in place of an *n*-propylsulfonyl group (Table 2). While little or no change was observed in the CYP450 inhibition profiles and the solubility, compound **2g** retained high affinity for the Y5 receptor along with further improved metabolic stabilities in liver microsomes (human 85.2%, rat 79.1%). Keeping the ethylsulfonyl group constant, we next turned our attention to the introduction of different substituents on the central phenyl ring of **2g** to disrupt molecular planarity and change the physicochemical profile.[23] As shown in Table 2, relatively bulky substituents, such as the chloro and methyl groups, at the 2′- or 4′-position (**4d**, **4e**, **4g** and **4h**) were not tolerated, probably due to prevention



Scheme 3. Representative synthetic routes of analogues **4**, **6**, **7** and **8**. Reagents and conditions: (a) $Pd(dtbpf)Cl_2$, $PhB(OH)_2$, K_2CO_3, DMF or EtOH, H_2O, rt to 60 °C; (b) **13**, **15**, **16** or **17**, HATU, Et_3N, DMF, 0 °C to rt; (c) AcOH, reflux; (d) MOMCl, NaH, DMF, 0 °C; (e) BnOH, NaH, DMF or NMP, 50 to 100 °C; (f) Y = Br: R^1SH, Hunig's base, $Pd_2(dba)_3$, xantphos, dioxane, MW(130 °C); (g) *m*-CPBA, CH_2Cl_2, rt; (h) Pd/C, H_2, THF, MeOH, rt; (i) CuI, *N*,*N*-dimethylglycine, PhBr, K_2CO_3, DMA, MW(170 °C); (j) 2 N HCl in MeOH, 60 °C; (k) 1,1-dimethoxy-*N*,*N*-dimethylmethanamine, dioxane, rt; (l) ethyl formate, *t*-BuOK, THF, 0 °C to rt; (m) $PhNH_2$, AcOH, 60 °C; (n) 2 N NaOH aq, MeOH, rt.

Table 1
IC_{50} values, CYP450 inhibition profiles and solubilities of **2a**–**f**



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Compd	R^2	Y5 IC_{50}[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)
2a	H	4657	2.9/13/9.5/>20/>20	>50
2b	ortho -Ph	4065	>20/>20/4.5/15/11	23.6
2c	*meta*-Ph	0.43	2.7/19/9.5/>20/6.0	0.50
2d	*para*-Ph	6.3	>20/5.9/2.4/15/2.9	0.50
2e	*meta*-CF3	44.5	0.4/17/7.4/>20/20	2.5
2f	*meta*-OCF3	1.9	1.9/10/5.6/>20/10	3.6

[a] Concentration of the compound that inhibited 50% of the 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.

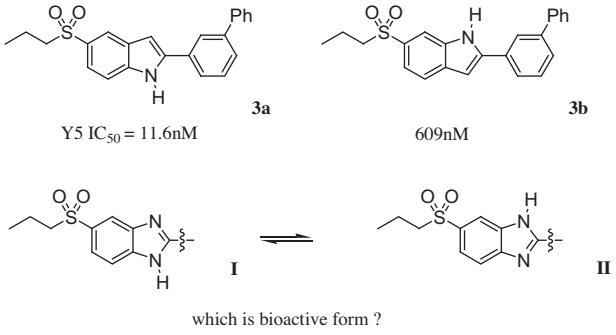


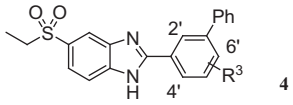
Figure 2. Elucidation of the preferred binding mode.

of the interaction between benzimidazole N–H and the Y5 receptor. In contrast, all substituents at the 6′-position (**4c**, **4f** and **4i**) maintained excellent binding affinity. However, they did not show acceptable improvement in the CYP450 inhibition profiles and the solubility. High lipophilicity due to the biphenyl moiety of derivative **2g** or **4** seemed to be the reason for the CYP450 inhibition and the low solubility.

To reduce the lipophilicity of **2g** ($c\mathrm{Log}P = 4.94$),[24] we sought to incorporate polar functionality into the phenyl linker. One strategy was insertion of a nitrogen atom to afford pyridine analogues. As shown in Table 3, the first example was **5a** ($c\mathrm{Log}P = 3.95$), which unfortunately resulted in significant decreases in the Y5 receptor binding affinity. We could explain the effect of the nitrogen atom in the pyridine linker of **5a** on the binding affinity as follow. Assuming that the bioactive form was the one described as form **IV** in Figure 3 and the pyridine nitrogen indirectly affected the binding affinity by changing the conformational preference, the decreased potency of **5a** would be reasonable. In case of **5a**, the proposed bioactive form **IV** would be unfavorable presumably because of lone pair repulsion between the benzimidazole nitrogen and the pyridine nitrogen, while the bio-inactive form **III** or **V** would be favored owing to the intramolecular hydrogen bond between the benzimidazole N–H and the pyridine nitrogen. To test our hypothesis, other pyridine analogues **5b–d**, which should have no undesirable intramolecular interaction and retain the high binding affinities, were explored. Compounds **5b–d** maintained similar Y5 receptor affinity for **2g** as expected. In addition, the pyridine nitrogen of **5c** or **5d** contributed to solubility presumably because of reduced lipophilicity (**5c** $c\mathrm{Log}P = 3.53$, **5d** $c\mathrm{Log}P = 3.74$). These investigations yielded additional information about the preferred binding mode. However, pyridine analogues did not show acceptable improvement in the CYP450 inhibition profiles.

In an attempt to resolve this issue, we next adopted less lipophilic pyridone rings as linkers. Dramatic improvement of the CYP450 inhibition profiles and the solubility without loss of targeted affinity was exhibited by the pyridone analogue **6a** ($c\mathrm{Log}P = 3.45$). While pyridone analogues **7a** and **8a** also had desirable CYP450 profiles, they showed dramatic loss of the Y5 receptor binding affinity, probably due to a change in the conformational preference, like pyridine analogue **5a**, and prevention of the interaction between benzimidazole N–H and the Y5 receptor, as observed for 4′-substituted biphenyl derivative **4h**.

Table 2
IC_{50} values, CYP450 inhibition profiles and solubilities of **2g** and **4a–i**



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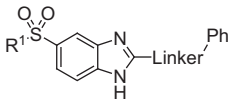
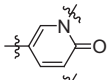
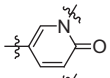
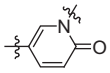
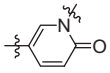
Compd	R^3	Y5 IC_{50}[a] (nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)
2g	H	0.46	0.4/17/9.9/>20/9.0	0.90
4a	2′-F	2.7	1.1/13/7.2/>20/6.6	0.60
4b	4′-F	0.54	2.2/19/17/>20/14	0.60
4c	6′-F	0.16	3.4/17/9.5/16/5.6	0.20
4d	2′-Cl	40.5	3.0/9.3/4.0/18/3.8	1.5
4e	4′-Cl	31.1	0.4/4.9/7.1/15/5.0	0.40
4f	6′-Cl	0.16	8.5/>20/5.4/18/2.6	N.D.
4g	2′-Me	21.8	7.8/14/4.4/>20/5.2	3.2
4h	4′-Me	383	0.4/4.2/6.8/14/5.9	0.50
4i	6′-Me	0.20	3.2/18/8.3/>20/5.7	N.D.

[a,b] See Table 1.

Table 3
IC_{50} values, CYP450 inhibition profiles and solubilities of **5a–d**, **6a–d**, **7a** and **8a**



Compd	R^1	Linker	Y5[a] IC_{50}(nM)	CYP450 inhibition (μM) 1A2/2C19/2C9/2D6/3A4	Solubility[b] (μM)
5a	Et		38.9	0.4/18/14/ 20/7.7	4.0
5b	Et		0.55	1.6/14/10/>20/7.0	1.6
5c	Et		1.8	6.3/15/9.8/>20/7.8	12.1
5d	Et		0.31	3.4/>20/>20/>20/8.7	20.3
6a	*n*-Pr		1.9	All>20	43.3
6b	*t*-Bu		7.5	All>20	9.4
6c	CF_3		2.5	>20/9.1/7.5/20/5.1	1.3
6d	CF_3CH_2		0.29	All>20	7.7
7a[c]	*n*-Pr		>1000	All>20	5.5
8a	*n*-Pr		579	All>20	2.0

[a,b] See Table 1.
[c] HCl salt.

We therefore chose pyridone analogue **6a** for further optimization. The influence of the R^1 group of the analogue is also shown in Table 3. Replacement with a *t*-butyl group led to retention of the binding affinity and improved CYP450 inhibition profiles with a slight decrease in the solubility (**6b**). While trifluoromethyl sulfone **6c** resulted in aggravation of the CYP450 profiles and reduction in solubility, consistent with high lipophilicity ($c\mathrm{Log}P = 4.50$), 2,2,2-trifluoroethyl sulfone **6d** retained desirable CYP450 profiles, consistent with low lipophilicity ($c\mathrm{Log}P = 3.18$) and showed sub-nanomolar Y5 receptor binding affinity. It appeared that the bulkiness of the β-position of the alkylsulfonyl group contributed to the binding affinity. In addition, pyridone analogues **6** exhibited high metabolic stabilities in liver microsomes (**6a** human 88.5%, rat 78.6%; **6b** human 94.2%, rat >99.9%; **6c** human 96.0%, rat 92.6%; **6d** human 94.7%, rat 93.6%).

In summary, the optimization of HTS hit **1** led to identification of the highly potent derivative **2c**. Modification of **2c** gave pyridone analogues **6** which had low to sub-nanomolar Y5 receptor binding

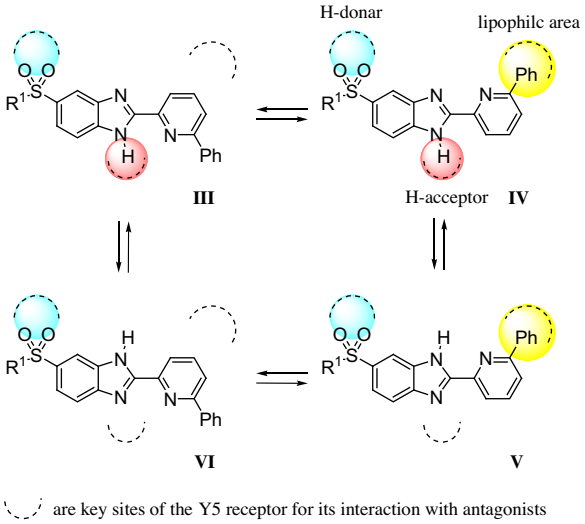


Figure 3. Proposed active conformation.

affinities with improved CYP450 inhibition profiles, good solubilities and high metabolic stabilities. Further investigations of these compounds are in progress and will be reported in due course.

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