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Hit to lead SAR study on benzoxazole derivatives for an NPY Y5 antagonist

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ABSTRACT

We report a hit to lead study on a novel benzoxazole NPY Y5 antagonist. Starting from HTS hit **1**, structure–activity relationships were developed. Compound **12** showed reduction of food intake and a tendency to suppress body weight gain over the 21-day experimental period.

Obesity is a major health issue in the 21st century. According to the Centers for Disease Control and Prevention (CDC), the percentage of obese adults has increased rapidly in the United States where the percentage of the obese population was 20% or more all states in 2010. This trend is also observed for children and adolescents among whom the percentage of the obese has tripled over the past 30 years.[1] While there are diverse treatment options for obesity, many patients do not comply with diet improvement and physical activity recommendations. Surgical procedures are becoming popular but are associated with risks such as mineral and vitamin deficiencies. Medication can offer an alternative treatment option, but Orlistat is the only medication currently approved in the US for long-term treatment of obesity and has only modest efficacy.

Neuropeptide Y (NPY), a 36 amino acid peptide widely expressed in the central and peripheral nervous system, is known to have various physiological roles including modulation of food intake, energy expenditure,[2] anxiety,[3,4] and depression.[3,4] NPY acts via five receptors (Y1, Y2, Y4, Y5, and y6).[5–7] The importance of the Y5 receptor on the regulation of food intake and body weight has been shown by several different lines of studies using NPY Y5 antisense oligodeoxynucleotides,[8] selective NPY Y5 agonists,[9] and antagonists. Recently Velneperit, a selective NPY Y5 antagonist under Phase II clinical trial has been shown to lead to clinically meaningful body weight loss in obese patients with one-year administration.[10,11] Therefore NPY Y5 antagonists have attracted considerable interest for the treatment of obesity and related diseases.[12–20]

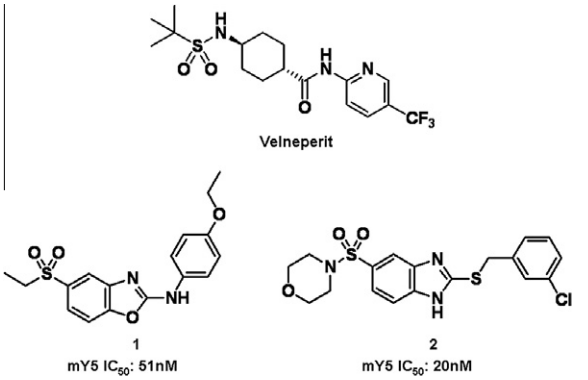
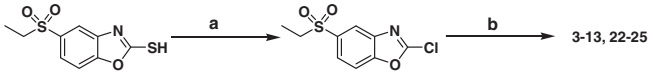


Figure 1. Velneperit and confirmed hit compounds in HTS.



Scheme 1. Synthesis of aminobenzoxazole via displacement reaction. Reagents and conditions: (a) $SOCl_2$, 120 °C; (b) RNH_2, *i*PrOH, reflux.

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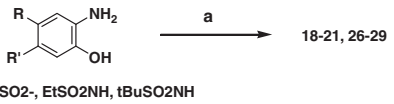
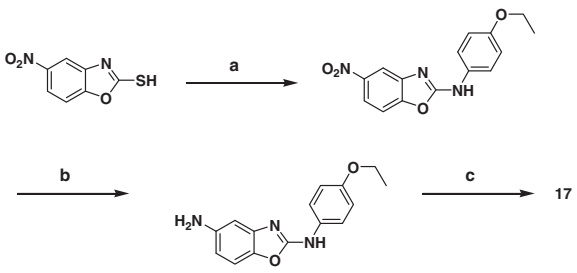
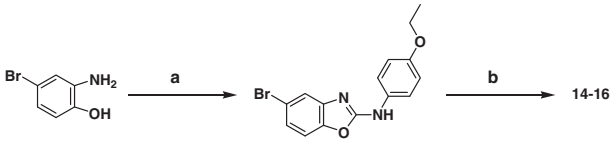


Scheme 2. Synthesis of **18–21**, **26–29** via oxidative annulation. Reagents and conditions: (a) (1) ArNCS, (2) LiOH, H_2O_2.



Scheme 3. Synthesis of **17**. Reagents and conditions: (a) (1) $SOCl_2$, (2) *p*-EtOPhNH$_2$ (b) Pd/C, H_2 (c) $EtSO_2Cl$, Et_3N.



Scheme 4. Synthesis of **14–16.** Reagents and conditions: (a) (1) ArNCS, (2) LiOH, H_2O_2 (b) CuI, RSO_2Na.

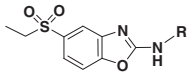
In order to search for novel NYP Y5 antagonists, we initiated HTS screening and identified several novel scaffolds namely a benzoxazole derivative (**1**) and a benzimidazole derivative (**2**). The presence of the same functional group (SO_2, aromatic NH) as in Velneperit convinced us that both are promising hits and should facilitate our hit to lead study (Fig. 1).[21]

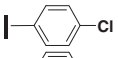
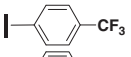
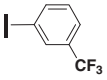
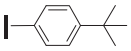
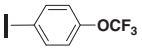
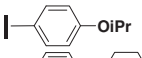
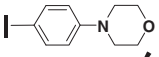
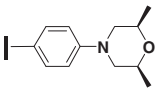
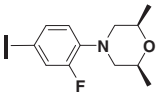
Herein we report our SAR study on benzoxazole derivative to identify the lead compound suitable for in vivo study.

The synthetic route for the preparation of benzoxazole derivatives is summarized in Schemes 1–4. Most benzoxazole derivatives were accessible in accordance with Scheme 1. The 2-thiobenzoxazole derivative was chlorinated in the presence of $SOCl_2$,[22] and subsequent displacement with amine led to **3–13**, **22–25**. Since electron deficient aryl amines such as pyridyl amines were not reactive in this condition, an alternative synthetic route was investigated. Thus Scheme 2 presents reaction of 2-aminophenol with arylated isothicyanate followed by oxidative annulation. This two step sequence enabled introduction of pyridyl amine at 2nd position of benzoxazole.[23] Scheme 3 illustrates the synthesis of ethyl sulfonamide **17**. Scheme 4 was designed to develop SAR on the left hand side moiety. Brominated benzoxazole obtained from oxidative annulation as presented in Scheme 2 was sulfonated with sodium salt of alkylsulfonate in the presence of CuI to give **14–16**.

In vitro mouse NPY Y5 binding activities, rat microsomal stabilities and kinetic solubilities at pH 6.8 of 5-$EtSO_2$ derivatives (**3–13**) are displayed in Table 1. *ortho*-CF_3 substitution caused a substantial drop in IC_{50}, while the difference in potency between *meta*- and *para*-CF_3s was subtle as shown in **5** and **6**. Alkoxy substituents such as *para*-OCF_3 and *para*-O*i*Pr improved potency. Incorporation of morpholine to improve solubility was tolerated as **12** displays

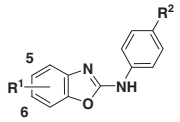
Table 1
SAR on right hand side moiety

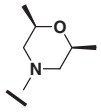


Compd	R	mY5 IC_{50}[a] (nM)	RatMS[b] (%)	Sol@pH6.8[c] (μM)
3		1470	53	10.2
4		329	81	0.7
5		100	101	0.9
6		144	63	0.8
7		>10000	68	0.9
8		728	27	0.6
9		31	97	0.8
10		9.1	73	4.6
11		219	88	13.8
12		71	85	11.8
13		155	61	5.3

[a] IC_{50} values are means of at least two experiments.
[b] Microsomal stability was measured as the percentage of drug remaining after 30 min incubation.
[c] Solubility was measured as kinetic solubility using 1% DMSO solution.

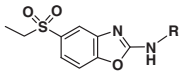
Table 2
SAR on left hand side moiety

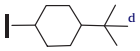
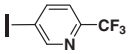
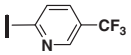
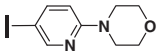
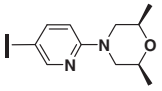


Compd	R^1	R^2	mY5 IC_{50}[a] (nM)	RatMS[b] (%)	Sol@pH6.8[c] (μM)
14	5-$MeSO_2$	OEt	221	62	3.1
15	5-$iPrSO_2$	OEt	99	49	1.5
16	5-$tBuSO_2$	OEt	643	46	1
17	5-$EtSO_2NH$	OEt	1680	59	7.8
18	6-$EtSO_2$	OEt	489	81	0.8
19	6-$EtSO_2NH$	OEt	35	86	9.2
20	6-$tBuSO_2NH$	OEt	2.3	57	1.9
21	6-$tBuSO_2NH$		5290	68	27

[a] IC_{50} values are means of at least two experiments.
[b] Microsomal stability was measured as the percentage of drug remaining after 30 min incubation.
[c] Solubility was measured as kinetic solubility using 1% DMSO solution.

Table 3
Replacement of aniline moiety



Compd	R	mY5 IC_{50}[a] (nM)	RatMS[b] (%)	Sol@pH6.8[c] (μM)
22		1400	45	>50
23	 [d]	723	1	3.5
24		1080	46	>50
25		>10000	35	13
26		795	99	4.6
27		1040	97	8.8
28		651	81	18
29		310	59	4.3

[a] IC_{50} values are means of at least two experiments.
[b] Microsomal stability was measured as the percentage of drug remaining after 30 min incubation.
[c] Solubility was measured as kinetic solubility using 1% DMSO solution.
[d] *Cis*/*trans* mixtures.

71 nM in IC_{50}. All compounds except **8** showed moderate to good rat microsomal stabilities.

Structure–activity relationships on the left hand side moiety are shown in Table 2. Conversion from 5-$EtSO_2$ to 5-$MeSO_2$ (**14**), 5-*i*Pr-SO_2 (**15**), and 5-*t*BuSO_2 (**16**) caused a drop in potency. 5-$EtSO_2NH$ (**17**) reduced potency by 30-fold, while very interestingly, the corresponding 6-$EtSO_2NH$ (**19**) gave comparable potency to **1**. Increasing steric bulk from 6-$EtSO_2NH$ (**19**) to *t*BuSO_2NH (**20**) further improved potency. However, dimethylmorpholine substitution at R^2 was not tolerated in this case with substantial loss of potency (**21**).

Increased demand for a good safety profile in anti-obesity medication prompted us to modify the aniline moiety in order to reduce the liability of reactive metabolite formation. Indeed **11** showed time-dependent CYP3A4 inhibition suggesting the formation of reactive metabolites derived from the aniline moiety.[24,25] Replacement of aniline with either cyclohexylamine (**22**) or benzylamine (**24**) resulted in slight gain in IC_{50}s compared to **3**, while there was solubility improvement as expected in both cases. In the case of cyclohexylamine, the substituent effect was moderate where *t*Bu substitution showed only 2-fold increase in IC_{50} (**23**).

Table 4
Rat PK profile of **10** and **12** upon iv 0.5 mg/kg and oral 1 mg/kg dosage

Compounds	Cl_{tot} (ml/mim/kg)	C_{max} (μg/ml)	$AUC_{(0-\infty)}$ (μg h/ml)	V_{dss} (l/kg)	BA (%)	B/P	CSF (nM)	fu (%)
10	66	0.006	0.04	2.62	16	2.63	8.8	3.8
12	33	0.026	0.26	1.74	52	0.64	15.9	7.1

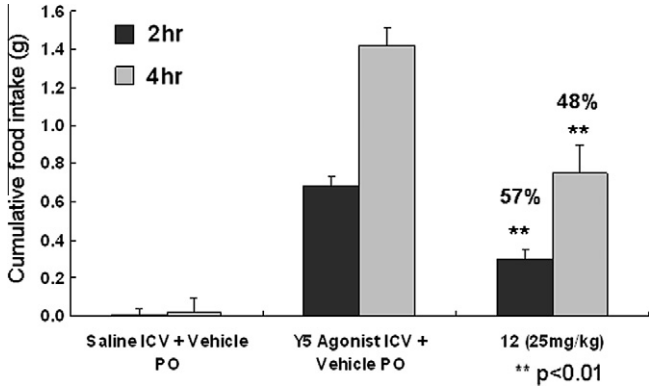


Figure 2. The effect of **12** on Y5 agonist-stimulated food intake in diet-induced obese mice.

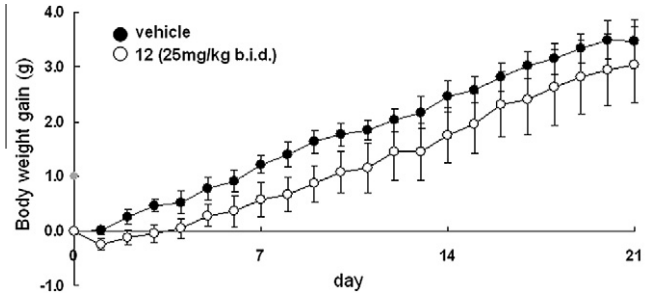


Figure 3. The effect of repeated administration of **12** on body weight gain in diet-induced obese mice.

Pyridine derivatives have affinities 3- to 10-fold less potent than the corresponding aniline derivatives (**26–29**) (see Table 3).

Selected compounds (**10** and **12**) were tested in a rat pharmacokinetic (PK) study (Table 4). Although **10** showed good brain to plasma (B/P) ratio, plasma exposure after oral administration at 1 mg/kg was limited and bioavailability was low. Compound **12** displayed moderate clearance and brain exposure with a B/P ratio of 0.64. The cerebrospinal fluid (CSF) concentration after intravenous administration at 0.5 mg/kg of **12** was nearly 16 nM and was higher than that of **10**.

Inhibitory effect of **12** on food intake induced by icv administration of NPY Y5 agonist ([cPP1–7, NPY19–23, Ala31, Aib32, Gln34]-human pancreatic polypeptide) was evaluated. As shown in Figure 2, 12 reduced food intake in both 2-h and 4-h periods at 25 mg/kg oral dosing. Based on these results, the effect of **12** on body weight gain in diet-induced obese (DIO) mice was further evaluated, and the results are summarized in Figure 3. Although not statistically significant, repeated administration of **12** to DIO mice showed a tendency towards suppression of body weight gain over the 21-day experimental period.

In summary, this hit to lead study identified a novel NPY Y5 antagonist which showed significant reduction of food intake induced by the NPY Y5 agonist. Further optimization efforts to improve reduction of body weight gain will be reported in due course.

Acknowledgments

The authors thank Kyoko Kadono, Tohru Mizutare, and Shigenori Yagi for conducting measurements on CYP3A4 inactivations, rat microsomal stabilities and solubilities, respectively.

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