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The identification and optimisation of novel and selective diamide neuropeptide Y Y2 receptor antagonists

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ABSTRACT

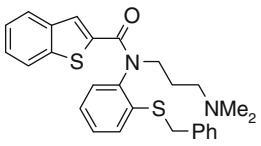
A novel small molecule NPY Y2 antagonist (**3**) identified from high throughput screening is described. A subsequent SAR study and optimisation programme based around this molecule is also described, leading to the identification of potent and soluble pyridyl analogue **36**.

Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family that also includes pancreatic polypeptide (PP) and peptide YY (PYY)[1] and is highly expressed in both the periphery and in a number of specific brain regions.[2,3] Its localisation implies that it may be involved in a variety of physiological responses (such as food intake, water consumption, anxiety, circadian rhythms, memory processing, endocrine and cardiovascular functions).[2] Six NPY receptors (Y1, Y2, Y3, Y4, Y5 and Y6), five of which have been cloned,[4] have been identified from pharmacological and molecular biology studies on NPY.[2,5]

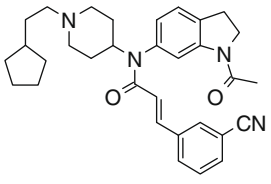
Whilst there are extensive literature reports of Y1 and Y5 antagonists,[1,6,7] and compounds have been progressed into the clinic (e.g., MK-0557[8]), small molecule Y2 antagonists have until very recently proved elusive, and hence the therapeutic potential of this receptor has remained relatively unexplored.[9] Doods et al.[10] reported the *pseudo*-peptide BIIE0246 as a potent and selective NPY Y2 antagonist (IC_{50} = 3.3 nM), and Caberlotto and co-workers subsequently administered BIIE0246 via intracerebroventricular injection to successfully demonstrate its anxiolytic-effect in rats in the elevated plus maze model.[11]

The first small molecule NPY Y2 receptor antagonists were disclosed by Grant-Young and co-workers,[12] who identified a series of amines (Fig. 1) from a high throughput screen (HTS) as exemplified by **1** (IC_{50} = 450 nM) although no comment was made on functional activity. Jablonowski et al.[13] disclosed a series of indolylpiperidines, again identified from a HTS, and optimised to obtain JNJ-5207787 (**2**), a compound with good affinity for the NPY Y2 receptor (IC_{50} = 100 nM in a binding assay) and demonstrated to be a functional antagonist via inhibition of PYY-stimulated [^{35}S]GTPγS binding (pIC_{50} = 7.2) (Fig. 1).

Herein we report the discovery and optimisation of a series of small molecule NPY Y2 functional antagonists based on diamide **3** (Fig. 2).



1



JNJ-5207787 (**2**)

Figure 1. Structures of known selective NPY Y2 antagonists.

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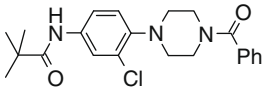


3

hNPY Y2 fpKi = 6.3
rNPY Y2 fpKi = 6.4
NPY Y1 fpKi <5.2
NPY Y5 fpKi <5.3
Clint mL/min/g = 0.5 (human)
1.6 (rat)
MW = 399
cLogP = 4.04
Solubility = 46 μg/mL

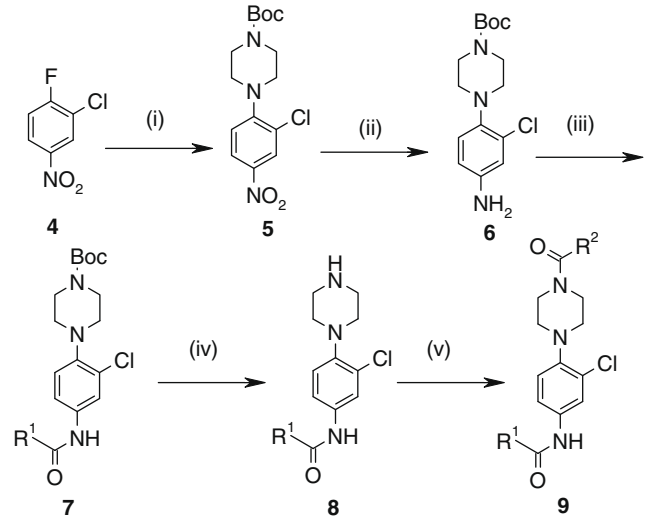
Figure 2. Initial HTS hit diamide **3**.

Diamide **3** was one of several chemotypes identified from a functional HTS of the GSK compound collection. The molecule is selective for NPY Y2[14,15] over both NPY Y1 and Y5 receptors,[16,17] exhibits moderate aqueous solubility,[18] and low intrinsic clearance/good in vitro metabolic stability (Clint), and hence represented a promising start point for a medicinal chemistry programme. The initial aim of this programme was to identify soluble, but more potent, NPY Y2 antagonists for use in Target Validation experiments in animal models of anxiety.

Compounds were readily synthesized in the general manner shown in Scheme 1. S_NAr displacement of 2-chloro-1-fluoro-4-nitrobenzene (**4**) with *N*-Boc-piperazine gave nitroarylpiperazine **5**, subsequent iron/ammonium chloride reduction of the nitro group afforded aniline **6**. Anilide formation under standard conditions afforded anilides **7**. Boc deprotection under acidic conditions gave aryl piperazines **8** which underwent subsequent amide formation to afford diamides **9**.[19]

Initially, the effect on potency of substituents on the piperazine amide was investigated. Electron donating and withdrawing substituents were well tolerated **10**–**14** (Fig. 3, Table 1). An *ortho* trifluoromethyl group as exemplified by **12** gave a modest increase in potency compared to diamide **3**.

The effect of replacing the *t*-butyl anilide with alternative aliphatic amides was also investigated (Fig. 4, Table 2). It was found that methylation of the amide nitrogen **15** resulted in a loss in potency although solubility was increased. As the steric bulk of the amide was increased, for example, by replacement of a methyl group of the *t*-butyl amide with an ethyl group to give amide **16**, or introduction of ring constrained analogues such as cyclohexane



Scheme 1. Representative synthesis of analogues. Reagents and conditions: (i) Boc-piperazine, Hünig's base, MeCN, 150 °C, microwave; (ii) iron, ammonium chloride, water, methanol, 80 °C; (iii) R^1CO_2H, HATU, Hünigs base, NMP; (iv) HCl in diethyl ether; (v) R^2CO_2H, HATU, Hünigs base, NMP.

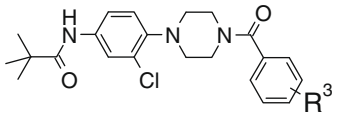


Figure 3. SAR for replacements of phenyl amide.

Table 1
Profiling results for phenyl amide SAR

	R^3	hNPY Y2 fpKi
3	H	6.3
10	2-OMe	6.7
11	3-OMe	6.5
12	2-CF_3	7.1
13	3-CF_3	6.9
14	4-CF_3	6.8

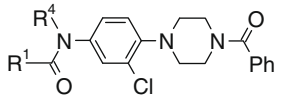


Figure 4. SAR for aliphatic replacements of the *t*-butyl amide.

Table 2
Profiling results for aliphatic amide SAR

	R^1	R^4	hNPY Y2 fpKi	Solubility (μg/mL)
15	*t*-Bu	Me	5.6	113
16	$C(CH_3)_2C_2H_5$	H	7.3	22
17	Cyclohexane	H	6.8	4
18	1-Methyl cyclohex-1-yl	H	7.7	4
±-19	2-Tetrahydropyran	H	6.5	NT
20	4-Tetrahydropyran	H	<5.2	NT

17, resulted in an increase in NPY Y2 potency. Replacement of the cyclohexane ring with 1-methylcyclohexane **18** resulted in nearly a 10 fold increase in the NPY Y2 potency compared to unsubstituted derivative **17**. Incorporation of more polar groups such as a tetrahydropyran moiety (**±-19** and **20**) did not benefit. In the case of the 2-tetrahydropyran derivative **±-19** the potency was only slightly reduced compared to the cyclohexyl amide **17**. However, in the case of the 4-tetrahydropyran amide **20**, the compound was inactive.

A range of substituted phenylacetamide replacements for the *t*-butyl anilide were also investigated (Fig. 5, Table 3). Phenylacetamides with a benzylic substituent, such as **22** or **23,** exhibited an increase in potency compared to the unsubstituted derivative **21**. Incorporation of a small lipophilic *meta* substituent such as methyl **24** resulted in a further small increase in potency. However, all of these compounds possessed poor solubility. The potency of 2-pyridyl derivatives **25** and **±-26** was slightly reduced compared to the parent phenyl analogues. Pyridyl **25** possessed improved aqueous solubility compared to the analogous phenyl derivative. Disappointingly, pyridine **±-26** was no more soluble than corresponding benzhydryl analogue **22**.

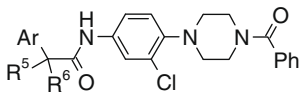


Figure 5. SAR for aromatic replacements of the *t*-butyl amide.

Table 3
Profiling results for replacement of the *t*-butyl amide

	Ar	R^5	R^6	hNPY Y2 fpKi	Solubility (μg/mL)
21	Ph	H	H	7.1	3
22	Ph	Ph	H	8.2	7
23	Ph	Me	Me	8.0	0
24	m-MePh	Me	Me	8.3	0
25	2-Py	H	H	6.5	70
±-26	2-Py	Ph	H	7.9	8

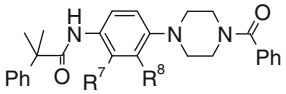


Figure 6. SAR for alternate aryl substitution.

Table 4
Profiling results for aryl substitution SAR

	R^7	R^8	hNPY Y2 fpKi
23	H	Cl	8.0
27	H	CF_3	6.2
28	H	Me	6.5
29	H	F	7.2
30	H	OMe	5.6
31	H	H	5.8
32	Cl	H	5.5

The effect of altering the substitution pattern in the central diamino aryl ring was also investigated (Fig. 6, Table 4). It was found that small lipophilic substituents such as trifluoromethyl **27,** methyl **28** and fluorine **29** were tolerated *ortho* to the piperazine but with reduced potency compared to the chloride **23**. More polar substituents such as in methoxy compound (**30**), no substituent (**31**), and substitution *meta* to the piperazine (e.g., **32**) were detrimental to potency. The above results suggest that there may be a small lipophilic pocket in the receptor that the chlorine atom, or to a lesser extent fluorine may access, and which may also require a 'twist' out of the planar geometry between the piperazine and aryl moiety.

The more potent anilides and aryl-*ortho* substituents described above were combined in a series of compounds (**33**–**36**) which displayed good potency (Fig. 7, Table 5). However, these potency enhancing combinations typically afforded highly lipophilic compounds with low solubility (**33** and **34**).

The incorporation of a pyridyl group allowed the lipophilicity to be reduced, for example, **35** and **36** and some aqueous solubility to be introduced.

On completion of the SAR study, a number of the more promising compounds with good NPY Y2 potency were progressed into in vitro DMPK screens (Table 6). A number of the compounds (**21, 23** and **±-26**) proved to be metabolically unstable in the rat intrinsic clearance assay (values >5 mL/min/g). Disappointingly, with the exception of pyridine amide **36**, these compounds also displayed high non-specific rat brain tissue binding[19] (BTB) which would predict very low free fraction in the brain in vivo.

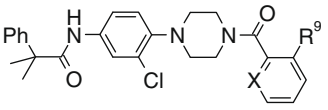


Figure 7. SAR for replacements of phenyl amide.

Table 5
Profiling results of combining potency enhancing modifications

	X	R_9	hNPY Y2 fpKi	*c*log *P*	Solubility (μg/mL)
33	C	CF_3	8.4	6.07	1
34	C	OMe	8.6	5.27	2
35	N	CF_3	7.9	4.90	11
36*	N	OMe	7.6	4.32	56

* Tested as the HCl salt.

Table 6
In vitro profiling results of selected compounds

	hNPY Y2 fpKi	*c*log *P*	Cli rat (mL/min/g)	Cli human (mL/min/g)	BTB (rat)%
21	7.1	4.34	13.8	0.5	99.0
23	8.0	5.05	7.4	0.6	99.5
±-26	7.9	4.19	15.3	<0.5	99.7
36	7.6	4.32	0.7	2.7	97.1

Table 7
Selected in vitro selectivity data for pyridyl **36**

	hERG pIC50	rNPY Y2 fpKi	NPY Y1 fpKi	NPY Y5 fpKi
36	4.3	7.3	<5.2	<5.0

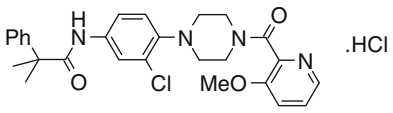


Figure 8. Compound **36** selected for selectivity profiling.

Pyridine **36** possessed the most promising profile of the compounds screened viz. moderate intrinsic clearance (Clint), good hNPY Y2 potency, moderate solubility and moderate brain free fraction. This compound also displays excellent selectivity against the other NPY receptors, good potency in the orthologue rat NPY Y2 assay and minimal activity at hERG (Table 7, Fig. 8). The compound had an excellent CYPEX bactosome P450 profile showing inhibition potencies greater than 10 μM against all isoforms tested and possessed no significant off-target activity when cross screened against an extensive panel of aminergic receptors (including dopamine, serotonin, adrenergic and histamine receptors) and liability targets (data not shown).

In summary, extensive SAR explorations were performed around highly promising HTS hit (**3**) and a number of potency enhancing modifications identified. A highly potent, soluble and selective NPY Y2 antagonist **36** suitable for in vitro profiling studies was identified. In vivo studies on this molecule will be published in due course.

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14. The functional activity (fpki) against human NPY Y2 was determined in a human neuroblastoma cell line (KAN-TS) which natively expresses NPY Y2, using a 384 well GTPγS35 assay, values quoted were a mean of 4 or more expts.
15. The functional activity (fpki) against rat NPY Y2 was determined in a human embryonic kidney (HEK) cell line transiently expressing the receptor, using a 384 well GTPγS35 assay.
16. The functional activity (fpki) against human NPY Y1 was determined in a Chinese hamster ovarian (CHO) cell line stably expressing the receptor, using a 384 well GTPγ^{35}S assay.
17. The functional activity (fpki) at the human NPY Y5 receptor stably expressed in HEK293 cells was assessed using FLIPR/Ca2+ methodology in a 384 well format.
18. Aqueous solubility was measured via Chemiluminescent Nitrogen Detection (CLND).
19. Compounds were characterised by ^{1}H NMR spectroscopy and hplc coupled-mass spectrometry. *Compound* **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.3 (s, 9H), 2.8–3.2 (br m, 4H), 3.6 (br s, 2H), 4.0 (br s, 2H), 6.9 (d, 1H), 7.4 (d, 1H), 7.8 (s, 1H). *Compound* **36**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.6 (s, 6H), 2.9 (m,2H), 3.1 (m, 2H), 3.3 (m, 2H), 3.9 (s, 3H), 3.9 (m, 2H), 7.1 (d, 1H), 7.2 (m, 1H), 7.3–7.4 (m, 5H), 7.5 (m,1H), 7.6 (s, 1H), 7.6 (m,1H), 8.2 (d, 1H).