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The identification of a series of novel, soluble non-peptidic neuropeptide Y Y2 receptor antagonists

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ABSTRACT

The identification and subsequent optimisation of a selective non-peptidic NPY Y2 antagonist series is described. This led to the development of amine **2**, a selective, soluble NPY Y2 receptor antagonist with enhanced CNS exposure.

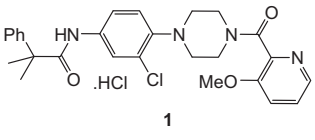
Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family that includes pancreatic polypeptide (PP) and peptide YY (PYY).[1] NPY is thought to be involved in regulating a number of physiological processes[2–4] including: food intake; water consumption; anxiety; circadian rhythm; memory processing; endocrine and cardiovascular functions via action at six specific receptors in mammalian systems (Y1, Y2, Y3, Y4, Y5 and Y6).[5,6]

We previously reported studies leading to the identification of pyridine **1** as a potent, selective NPY Y2 receptor antagonist (Fig. 1, Table 1).[7]

Whilst compound **1** has a promising in vitro pharmacokinetic (PK) profile, it exhibited disappointing in vivo rat PK with a brain to blood ratio of 0.6 and low exposure in the brain (6 ng/g) following a single subcutaneous (sc) administration of 3 mg/kg (Table 1).

Herein we report further optimisation studies of the diamide series exemplified by **1** resulting in the identification of amine **2** (Fig. 2) as a selective NPY Y2 antagonist with improved in vivo CNS exposure.

A number of selective small molecule NPY Y2 antagonists have now been described.[4,7–10] Unfortunately, the utility of these to date for in vivo target validation studies has been limited by their generally poor physiochemical properties and only one such study has been described. Caberlotto and co-workers have demonstrated



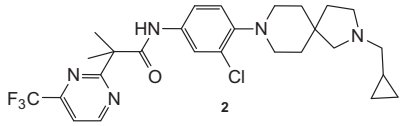
1

hNPY Y2 fpK_i = 7.6
rNPY Y2 fpK_i = 7.3
BTB (rat) = 97.1%
Clint mL/min/g = 0.7 (human), 2.7 (rat)
cLogP = 4.32
Solubility = 56 μg/mL

Figure 1. Structure and profile of pyridine **1**.

Table 1
In vivo PK profile of pyridine **1** after sc administration

Dose (mg/kg)	Br:Bl	C_{max} (Br) (ng/g)	C_{max} (Bl) (ng/mL)	Sample time (h)
3.0	0.6	6	13	1.0



2

Figure 2. Selective, soluble NPY Y2 antagonist **2**.

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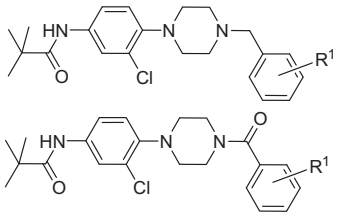
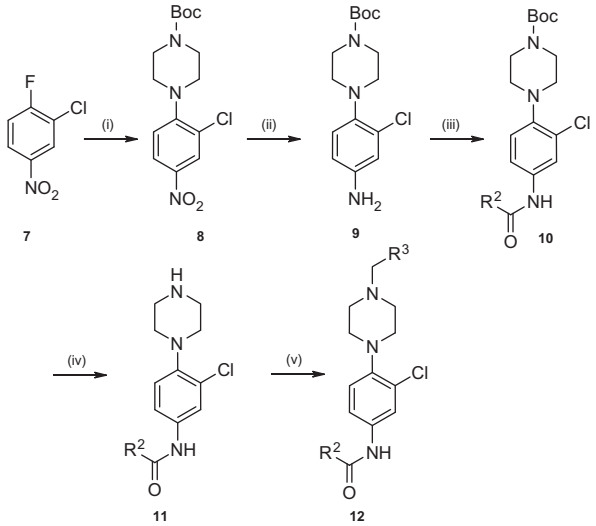


Figure 3. SAR for the comparison of amines and amides.

Table 2
Profiling results for the comparison of amine and amide analogues

	R^1	hNPY Y2 (fpK_i)[a]	Solubility[a] (μg/mL)
3	H	6.6 (6.3)	38 (40)
4	2-F	6.4 (6.9)	25 (53)
5	3-F	6.7 (6.6)	4 (52)
6	4-F	6.7 (6.3)	2 (36)

[a] Data in parentheses is for the amide analogue.

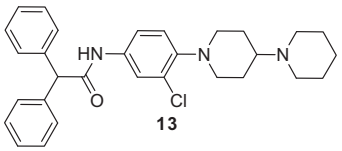


Scheme 1. Representative synthesis of amine analogues. Reagents and conditions: (i) Boc-piperazine, Hünigs base, MeCN, 150 °C, microwave; (ii) iron, ammonium chloride, water, methanol, 80 °C; (iii) R^2CO_2H, HATU, Hünigs base, NMP; (iv) HCl in diethyl ether; (v) R^3CHO, AcOH, NMP, $NaBH(OAc)_3$.

that the potent and selective *pseudo*-peptide BIIE0246 (NPY Y2 IC_{50} = 3.3 nM), when administered via intracerebroventricular (icv) injection, induced an anxiolytic effect in rats in the elevated plus maze model.[11] Whilst encouraging this result is open to interpretation as the use of icv administration in an anxiety model is not ideal.

The aim of this study was to identify selective NPY Y2 antagonists suitable for in vivo target validation studies when administered either orally or via sc dosing. Additionally, we sought to investigate whether total or free brain concentration of a selective NPY Y2 antagonist correlated with in vivo receptor occupancy and hence pharmacodynamic (PD) effects. It was anticipated that by synthesizing compounds with a range of brain tissue binding (BTB)[12] that suitable tool molecules could be identified to study the interplay between potency and brain free fraction in achieving receptor occupancy for the NPY Y2 receptor.[13]

Using our previously reported diamide series[7] as a starting point the effect of modifying the piperazine amide was examined (Fig. 3, Table 2). It was found that the amines (**3**–**6**) were of



hNPY Y2 fpK_i = 7.3
rNPY Y2 fpK_i = 7.1
BTB (rat) = 99.6%
Clint mL/min/g = <0.53 (human), <0.53 (rat)
MW = 488
cLogP = 5.88
Solubility = 27 μg/mL

Figure 4. Profile of piperidine **13**.

comparable potency[14] to the corresponding amides but exhibited lower aqueous solubility.

Amines (**3**–**6**) were synthesized using the synthetic route detailed in Scheme 1. A S_NAr reaction of Boc-piperazine and 2-chloro-1-fluoro-4-nitrobenzene (**7**) gave nitro compound **8**. Subsequent reduction using iron/ammonium chloride of the nitro group gave aniline **9** with retention of the aromatic chloride. Anilide formation using HATU afforded **10**. Deprotection of the Boc group under acidic conditions gave aryl piperazine **11** which was subsequently reductively alkylated to afford the desired compounds **12**.[15]

Alternatives to the piperazine ring including 3 and 4-substituted piperidines, morpholine, homopiperidine and 3-substituted pyrrolidines were then investigated. Using a synthetic strategy similar to that described in Scheme 1 a diverse range of amines were substituted on to **7** and then elaborated to give analogues of **12**. Throughout this study the benzhydryl group was used as the R2 group of choice. The majority of compounds prepared displayed low potency at NPY Y2 with the exception of piperidine **13** (Fig. 4). Compound **13** displayed encouraging potency (hNPY Y2 pIC_{50} = 7.3), low intrinsic clearance and promising aqueous solubility despite its high lipophilicity.

Initially, SAR explorations around **13** focused on replacing the benzhydryl group to reduce lipophilicity (Fig. 5, Table 3). As previously observed for the diamide series, it was found that the benzhydryl group could be replaced with substituted phenylacetamides for example **14–17**. Additionally, *meta* substitution of the phenylacetamide with a lipophilic group, for example methyl **16** or trifluoromethyl **17**, gave compounds with enhanced NPY Y2 potency compared to the unsubstituted derivative **14**. Increasing the bulk of the benzylic substituents as in cyclopentyl **15** also resulted in a potency increase. All the phenylacetamide compounds tested displayed improved aqueous solubility compared to benzhydryl **13**. Attempts at reducing the lipophilicity by introduction of a heteroatom into the benzhydryl group (e.g., **±-18**) resulted in a reduction in the NPY Y2 potency.

Modification of the distal piperidine group was then studied and a series of mono substituted 4-amino piperdines were prepared (Fig. 6, Table 4). It was shown that the size of the 4-amino substituent affected NPY Y2 potency and for an alkyl chain potency increased from ethyl to *i*-butyl (**19**–**22**). Methylene cyclopropyl substitution was well tolerated, as in **23**, and inferred similar potency to an *i*-butyl chain but with reduced lipophilicity. As previ-

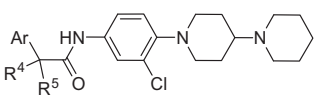


Figure 5. SAR for replacements of the benzhydryl amide.

Table 3
Profiling results for replacements of the benzhydryl amide

	Ar	R^4	R^5	hNPY Y2 (fpK_i)	*c* log *P*	Solubility (μg/mL)
14	Ph	Me	Me	6.5	5.24	92
15	Ph	$-(CH_2)_3-$		7.1	5.99	114
16	*m*-MePh	Me	Me	7.2	5.73	131
17	*m*-CF_3Ph	Me	Me	7.8	6.12	96
±-18	2-Pyridyl	Ph	H	6.5	4.38	NT

ously observed addition of a *meta* substituent resulted in increased potency, for example **24** and **25**. Pleasingly, addition of a *meta* substituent also resulted in greatly enhanced aqueous solubility.

Physiochemical profiling of project compounds established a linear relationship between *c* log *P* (calculated using ACD software) and measured log *D* at pH 7.4 for both the diamide and amine series. Both parameters were also shown to be predictive of measured BTB with high *c* log *P* correlating with high BTB. We were therefore able to specifically target compounds for synthesis with low *c* log *P* that were predicted to have a high fraction unbound in brain tissue.

Consequently, heterocyclic acetamides including the pyridine **26** and pyrimidines **27**, **28** and **±-29** were synthesized and tested (Fig. 7, Table 5). Unfortunately, these compounds displayed significantly reduced NPY Y2 potency compared to the corresponding phenylacetamide derivatives.

In an attempt to increase potency, to allow the incorporation of heteroatoms, optimisation of the position of the basic nitrogen was reinvestigated and a number of extended and spirofused amines synthesized. The majority of compounds prepared were poorly active (data not shown) with the exception of spiroamine **30** (Fig. 8, Table 6).

Further gains in potency were achieved when spiroamine **30** was reductively alkylated to afford amine **31** which demonstrated excellent potency at the NPY Y2 receptor but unsurprisingly with reduced aqueous solubility (Fig. 9, Table 7). To reduce the lipophilicity of **31**, the corresponding pyrimidine analogue was prepared. Pleasingly pyrimidine **2** still maintained significant potency (hNPY Y2 $pIC_{50} = 6.8$) coupled with considerable aqueous solubility.

Profiling of exemplar compounds to determine in vitro intrinsic clearance (Clint) revealed promising metabolically stability in both rat and human liver microsomes (Table 8). Notably, the introduction of a trifluoromethyl group in **25** increased metabolically stability and enhanced potency at the NPY Y2 receptor. As predicted, BTB values increased with lipophilicity and incorporation of a pyrimidine acetamide led to a significant reduction in BTB as exemplified by **28** and **±-29**.

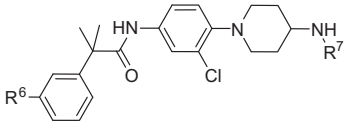


Figure 6. SAR for piperidine replacements.

As pyrimidine **2** demonstrated a balanced profile of NPY Y2 potency, solubility, Clint and BTB (Tables 7 and 8) it was selected for further profiling. In vitro cross screening showed **2** exhibited comparable potency at both human and rat[16] NPY Y2 receptors and selectivity against human NPY Y1[17] and Y5[18] receptors (Table 9, Fig. 10). Additionally, **2** had an excellent CYP-EX bactosome p450 inhibition profile showing inhibition of >10 μM against the 1A2, 2C9, 2C19, 2D6 and 3A4 (7BQ and DEF) isoforms.

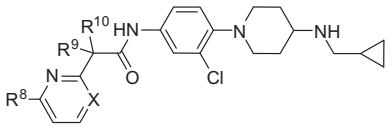
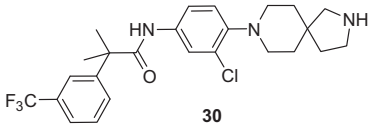


Figure 7. SAR for heterocyclic amide replacements.

Table 5
Profiling results of heterocyclic amide replacements

	R_8	X	R_9	R_{10}	hNPY Y2 (fpK_i)	*c* log *P*	Solubility (μg/mL)
26	H	C	Me	Me	5.9	2.90	NT
27	CF_3	N	Me	Me	6.4	2.90	112
28	Me	N	Me	Me	6.1	2.44	248
±-29	H	N	Et	H	6.2	2.08	177



30

Figure 8. Spiroamine replacement.

Table 6
Profiling results for spiroamine **30**

	hNPY Y2 (fpK_i)	*c* log *P*	Solubility (μg/mL)
30	7.8	5.16	96

Table 4
Profiling results for piperidine replacements

	R^6	R^7	hNPY Y2 (fpK_i)	*c* log *P*	Solubility (μg/mL)
19	H	Et	5.7	3.96	NT
20	H	*i*-Pr	6.0	4.26	NT
21	H	*n*-Pr	6.6	4.48	NT
22	H	*i*-Bu	7.1	4.88	NT
23	H	Methylene cyclopropyl	6.9	4.40	121
24	Me	Methylene cyclopropyl	7.6	4.90	268
25	CF_3	Methylene cyclopropyl	7.7	5.28	219

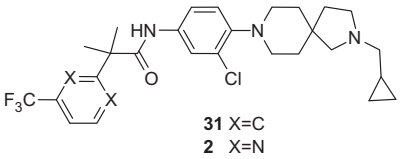


Figure 9. SAR of substituted spiroamines.

Table 7
Profiling results of substituted spiro amines

	hNPY Y2 (fpK_i)	*c* log *P*	Solubility (μg/mL)
31	8.7	6.41	53
2	6.8	4.03	225

Table 8
Sample compounds selected for in vitro pharmacokinetic profiling

	hNPY Y2 (fpK_i)	*c* log *P*	Clint rat (mL/min/g)	Clint human (mL/min/g)	BTB (rat) %
23	6.9	4.40	5.7	0.8	97.6
24	7.6	4.90	16.0	4.8	99.1
25	7.7	5.28	1.4	<0.5	99.6
28	6.1	2.44	1.9	<0.5	88.2
±-29	6.2	2.08	0.8	0.7	75.9
2	6.8	4.03	1.8	0.8	97.2

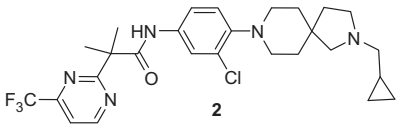


Figure 10. Pyrimidine **2**.

Table 9
Selected in vitro selectivity data for pyrimidine **2**

	hNPY Y2 (fpK_i)	rNPY Y2 (fpK_i)	NPY Y1 (fpK_i)	NPY Y5 (fpK_i)
2	6.8	7.2	<5.2	<5.4

Table 10
In vivo DMPK profile of **2** after sc administration

Dose (mg/kg)	Br:Bl	C_{max} (Br) (ng/g)	C_{max} (Bl) (ng/mL)	Sample time
2.0	2.3	73	32	1.0

In vivo pharmacokinetic profiling in the rat showed **2** displayed a brain to blood ratio of 2.3 with 73 ng/g in brain and 32 ng/mL in blood after a single sc administration at 2 mg/kg (Table 10).

In summary, we have identified a novel series of soluble, selective small molecule NPY Y2 antagonists exemplified by amine **2**. Compound **2** has a significantly improved in vivo PK profile compared to our previously described diamide series, with comparable potency at the rat NPY Y2 receptor in vitro, making it a suitable candidate for in vivo target validation studies.

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14. The functional activity (fpK_i) 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 four or more experiments.
15. Compounds were characterised by ^{1}H NMR spectroscopy and LC–MS.
16. The functional activity (fpK_i) against rat NPY Y2 was determined in a human embryonic kidney (HEK) cell line transiently expressing the receptor, using a 384 well GTPγ35S assay.
17. The functional activity (fpK_i) against human NPY Y1 was determined in a Chinese hamster ovarian (CHO) cell line stably expressing the receptor, using a 384 well GTPγ35S assay.
18. The functional activity (fpK_i) at the human NPY Y5 receptor stably expressed in HEK293 cells was assessed using FLIPR/Ca2+ methodology in a 384-well format.