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Synthesis and structure–activity relationship of *N*-(3-azabicyclo[3.1.0]hex-6-ylmethyl)-5-(2-pyridinyl)-1,3-thiazol-2-amines derivatives as NPY Y5 antagonists

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ABSTRACT

A novel class of small molecule NPY Y5 antagonists based around an azabicyclo[3.1.0]hexane scaffold was identified through modification of a screening hit. Structure–activity relationships and efforts undertaken to achieve a favourable pharmacokinetic profile in rat are described.

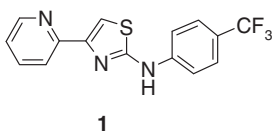
Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family that also includes pancreatic polypeptide (PP) and peptide YY (PYY).[1–3] NPY is one of the most abundant neuropeptides in the CNS of both humans and rodents[4,5] and is a potent orexigenic factor.[6,7]

Its anatomical localisation and preclinical studies suggest that NPY may be involved in a variety of physiological responses such as food intake, water consumption, emotional behaviour, circadian rhythms, endocrine and cardiovascular functions.[8–10] In particular a wealth of preclinical evidence points to a key role of NPY in the regulation of both feeding behaviour and energy expenditure, and hence agents modulating the interactions of NPY and its receptors could provide novel centrally mediated treatments for eating disorders.[11–16] Of the five NPY receptors cloned to date (Y1, Y2, Y4, Y5 and y6), the Y5 subtype has been one of the main candidates implicated in mediating this physiological role of NPY. Accordingly, many pharmaceutical companies have targeted antagonists of the Y5 receptor for the treatment of obesity,[17–28] although the results of clinical trials reported to date have shown modest efficacy.[29,30] Recently, emerging studies address a further role of NPY Y5 receptors in the regulation of addictive behaviours.[31] A screening campaign of our corporate compound collection generated a number of promising chemotypes from which the aminothiazolepyridine **1** was identified as an efficient, orally bioavailable and brain penetrant Y5 antagonist (Fig. 1). A direct comparison of compound **1** with the structure of known Y5 antagonists[21,23] revealed some recurrent pharmacophoric features and suggested that the opportunity to further optimise this molecule lay with the modification of the *N*-phenyl side chain.

Herein, we report structure–activity relationship (SAR) studies undertaken in an effort to develop a novel class of NPY Y5 antagonists from **1**.

Amongst a number of compounds prepared from commercially available 4-(2-pyridinyl)-1,3-thiazol-2-amine **2**, the azabicyclohexane derivatives **3** showed appealing Y5 antagonist activities (Fig. 2).

Despite the specific orientation of the end-capping carbamate moiety dictated by the rigid [3.1.0] bicyclic linker both the *endo* and the corresponding *exo* isomer of **3** were found to demonstrate low-nanomolar NPY Y5 activity.



1

hNPY Y5 fpKi 7.8
hNPY Y1/2 fpKi < 4.5
CYP450 1A2 < 0.1 uM
Cl_b 27 ml/min/kg
F = 30%
B/B 4.2

Figure 1. Structure of hit compound **1**.

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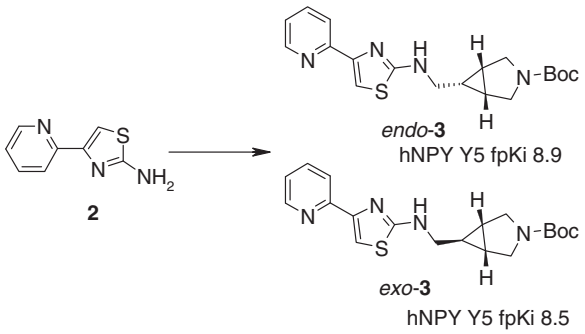
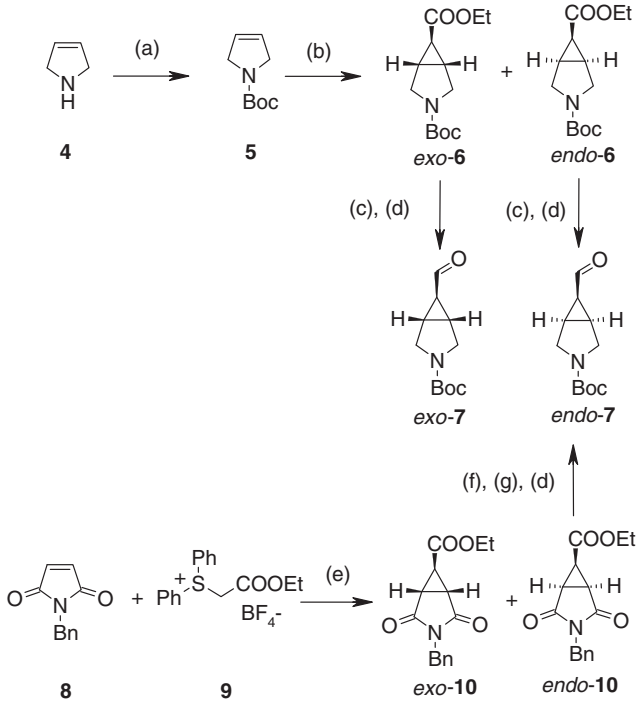


Figure 2. Structure of lead compound *endo*-**3**.

Although a preference is evident for the *endo* isomer, the fact that the receptor is able to accommodate such differing conformational limitations substantiates the original proposition that this area of the pharmacophore is the most tolerant to modification.

The stereoselective synthesis of the 3-azabicyclo[3.1.0]hexane linker was achieved by two different synthetic strategies (Scheme 1). Commercially available 3-pyrroline (**4**) was protected with Boc anhydride, and then reacted with ethyl diazoacetate (EDA), added over 60 h by means of a syringe pump, in the presence of 5 mol % of $[Rh(OAc)_2]_2$ to afford a mixture of the two isomers *exo*-**6** and *endo*-**6** in a ~3:1 ratio, that could be separated by silica gel chromatography. Reduction of compounds *exo*-**6** and *endo*-**6** with $LiAlH_4$ followed by oxidation with the Dess–Martin reagent afforded the required aldehydes *exo*-**7** and *endo*-**7** with good yields. The replacement of hazardous EDA with diphenylsulfonium (methoxycarbonyl)methylide allowed inversion of the stereochemical outcome of the cyclopropanation step to favour the more active *endo* isomer. Indeed, the reaction of sulfonium salt[32] **9** with maleimide **8** in the presence of *N*-phenyl-tris(dimethylamino)iminophosphorane (BEMP) produces a readily separable mixture of *exo*-**10** and *endo*-**10** in a ~1:3.5 ratio. It is noteworthy that while the use of a number of both organic and inorganic bases (Table 1, entries 1–5) resulted ineffective to promote the cyclopropanation of maleimide **8**, as previously reported by Mikołajczyk and co-workers.[33] DBU successfully afforded the *endo* isomer (entry 6), albeit with lower yield compared to BEMP (entry 7) (Table 1).



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$ (1.05 equiv), DCM, rt 80%; (b) ethyl diazoacetate (1.3 equiv), $[Rh(OAc)_2]_2$ 5 mol %, DCM, syringe pump over 60 h, 3:1 *exo*:*endo*, 38% combined yield; (c) $LiAlH_4$ (0.75 equiv), THF, rt, 1 h, 90%; (d) Dess–Martin periodinane (1.2 equiv), DCM, rt, 90%; (e) BEMP (1.4 equiv), DCM, rt, 18 h, 1:3.5 *exo*:*endo*, 78% combined yield; (f) $LiAlH_4$ (2.4 equiv), THF, 70 °C, 30 min, 83%; (g) $Pd(OH)_2$ (20% w/w), H_2, $(Boc)_2O$ (1.2 equiv) EtOH, rt, 5 h, 100%.

endo-**10** was readily converted into the Boc protected derivative *endo*-**7** by reduction with $LiAlH_4$, then Pd-catalysed removal of the benzyl group under a H_2 atmosphere and in situ protection with Boc anhydride, followed by oxidation with Dess–Martin periodinane.

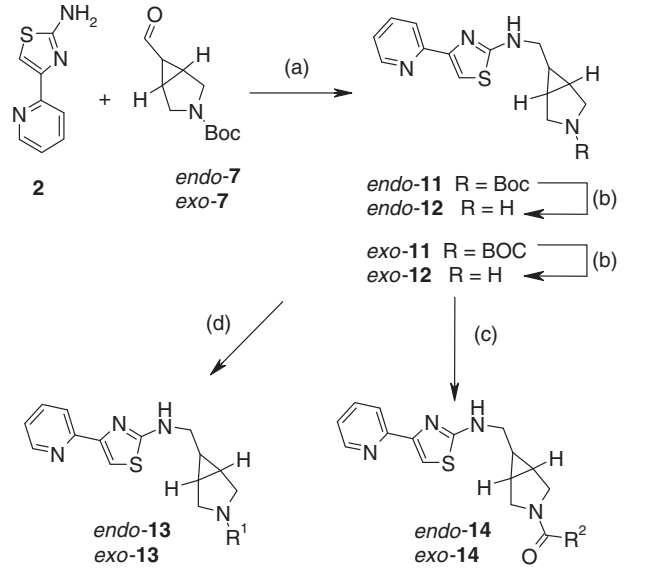
Finally, aldehydes *exo*-**7** and *endo*-**7** led to the required scaffolds *exo*-**11** and *endo*-**11** through reductive amination promoted by $Ti-Cl(OiPr)_3$. Removal of the Boc groups afforded amines *exo*-**12** and *endo*-**12**, that were converted into the corresponding amides *exo*-**14** and *endo*-**14** under standard conditions. Reductive amination in the presence of a suitable aldehyde gave amines *exo*-**13** and *endo*-**13** (Scheme 2).

The SAR exploration mainly focused on the amide decoration and resulted in a fair number of potent derivatives supporting the interesting potential of the new [3.1.0] linker (Table 2).

Indeed, it was found that a wide diversity of capping amides was well tolerated. With the exception of the inactive methyl derivative *endo*-**15**, both aliphatic and aromatic amides showed very high NPY Y5 activities in the low- to sub-nanomolar range. The profile of the amide *endo*-**16** was also supported by a reasonable solubility (120 μg/ml, determined using a high throughput

Table 1
Reaction of the diphenylsulfonium salt **9** with maleimide **8**

Entry	Base	Solvent	*T* (°C)	Yield (*exo*-**10**/*endo*-**10**)
1	LDA	THF	rt	—/—
2	NaH	THF	rt	—/—
3	K_2CO_3/NaOH	Acetone	45	—/—
4	Et_3N	DCM	rt	—/—
5	LHMDS	THF	rt	0/14
6	DBU	DCM	rt	10/37
7	BEMP	DCM	rt	17/61



Scheme 2. Reagents and conditions: (a) $TiCl(O^iPr)_3$ (1.5 equiv), $NaBH(OAc)_3$ (4 equiv), DCM, rt; (b) TFA (10 equiv), DCM, rt, 95%; (c) acid chloride R_2COCl (1.05 equiv), Et_3N (2 equiv), DCM, 0 °C; (d) aldehyde R_1CHO (1.1 equiv), NaBH(OAc), DCM, rt.

Table 2
Y5 activities of amide derivatives, clog *P* and in vitro metabolic stability

Compds	R^2	hNPY Y5 fpKi[a]	Clint h/r[b]	clog *P*[c]
endo-**15**	Me	5.6	na	0.4
endo-**16**	*i*Pr	8.6	3/2.3	1.3
exo-**17**	Phenyl	7.9	0.9/0.6	2.3
endo-**17**	Phenyl	8.6	7/3	2.3
endo-**18**	3-F-phenyl	9.0	9/7	2.5
endo-**19**	3,5-DiF-phenyl	8.8	17/11	2.6
endo-**20**	3-Pyridyl	8.3	10/1.9	1.2
endo-**21**	4-Pyridyl	7.3	na	1.2
exo-**22**	Benzyl	9.0	6/3.7	2.7
endo-**22**	Benzyl	9.0	15/15	2.7
endo-**23**	1-Phenylethyl	9.4	37/48	3.0
endo-**24**	1-Phenylcyclopropyl	9.1	31/44	3.0
exo-**25**	2-Phenylethyl	8.2	23/17	2.5
endo-**25**	2-Phenylethyl	9.2	na	2.5
endo-**26**	1-Methyl-2-phenylpropyl	8.3	50/50	3.2
endo-**27**	*trans*-2-Phenylcyclopropyl	9.1	23/22	2.6
endo-**28**	3-Tetrahydrofuranyl	8.1	0.7/0.7	0.9
endo-**29**	2-Tetrahydrofuranyl	8.3	1.6/0.9	1.3
endo-**30**	4-Tetrahydropyranyl	7.5	na	0.1

[a] The functional activity (fpKi) at the human NPY Y5 receptor stably expressed in HEK293 cells was assessed using FLIPR/Ca^{2+} methodology in a 384 well format. Each determination lies within 0.3 log units of the mean with a minimum of two replicates.
[b] Intrinsic clearance values (Clint) expressed as ml/min/g liver were determined in human (h) and rat (r) liver microsomes; na = not available.
[c] ACD log *P* version 11.

chemiluminescent nitrogen detection method) low CYP450 inhibition potential ($IC_{50} \geqslant 7$ μM at 1A2, 2C9, 2C19 and 3A4 isoforms), and a suitable selectivity profile over a panel of 22 other receptors and ion channels. Compound *endo*-**16** was further characterised in in vivo rat cassette dosing PK studies revealing a blood clearance in excess of liver blood flow (Cl_b = 137 ml/min/kg) and low oral bioavailability (*F* = 6%) (Table 3).[34]

The *endo*-phenyl amide **17** confirmed to be more potent than the corresponding *exo*-**17** analogue but surprisingly it had drastically higher human and rat Clint values (Clint h/r = 7/3 ml/min/g liver). Although the introduction of fluorine atoms on the phenyl ring, as in *endo*-**18** and *endo*-**19**, was unable to increase significantly the potency, replacement with a less lipophilic pyridine group, *endo*-**20** and *endo*-**21**, resulted in a reduction of Y5 activity. Interestingly, while both benzylamides *exo*-**22** and *endo*-**22** displayed equipotent Y5 antagonism, further homologation, leading to *exo*-**25** and *endo*-**25**, restored the prevalent activity of the *endo*-analogue observed previously. Unfortunately, all these modifications were marked by a worsening Clint profile. Attempts to overcome metabolic liabilities by hindering the benzylic position, as in *endo*-**23**, *endo*-**24**, *endo*-**26** and *endo*-**27**, were unsuccessful, leading to higher intrinsic clearances and poor CYP450 profiles with potent inhibition of the 3A4 isoform.

Disappointingly, even compound *endo*-**28**, characterised by an attractive in vitro profile, having low intrinsic clearance (Clint h/r = 0.7/0.7 ml/min/g liver), low CYP450 inhibition potential (IC_{50} >10 μM at 1A2, 2C9, 2C19 and 3A4 isoforms), low protein binding (fraction unbound = 26%), excellent selectivity against NPY Y1 and Y2 receptors (fpKi <5) and modest activity at the hERG channel

Table 3
Selected DMPK data[a] for compounds *endo*-**25** and *endo*-**28**

Compds	Cl_b (ml/min/kg)	B/B rat	WBB%[b] rat	*F*%
endo-**16**	137	0.7	—	6
endo-**28**	98	<0.5	74	15

[a] In vivo data determined by 0.5 mg/kg iv and 1 mg/kg po administration in rat. Brain/blood ratio (B/B) determined at 1 h following iv dosing.
[b] Whole blood binding was determined in an equilibrium dialysis assay.

Table 4
Y5 activities of amine derivatives, clog *P* and in vitro metabolic stability

Compds	R^1	hNPY Y5 fpki[a]	Clint h/r[b]	clog *P*[c]
exo-**31**	H	6.0	na	1.4
endo-**32**	*i*Bu	8.4	2.6/15	1.3
endo-**33**	Benzyl	8.8	na	3.6
endo-**34**	3,5-Dimethyl-benzyl	8.4	6/14	4.6
endo-**35**	3,5-DiF-benzyl	9.2	5/39	3.9
endo-**36**	1-Phenylethyl	8.9	20/31	3.9

[a,b,c] See Table 2.

(pIC_{50} = 5.7), resulted highly cleared in a rat cassette dosing study (Cl_b 98 ml/min/kg). The poor correlation between the Clint values and the in vivo blood clearance observed for both *endo*-**16** and *endo*-**28** hints that extrahepatic clearance routes may be operating.

In an attempt to better understand the factors responsible for the high clearance observed with the amide derivatives, and to generate further insights into the SAR, a number of N-alkylated derivatives were prepared (Table 4).

Generally, these derivatives were as potent as the corresponding amides, suggesting that the carbonyl group most likely was not involved in a specific interaction within the binding pocket, or in imparting the active conformation. However, further in vitro characterisation of these amines showed moderate to high Clint values and an unacceptable recurring CYP450 inhibition at the 2D6 isoform, probably attributable to the basic center.[35] It is, however, noteworthy that these compounds, containing a basic tertiary amine residue, maintain high Y5 potency as the majority of Y5 antagonists reported in the scientific literature are neutral at physiological pH.

In summary, a novel series of highly potent, functional NPY Y5 antagonists has been prepared based around a bicyclic [3.1.0] scaffold. An extensive SAR exploration revealed a preference for the *endo*-diastereoisomer and lipophilic capping groups alongside a penchant for high intrinsic clearance. Optimisation of the in vitro DMPK profile led to the identification of compound *endo*-**28**. Notwithstanding its low intrinsic clearance and respectable physicochemical properties *endo*-**28** was found to be highly cleared in rat, revealing a poor in vitro/in vivo correlation which is currently under further investigation.

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