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Design, synthesis and SAR of a novel series of benzimidazoles as potent NPY Y5 antagonists

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ABSTRACT

A novel class of benzimidazole NPY Y5 receptor antagonists was prepared exploiting a privileged spirocarbamate moiety. The structure–activity relationship of this series and efforts to achieve a profile suitable for further development and an appropriate pharmacokinetic profile in rat are described. Optimisation led to the identification of the brain penetrant, orally bioavailable Y5 antagonist **9b** which significantly inhibited the food intake induced by a Y5 selective agonist with a minimal effective dose of 30 mg/kg po.

Neuropeptide Y (NPY) is a 36-amino acid peptide originally discovered in extracts of porcine brain.[1] The peptide belonging to a broad family of peptides (which includes peptide YY and pancreatic polypeptide PP) has a wide distribution in the central[2–4] and peripheral nervous system.[5–7] NPY is expressed in hypothalamic regions and this suggests that it may be implicated in a variety of physiological functions, notably the central regulation of feeding behaviour and energy homeostatis.[8,9] The biological effects of NPY are mediated through its interaction with five G-protein coupled receptors (Y1, Y2, Y4, Y5 and y6).[10] A number of pharmacological studies conducted with transgenic mice and/or subtype selective agonists and antagonists have suggested that the Y5 receptors are involved in the regulation of food intake.[11–14] Additionally, small molecule Y5 antagonists have proven efficacious in animal models of addiction to drugs of abuse[15] and in animal models of depression.[16] Hence, antagonism of the Y5 receptor represents an interesting pharmacological target with potential therapeutic applications for the treatment of eating disorders, drug dependency or depression.

Herein we report our efforts to elaborate a proprietary series of NPY Y5 antagonists that led to the discovery of a novel class of benzimidazoles, endowed with potent Y5 antagonist activity and a profile suitable for further development.

As part of a campaign to identify novel, orally bioavailable and brain penetrant Y5 antagonists, the urea lead **1**, characterised by a spirocyclic carbamate moiety, was discovered from a number of prospective diversity arrays (Fig. 1).[17]

Considering that a spirocyclic lactone group, such as that shown in structure **2**, appears to be a privileged substructure, recurring in several series of Y5 antagonists reported in literature,[18–22] we hypothesised that the spirocarbamate group may represent an isosteric privileged substructure that could bestow Y5 affinity upon other templates. Moreover, if this hypothesis held true, then judicious selection of the new template may potentially lead to structures with improved brain penetration with respect to **1**.

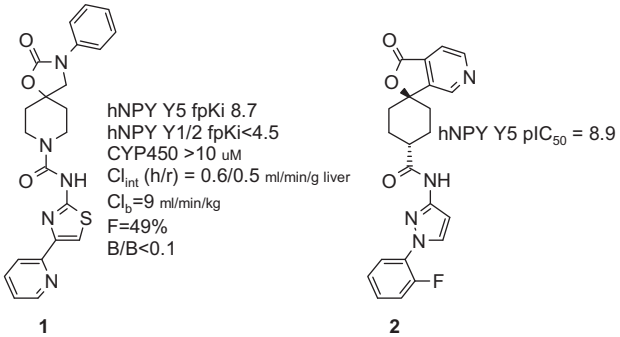

Figure 1. Structure of lead compound **1** and spirolactone **2**.

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Consequently, we set about transferring the novel spirocarbamate building block, present in derivative **1**, to a benzimidazole scaffold to give compound **3a** (Fig. 2).

Gratifyingly, compound **3a** demonstrated good Y5 activity verifying our hypothesis. In addition, the in vivo pharmacokinetic profile[23] of **3a** was characterised by exceptionally low clearance and high bioavailability; as hoped brain penetration improved with respect to **1** although it remained moderate in absolute terms ($B/B = 0.2$). The in vitro profile was tarred by potent CYP450 inhibition of the 2C9 isoform and limited selectivity with respect to α_{1A} ($fpK_i = 7.0$). The initial optimisation strategy was directed at increasing the brain penetration of this class whilst exploring the structure–activity relationships (SARs) at CYP450 and the α_{1A} receptor. Three different approaches were followed for the optimisation: (i) variation of the substituent on the benzimidazole; (ii) reduction of the polar surface area (PSA) via removal of heteroatoms, switching from a N-linker to a C-linker between the spirocarbamate building block and the benzimidazole; (iii) introduction of an 'ortho-substituent' on the *N*-phenyl ring of the spirocarbamate to help mask the polarity of carbamate group.

The synthetic route used to access the N-linked compounds **3** and the C-linked derivatives **9** is outlined in Scheme 1.

To prepare the N-linked benzimidazoles, amine **4**[24] and 2-chlorobenzimidazoles **5**, which were either purchased or prepared according to reported methods,[19] were reacted in the presence of Hunig's base, in DMSO at 180 °C in a microwave reactor for 40 min, to give the products **3**. Additionally, the bromo derivative **3c** was arylated under Suzuki conditions to afford **3e**.

For the C-linked benzimidazoles, the *trans*-carboxylic acids **6**[17] and the substituted phenyl-1,2-diamines **7** were easily converted into a mixture of regioisomeric amides **8** with EDC·HCl in pyridine at room temperature. The amides were subsequently cyclised with *p*-toluenesulphonic acid, in a mixture of toluene and dioxane, heating the mixture to 150 °C in a microwave reactor for 30 min, to afford the final derivatives **9**. Initially, a similar synthetic sequence was followed using the corresponding *cis*-isomer of **6** (R = Ph) to give the *cis*-analogue of **9a**, however, this derivative had low target activity (NPY Y5 $fpK_i = 5.6$ vs 8.8 for **9a**) hence SAR studies were conducted exclusively with the *trans*-isomer.

The SAR exploration focused on the introduction of different substituents on the benzimidazole resulted in a fair number of potent derivatives (Table 1). It was found that a substituent at the 5-position was essential for high target activity and that larger, more lipophilic substituents gave the highest Y5 activities; compounds **3e** and **3f** were most active, showing 10-fold higher potencies than the lead **3a**. Most substituents improved the P450 inhibition profile at the 2C9 isoform, with the fluorophenyl derivative **3e** being of particular note (>10 μM at all isoforms). Little or no change was observed in α_{1A} activity, however, metabolic stability proved highly susceptible to changes in the substituent

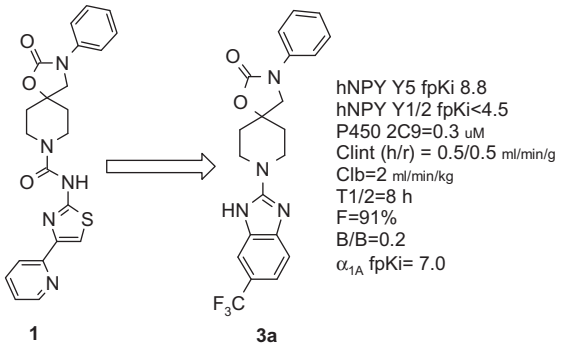
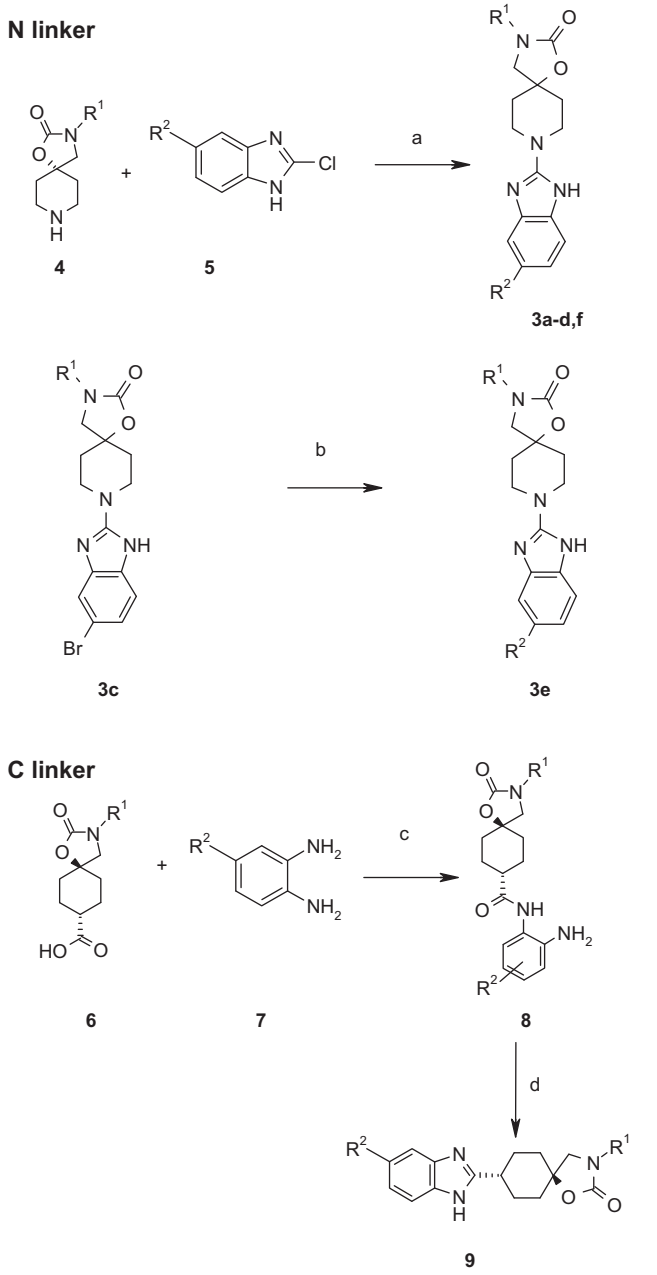


Figure 2. Structure of novel lead compound **3a**.



Scheme 1. Reagents and conditions: (a) DIPEA, DMSO, 180 °C, microwave reactor, 40 min; (b) $R^2B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, dioxane:water (5:1), 140 °C, microwave reactor, 10 min; (c) EDC·HCl, Py, rt, 1.5 h; (d) TsOH, dioxane:toluene (1:1), 150 °C, 30 min.

in the 5-position, where only trifluoromethyl (**3a**) or trifluoromethoxy (**3f**) groups gave acceptable intrinsic clearances (Cl_{int}) values. Compound **3f** was progressed to rat PK studies where it demonstrated a similar profile to **3a** but with slightly improved brain penetration ($Cl_b = 3$ ml/min/kg, $F = 90\%$, $B/B = 0.3$) although this was at the cost of higher protein binding—the unbound fraction in the brain (Fu_{br}) was determined as this is considered an influential parameter in driving the in vivo efficacy of a compound.

The strategy to exchange the N-linker of the aminobenzimidazole **3a** for a C-linker led to compound **9n**; as well as maintaining excellent Y5 activity this modification also led to an improved P450 inhibition profile on the 2C9 isoform and a slight increase in brain penetration ($B/B = 0.3$),[25] but, more importantly, activity at the α_{1A} receptor dropped 10-fold to give >1000-fold selectivity against Y5.

Table 1
NPY Y5, α_{1A} and hERG activities, in vitro metabolic stability, *c* log *P*, tPSA, CYP450 inhibitions, Fu_{br} and brain penetration of benzimidazole derivatives **3a–f** and **9a–s**

Compds	R^1	R^2	hNPY Y5 fpK_i[a]	Cl_{int} *h*/*r*[b] (ml/min/g liver)	*c* log *P*[c]	PSA[d] (Å^2)	α_{1A} fpK_i[e]	hERG pIC_{50}[f]	CYP450 inh. (μM)[g]	Fu_{br}[h]	*B*/*B*[i]
3a	Ph	CF_3	8.8	0.5/0.5	3.75	61	7.0	5.7	>10/0.3/2/5/>10	1.0	0.2
3b	Ph	H	6.5	9.8/1.0	2.73	61	7.0	6.0	>10/>10/>10/2/>10		
3c	Ph	Br	8.9	2.5/0.5	3.67	61	7.4	na	>10/-/2/3/>10		
3d	Ph	F	7.7	7/0.5	2.95	61	7.0	na	>10/-/7/2/>10		
3e	Ph	4-F-phenyl	9.7	7/5	4.76	61	na	na	All >10		
3f	Ph	OCF_3	9.8	0.5/0.5	4.14	71	6.7	na	>10//2/2/1/>10	0.6	0.3
9a	Ph	OCF_3	9.5	0.5/0.5	5.15	67	5.9	5.6	>10/3/2/4/>10	0.3	0.6
9b	2-F-phenyl	OCF_3	9.2	0.5/0.5	5.29	67	6.1	5.2	>10/4/2/>10/>10	0.9	0.8
9c	4-F-phenyl	OCF_3	9.5	0.5/0.5	5.29	67	6.1	6.1	>10/4/3/1/>10		
9d	2-Me-phenyl	OCF_3	8.3	0.5/0.5	5.65	67	na	na	>10/7/3/>10/>10		
9e	2-Cl-phenyl	OCF_3	8.6	0.5/0.5	5.86	67	na	na	>10/5/2/6/10		
9f	2-Py	OCF_3	8.9	0.5/0.5	3.65	80	5.7	5.6	>10/3/2/4/>10	1.0	0.5
9g	6-Me-2-Py	OCF_3	9.4	0.6/0.7	4.15	80	5.8	5.7	>10/2/2/1/>10		
9h	2-Me-3-Py	OCF_3	7.1	0.6/0.7	4.15	80	<5.6	4.3	All >10		
9i	6-F-2-Py	OCF_3	8.9	0.5/0.5	3.88	80	6.3	5.4	>10/5/2/3/-		
9l	3-Py	OCF_3	7.6	0.5/0.5	3.65	80	<5.6	5.1	>10/8/5/2/>10		
9m	2-F-3-Py	OCF_3	7.7	na	3.88	80	na	4.8	>10/10/5/>10/>10		
9n	Ph	CF_3	9.2	0.5/0.5	4.75	58	5.7	5.4	>10/4/2/3/>10	0.7	0.3
9o	2-Py	CF_3	8.3	0.5/0.5	3.26	71	5.9	5.4	>10/2/2/8/>10		
9p	6-F-2-Py	CF_3	8.4	na	3.48	71	na	na	na		
9q	Ph	CN	9.5	0.5/0.5	3.35	82	<5.6	5.0	>10/>10/7/>10/>10	3.4	<0.03
9r	4-F-phenyl	CN	9.2	0.5/0.5	3.49	82	<5.6	5.8	>10/10/5/7/>10		
9s	2- Py	CN	8.1	15/14	1.85	95	<5.6	4.9	All >10		

[a] The functional activity (fpK_i) 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 (Cl_{int}) were determined in human (h) and rat (r) liver microsomes and are expressed as ml/min/g liver.
[c] ACD log *P* version 11.
[d] Topological polar surface area (PSA) calculated using freeware by Peter Ertl.
[e] The functional activity (fpK_i) at the human α_{1A} receptor stably expressed in rat 1 fibroblast 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.
[f] The binding affinity (pIC_{50}) for the human ether-a-go-go related gene (hERG) ion channel stably expressed in CHO cells was assessed using [^{3}H]-dofetilide in a SPA displacement assay in a 384-well format. Each determination lies within 0.3 log units of the mean with a minimum of two replicates.
[g] CYP450 inhibition potential at 1A2/2C9/2C19/2D6/3A4 isoforms, respectively, determined in Cypex bactosomes.
[h] Fraction unbound determined in an equilibrium dialysis assay using homogenised rat brain.
[i] Brain/blood partition determined 1 h after iv administration of 0.5 mg/kg test compound to male rat; na = not available.

The combination of OCF_3 on the benzimidazole and the C-linker afforded compound **9a**, with high target activity and high selectivity. The PK profile of this compound in rat confirmed the high metabolic stability and good oral absorption typically seen in this series (Cl_b = 7 ml/min/kg, *F* = 69%) and brain penetration was significantly higher than previous analogues (*B*/*B* = 0.6) although, again, this was compensated by a lower free fraction in the brain (Fu_{br} = 0.3%). In an effort to reduce brain tissue binding, while maintaining the desirable characteristics of **9a**, an attempt was made to reduce the lipophilicity by exchanging the 5-trifluoromethoxy group on the benzimidazole for a 5-cyano group. This gave compound **9q** which was endowed with the best in vitro profile observed in the series offering high Y5 activity (fpK_i = 9.5), high metabolic stability (Cl_{int} *h*/*r* = 0.5/0.5 ml/min/kg), high selectivity and greatly improved P450 inhibition profile (all isoforms >7 μM) and protein binding (Fu_{br} = 3.4%). As usual the rat PK profile showed low clearance and good bioavailability (Cl_b = 7 ml/min/kg, *F* = 66%), but brain penetration was exceedingly low (*B*/*B* <0.03%) most likely due to active efflux.[26] Attempts to reduce lipophilicity via replacement of the phenyl group in **9a,n,q** with a pyridyl group in **9f,o,s** were accompanied by a modest reduction in Y5 potency, and in the case of **9s** a dramatic increase in intrinsic clearance (Cl_{int} *h*/*r* = 15/14 ml/min/g liver).

Finally, a number of *ortho*-substituents were introduced on the *N*-phenyl ring of the spirocarbamate with the intent to mask the polarity of the carbamate and increase the brain penetration. Y5 activities tended to decrease (**9d,e**) but compound **9b**, with a small *ortho*-fluoro substituent, maintained acceptably high NPY Y5 activity (fpK_i = 9.2) and good selectivity including NPY Y1 and Y2 receptors (all compounds **3** and **9** showed fpK_i <5.3 in Y1 and Y2 GTPγS functional assays). In addition an *ortho*-fluoro or methyl group had a beneficial effect on CYP 2D6 inhibition. Compound **9b** was further characterised in rat in vivo PK studies, where it exhibited the best overall profile seen within the series displaying good brain penetration (Cl_b = 8 ml/min/kg, *F* = 75%, *B*/*B* = 0.8) without erosion of the free fraction in the brain (Fu_{br} = 0.90%). Considering its whole profile, compound **9b** was selected for further characterisation in a pharmacodynamic model to verify its in vivo efficacy as a NPY Y5 antagonist. Compound **9b** dose dependently reversed the food intake induced by icv administration of 0.6 nM[27] of the NPY Y5 selective agonist, [cPP1-7,NPY19-23,Ala31,Aib32,Gln34]hPP, with a minimal effective dose of 30 mg/kg po.

In conclusion, rational design exploiting a privileged spirocarbamate building block led to the discovery of a novel class of benzimidazole NPY Y5 receptor antagonists. Optimisation of the lead structure for α_{1A} selectivity, CYP450 inhibition and brain penetration led to the identification of compound **9b** which combines potent NPY Y5 antagonist activity with high selectivity, good developability, high metabolic stability, high bioavailability and brain penetration.

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25. It is noteworthy that various factors may be influencing the modest brain/blood partitions observed with some of the derivatives **3** and **9**. For instance, compound **3f** has an observed B/B ratio ($B/B = 0.3$) close to its K_{bb} ($K_{bb} = 0.4$) (K_{bb} = fraction unbound in blood/fraction unbound in brain) and it tested negative in an in vitro *p*-glycoprotein assay, suggesting its observed B/B ratio is merely a consequence of its natural disposition at equilibrium to distribute into these two compartments. In contrast compound **9n** has an observed B/B ratio ($B/B = 0.3$) much lower than its K_{bb} ($K_{bb} = 2.0$) and it tested positive in an in vitro *p*-glycoprotein assay, implying its observed B/B ratio is strongly influenced by active efflux.
26. Active efflux is assumed to be responsible for the low observed brain penetration for compound **9q** as its B/B ratio ($B/B < 0.03$) is much lower that its K_{bb} ($K_{bb} = 1.1$) and the series is known to be liable to *p*-glycoprotein efflux (see Ref. 25).
27. The dose of 0.6 nM of [cPP1-7,NPY19-23,Ala31,Aib32,Gln34]hPP used in the pharmacodynamic model was chosen based on the findings of a dose–response curve which indicated this dose induced a near-maximal response.