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Aryl urea derivatives of spiropiperidines as NPY Y5 receptor antagonists

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ABSTRACT

Continuing medicinal chemistry studies to identify spiropiperidine-derived NPY Y5 receptor antagonists are described. Aryl urea derivatives of a variety of spiropiperidines were tested for their NPY Y5 receptor binding affinities. Of the spiropiperidines so far examined, spiro[3-oxoisobenzofurane-1(3*H*),4′-piperidine] was a useful scaffold for producing orally active NPY Y5 receptor antagonists. Oral administration of **5c** significantly inhibited the Y5 agonist-induced food intake in rats with a minimum effective dose of 3 mg/kg. In addition, this compound was efficacious in decreasing body weight in diet-induced obese mice.

Neuropeptide Y (NPY) is a highly conserved C-terminus amidated peptide consisting of 36 amino acid residues which has been shown to have potent, centrally-mediated orexigenic effects.[1–4] NPY is abundant in the central nervous system throughout the cerebral cortex, forebrain, hypothalamus, brain stem and spinal cord. In the periphery, NPY is present in most sympathetic nerve fibers, especially around blood vessels. Reports of NPY activity demonstrate a wide range of potential effects at both central and peripheral targets, acting either alone or in combination with other neurotransmitters such as norepinephrine and glutamate. The effects of the NPY family of peptides are mediated via a family of GPCRs, providing opportunities for subtype selective therapeutics. At least six receptor subtypes of the NPY family have been characterized based on cloning and/or their pharmacological properties.[5–19] Various pharmacological studies, employing receptor deficient mice and/or subtype-selective agonists and antagonists, have suggested that the Y1 and Y5 receptors are involved in body weight regulation.[14,16,20–32] Thus, the antagonism of the Y1 and/or Y5 receptors may have considerable therapeutic benefits for the treatment of obesity.

In our preceding Letter,[33] we reported discovery of potent and orally active NPY Y5 receptor antagonists based on a spiroindoline-3,4′-piperidine scaffold, exemplified by **1a** and **1b**. Compound **1a** was highly potent and well brain penetrant, but lacked oral bioavailability. In contrast, while **1b** was orally bioavailable and active in a Y5 agonist-induced food intake model in rats, **1b** was poorly brain penetrant. We were intrigued by these results as the spiroindoline-3,4′-piperidine structure is a so-called 'privileged structure'.[34–38] It is known that certain 'privileged structures' are capable of providing useful ligands for more than one receptor and that judicious modification of such structures could be a viable alternative in the search for new receptor agonists and antagonists.[39] We assumed that replacement of the spiroindoline-3,4′-piperidine with other spiropiperidine-based privileged structures may yield potent NPY Y5 receptor antagonists. In addition, some of them could possess more desirable profiles than the spiroindoline-3,4′-piperidine Y5 antagonists from the viewpoint of clinical development candidates, such as improvements in pharmacokinetics, brain penetration and safety. We herein report structure–activity relationships of spiropiperidine–urea derivatives NPY Y5 receptor antagonists and identification of potent spiro[3-oxoisobenzofurane-1(3*H*),4′-piperidine]–urea derivatives Y5 receptor antagonists.

The spiroindoline phenylpyrazine urea derivative **1c** was as potent and as brain penetrant as **1a** (Fig. 1). We employed a 2-amino-5-phenylpyrazine structure as a surrogate of the biphenylamine moiety in **1a** for initial exploration of the structure–activity studies at the spiropiperidine moiety due to safety concerns.[40] 2-Amino-3-phenylpyrazine **2** was reacted with phenyl chloroformate in pyridine to afford phenyl carbamate **3**, which was reacted with spiropiperidines **4a–h** to give the corresponding urea derivatives of spiropiperidines **5a–h**. Two conditions were employed for the urea formation reaction; (a) the piperi-

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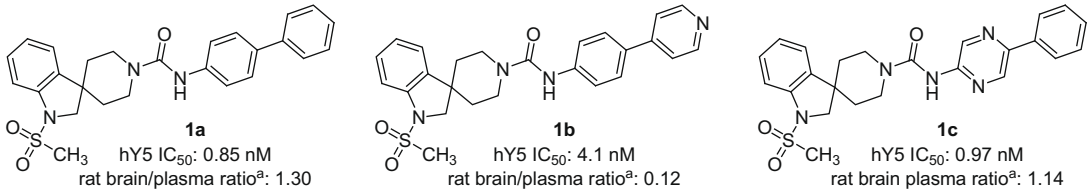


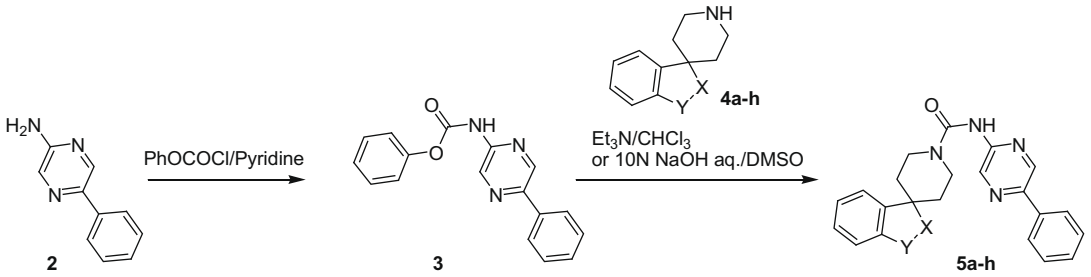
Figure 1. Spiroindoline-3,4′-piperidine Y5 leads. At 10 min after 3 mg/kg iv. $n = 2$.

dine derivative and the phenyl carbamate were refluxed in $CHCl_3$ in the presence of Et_3N or (b) the piperidine derivative and the phenyl carbamate were reacted in DMSO in the presence of 10 N aqueous NaOH at room temperature, and the reaction condition was chosen in accordance with solubility of the substrates (Scheme 1). The general synthetic method was applied for replacement of the 4-phenylpyrazine group with various groups.

Compounds **5a–h** were evaluated for their Y5 binding affinities.[41] Compounds **5b**, **5c**, **5e** and **5g** showed potent Y5 receptor binding affinities (although other analogs were very weak) suggesting that privileged structures are applicable to produce potent Y5 antagonists. The potent compounds **5b**, **5c** and **5e** were tested for their oral bioavailability in rats. Although **5g** showed a potent Y5 binding affinity, this compound exhibited unacceptable CYP3A pre-incubation time dependent inhibition,[42] and we did not further investigate this scaffold. Of the three compounds tested for their oral bioavailability, **5c** showed the best oral bioavailability (49%). Compound **5c** also exhibited a high brain level as well as a high cerebrospinal fluid (CSF) level at 2 h after oral administration in rat (Table 1). Compound **5c** showed potent binding affinities to rat and mouse Y5 receptors[43] (IC_{50} values of 1.7 nM and 1.7 nM, respectively) while the compound was very weak at human Y1, Y2 and Y4 receptors ($IC_{50} > 10\ \mu M$). The antagonistic activity of **5c** was assessed by its ability to inhibit NPY-induced $[Ca^{2+}]_i$ increases in LMtk-cells expressing the recombinant human Y5 receptor.[43] In this Y5 functional assay, **5c** inhibited the $[Ca^{2+}]_i$ increase with IC_{50} of 6.1 nM, demonstrating that **5c** is a Y5-selective antagonist. Oral administration of **5c** strongly inhibited the Y5 agonist, D-Trp^{34}NPY-induced food intake[44] in rats with a minimum effective dose of 3 mg/kg. In contrast, **5c** did not significantly inhibit NPY-induced food intake even at 10 mg/kg, demonstrating that the inhibitory effect of **5c** in D-Trp^{34}NPY-induced food intake was due to the Y5 receptor-selective antagonism (Fig. 2).[43,45]

As the spiro[3-oxoisobenzofurane-1(3*H*),4′-piperidine] was identified as a particularly useful scaffold for potent and selective Y5 antagonists, we further explored further modifications of this scaffold. Although compound **5c** was in vivo active, its solubility in aqueous media was limited (1.9 μg/mL and 1.2 μg/mL in JP1 at pH 1.2 and JP2 at pH 6.8, respectively).[46] More soluble compounds may be more preferable in terms of oral absorption and developability, so the 4-phenylpyrazinyl group in **5c** was replaced with various biaryl groups to investigate structure-activity relationships at this moiety. While various biaryl groups were tolerable in terms of the Y5 binding affinity although most compounds showed limited solubility, only the 4-phenylpyrimidine analog **6a** was particularly interesting for its good solubility. Introduction of substituents, such as Cl, CH_3O, F and CF_3, on the outer phenyl ring in **6a** was examined. In all cases, introduction of substituents at the *para*-position decreased the Y5 binding affinity whereas those at the ortho or *meta*-positions produced similar or modestly increased Y5 binding affinity. The phenylisoxazole analog **9a** was another interesting compound in terms of good Y5 binding affinity, and introduction of Cl on the outer phenyl ring was examined. In this case, the *p*-Cl substituent was not detrimental, and *o*-Cl, *m*-Cl and *p*-Cl analogs **9b–d** were as potent as **9a** unlike the prior case of 4-phenylpyrimidine analogs. (Table 2) Of the 4-phenylisoxazole analogs **9a**–**d**, **9d** was orally available in rats while compounds **9a**–**c** were very poorly available presumably due to their insufficient metabolic stability (data not shown).

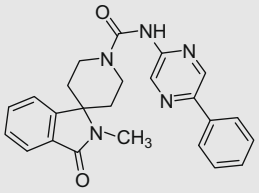
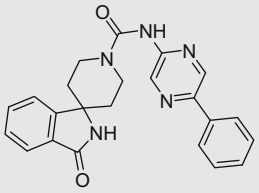
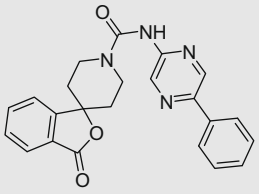
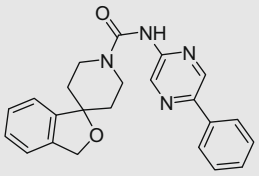
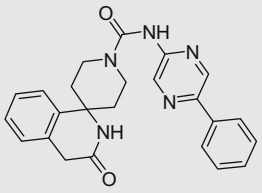
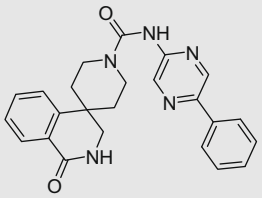
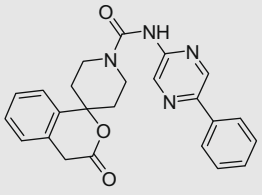
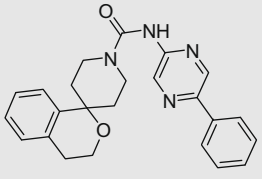
Compounds **6a** and **9d** were tested for their inhibitory effects on D-Trp^{34}NPY-induced food intake in rats after 10 mg/kg po dosing; plasma, brain and CSF exposures were also determined. The exposure data at 2 h after 10 mg/kg oral administration together with rat Y5 receptor binding affinities are listed in Table 3. Compounds **5c**, **6a** and **9d** showed equally potent binding affinities to the rat Y5 receptor as well as to the human Y5 receptor. In the



4a, **5a**: X = N-CH_3, Y = C(O)
4b, **5b**: X = NH, Y = (CO)
4c, **5c**: X = O, Y = C(O)
4d, **5d**: X = O, Y = CH_2
4e, **5e**: X = NH, Y = $CH_2C(O)$
4f, **5f**: X = CH_2NH, Y = (CO)
4g, **5g**: X = O, Y = $CH_2C(O)$
4h, **5h**: X = O, Y = CH_2CH_2

Scheme 1. Synthesis of urea derivatives of spiropiperidines.

Table 1
Human Y5 receptor binding affinities, bioavailability, and plasma, brain and CSF exposure data in rats

Compounds	Structure	hY5 binding IC_{50}[a] (nM)	Rat F[b]	Brain, plasma and CSF levels at 2 h after 10 mpk po in rats[c]		
				Plasma level (μM)	Brain level, nmol/g tissue	CSF level (μM)
5a		>1000[d]	NT	NT	NT	NT
5b		3.7	8%	0.36	0.029	ND
5c		2.4	49%	2.98	2.92	0.10
5d		>1000[d]	NT	NT	NT	NT
5e		1.7	3%	NT	NT	NT
5f		>1000[d]	NT	NT	NT	NT
5g		5.7	NT	NT	NT	NT
5h		>1000[d]	NT	NT	NT	NT

[a] In vitro data in nM are the average of at least two experiments.
[b] 3 mg/kg iv ($n = 2$) vs 10 mg/kg po ($n = 2$).
[c] $n = 3$.
[d] $n = 1$. NT, not tested. ND, not detected.

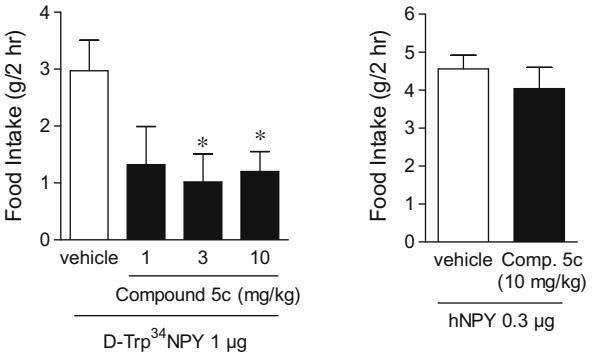
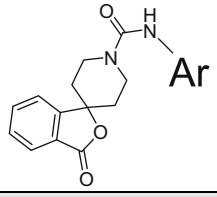


Figure 2. Inhibitory effect of compound **5c** on D-Trp^{34}NPY—or NPY-induced food intake in rats. $^{*}P < 0.05$ versus vehicle treated group. $n = 13–16$.

Table 2
Human Y5 binding affinities and solubility data



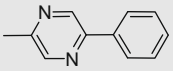
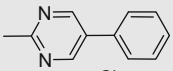
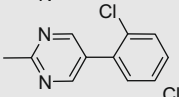
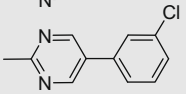
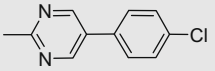
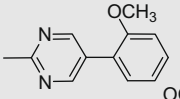
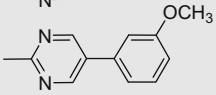
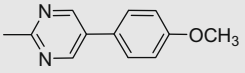
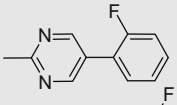
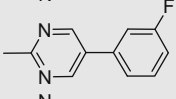
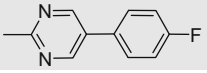
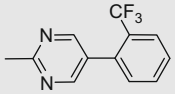
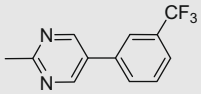
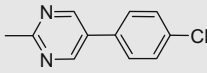
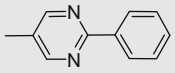
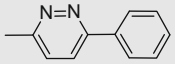
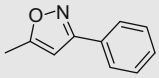
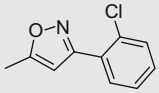
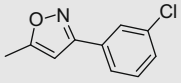
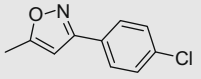
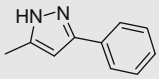
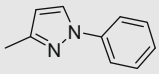
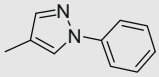
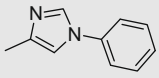
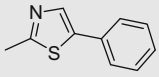
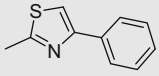
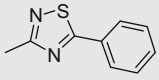
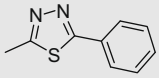
Compounds	Ar	hY5 binding IC_{50}[a] (nM)	Solubility (μg/mL)	
			JP1 at pH 1.2	JP2 at pH 6.8
5c		2.4	1.9	1.2
6a		1.4	>100	85
6b		1.1	2.6	0.2
6c		0.91	7.1	0.7
6d		3.9	NT	NT
6e		1.1	3.1	0.1
6f		1.8	NT	NT
6g		14	NT	NT
6h		0.99	5.9	0.6
6i		1.1	43	4.5
6j		2.4	48	4.0

Table 2 (*continued*)

Compounds	Ar	hY5 binding IC_{50}[a] (nM)	Solubility (μg/mL)	
			JP1 at pH 1.2	JP2 at pH 6.8
6k		1.3	0.8	0.1
6l		0.85	4.4	0.3
6m		20	NT	NT
7		2.2	0.6	0.2
8		6.1	NT	NT
9a		0.84	0.1	0.2
9b		0.83	NT	NT
9c		1.0	NT	NT
9d		0.69	<0.1	<0.1
10		1.9	2.5	17
11		2.9	27	23
12		2.3	0.8	0.7
13		3.5	>100	4.3
14		1.8	0.4	<0.1
15		7.9	NT	NT
16		1.2	0.6	0.5
17		3.4	NT	NT

NT, not tested.
[a] In vitro data in nM are the average of at least two experiments.

Table 3
Rat Y5 receptor binding affinities, inhibitory effect in D-Trp34NPY-induced food intake, and plasma, brain and CSF exposure data in rats

Compounds	rY5 agbinding IC_{50}[a] (nM)	Minimum effective dose (mg/kg po)[b]	Brain, plasma and CSF levels at 2 h after 10 mpk po in rats[c]		
			Plasma level (μM)	Brain level, nmol/g tissue	CSF level (μM)
5c	1.7	3	2.98	2.92	0.10
6a	0.67	3	3.86	0.26	0.13
9d	1.3	>30	1.42	1.09	<0.01

[a] In vitro data in nM are the average of at least two experiments.
[b] Inhibitory effect in D-Trp^{34}NPY-induced food intake.
[c] $n = 3$.

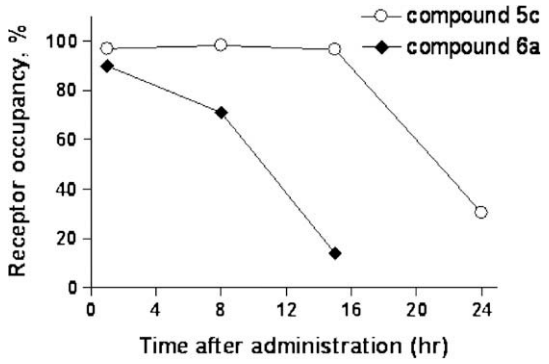


Figure 3. Receptor occupancy data in mice.

food intake assay, D-Trp^{34}NPY was intracerebroventricularly injected at 1 h after oral administration of the compounds, and food intake was measured for 2 h after the D-Trp^{34}NPY injection. Thus, the exposure data at 2 h reasonably represents exposure during the food intake assay. Compound **6a** showed lowest brain level among the three compounds while **6a** showed as high CSF level as **5c**. In contrast, **9d** showed a relatively high brain level, one third of **5c** but 4 times higher than **6a**, but very low CSF levels of less than 0.01 μM. In the D-Trp^{34}NPY-induced food intake assay, **9d** did not inhibit the food intake even at 30 mg/kg while **6a** potently inhibited food intake comparable to **5c**, suggesting that the CSF exposure level is a better indicator than the brain exposure level to predict Y5-mediated pharmacological effect and brain Y5 receptor occupancy.

Compound **5c** was efficacious at 10 mg/kg once daily oral treatment in the diet-induced obesity mice model as reported separately.[47] In contrast, **6a** was not efficacious even at 30 mg/kg in the same model (data not shown). We compared the two compounds for their brain Y5 receptor occupancy after oral administration in mice.[48] Compound **5c** showed almost full receptor occupancy until 16 h after 10 mg/kg oral administration. In contrast, **6a** showed transient receptor occupancy under the same conditions, suggesting that sustained receptor occupancy is required to show the anti-obesity effect in the DIO mice model (Fig. 3).

In summary, we identified a series of spiro[3-oxoisobenzofurane-1(3*H*),4′-piperidine]–urea derivatives that are potent, selective and orally active NPY Y5 receptor antagonists. In particular, **5c** significantly inhibited D-Trp^{34}NPY-induced food intake in rats with a minimum effective dose of 3 mg/kg. The CSF exposure level was a useful indicator to predict efficacy in the rat D-Trp^{34}NPY-induced food intake model. Compound **5c** showed sustained brain Y5 receptor occupancy in mice, and oral once-daily administration of **5c** at 10 mg/kg was efficacious in diet-induced obesity mice. Thus, **5c** was an attractive candidate for clinical development.

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