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Discovery of substituted 2,4,4-triarylimidazoline derivatives as potent and selective neuropeptide Y Y5 receptor antagonists

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ABSTRACT

Novel imidazoline derivatives were discovered to be potent neuropeptide Y Y5 receptor antagonists. High-throughput screening of Merck sample collections against the human Y5 receptor resulted in the identification of 2,4,4-triphenylimidazoline (**1**), which had an IC_{50} of 54 nM. Subsequent optimization led to the identification of several potent derivatives.

NPY is a 36 amino acid peptide neurotransmitter which was discovered in 1982 as a member of the pancreatic polypeptide family, like peptide YY, and a pancreatic polypeptide.[1] NPY is a widely distributed peptide in both the central and the peripheral nervous systems and has various physiological functions. Concentrations of NPY and mRNA levels in the hypothalamus respond to feeding status, including food deprivation and refeeding.[2,3] Chronic central infusion of NPY in rodents results in a syndrome similar to that in some genetic obesity models, characterized by hyperphagia, insulin resistance, hyperinsulinemia, and reduced thermogenic activity in brown adipose tissue.[4] Furthermore, NPY-deficient ob/ob mice are less obese and have reduced food intake compared with ob/ob mice.[5] Therefore, NPY is thought to have a major role in the physiological control of energy homeostasis.

Five distinct NPY receptor subtypes (Y1, Y2, Y4, Y5, and mouse Y6) have been cloned to date,[6] and pharmacological data suggest that the NPY Y5 receptor is involved in feeding regulation. Administration of Y5 antagonists suppresses Y5 agonist-induced food intake and diet-induced body weight gain,[7,8] and mice lacking the Y5 show a reduced response to exogenously administered Y5 agonists.[9] In addition, chronic intracerebroventricular administration of a Y5-specific agonist, D-Trp^{34}NPY, produces obesity in rodents.[10] These results suggest that Y5 is a key regulator involved in the development of obesity in rodents; hence, Y5 antagonists have been targeted by many pharmaceutical companies as potential anti-obesity drugs.[11]

Our in-house chemical collection was screened against the human Y5 receptor, resulting in the identification of 2,4,4-triphenylimidazoline (**1**). Subsequently, the 2-phenyl portion was extensively optimized to identify several potent derivatives. In this Letter, the synthesis and structure–activity relationships of the potent and selective imidazoline class of Y5 antagonists are described.

The synthetic route for the derivatives reported herein is described in Schemes 1 and 2. The synthesis of diamine intermediates **38a** and **38b** is outlined in Scheme 1. The diamine **38a** was prepared from 4,4′-difluorobenzophenone **35** by formation of corresponding imine **36a**, followed by the addition of cyanide and subsequent reduction of the nitrile. Benzophenone **35** was converted to the imine **36a**,[12] which was treated with trimethylsilyl cyanide in the presence of catalytic amounts of zinc iodide followed by reduction with DIBAL to give the diamine **38a**.[13] Commercially available imine **36b** was converted to the corresponding diamine **38b** in the same manner. Imidazoline rings of **1**–**12**, **14**–**16**, **18**, **20**–**32**, and **34** were prepared either by direct condensation of the diamines with aryl imidates (**1**, **2**, **10**–**12**, **15**, **26**, **27**, and **29**),[14] thermal condensation of the diamines with aryl esters (**3**–**9**, **16**, **18**, **20**–**22**, **24**, **31**, and **32**),[15,16] or coupling of diamines with aryl carboxylic acid followed by thermal cyclization of the resultant amides (**14**, **23**, **25**, **28**, **30**, and **34**)[15] as shown in Scheme 2. Ester **15** was converted to alcohol **17** by reduction with DIBAL followed by oxidation using MnO_2 to afford aldehyde **13**.

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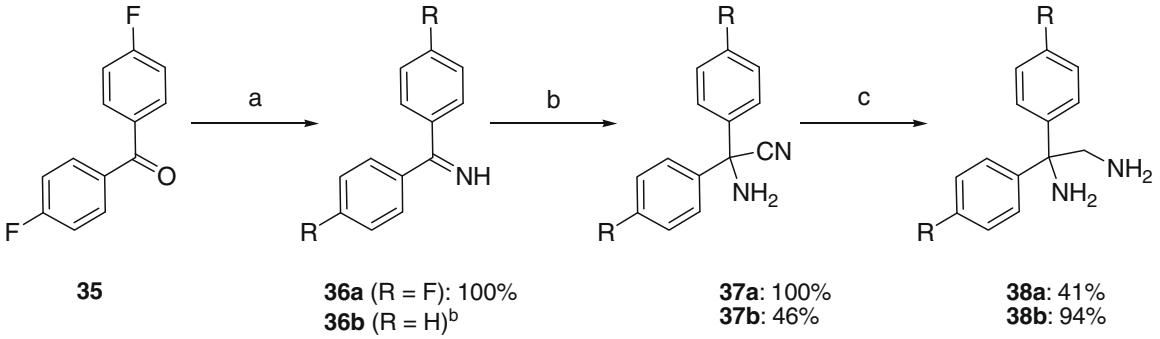
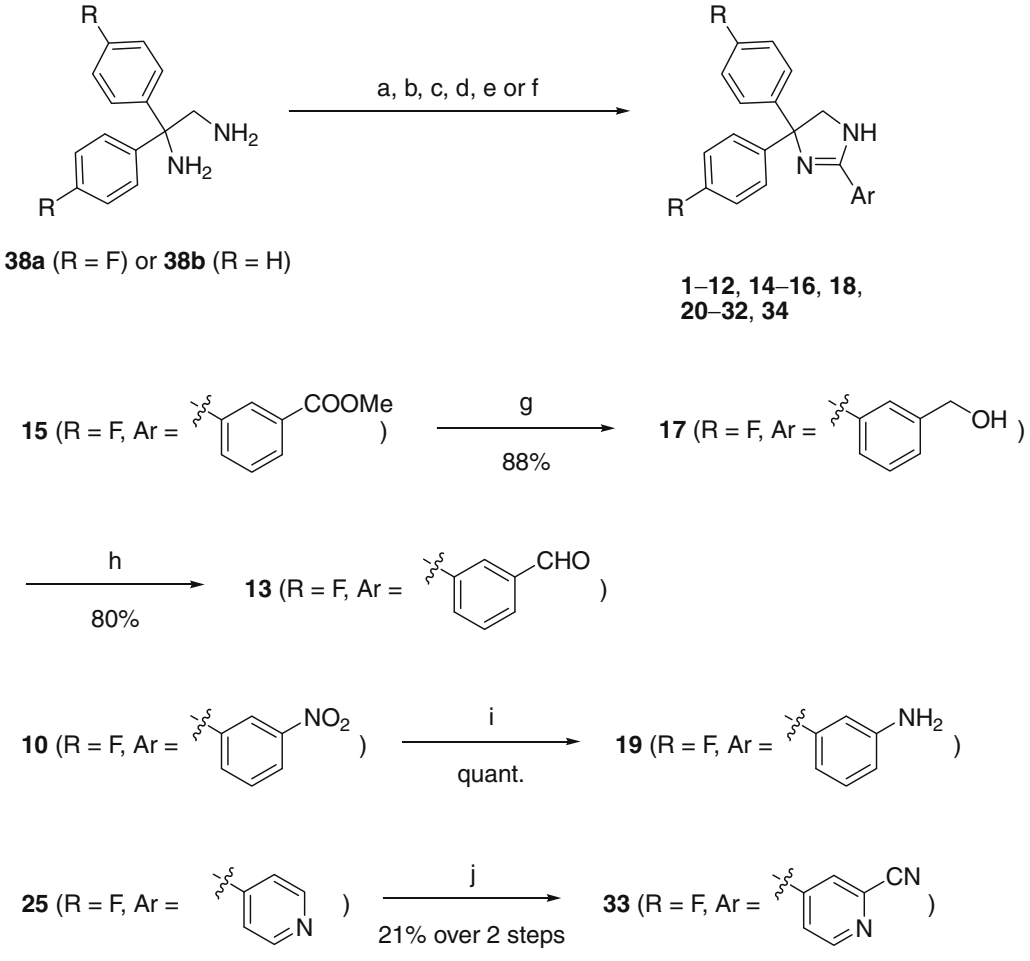


Scheme 1. Synthesis of diamine intermediates. Reagents and conditions: (a) $TiCl_4$, NH_3, toluene, −30 °C; (b) TMSCN, cat. ZnI_2, toluene, rt; (c) DIBAL, toluene, −78 °C → rt. [b]Commercially available reagent.

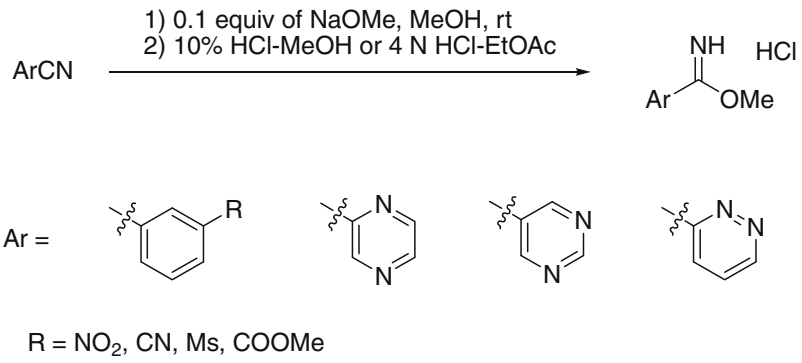


Scheme 2. Synthesis of derivatives **1**–**34**. Reagents and conditions: (a) Ar(C=NH)OMe·HCl, MeOH, rt; (b) ArCOOMe, $AlMe_3$, toluene, 80–110 °C; (c) (i) ArCOOH, CDI, Et_3N, THF, rt, then **38a**, rt, (ii) neat, 180 °C; (d) (i) ArCOOH, EDCI, Et_3N, $CHCl_3$, rt, (ii) PCl_5, toluene, reflux; (e) (i) ArCOOH, CDI, Et_3N, THF, rt, then **38a**, rt, (ii) $POCl_3$, reflux; (f) ArCOOMe, neat or xylene, 180–200 °C; (g) DIBAL, CH_2Cl_2, 0 °C; (h) MnO_2, $CHCl_3$, rt; (i) $NH_2NH_2·H_2O$, Raney-Ni, EtOH, THF, rt; (j) (i) *m*CPBA, $CHCl_3$, rt, (ii) TMSCN, Et_3N, CH_3CN, reflux.

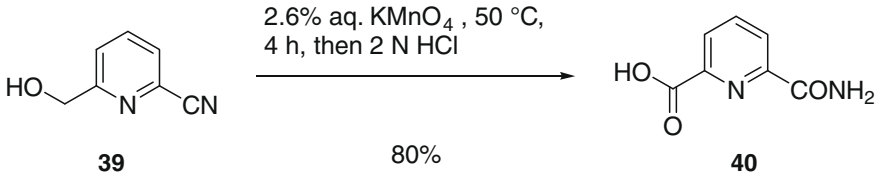
The nitro group of **10** was reduced to give the desired amine **19**. Oxidation of pyridine **25** with *m*CPBA and successive treatment of the resulting pyridine *N*-oxide with trimethylsilyl cyanide afforded **33**.[17]

Imidate hydrochlorides employed in the present study were prepared from corresponding aryl cyanides by treatment with 0.1 equiv of sodium methoxide followed by addition of hydrogen chloride solution as shown in Scheme 3,[18] except for commercially available methyl benzenecarboximidoate hydrochloride. Synthesis of carboxylic acid **40** used for preparing compound **30** is shown in Scheme 4. Oxidation of the hydroxymethyl group of **39** followed by hydrolysis of the nitrile group with 2 N hydrochloric acid afforded the desired carboxylic acid **40**.

The series of imidazoline compounds was tested in a [^{125}I]PYY binding assay using LMtk$^-$ cell membranes expressing human recombinant Y5 receptors.[19] High-throughput screening of Merck sample collections against the human Y5 receptor resulted in the identification of 2,4,4-triphenylimidazoline (**1**), which has an IC_{50} of 54 nM. In vitro metabolism of compound **1** was studied in rat and human microsomes, and oxidation of the 4,4-diphenyl moie-



Scheme 3. Synthesis of the imidates.



Scheme 4. Synthesis of carboxylic acid intermediate **40**.

ties was found to be a major metabolic pathway (data not shown). Therefore, the fluorine substituted derivative **2** was synthesized and evaluated. The 4,4-difluoro derivative **2** was found to have an improved activity and utilized as a template for the present SAR study (Fig. 1).

Substituent effects on the 2-phenyl ring of **2** were studied initially (Table 1). Positional scanning with chlorine as in the derivatives **3**–**5** showed a clear result. The 3-chloro derivative **4** was sixfold more potent than the parent **2**, while the 2-chloro derivative **3** displayed a 20-fold decrease in potency. The 4-chloro derivative **5** exhibited a fourfold decrease in activity. With trifluoromethyl substitution, the same positional effect was observed where the 3-substituted derivative **6** was fivefold more potent, and the 4-subsituted derivative **7** was fivefold less potent than **2**. Based on these observations, additional 3-susbstitued derivatives were prepared and evaluated. The bromo derivative **8** was equipotent to the chloro derivative **4**, and the fluoro derivative **9** showed a slightly decreased potency. The 3-nitro and cyano derivatives **10** and **11** were more potent than the chloro derivative **2**, and the methanesulfonyl derivative **12** was slightly less potent than the chloro derivative **4**.

A set of carbonyl derivatives showed variable activities. The formyl derivative **13** showed good activity, and the acetyl derivative **14** was equipotent to the parent **2**. The methoxycarbonyl derivative **15** displayed a significant loss of potency. With the exception of the methoxycarbonyl group as in **15**, a range of electron-with-

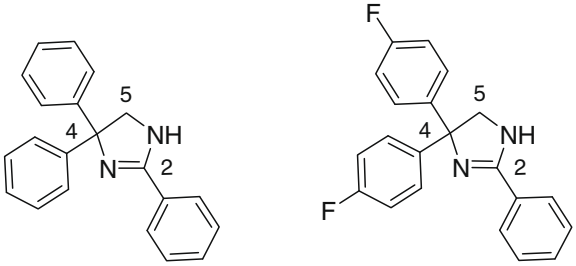
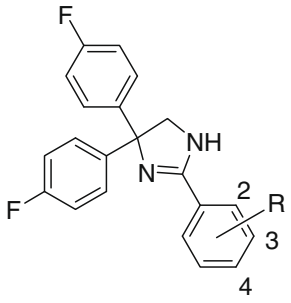


Figure 1. Structure and hY5 activity of compounds **1** and **2**.

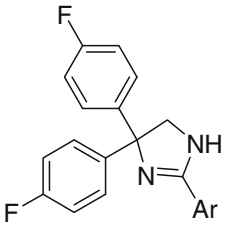
Table 1
SAR of derivatives **2**–**20**[a]

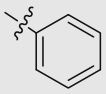
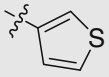
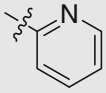
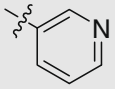
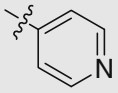
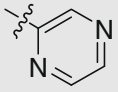
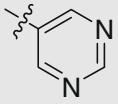
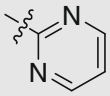
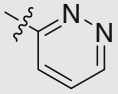
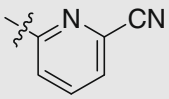
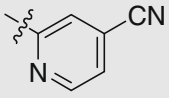
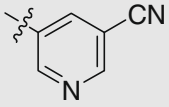
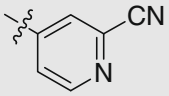
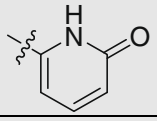


Compound	R	hY5R binding[b] IC_{50} (nM)	Cyclization condition and yield[c]
2	H	32 ± 8	a , 75%
3	2-Cl	630 ± 29	b, 31%
4	3-Cl	5.4 ± 0.5	b, 4%
5	4-Cl	130 ± 25	b, 13%
6	3-CF_3	6.2 ± 0.6	b, 28%
7	4-CF_3	160 ± 24	b, 55%
8	3-Br	4.8 ± 0.5	b, 20%
9	3-F	10 ± 2	b, 24%
10	3-NO_2	4.3 ± 0.6	a, 85%
11	3-CN	2.7 ± 0.1	a, 51%
12	3-SO_2CH_3	9.2 ± 0.9	a, 88%
13	3-CHO	8.7 ± 2.3	—
14	3-$COCH_3$	44 ± 8	c, 34%
15	3-CO_2CH_3	270 ± 26	a, 91%
16	3-CH_3	30 ± 6	b, 42%
17	3-CH_2OH	59 ± 15	—
18	3-OCH_3	51 ± 8	b, 15%
19	3-NH_2	300 ± 91	—
20	3-$N(CH_3)_2$	>1000	b, 20%

[a] The values represent the mean ± SEM (n = 3).
[b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors.
[c] See Scheme 2 for the cyclization conditions.

Table 2
SAR of derivatives **21**–**34**[a]



Compound	Ar	hY5 binding[b] IC_{50} (nM)	Cyclization condition and yield[c]
2		32 ± 8	a, 75%
21		93 ± 20	b, 50%
22		54 ± 6	b, 34%
23		160 ± 39	c, 38%
24		7.2 ± 0.6	b, 36%
25		160 ± 23	c, 8%
26		4.3 ± 0.2	a, 64%
27		47 ± 11	a, 2%
28		350 ± 85	d, 2%
29		560 ± 130	a, 32%
30		39 ± 7	e, 42%
31		2.0 ± 0.4	f, 49%
32		17 ± 3	f, 29%
33		4.8 ± 0.5	—
34		8.1 ± 1.5	c, 42%

[a] The values represent the mean ± SEM (n = 3).
[b] [^{125}I]PYY binding assay in LMtk$^-$ cells expressing human recombinant Y5 receptors.
[c] See Scheme 2 for the reaction conditions.

drawing substituents introduced to the 3-position was effective to enhance potency.

Next, electron-donating substituents were examined. The 3-methyl derivative **16** was equipotent to the parent **2**. The hydroxymethyl and methoxy derivatives **17** and **18** were slightly less potent than the parent **2**. Introduction of more electron-donating amino and dimethylamino groups as in **19** and **20** were detrimental to potency. In this study, it became apparent that substitution of the 3-position by electron-withdrawing groups increases potency. Of them, the 3-cyano derivative **11** was identified as the most potent compound.

Finally, heteroaromatics were investigated on the 2-position of the imidazoline ring of compound **2** (Table 2). The thiophene derivatives **21** and **22** were less potent than **2**. As a result of positional scanning with a pyridine, the significantly potent 3-pyridyl derivative **24** was identified. The 2- and 4-pyridyl substitutions as in **23** and **25** resulted in noticeable decreases in potency. Among the diazine analogs **26**–**29**, the potent pyrazine derivative **26** was identified. The 5-pyrimidine derivative **27** was equipotent to the parent **2**, and the substitution with 2-pyrimidine and 3-pyridazine groups as in **28** and **29** resulted in significant loss of potency. A nitrogen atom was introduced to the potent cyano derivative **11** as in **30**–**33**. The 4-cyanopyridin-2-yl and 2-cyanopyridin-4-yl derivatives **31** and **33** displayed retained activities. It was interesting to find that the 2-pyridon-6-yl derivative **34** exhibited potent activity.

Among the derivatives possessing potent Y5 activity, compounds **11**, **26**, and **31** were evaluated for their brain and cerebrospinal fluid (CSF) penetrability in Sprague–Dawley (SD) rats (Table 3). All three compounds showed very good brain permeability based on the brain-to-plasma ratios. Brain unbound concentration is one of the critical factors that should be taken into consideration for CNS targeting agents, and the brain unbound concentration is estimated by CSF concentration for highly brain permeable compounds.[20] Compound **26** displayed the highest CSF concentration (0.064 μM) and brain free fraction (CSF-to-brain ratio: 0.01) among the three tested compounds. Hence, compound **26** was selected for further in vivo studies. In addition to its good brain and CSF permeability profile, compound **26** showed a potent antagonistic activity

Table 3
Brain penetration of compounds **11**, **26**, and **31**[a]

	Plasma (μM)	Brain (nmol/g)	CSF (μM)	Brain-to-plasma ratio	CSF-to-brain ratio
11	1.4	8.1	0.027	5.8	0.003
26	1.2	5.9	0.064	4.9	0.01
31	1.3	6.4	0.025	4.9	0.004

[a] The brain, plasma, and CSF concentrations were measured 2 h following oral administration of 10 mg/kg of the compounds in rats. The values represent the mean for n = 3.

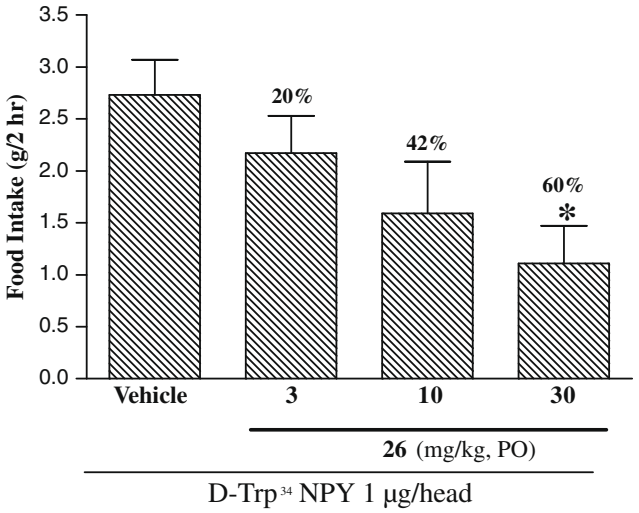


Figure 2. Effect of **26** on food intake induced by D-Trp^{34}NPY. Compound **26** was orally administered 2 h before third ventricle injection of D-Trp^{34}NPY (1 μg/head). The values represent the mean ± SEM (n = 7–8). $^{*}P < 0.05$ (compared with only the D-Trp^{34}NPY treated group).

(IC_{50} = 2.4 ± 0.5 nM)[21] and selectivity over other NPY receptor subtypes (hY1, hY2, and hY4 binding: >10 μM).[19]

Compound **26** was tested in an agonist-induced food intake model (Fig. 2).[22] The compound was orally administered 2 h before animals were treated with either a Y5 selective agonist D-Trp^{34}NPY or artificial CSF, and cumulated food intake was measured for the following 2 h. Compound **26** showed dose-dependent inhibition of food intake in this feeding model.

In summary, modification of the 2-aryl portion of lead **1** resulted in several potent derivatives. Of them, compound **26** showed potent antagonistic activity and a suitable brain penetration profile. Compound **26** showed dose-proportional inhibition of food intake in the agonist-induced food intake model.

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