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Structure–Activity Relationships in a Series of NPY Y5 Antagonists: 3-Amido-9-ethylcarbazoles, Core-Modified Analogues and Amide Isosteres

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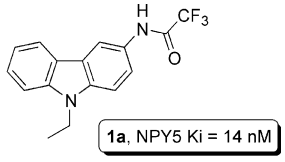
Abstract—Beginning with carbazole **1a**, the amide and alkyl substituents were optimized to maintain potency while adding solubilizing groups. Efforts to replace the 3–amino-9-ethylcarbazole core, a known carcinogen, used the SAR generated in the carbazole series for guidance and led to the synthesis of a number of core-modified analogues. In addition, an isosteric series, in which the amide was replaced with an imidazole, was prepared. Two potent new series lacking the putative toxicophore were identified from these endeavors.

Neuropeptide Y (NPY), a member of the PYY family of peptides, exerts its physiological effects through at least 6 receptor subtypes, the Y1 through Y5 and y6 receptors.[1] Of interest to us has been the central orexigenic effect observed when NPY is given ICV to rodents.[2] It is believed that NPY exerts these effects primarily through the Y1 and Y5 receptor subtypes. Peptide agonist analogues of NPY having selectivity for the Y5 receptor have been found to be orexigenic, with the food intake effect directly correlated to the affinity for the receptor.[3] In addition, it has been postulated that blockade of either or both of Y1 or Y5 receptors might result in an anorectic effect. In fact, a number of studies have shown that both Y1 and Y5 antagonists are capable of blocking the orexigenic effects of selective Y1 and Y5 peptide agonists.[4]

As part of a program directed at discovering novel, potent antagonists of the Y5 receptor, we (and others)[5] have identified aminocarbazole-based Y5 antagonists. The initial lead was 3-trifluoroacetamido-9-ethylcarbazole **1a**, a high-throughput screening hit with an affinity for the Y5 receptor of 14 nM. In an effort to improve the potency and solubility within the series, several analogues of **1a** were prepared as shown in Scheme 1. Those compounds having $R^1 = Et$ were synthesized beginning with the commercially available 3-amino-9-ethylcarbazole (**4**, $R^1 = Et$) by acylation at the 3-amino position. Intermediate **4** for analogues in which R^1 was modified from ethyl were prepared by nitration and reduction of intermediate **3**, which was either commercially available ($R^1 = i$-Pr) or prepared by alkylation of carbazole (**2**). The aminocarbazoles **4** were then acylated following the same protocol as for the commercial aminocarbazole.



Analogues of **1a** were screened against human Y5 receptor using ^{125}[I]-PYY as the radioligand.[6] Selected binding data for this series is given in Table 1, and shows that solubilizing amines present on the amide substituents are well tolerated. For example, the 4-pyridylacetamide-containing aminocarbazole **1b** was found to have a Y5 K_i of 4.7 nM. More striking was the effect seen when the alkyl group at the 9-position was modified. Replacement of the ethyl group with a methyl group resulted in a substantial loss in binding potency (**1e**), while adding

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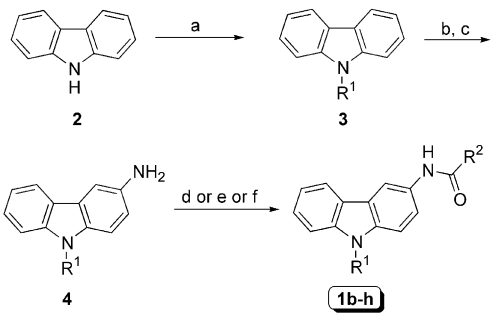
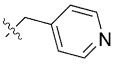
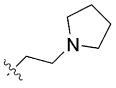
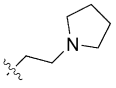
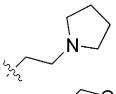
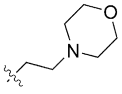
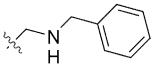


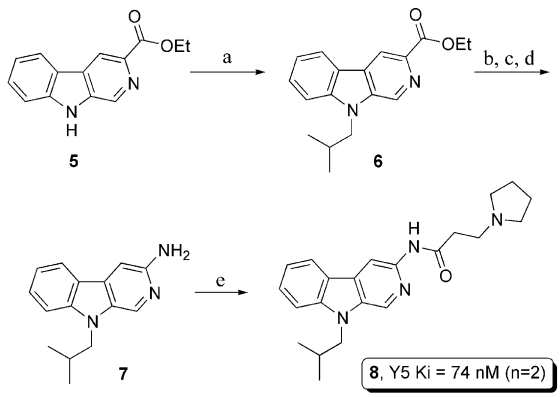
Scheme 1. Reagents and conditions: (a) R^1Br, NaH, DMF, 23 °C; (b) HNO_3, HOAC, 60 °C; (c) H_2, Pd/C, EtOH, 23 °C; (d) trifluoroacetic anhydride, Et_3N, CH_2Cl_2, 23 °C; (e) *N*,*N*-dimethylglycine or 4-pyridylacetic acid, EDC, Et_3N, DMAP, CH_2Cl_2, 23 °C; (f) Me_3Al, ethyl 3-*N*-pyrrolidinopropionate, 1,2-dichloroethane, 50 °C.

Table 1. Y5 binding data for **1a**–**1 h**

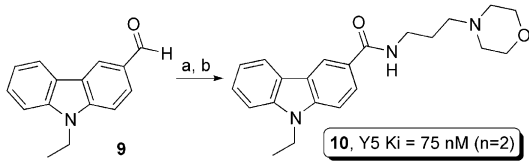
Compd	R^1	R^2	Avg. Y5 K_i, nM (n)
1a	Et		14 (2)
1b	Et		4.7 (5)
1c	Et		21 (2)
1d	Et		11 (7)
1e	Me		93 (2)
1f	*i*-Pr		15 (2)
1g	*i*-Pr		3.4 (2)
1h	*i*-Bu		0.3 (2)
1i	Et		0.84 (2)
1j	Et		2.6 (7)

the more bulky and lipophilic substituents isopropyl and isobutyl resulted in 3- and 36-fold increases in potency, respectively (cf. **1d**, **1g**, and **1h**).

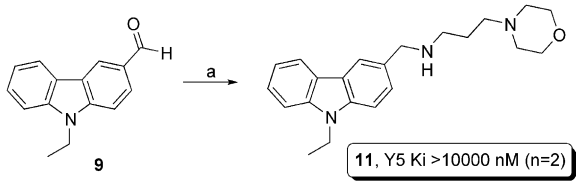
Although potent antagonists of the Y5 receptor having a core derived from 3-aminocarbazole were readily identified, we were acutely aware of the risks associated with this series, as 3-amino-9-ethylcarbazole is a known carcinogen.[7] Although the structural origin of this toxicity (the 'toxicophore') has not been definitively identified, we targeted the 1,4-phenylenediamine substructure as a likely culprit. Thus, guided by the SAR we had developed in this original series, we began to search for a non-aminocarbazole core. We investigated a variety of modified cores, as shown in Schemes 2–6. The β-carboline-derived analogue **8** (Scheme 2) had nearly 250-fold reduced potency relative to the analogous carbazole **1h**. Both the 'reverse amide' **10** (Scheme 3) and the amino-



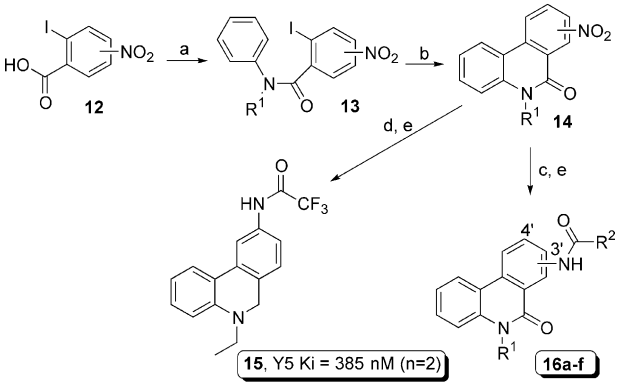
Scheme 2. Reagents and conditions: (a) 1-bromo-2-methylpropane, potassium *tert*-butoxide, THF, 60 °C; (b) H_2NNH_2, CH_3OH, reflux; (c) $NaNO_2$, HCl, 0 °C; (d) HOAc, H_2O, reflux; (e) Me_3Al, ethyl 3-*N*-pyrrolidinopropionate, 1,2-dichloroethane, 50 °C.



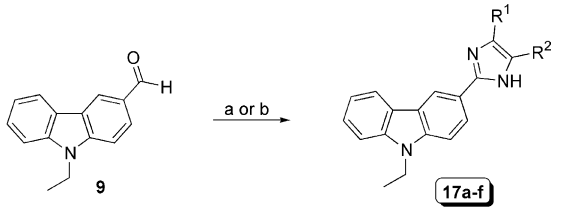
Scheme 3. Reagents and conditions: (a) $KMnO_4$, H_2O/acetone, 70 °C; (b) CDI, CH_2Cl_2, 23 °C; 1-(3-aminopropyl)morpholine, DMAP, 23 °C.



Scheme 4. Reagents and conditions: (a) 3-morpholino-1-propanamine, $NaBH(OAc)_3$, 1,2-dichloroethane, 23 °C.



Scheme 5. Reagents and conditions: (a) $(CO)_2Cl_2$, CH_2Cl_2, DMF 23 °C, then $PhNHR^1$, Et_3N, DMAP, CH_2Cl_2, 0 °C; (b) $Pd(OAc)_2$, Ag_2CO_3, PPh_3, CH_3CN, reflux; (c) H_2, Pd/C, EtOH, 23 °C; (d) LAH, THF, reflux; (e) trifluoroacetic anhydride, pyridine, CH_2Cl_2, 23 °C or R_2CO_2H, EDC, Et_3N, CH_2Cl_2, 23 °C.

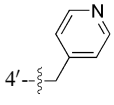
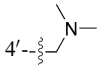
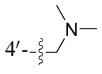
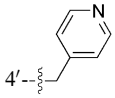
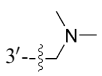


Scheme 6. Reagents and conditions: (a) ii. $R^1(CO)_2R^2$, NH_4OAc, AcOH, 100 °C; (b) aryl 1,2-diamine; CH_3NO_2, 145 °C.

methylcarbazole **11** (Scheme 4) also demonstrated a significant loss in binding affinity relative to the close-in carbazole analogue **1i**.

Binding potency was ultimately restored to the range of the original aminocarbazole series when we prepared a series of phenanthridinone-based analogues. The phenanthridinones were prepared as shown in Scheme 5, starting with the appropriately substituted nitro 2-iodobenzoic acid **12**. Amide formation under standard conditions afforded intermediate **13**, which underwent facile Pd-catalyzed ring-closure to afford phenanthridinones **14**.[8] Reduction of both the nitro group and the amide with LAH followed by acylation afforded phenanthridine **15**, which showed sharply decreased potency over the parent carbazoles. Chemoselective reduction of the nitro group followed by acylation provided the phenanthridinones **16a–f** (Table 2). Compounds were examined for binding activity and were found to be of comparable potency to the aminocarbazoles. Compounds from this series were also submitted to a functional assay (Ca^{2+} mobilization),[6] where they showed 3-fold or more reduction in potency relative to the binding assay. The most potent of these analogues were **16b** and **16e**, each containing a 4-pyridylacetamide side-chain. The R^1 alkyl group appeared to have less influence over binding potency, as evidenced by the similarities in potency between pairs **16b**, **16e** and **16c**, **16d**. The regioisomeric 3′-substituted phenanthridinone **16f** was essentially inactive relative to its 4′-substituted isomers.

Table 2. Y5 binding and functional data for **16a–f**

Compd	R^1	R^2	Avg. Y5 K_i, nM (n)	Avg. Ca^{2+} IC_{50}, nM (n)
16a	Et		43 (2)	166 (1)
16b	Et		12 (4)	37 (3)
16c	Et		41 (4)	362 (3)
16d	*i*-Pr		38 (4)	351 (3)
16e	*i*-Pr		16 (4)	46 (3)
16f	Et		2165 (2)	

Another potent series arose from replacement of the amide functionality in the 3-position of the carbazole core with an isosteric imidazole. Compounds were prepared as depicted in Scheme 6, starting with aldehyde **9**. Heating of **9** and a 1,2-diketone in acetic acid with ammonium acetate provided imidazolyl carbazoles **17a–c**, while heating of **9** and the appropriate aryl 1,2-diamine in nitromethane provided analogues **17d–f** (Table 3). With respect to binding potency, a range of imidazole substitution was well-tolerated within these analogues, including alkyl (**17a**) and aryl (**17b**). Incorporation of a more polar, potentially solubilizing group (**17c**) initially resulted in a drop in potency, but by incorporating the basicity in a fused system, potency returned (**17d–f**). Interestingly, compounds from this series examined in the Ca^{2+} functional assay demonstrated a 10- to 20-fold reduction in potency (**17a** and **17c**) relative to binding affinity.

Several of the Y5 antagonists from the carbazole, phenanthridinone and imidazolylcarbazole series were examined in vivo in a feeding model using 24-h fasted rats. Compounds were dosed orally in a methylcellulose vehicle, food was re-introduced, and total food intake was measured 3 h later. As seen in Table 4, compounds considered active in this assay were those that produced statistically significant reductions in food intake of $\geqslant$25% relative to control animals. With the exception

Table 3. Y5 binding and functional data for **17b–17e**

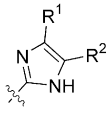
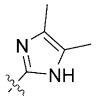
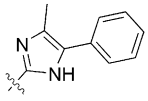
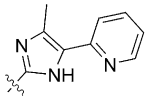
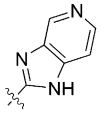
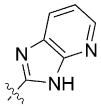
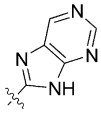
Compd		Avg. Y5 K_i, nM (n)	Avg. Ca^{2+} IC_{50}, nM (n)
17a		10 (2)	242 (1)
17b		15 (4)	
17c		101	967 (1)
17d		15 (2)	
17e		1.5 (2)	
17f		8 (2)	

Table 4. Food intake effects of analogues in rats[a]

Compd	24 h Food-Deprived FI (%)
1j	−7/−59*/−16
16b	−20/−20/−51*
16e	−14/−23/−40*
17a	−34*/−40*
17b	+18/+25
17e	−20/+4/−7

*Considered active in this assay.
[a]All compounds dosed orally at 40 mg/kg in male Sprague–Dawley rats. Data presented as percent change (− = reduction; + = increase) in food intake relative to control animals.

of the imidazolylcarbazole **17a**, which produced significant reductions in food intake both times it was tested, the other five antagonists, **1j**, **16b**, **16e**, **17b** and **17e**, did not have consistent anorectic effects (n of at least 2). Compound **17b** was also tested in an overnight feeding model wherein the compound was dosed 2 h before the start of the dark cycle and food intake was measured 13 h later, and was found to have no effect on food intake.

To determine whether the variable activity of these compounds could be due to poor bioavailability, we examined the pharmacokinetic properties of the carbazole **1j** and the imidazolylcarbazole **17b** in separate studies. Carbazole **1j** was found to have excellent exposure, having a C_{max} of 3133 ng/mL and a bioavailability of >50% when dosed orally at 10 mg/kg. When dosed at 40 mg/kg, **17b** was found to have a moderate plasma exposure of 350 ng/mL and a bioavailability of 13%, suggesting that poor exposure could account for the lack of activity in some cases, but not all. Although CSF and/or brain exposure data is not available for these compounds, we know that in other chemically distinct Y5 antagonist series known to be well-exposed in both the CNS and periphery we have observed a similar absence of in vivo activity.[9] By contrast, we have been able to demonstrate that these compounds will block the orexigenic effect of a Y5 selective agonist.[9]

The ability of small molecule Y5 antagonists to block food intake under 'normal' (non agonist-induced) conditions has yet to be demonstrated unambiguously. Although selective Y5 antagonists with potent anorectic effects have been reported,[10] recently there have been studies disclosed in which potent, selective Y5 antagonists with good central exposure are capable of blocking agonist-induced feeding but have no effect on normal food intake.[11] Other Y5 antagonists with good in vivo anorectic potency have more recently been found to lack selectivity for the Y5 receptor.[12] While it is clear that NPY and its peptide agonists produce robust orexigenic effects in rodents, blockade of the Y5 receptor does not appear to unequivocally produce anorectic effects.

In summary, two series of potent Y5 antagonists have been developed from an aminocarbazole lead structure. Neither of the new series contains the toxic 3-amino-9-ethylcarbazole core, yet each matches the lead series in affinity for the Y5 receptor. Although compounds were found to be potent Y5 antagonists, their in vitro affinity did not translate into consistent in vivo activity.

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