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Novel carbazole derivatives as NPY Y1 antagonists

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Abstract—The synthesis of a series of carbazole derivatives and their SAR at the NPY Y1 receptor is described. Modulation of physicochemical properties by appropriate decoration led to the identification of a high-affinity NPY Y1 antagonist that shows high brain penetration and modest oral bioavailability.

Neuropeptide Y (NPY) is a 36 amino acid peptide which was first isolated in 1982 from porcine brain.[1] It is widely distributed in both the central nervous system (CNS)[2] and in peripheral neurones[3] and has a range of biological actions. These include stimulation of food and water intake,[4] regulation of vascular tone[5] and control of mood.[6] Several NPY receptor subtypes have been characterised at a molecular level (Y1, Y2, Y4, Y5 and Y6) but to date it has not been possible to unambiguously ascribe the role of each subtype in the pharmacological responses to NPY.

Over the past decade many researchers have strived to discover potent selective NPY Y1 receptor antagonists in order to elucidate the role of this receptor subtype and evaluate the potential of such agents to treat obesity in man. Although a number of such ligands have been reported (BIBP3226,[7] SR120819A,[8] PD160170,[9] LY357897,[10] J-115814[11]), none are ideal tools as they all share pharmacokinetic weaknesses that limit CNS exposure following oral administration.

As part of our efforts to prepare novel NPY Y1 ligands with improved pharmacokinetic properties, we recently described the preparation and structure–activity relationships (SAR) of a series of tetrahydrocarbazole NPY Y1 antagonists.[12] Herein, we disclose a complementary series of highly brain penetrant, bioavailable carbazole derivatives that are potent and selective NPY Y1 antagonists.

The core carbazole scaffold was synthesised as outlined in Scheme 1. Oxidation of the functionalised toluene **1**, followed by esterification, gave **2**. The aryloxy group was introduced by nucleophilic aromatic substitution to give **3**. Reduction of the nitro group was followed by iodination to give the key aniline intermediate **5**. Carbazole formation was accomplished via sequential palladium-catalysed Suzuki coupling, with 2-bromophenylboronic acid, and intermolecular Buchwald amination to give **7**. Deprotonation of **7** with sodium hydride or cesium carbonate followed by treatment with a suitable alkylating agent gave **8**. Ester hydrolysis was followed by amide coupling and any eventual deprotections to give final products **9**. For convenience, the order in which the two points of diversity were elaborated was inverted (hydrolysis and amide formation prior to alkylation) when exploring the SAR of the *N*-carbazole sidechain.

Binding affinities for all new compounds were determined by measuring their ability to displace [^{125}I]peptide YY from cloned human Y1 receptors expressed in CHO cells. The affinity of selected compounds

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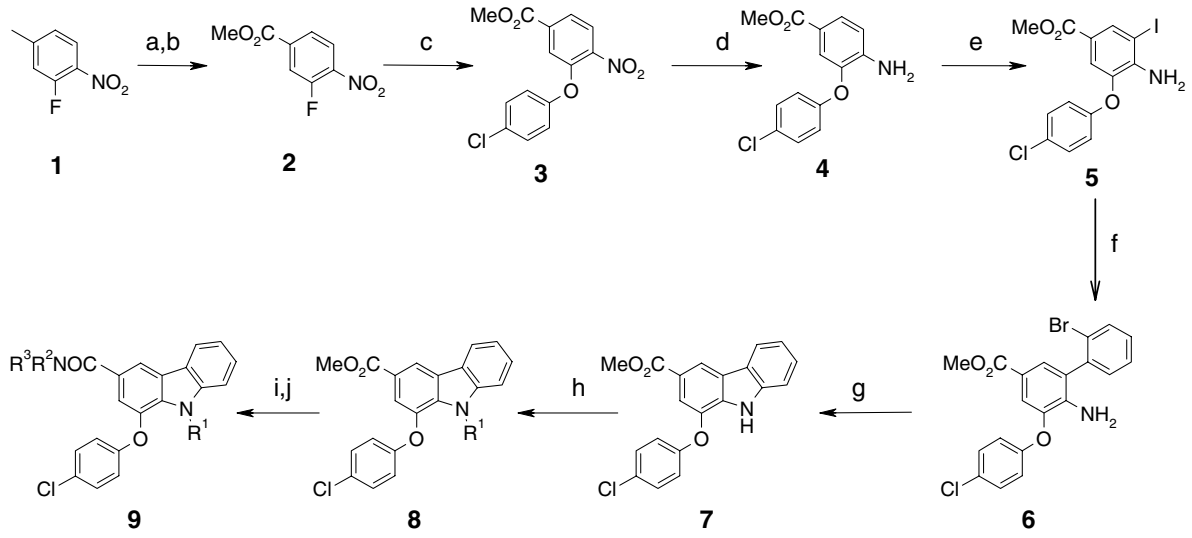


Scheme 1. Reagents and conditions: (a) $Na_2Cr_2O_7$, H_2SO_4, H_2O, 0–100 °C, 0.5 h, 62%; (b) $SOCl_2$, MeOH, rt, 18 h, 93%; (c) 4-chlorophenol, K_2CO_3, *o*-xylene, reflux, 2 h, 97%; (d) Fe, NH_4Cl, EtOH/H_2O, reflux, 3 h, 95%; (e) $IPyBF_4$, CF_3CO_2H, CH_2Cl_2, rt, 0.5 h, 84%; (f) 2-bromophenylboronic acid, cat. $Pd(PPh_3)_4$, Na_2CO_3, toluene, 85 °C, 3 h, 99%; (g) stoich. $Pd(PPh_3)_4$, Na_2CO_3, toluene, reflux, 6 h, 74% ; (h) NaH or Cs_2CO_3, DMF, 90 °C, R^1Br or R^1OMs; (i) NaOH, THF/MeOH, 60 °C, 2 h, 98%; (j) EDC, HOBt, R^2R^3NH, DMF, rt.

for human Y2 and Y5 receptors was measured analogously, giving IC_{50} values greater that 10 μM in all cases. In vitro functional antagonism was confirmed for selected compounds by measuring their ability to inhibit [^{35}S]GTPγS binding.

Clearance, half-life, volume of distribution and area under the curve were measured after intravenous administration of a 1 mg/kg DMSO/PEG400/H_20 5:50:45 solution of the test compound to male Sprague–Dawley rats. Brain penetration was determined by measuring the blood/plasma partition ratio (B/P) 5 min after the administration. Oral bioavailability (F) was calculated using the normalised area under the curve measured following oral administration of a 5 mg/kg methocel suspension of the test compound to male Sprague–Dawley rats.

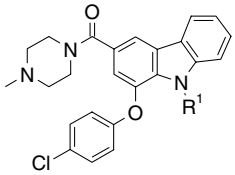
Throughout our SAR explorations special attention was given to a number of physicochemical parameters which we believed would be important in confering good permeability properties. In particular (i) the overall size of the molecule was to be kept as low as possible, (ii) log *P* should be maintained in the range of 2–6, (iii) the pK_a of any basic residues should be kept below 9, (iv) the pK_a of any acidic residues should be kept above 5, (v) the number of rotatable bonds was to be kept as low as possible. We began by examining the SAR of the *N*-carbazole sidechain. For this investigation the amide substitution was fixed as piperazine in light of the SAR that had emerged around the tetrahydrocarbazole and related scaffolds.[12,13]

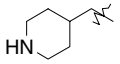
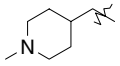
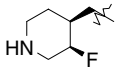
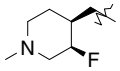
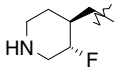
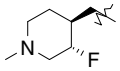
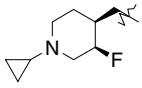
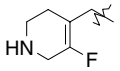
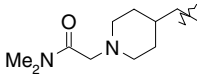
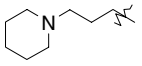
The piperidinylmethyl sidechain (Table 1, **10**) was found to give high affinity whilst maintaining a relatively compact molecular footprint. Unfortunately, **10** showed poor permeability properties in vivo (F = 0%, B/P = 0.12). It was hypothesised that the strong basic character of the secondary amine (calculated pK_a > 10) of the piperidinylmethyl chain was a major contributing factor to the poor pharmacokinetic profile of **10**. Consequently, a focused exploration around the parent piperidylmethyl sidechain was made with modifications targeting reduction of the pK_a of the amine. These studies indicate that affinity can be maintained or even improved whilst reducing the basicity of the nitrogen (Table 1, compounds **11** and **19**). However, it was found that affinity dropped off sharply when the basicity was reduced below a certain limit. This can be noted by contrasting the gain in potency upon methylation of **10** or **12** (Table 1, compounds **11** and **13**, respectively) with the drop in affinity observed upon both methylation of **14** and cyclopropanation of **12** (Table 1, compounds **15** and **17**, respectively). In agreement with this evidence, all attempts to replace the amino group with alternative hydrogen bond donors or acceptors led to inactive products (data not shown). This behaviour is consistent with a cationic charge interaction of the amine with acidic residues in the receptor.

As expected an improvement in the pharmacokinetic parameters was seen with compounds bearing less basic amine groups. Indeed, with other parameters effectively constant a trend towards greater brain penetration was observed with decreasing pK_a: for example, the brain/plasma partitions of compounds **11**, **13** and **15** were measured as 0.8, 4.2 and 8.2, respectively.

From this series of compounds the best compromise between high receptor affinity and good pharmacokinetic properties was reached with compound **13**, which offers potent antagonist activity and good CNS exposure after oral administration (F = 19%, B/P = 4.2). Furthermore,

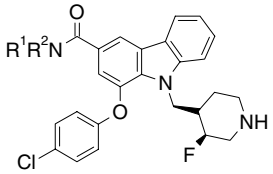
Table 1. Structure–activity relationship of *N*-carbazole sidechain

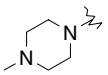
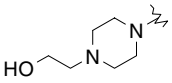
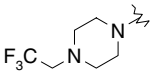
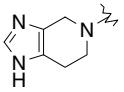
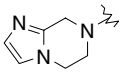
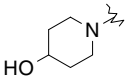


Compound	R^1	NPY Y1 binding affinity IC_{50} (nM)	Calculated pK_a for R^1[a]
10		16	11.0
11		11	9.7
12		51	9.2[a]
13		28	7.9[a]
14		53	7.8[a]
15		95	6.5[a]
16		107	11.0
17		186	8.0[a]
18		43	7.9
19		14	7.4
20		50	9.7

[a] see Ref. 14.

Table 2. Structure–activity relationship of the amide group



Compound	NR^1R^2	NPY Y1 binding affinity IC_{50} (nM)	Calculated pK_a for NR^1R^2
12		51	8.3
21		832	8.1
22		9772	7.9
23		2882	7.0
24		1905	7.3
25		7762	—

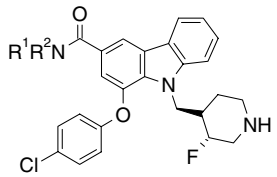
the plasma clearance of **13** in rat is moderately low (Clp = 15 mL/min/kg), leading to a long half-life (11 h) in this species.

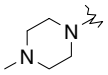
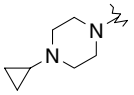
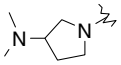
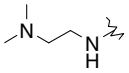
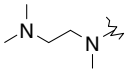
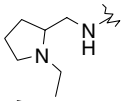
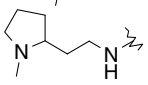
Our attention then turned to the exploration of the amide substitution. During this investigation we chose to maintain an emphasis on reducing the basicity of the amine group in the light of the positive impact that this strategy had made on permeability properties when applied to the *N*-carbazole sidechain. The SAR was constructed in part with the carbazole bearing the sidechain of **12** (Table 2) and in part with the carbazole bearing the epimeric sidechain of **14** (Table 3). Despite an extensive exploration of the amide position, no improvement could be made over the originally chosen piperazine, with most alternatives leading to significantly less potent analogues. Only the open-chain derivative **28** (Table 3) maintained a similar binding affinity, suggesting that the binding pocket occupied by the piperazine is highly demanding in terms of the size and orientation of the amide substitutent which it can accommodate. Simple methylation of **28** is sufficient to give a fourfold drop in affinity. The requirement for a basic amine in this region was confirmed by the inactivity of amide derivatives such as **25** and **32** (Tables 2 and 3, respectively).

In summary, a novel series of carbazole NPY Y1 receptor antagonists were prepared and characterised. The SARs at the *N*-carbazole sidechain and at the C4 amide substituent have been explored, evidencing the requirement of a basic nitrogen in each position. A trend towards improved bioavailability and brain penetration was observed upon reduction of the basicity of the amine on the *N*-carbazole sidechain. Reduction of basicity below a certain limit led to a dramatic drop in binding affinity.

The culmination of the present study was the identification of compound **13**, a potent, orally bioavailable, highly brain penetrant antagonist of the NPY Y1 receptor. As such **13** represents an invaluable tool for studying the centrally mediated effects of this receptor and should help clarify the scope of Y1 antagonists as pharmaceutical agents.

Table 3. Structure–activity relationship of the amide group



Compound	NR^1R^2	NPY Y1 binding affinity IC_{50} (nM)	Calculated pK_a for NR^1R^2
14		53	8.3
26		871	8.2
27		467	9.0
28		50	8.9
29		191	9.0
30		263	9.0
31		1862	10.3
32		12022	—

Acknowledgments

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13. Unpublished results, GSK.
14. pK_a's were calculated using ACD/Labs software (http://www.acdlabs.com). As this software does not discriminate between the axial and equatorial fluoropiperidine isomers, pK_a's for compounds **12**, **13**, **14**, **15** and **17** were calculated by correcting the values for the corresponding non-fluorinated piperidines according to van Niel, M. B.; Collins, I.; Beer, M.S.; Broughton, H. B.; Cheng, S. K. F.; Goodacre, S. C.; Heald, A.; Locker, K. L.; MacLeod, A. M.; Morrison, D.; Moyes, C. R.; O'Connor, D.; Pike, A.; Rowley, M.; Russel, N.; Sohal, B.; Stanton, J. A.; Thomas, S.; Verrier, H.; Watt, A. P.; Castro, J. L. *J. Med. Chem.* **1999**, *42*, 2087 (−1.83 pK_a units for an axial fluorosubstituent and −3.24 pK_a units for an equatorial fluorosubstituent). The authors believe that the calculated pK_a for compound **17** may be an overestimation as experimentally determined pK_a values of cyclopropanated amines can show a 2 pK_a unit drop compared to the non-alkylated amines (for example, see Gillaspy, M. L.; Lefker, B. A.; Hada, W. A.; Hoover, D. J. *Tetrahedron Lett.* **1995**, *41*, 7399).