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Synthesis and SAR of substituted tetrahydrocarbazole derivatives as new NPY-1 antagonists

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Abstract—The SAR of a new series of tetrahydrocarbazole derivatives is described: the appropriate decoration of this template led to the identification of a new class of NPY-1 antagonists showing good in vitro potency and a promising in vivo pharmacokinetic profile in rat.

Neuropeptide Y (NPY) is a 36-amino acid peptide isolated from porcine brain in 1982 by Tatemoto et al.[1] NPY belongs to a family of biologically active polypeptides such as peptide YY (PYY) and pancreatic polypeptide (PP)[2] widely distributed in the central and peripheral nervous system of many mammalian species including humans.

Different receptor subtypes exhibiting high affinity for NPY have been identified.[3] These receptors have been hypothesized to be involved in different physiological processes[4] such as cardiovascular regulation, food intake, and pain. However, their real pharmacological potential remains to be fully elucidated, depending upon the availability of suitable receptor ligands exhibiting appropriate in vitro potency and pharmacokinetics. In particular, as far as the NPY-1 receptor is concerned, although several in vitro potent antagonists have been reported in the literature, namely BIBP3226,[5] SR120819A,[6] PD-160170[7] and LY357897,[8a] compounds with an appropriate pharmacokinetic profile have not yet been identified.

As part of a broad chemical strategy aimed toward the discovery of new series of NPY-1 antagonists, we focussed our attention on the exploration of the tetrahydrocarbazole template, as depicted in Figure 1, suitably designed according to the proposed pharmacophore model for the NPY-1 antagonists.[8b] In particular, we managed to optimize in parallel both the in vitro activity at the NPY-1 receptor binding site as well as the physicochemical properties/pharmacokinetic profile depending upon the decoration of the tetrahydrocarbazole scaffold by the appropriate choice of the substituents R^1 and R^2. This exploration was performed in two steps: a preliminary phase to understand the potential of the template designed, gathering basic elements of SAR on substituents R^1 and R^2 (compounds **1a**–**g**, shown in Table 1); an optimization phase aimed at maximizing the affinity to the receptor and improve the *drug-like* profile, fine-tuning the physicochemical properties of the substituents R^1 and R^2 (compounds **1h**–**s**, shown in Table 2).

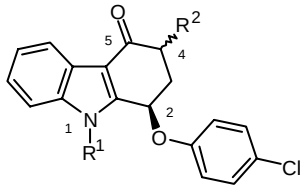

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Figure 1. The tetrahydrocarbazole series explored.

The synthetic route set up to prepare compounds **1a**–**s** is shown in Scheme 1. Oxidation of the commercially

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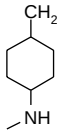
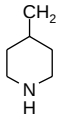
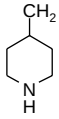
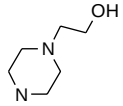
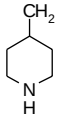
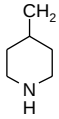
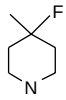
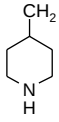
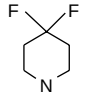
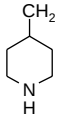
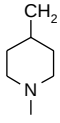
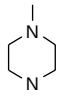
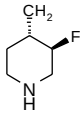
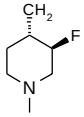
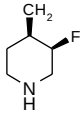
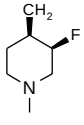
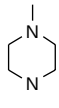
Table 1. Preliminary exploration of R^1 and R^2 substituents

Entry	R^1	R^2	pIC_{50}
1a	CH_3		4.60
1b			5.88
1c		H	5.14
1d			4.80
1e			5.47
1f			6.19
1g			6.48

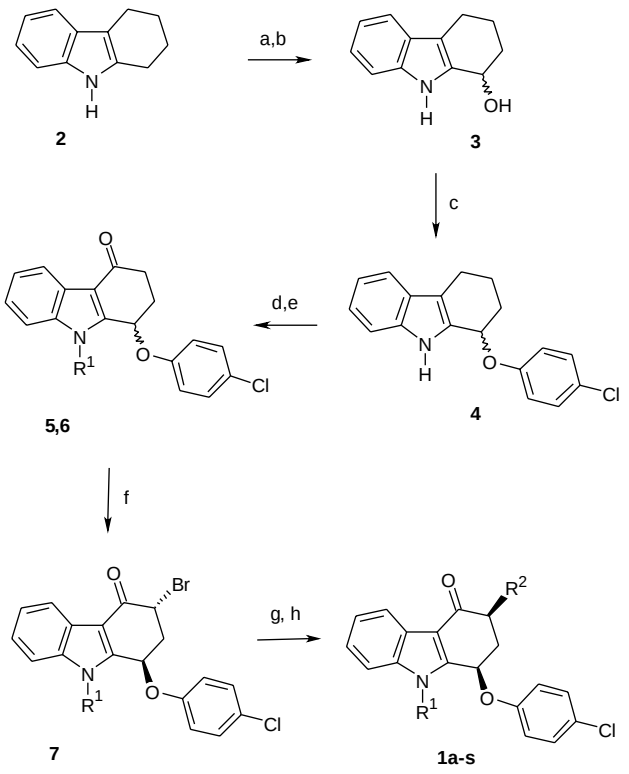
available tetrahydrocarbazole[9,10] **2** at the C-2 position with H_5IO_4 in THF/H_2O 3:1 at 0 °C gave the corresponding ketone derivative, which was reduced with $NaBH_4$ in MeOH to afford the alcohol intermediate **3** in 68% total yield from **2**. Then, the aryl ether derivative **4** was prepared by the Mitsunobu-type reaction; in particular, after some preliminary promising attempts, a careful analysis of the reaction conditions was performed to optimize the chemical yield. To this end, the use of polymer-bound triphenylphosphine gave the best yield (65%, after purification by flash chromatography), avoiding laborious work-up when the reaction was run on multigram scale.[11] The following oxidation reaction at the C-5 position, performed with DDQ, furnished smoothly the 6-keto derivative **5** (R^1 = H), which was efficiently transformed into compounds **6** by treatment with NaH and suitable alkylating agents in DMF at 60 °C for 12 h. Then, the bromination reaction at the position α to the ketone (LHMDS, THF, −70 °C, 1 h, then Br_2, 30 min) gave a 9:1 mixture of *anti*/*syn* diastereoisomers obtained in 70% and 8% yield, respectively, after purification by flash chromatography. The *anti* diasteroisomer **7** was then reacted with a selected number of tertiary amines. Final compounds **1a**–**g** and **1h**–**s** were obtained in good yield with complete inversion of configuration at the C-4 position.[12]

The fluorinated monomers R^1 (see compounds **1p**, **1q**, **1r** and **1s** in Table 2) were prepared in four steps and good total yield starting from the known fluoro ketone derivative **8**,[13] as shown in Scheme 2. The sequential addition of the Grignard reagent to the carbonyl group, followed by the Peterson-type elimination reaction afforded the olefin intermediate **10** in good yield. Then, the hydroboration reaction of the double bond furnished a mixture of *cis* and *trans* isomers **11** and **12**, which were isolated in 47% and 25% yield, respectively, after separation by

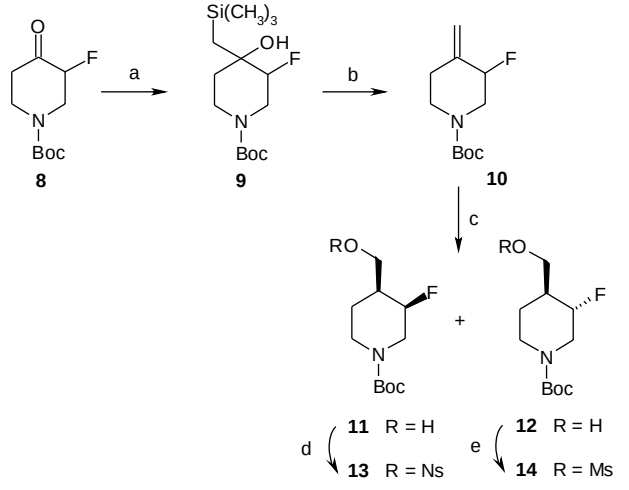
Table 2. Optimization strategy using constrained R^1 and R^2 substituents

Entry	R^1	R^2	pIC_{50}
1h			7.03
1i[a]			7.31
1j			7.43
1k[a]			7.65
1l			7.00
1m			5.73
1n			5.41
1o			7.47
1p			6.62
1q			6.36
1r			6.73
1s[b]			6.77

[a] pIC_{50} = 6.11 and 7.01 for the *anti* diastereoisomers of **1i** and **1k**.
[b] pK_i = 7.00.[15]



Scheme 1. Reagents and conditions: (a) H_5IO_6, THF/H_2O, 0 °C, 80%; (b) $NaBH_4$, MeOH, 0 °C, 85%; (c) DEAD, PPh_3, *p*-Cl-phenol, THF, rt, 2 h, 65%; (d) DDQ, THF/H_2O, rt, 60%; (e) NaH, DMF, R^1X, 60 °C, 12 h, 60–80%; (f) LHMDS, THF, −70 °C, 1 h, then Br_2, 30 min, 70%; (g) amine, K_2CO_3, CH_3CN, 70 °C, 70%; (h) HCl, Et_2O, rt, 12 h.



Scheme 2. Reagents and conditions: (a) $(CH_3)_3SiCH_2MgCl$, THF, 0 °C, 1.5 h, 57%; (b) KH, THF, 0 °C, 1 h, 89%; (c) BH_3·THF, THF, 0 °C, 3 h, then NaOH, H_2O_2, rt, 72%; (d) NsCl, CH_2Cl_2, TEA, rt, 5 h, 96%; (e) MsCl, CH_2Cl_2, TEA, 0 °C to rt, 4 h, 90%.

flash chromatography. Finally, the *trans* isomer **12** was transformed into the mesylate derivative **14**, whereas the *cis* isomer **11** was transformed into nosylate derivative **13**, more reactive than the corresponding mesilate in the following alkylation reaction. All the compounds synthesised were characterized as racemates in terms of their in vitro binding affinities in CHO cells expressing human recombinant NPY-1 receptors.[14]

On the basis of the results obtained the following comments can be addressed: (a) affinity at the receptor is improved when final compounds exhibited a *di*-basic character (entries **1b** vs **1a** and **1c** in Table 1); (b) the in vitro affinity of the *sub*-series of compounds shown in Table 1 was maximized modulating both the positioning of the nitrogen within the R^2 substituent and/or its basic character; (c) more constrained R^1 substituents enabled further increases of the in vitro affinity (see compounds **1h**, **1i** and **1o** in Table 2 vs **1b** in Table 1); (d) both substituted piperidines and N-substituted piperazines are tolerated at the C-4 position; in particular, the 4-methyl piperidine seems to be the R^2 substituent of choice to maximize the in vitro potency (entry **1k**); (e) the nitrogen atoms present both in the R^1 and R^2 substituents seem to be key pharmacophoric points, as demonstrated by the decrease of pIC_{50} values observed reducing their basicity (entry **1k** vs **1l**, **1m**, and **1n**; **1p** and **1q** vs **1i** and **1o**, respectively, in Table 2); (f) *syn* diastereoisomers are more potent than the corresponding *anti* (see footnote entries **1i** and **1k**); (g) as far as the R^1 substituent is concerned both secondary and tertiary amines are tolerated; tertiary amines are preferred for the better pharmacokinetic profile[16] (entry **1o** vs **1i**).

In summary, an appropriate choice of substituents R^1 and R^2 enabled to get in vitro potent NPY-1 antagonists associated with improved *drug-like* properties with respect to previous series of known NPY-1 antagonists. According to these features the in vivo pharmacokinetic profile of compound **1o** was assessed in CD male rats. Moderate oral bioavailability ($F = 27\%$) was observed when the compound was given po at 5 mg/kg and at 1 mg/kg, iv, associated to moderate/high Clp (59 ml/min/kg) and high Vd (>20 L), probably due to the *di*-basic character of the molecule. The brain penetration was evaluated 5 min after the administration of 1 mg/kg dose, iv, giving promising results (B/P = 0.8).[16]

In conclusion, a novel class of drug-like NPY-1 antagonists has been identified by exploration of the tetrahydrocarbazole template by suitable decoration at the position N-1, C-2 and C-4. The preliminary exploration performed allowed to get positive results both in terms of in vitro potency and improved developability features. Additional studies to expand the SAR information and to optimize pharmacokinetic properties are currently ongoing and results will be reported in due course.

Acknowledgments

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11. Following this procedure the reaction was performed on a large scale starting from 20 g of alcohol **3** affording **4** in the same yield. It is worth mentioning that in our hands signs of chemical instability of this compound were observed during the purification by flash chromatography on silica gel. This issue was solved reducing the acid character of the silica gel by pre-eluting the chromatographic column with 5% TEA in AcOEt.
12. In these reaction conditions, the *syn* diasteroisomer gave rise to a complex mixture of products: the final compounds *anti* **1** were isolated in poor yield, after difficult purification by flash chromatography. ^{1}H NMR **1k** (*syn*): $CDCl_3$ δ 8.36 (dd, 1H); 7.40–7.30 (m, 5H); 7.03 (d, 2H); 5.90 (dd, 1H); 4.29 (dd, 1H); 4.09 (dd, 1H); 3.57 (dd, 1H); 3.04 (bt, 1H); 3.00–2.30 (m, 8H); 2.01 (m, 1H); 1.50–1.20 (m, 9H); 0.93 (d, 3H). ^{1}H NMR **1k** (*anti*): $CDCl_3$ δ 8.36 (dd, 1H); 7.35 (m, 5H); 6.99 (d, 2H); 5.80 (t, 1H); 4.05–3.95 (m, 3H); 3.27 (d, 2H); 3.00–2.50 (m, 8H); 2.20–1.20 (m, 10H); 0.93 (d, 3H).
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14. The in vitro affinity for human recombinant NPY-1 receptors was assessed in the present study by competition binding of [^{125}I]PYY, by scintillation proximity assay (SPA) technology.
15. Functional activity was measured in homogenates of CHO cells expressing the human NPY-1 receptor in GTPγS binding assay using LEADSeeker beads.
16. When evaluated in vivo in the same experimental conditions, the corresponding N-demethylated analogue **1i** was not orally bioavailable ($F\% = 0$), due to the high basicity of the nitrogen.