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Synthesis of new thiophene and benzo[*b*]thiophene hydrazide derivatives as human NPY Y_5 antagonists

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Abstract—Neuropeptide Y is one of the most potent appetite stimulating hormones known. Novel thiophene and benzo[*b*]thiophene hydrazide derivatives were synthetized and evaluated biologically as NPY Y_1 and Y_5 receptor subtype antagonists. They were found to have nanomolar binding affinities for human NPY Y_5 receptor, obtaining the lead compound, *trans*-*N*-4-[*N*′-(thiophene-2-carbonyl)hydrazinocarbonyl]cyclohexylmethyl-4-bromobenzenesulfonamide, which binds with a 7.70 nM IC_{50} to the hY_5 receptor.
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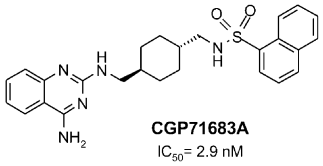
Neuropeptide Y (NPY), a 36 amino acid peptide, is widely expressed in both the peripheral and central nervous system.[1] NPY regulates a variety of physiological functions, such as food intake, intestinal motility, vasoconstriction, blood pressure, nasal congestion, anxiety, depression, pain, motor and sexual behaviour.[2,3] Five distinct types of G-protein coupled NPY receptors (Y_1, Y_2, Y_4, Y_5 and Y_6) have been cloned.[4] NPY Y_1 and Y_5 receptor subtypes have been implicated in mediating the orexigenic effects of NPY and in regulating food intake and body weight. On the basis of the potent orexigenic effects of NPY in vivo,[5,6] a number of papers have been published with NPY receptor-specific ligands as a target for developing antagonists for the treatment of obesity.[7] Diverse structural series of arylsulfonamidomethylcyclohexyl derivatives as **CGP71683A**[8] and Synaptic Pharmaceutical's tetralin derivative **1**[9] (Fig. 1) have been reported to antagonize the NPY Y_5 receptor, reducing food intake in *ob*/*ob* mice and Zucker obese rat models.[10] Consequently, the aim of this work was the synthesis and biological evaluation of new thiophene and benzo[*b*]thiophene hydrazide derivatives, structurally related to the previous arylsulfonamidomethylcyclohexyl derivatives, as part of a program to discover a novel antiobesity agent with antagonistic activity for human NPY Y_5 receptor subtype.

Synthesis of new thiophene and benzo[*b*]thiophene hydrazide derivatives have been carried out following the synthetic route shown in Scheme 1.

The formation reaction of the primary sulfonamides **2a–g** consists of a nucleophilic attack on the part of amine **1** (1.00 equiv) against different sulfonyl chlorides (1.50 equiv),[11] following a S_N2 mechanism, by means of NaOH 2M (150 mL) is used as a solvent. The reaction is



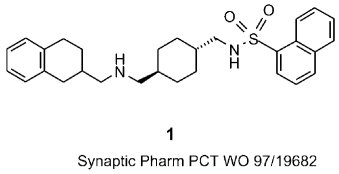


Figure 1.

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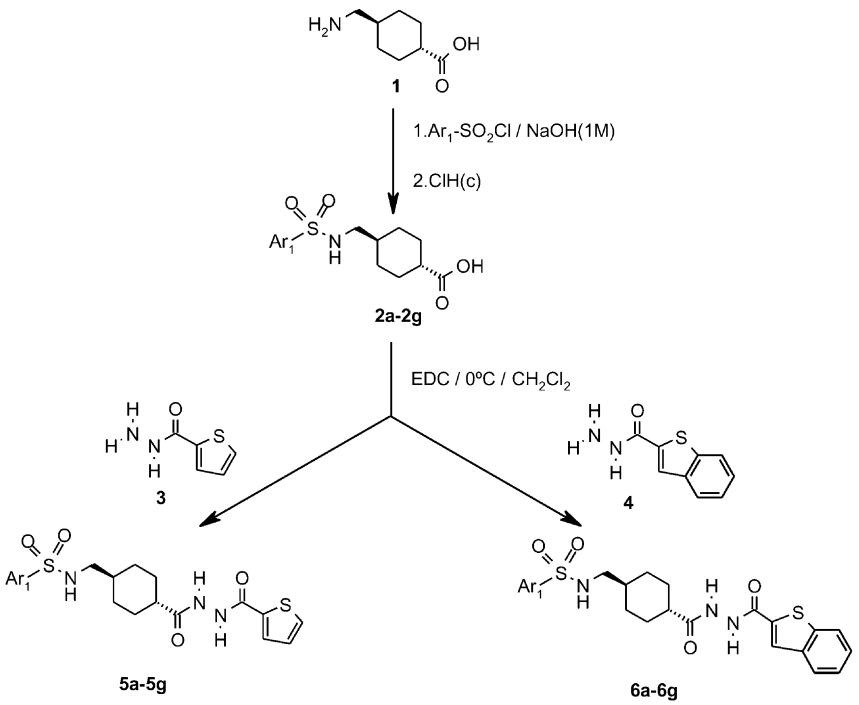


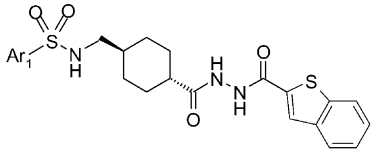
Scheme 1.

stirred at room temperature for 48 h. Sulfonamide derivatives are obtained by precipitation, adding HCl (c) until pH 1–2 and then washing with H_2O and *n*-hexane.

The sulfonamides **2a–g** are maintained at 0 °C, for 1 h, in dry CH_2Cl_2 (150 mL), under N_2 atmosphere, and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide·HCl (EDC) (1.13 equiv)[12] is used for the activation of the carboxylic group (1.00 equiv). Next, hydrazide derivatives **3** or **4** (1.13 equiv) is added and the reaction is stirred at room temperature for 24 h. The solvent is evaporated and the residue is washed with H_2O (20 mL) and diethyl ether (5 mL), yielding the corresponding thiophene and benzo[*b*]thiophene hydrazide derivatives **5a–g**, **6a–g**. All of the compounds were chemically characterized by thin layer chromatography (TLC), melting point, infrared, nuclear magnetic resonance (^{1}H NMR), elemental microanalysis and HPLC.

Binding assays for both receptors NPY_1 and NPY_5 were carried out as described by Duhault et al.[13] For the human Y_1 receptor binding assay, using iodinated peptide YY (NEN), incubations were performed at 30 °C for 90 min with various competitors concentrations in Buffer A (Hepes/NaOH 20 mM, pH 7.4, NaCl 10 mM, KH_2PO_4 220 μM, $CaCl_2$ 1.26 mM, $MgSO_4$ 0.81 mM and bovine serum albumin 0.1%) with SK-N-MC cell membranes (50 μg of protein/mL of assay) in a total volume of 500 μL. Non-specific binding was determined in the presence of 1 μM NPY. The reaction was then stopped by filtration. The filters (GF/B, Whatman, precoated in 0.3% PEI) were extensively washed with buffer A, and counted in a gamma counter (Packard). For human Y_5 receptor binding assay, the binding was carried out with iodinated peptide YY (NEN) as follows: COS cells transfected with the human Y_5 NPY receptor were lysed and the membranes were prepared by differential centrifugation. These membranes contained about 2 pmol per mg of protein of this receptor. Incubations were perfomed in 500 μL comprising, 20 pM final of [^{125}I]PYY in 50 μL, 400 μL of membrane suspension (0.15 mg/mL) and competitor dilutions in 50 μL, at 30 °C for 2 h. The reaction was stopped by filtration through GF/C filters (Whatman) and the results were expressed in IC_{50} (Tables 1 and 2).

Table 1. Y_1 and Y_5 receptor binding affinities of compounds **5a–g**



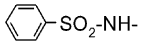
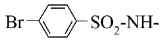
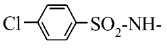
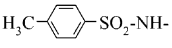
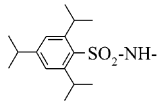
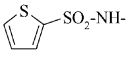
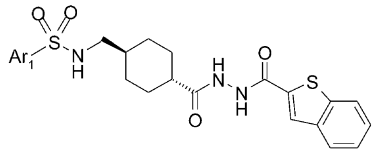
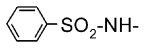
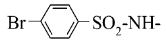
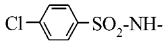
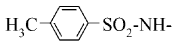
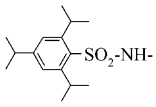
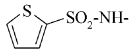
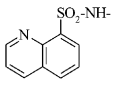
Compd	Ar_1–SO_2–NH–	Y_1 IC_{50} (nM)	Y_5 IC_{50} (nM)
5a		$>10^4$	32.2
5b		$>10^4$	7.70
5c		$>10^4$	48.4
5d		$>10^4$	20.4
5e		$>10^4$	47.7
5f		$>10^4$	12.5
5g		$>10^4$	147

Table 2. Y_1 and Y_5 receptor binding affinities of compounds **6a–g**



Compd	Ar_1–SO_2–NH–	Y_1 IC_{50} (nM)	Y_5 IC_{50} (nM)
6a		$>10^4$	175
6b		$>10^4$	28.4
6c		$>10^4$	279
6d		$>10^4$	42.9
6e		$>10^4$	142
6f		$>10^4$	11.5
6g		$>10^4$	92.2

Fourteen new compounds, derivatives of the thiophene and benzobthiophene hydrazide, have been synthetized. The NPY Y_1 and Y_5 receptor binding affinities for these compounds are reported in Tables 1 and 2.

The results of the in vitro evaluation of the antagonist activity on the NPY Y_5 receptor have shown that all the compounds present good affinity on said receptor leading compound **5b** with an IC_{50} value of 7.7 nM and selectivity as neither of them were found to antagonize the NPY Y_1 receptor ($IC_{50}10^4$ nM).

Structure–activity relationships have been studied, introducing different substituents Ar_1 of the molecule's general structure. The results showed that the thiophene derivatives (**5a–f**) present better affinity than their benzo[*b*]thiophene analogues (**6a–f**). Three compounds, **5b**, **5f** and **6f** with IC_{50} values of 7.7, 12.5 and 11.5 nM, showed the best activity. All of these potent hY_5 antagonists possess a tiophene ring in their structures, as either an Ar_l substituent or a hydrazide rest. The presence of the ring of thiophene could be determinant in their link to the receptor.

We are continuing our search for in vivo assays to that will allow us to clearly understand the SAR trends and the absorption and permeability to brain.

In summary, we have developed a new series of thiophene and benzo[*b*]thiophene derivatives as potent and selective antagonists at the human NPY Y_5 receptor. Based on the obtained data, we propose a more thorough research program regarding the determination of the importance of the substituents Ar_1 with in vivo assays, the biological significance of the results obtained, and the role played by neuropeptide Y in food intake response.

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References and notes

1. Hipskind, P.; Gehlert, D. *Annu. Rep. Med. Chem.* **1996**, *31*, 1.
2. Kalra, S. P.; Dube, M. G.; Fournier, A.; Karla, P. S. *Physiol. Behav.* **1992**, *50*, 5.
3. Gehlert, D. *Proc. Soc. Exp. Biol. Med.* **1998**, *218*, 7.
4. Blomqvist, A. G.; Herzog, H. *Trends Neurosci.* **1997**, *20*, 294.
5. Clark, J. T.; Kalra, P. S.; Crowley, W. R.; Kalra, S. P. *Endocrinology* **1984**, *115*, 427.
6. Stanley, B. G.; Leibowitz, S. F. *Life Sci.* **1984**, *35*, 2635.
7. Islam, I.; Dhanoa, D.; Finn, J.; Du, P.; Walker, M. W.; Salon, J. A.; Zhang, J.; Gluchowski, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1767.
8. Rüeguer, H.; Schmidlin, T.; Rigollier, P.; Yamaguchi, Y.; Tintelnot-Blomley, L.; Schilling, W.; Criscione, L.; Mah, R. PCT WO 97/20823.
9. Islam, I.; Dhanoa, D. S.; Finn, J. M.; Du, P.; Gluchowski, C.; Jean, Y.T. PCT WO 97/19682.
10. Hofbauer, K. G.; Schaffhauser, A. O.; Batzl-Hartmann, C.; Stricker-Krongrad, A.; Whitebread, S.; Cumin, F.; Rigolier, P.; Yamaguchi, Y.; Chiesi, M.; Levens, N.; Schilling, W.; Walker, M. W.; Gerald, C.; Rüeger, H.; Criscione, L. *Regul. Pept.* **1997**, *71*, 211.
11. Smith, M. B., March, J., Eds. *March's Advanced Chemistry,* 5th Ed; Wiley-Interscience: New York, 2001; p 574.
12. Sheehan, J.; Preston, J.; Cruickshank, P. A. *J. Am. Chem. Soc.* **1965**, *87*, 2492.
13. Duhault, J.; Boulanger, M.; Chamorro, S.; Boutin, J. A.; Della Zuana, O.; Douillet, E.; Fauchere, J. L.; Feletou, M.; Germain, M.; Husson, B.; Renard, P.; Tisserand, F. *Can. J. Biochem. Physiol.* **2000**, *78*, 173.