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Synthesis and pharmacological characterization of 5-phenyl-2-[2-(1-piperidinylcarbonyl)phenyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*c*]imidazol-1-ones: A new class of Neuropeptide S antagonists

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ABSTRACT

A new class of selective NPS antagonist was developed starting from a commercially available product identified by screening activities. Experimental NMR observations and computational experiments allowed the discovery of a new class of derivatives. 5-Phenyl-2-[2-(1-piperidinylcarbonyl)phenyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*c*]imidazol-1-one represents a new lead compound in the NPS antagonist field.

Neuropeptide S (NPSa)[1–4] was the last endogenous peptide identified via the reverse pharmacology approach. Human NPS is a 20 residue peptide showing the following primary sequence: SFRNGVGTGMKKTSFQRAKS. This sequence is also well conserved among species; in particular, the serine (S) N-terminal residue of NPS is conserved among all species examined so far.[2]

After its pairing with NPS, the previously orphan G-protein coupled receptor (GPCR) GPR154 was named the NPS receptor and abbreviated as NPSR.

This receptor exhibits overall low homology to other members of the GPCR family. However, phylogenetic tree analysis of 7TM receptors has shown close proximity of NPSR to the vasopressin/oxytocin receptor family.[5]

In situ hybridization studies[1,6] showed that NPS mRNA is heavily expressed in a few brain regions only. These include the locus coeruleus area and a few nuclei of the brainstem. The medicinal chemistry interest in this field was elicited by the high number of potential applications for NPS ligands, in particular due to its potential involvement in several biological processes such as arousal, anxiety, and food intake.[7–9]

A series of studies were recently reported[10] on the neuropeptide modification, while nonpeptide NPRS antagonists are quite limited.[11–13] Molecular modeling studies dealing with potential binding sites in the NPS receptor model for non peptide antagonists were also recently reported.[14]

Considering that non peptide antagonists like SHA-66 and SHA-68 (Fig. 1) show high MW and lipophilicity (MW = 427 and *C* log *P*[15] of 4.6 for SHA-66; MW = 445 and *C* log *P* = 4.7 for SHA-68), it might be hypothesized that these structures are not ideal starting points for a medicinal chemistry exploration considering their potential developability issues (e.g., potential low solubility) for CNS indications. Screening activities performed in house in recombinant system (hGPR154 HEK) using FLIPR assay[16,17] allowed for the identification of the competitive antagonist compound **1**, which shows a pIC_{50} value of 7.5, comparable to SHA-66, but lower

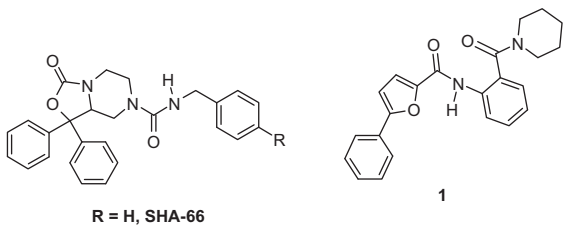

Figure 1. NPS antagonists.

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MW and lipophilicity (MW = 374 and *C* log *P* = 4). Further selectivity studies confirmed its selectivity for this target and a detailed chemical exploration around this lead was performed.

In this Letter, we report how an experimental observation led to the discovery of an alternative lead series.

The high value of the amide proton chemical shift in the NMR spectra of compound **1** clearly suggested the existence of a hydrogen bond interaction between such proton and an appropriate acceptor.[18] The value of the chemical shift remained unchanged upon dilution of the sample and upon titration of DMSO to the sample dissolved in chloroform (data not shown). This demonstrated the intra-molecular character of this interaction.[19] In order to test whether or not the receptor affinity was linked to the aforementioned folded conformation, a series of aromatic/heteroaromatic rings with different H-bond capabilities either as donor or acceptor were prepared. The results of this exploration are reported in Table 1.

In agreement with the working hypothesis, the pyridyl scaffold (**2**) demonstrated the best potency at the receptor, while the phenyl (**4**) and thienyl (**3**) derivatives showed a substantial drop in the NPSR potency. Finally, the pyrrolyl derivative (**5**) resulted completely inactive; this might be due to a potential clash between the two acidic hydrogens.

To further refine this working hypothesis and to provide rational basis for the exploitation of such results, in silico conformational analyses[20] were performed with low-mode conformational sampling in implicit water model.

Results of these studies demonstrated the capability of the acidic hydrogen to interact with the furane oxygen rather then with the carbonyl group of the piperidine amide because of the particular geometry adopted by the template in the space as reported in Figure 2.

Within a 3 kcal/mol energy window, the 37% of the conformations sampled for compound **1** are characterized by the presence of such interaction.

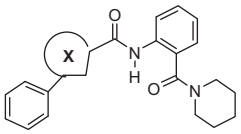
The same computational analysis was performed for the pyridyl derivative **2** and results are reported in Figure 3.

In this case, within a 3 kcal/mol energy window, 100% of the conformations sampled had the possibility to show H-bonding capabilities. As shown in the left panel of Figure 3, only the conformation with the amide NH and the pyridyl N close in space were populated. The same analysis demonstrate that, for the phenyl derivative **4**, an almost equal distribution of cis- and trans-conformations were obtained with in silico calculations.

Based on these evidences, conformationally locked analogues of derivative **1** were designed.

Three main structures were identified as 'ideal' candidates for this task supported by conformational analyses. Their structures

Table 1
Affinity results for derivatives **1**–**5**[16]



Entry	X	hNPS pIC_{50}
SHA-66	NA	8.7 ± 0.1
1	2,5-Furanyl	7.5 ± 0.2
2	2,6-Pyridyl	7.7 ± 0.1
3	2,5-Thienyl	6.1 ± 0.1
4	2,6-Phenyl	6.6 ± 0.2
5	2,5-Pyrrolyl	<5.3

Figure 2. Conformational studies performed on compound **1**. In the left panel, the lowest energy conformation is reported. Conf #1 $\Delta E = 0$. $\tau 1 = -1.0$ (O–C–C–N), $\tau 2 = 22.8$ (H–N–C=C), $\tau 3 = -112.6$ (C=C–C=O). Boltzmann factor[21] ($B_f = 2.7 \times 10^{-1}$). In the right panel, the first 'trans' conformation is reported. Conf #2 $\Delta E = 0.56$ kcal/mol (2.35 kJ/mol). $\tau 1 = -175.9$, $\tau 2 = -26.7$, $\tau 3 = 112.2$. $B_f = 1.0 \times 10^{-1}$.

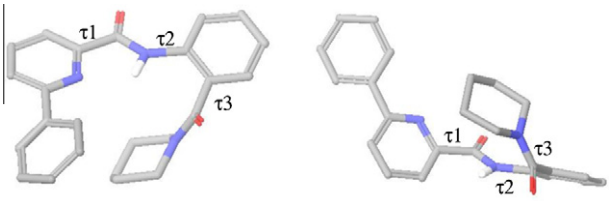


Figure 3. Conformational studies performed on compound **2**. In the left panel, the lowest energy conformation is reported. Conf #1 $\Delta E = 0$. $\tau 1 = -2.9$ (N–C–C–N), $\tau 2 = 17.4$ (H–N–C=C), $\tau 3 = -111.7$ (C=C–C=O). Boltzmann factor[21] ($B_f = 4.3 \times 10^{-1}$). In the right panel, the first 'trans' conformation is reported. Conf #17 $\Delta E = 3.24$ kcal/mol (13.53 kJ/mol). $\tau 1 = 137.6$, $\tau 2 = -24.9$, $\tau 3 = 112.6$. $B_f = 3.4 \times 10^{-2}$.

are reported in Figure 4 in order of synthetic feasibility from left to right.

As it can be clearly appreciated from Figure 5 (left panel), the compound **8** can be nicely overlapped on derivative **2**, while for structure **7** (Fig. 5, right panel) and structure **6** (data not shown), the steric bulk of the newly formed ring might cause a clash within the receptor pocket.

In agreement with the synthetic feasibility, the first compound to be prepared was derivative **6**.[22] Results for this compound are reported in Table 2.

Unfortunately, in agreement with the computational suggestions, the compound was completely inactive when tested versus the hNPSR.

The second compound to be prepared was derivative **7**.[23] Once again, in agreement with what reported in the right part of Figure 5, a negative result was observed, potentially suggesting that either the steric bulk of the core ring or the change in the spatial orientation of the pendant phenyl group prevented their interaction in the receptor binding site.

Finally, derivative **8**[24] was prepared, in agreement to Scheme 1. In this case, the compound showed the expected potency at the hNPS receptor, confirming the working hypothesis and the computational predictions.

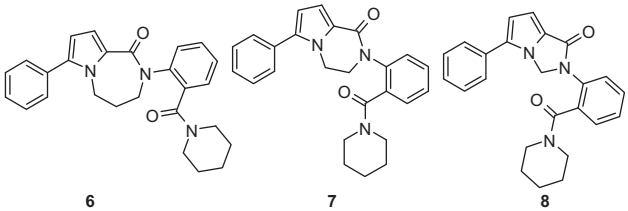


Figure 4. The proposed conformationally locked templates.

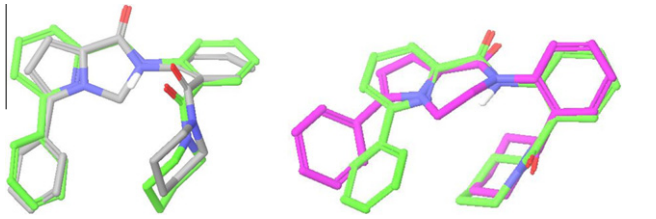
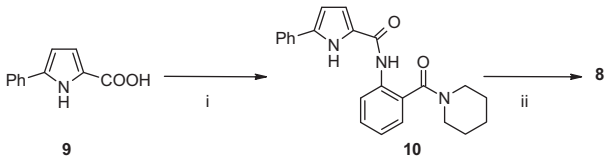


Figure 5. Left: Atom-based superimposition of the lowest energy conformation of compound **8** (atom type colored) on the 4th energy ranked conformation of derivative **2** (green). Right: Atom-based superimposition of the lowest energy conformation of compound **2** (green) on the 3rd energy ranked conformation of derivative **7** (purple).

Table 2
Affinity results for derivatives **6**–**8**[16]

Entry	hNPS pIC_{50}
6	<5.3
7	<5.3
8	8.0 ± 0.1



Scheme 1. Preparation of compound **8**. (i) $SOCl_2$, 2-(1-piperidinylcarbonyl)aniline; (ii) ICH_2Cl, DMF.

Considering the results reported in Table 2, it might be hypothesized that, within the NPS receptor, very stringent conformational requirements have to be met to achieve the desired interactions and that minimal variations to the structure might lead to potential steric clashes with the amino-acid residues in the binding site.

The identification of a small, rigid, non peptidic hNPSR antagonist elicited the interest to derivative **8**.

Accordingly, the molecule was better characterized in the programme screening cascade; the CYPEX bactosome P450 inhibition and rat and human in vitro clearance in liver microsomes were performed to further evaluate its developability potential.

IC_{50} values for all major P450 isoforms tested (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were greater than 5 μM. Intrinsic clearance (Cli) values both in human and in rat resulted relatively high (6.9 and 26.3) ml/min/g of protein. This result was quite expected considering that the template was completely not substituted and therefore potentially prone to metabolic degradation.

In vitro results found confirmation in in vivo studies in rat.[25] Derivative **8** (1 mg/kg, po, 5%DMSO + 0.5% HPMC in water) actually showed high blood clearance (69 ml/min/kg), a relatively low half-life (0.7 h), a moderate distribution volume (2.4 l/kg) and a low bioavailability (F = 1%). Brain/blood ratio was 0.3.

In summary, the exploitation of the experimental observations relative to a HTS hit allowed the identification of a promising new scaffold. The new compound is a low molecular weight, non peptidic hNPSR antagonist.

The use of computational chemistry was critical to design the new scaffold and a good agreement between experimental findings and calculations was observed.

Further refinements have to be performed to transform the new scaffold in a potential lead series for in vivo experimental activities.

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16. The compounds functional activity was tested on recombinant human GPR154 receptors, transiently expressed in HEK293 cells. Briefly: Cells were seeded at a density of 18.000 cells/well in 384-well plates and incubated at 37 °C, 5% CO_2 for 24 h before the experiment.
On the day of the experiment, cells were loaded with the fluorescent calcium indicator dye FLUO-4-AM for 1 h at 37 °C, after which the dye was removed by washing cells with HBSS. Compound modulation of intracellular calcium levels, induced by an EC_{80} concentration of Neuropeptide S, was assessed using FLIPR.[17] Concentration response data are expressed in terms of percentage response relative to a maximum test concentration of NPS (30 nM final concn) calculated from a maximum minus minimum of the relative fluorescence units, and pIC_{50} values were determined by nonlinear regression analysis.
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20. Conformational analyses were carried out with BatchMin V9.5 and V9.6 (Schrodinger, http://www.schrodinger.com). Conformational sampling was done with Low-frequency-Mode Conformational Search (LMCS keyword), OPLS_2005 force field, implicit water model. 1000 Monte Carlo steps per rotatable bond, 10,000 minimization steps and 50 kJ/mol energy window were set. A root mean square deviation cutoff of 0.5 Å was utilized to discard duplicated conformations. Conformations were visually inspected within Maestro (Schrodinger). Atom-based superimpositions were performed with the use of the routines available within Maestro.
21. The Boltzmann factor (B_f) of each conformation j was calculated as the ratio between $p(j)$/totp where $p(j) = \exp(-1\ ((\text{energy}(j) - \text{energy}(\text{lowest})/(R^* T)))$ and totp = sum($p(j)$) over all the conformations sampled within 50 kJ/mol energy window, energy(j) is the energy of the conformation j, energy(lowest) is the energy of the lowest energy conformation, R = 8.31434/1000 and T = 300 K.
22. 7-Phenyl-2-[2-(1-piperidinylcarbonyl)phenyl]-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*a*][1,4]diazepin-1-one **6**. To a solution of the commercially available methyl 5-phenyl-1*H*-pyrrole-2-carboxylate, in dry DMF at 0 °C under inert atmosphere, NaH was added. Subsequently, 1-bromo-3-chloropropane was added and the resulting solution was stirred at 60 °C for 3 h. After quenching and work-up, the intermediate compound was dissolved in ammonia (7 N in MeOH) and stirred at 65 °C for 20 h. The reaction intermediate was cyclized with EtONa in EtOH (2 h, 60 °C) and the resulting 7-phenyl-2,3,4,5-tetrahydro-1*H*-pyrrolo[1,2-*a*][1,4]diazepin-1-one was coupled to 1-[(2-bromophenyl)carbonyl]piperidine in a mixture of dry 1,4-dioxane/DMSO at 120 °C for 4 h using CuI, K_3PO_4 and dimethylethane diamine to give the desired product.

23. 6-Phenyl-2-[2-(1-piperidinylcarbonyl)phenyl]-3,4-dihydropyrrolo[1,2-*a*]pyrazin-1(2H)-one **7**. To a solution of the commercially available methyl 5-phenyl-1*H*-pyrrole-2-carboxylate, in dry DMF at 0 °C under inert atmpsphere, NaH was added. Subsequently, bromoacetonitrile was added and the resulting solution was stirred at rt °C for 3 h. After quenching and work up, the resulting nitrile was reduced using $NaBH_4$ and $CoCl_2$ in MeOH. The resulting amino derivative was cyclized with EtONa and EtOH to give 6-phenyl-3,4-dihydropyrrolo[1,2-*a*]pyrazin-1(2*H*)-one. This intermediate was coupled with 1-[(2-bromophenyl)carbonyl]piperidine as described in Ref. 14 to give the title material.
24. 5-Phenyl-2-[2-(1-piperidinylcarbonyl)phenyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*c*]imidazol-1-one **8**. Thionyl chloride was added to the commercially available 5-phenyl-1*H*-pyrrole-2-carboxylic acid and the resulting mixture was stirred at rt for 2 h. After work-up, the resulting acyl chloride was dissolved in dry THF and the solution was added drop-wise to a previously prepared solution of [2-(1-piperidinylcarbonyl)phenyl]amine (commercially available) and Et_3N in dry THF at 0 °C. After quenching and work-up, the resulting -phenyl-*N*-[2-(1-piperidinylcarbonyl)phenyl]-1*H*-pyrrole-2-carboxamide was suspended in DMF at 0 °C under a N_2 blanket. NaH was added and the mixture stirred for 0.3 h; iodochlorometane was added drop-wise and the reaction was stirred for 24 h at 60 °C to give, after quenching and work-up, the title compound.
25. All the works involving animals were carried out in accordance with European directive 86/609/EEC governing animal welfare and protection, which is acknowledged by Italian Legislative Decree no. 116, 27 January 1992, and according to internal review performed by the GlaxoSmithKline Committee on Animal Research & Ethics (CARE) and to the company Policy on the Care and Use of Laboratory Animals.