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Coumarinyl pyranopyrimidines as new neuropeptide S receptor antagonists; design, synthesis, homology and molecular docking

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ABSTRACT

In this work, we described the design, synthesis and characterization of a new class of NPSR antagonists bearing the tetracyclic coumarinyl pyranopyrimidine scaffold incorporated with different acyclic and/or heterocyclic moieties. These compounds are highlighted in this study as never being used as NPSR antagonists before which provides a model for the discovery of new bioactive inhibitors that may hold potential for drug development towards anxiety, food, and addiction disorders. Synthetic and medicinal chemistry studies led to the identification of four potent antagonists, compounds **7d**, **10**, **12** and **13,** which were able to significantly inhibit the stimulatory effect of NPS through counteracting the increased intracellular Ca^{2+} accumulation. The target compound **7d** was the most active derivative behaving as a pure NPSR antagonist and displaying IC_{50} value of 2 μM. Homology model of NPSR was built based on bovine rhodopsin structure. Modeling studies were carried out to further rationalize the NPSR binding mode of the target compounds. Moreover, molecular dynamics simulation study was performed for compounds **7d**, **10** and **12** which revealed the stability of the ligand-protein complex and the reliability of the docking studies.

1. Introduction

Neuropeptide S is a peptide produced predominantly in the brain in a group of neurons that are located between the locus coeruleus, the Barrington nucleus, and the parabrachial nuclei. Neuropeptide S receptor (NPSR), a G-protein coupled receptor expressed in several brain areas, is functionally coupled with Gq and Gs G-proteins and its activation leads to mobilization of cytoplasmic Ca^{2+} and formation of cyclic adenosine monophosphate (cAMP) [1,2]. NPS/NPSR system proved to be an interesting target in the medicinal chemistry field. The pharmacological effects of NPS administration include stimulation of locomotor activity, inhibition of food intake, antinociception, wakefulness and arousal-promoting action associated with anxiolytic-like effects [3,4]. The roles of NPS in respiratory function, olfaction, inflammation, alcohol and cocaine seeking, panic disorders and hippocampus-dependent learning and memory, have been reported [5,6].

Structure activity relationship analysis of NPS has reported neuropeptide analogues with antagonist or partial agonist profiles [7,8]. An extended overview of NPS analogues provided several series of nonpeptide NPSR ligands based on various scaffolds as quinolinone **I** [9], bicyclic piperazine **II** [10], tricyclic imidazole **III** [11], naphthopyranopyrimidine **IV** [12] and pyrroloimidazole derivatives **V** [13], which exhibited antagonist activity at the receptor site (Fig. 1).

Although the aforementioned structural classes of NPS antagonistic molecules are currently described, comprehensive information of the structure activity relationships is still limited and more detailed understanding of the molecular requirements for antagonistic activity at the NPS receptor is required. The development of target NPS antagonists with improved potency and better pharmacokinetic properties could provide a better understanding of the NPSR system.

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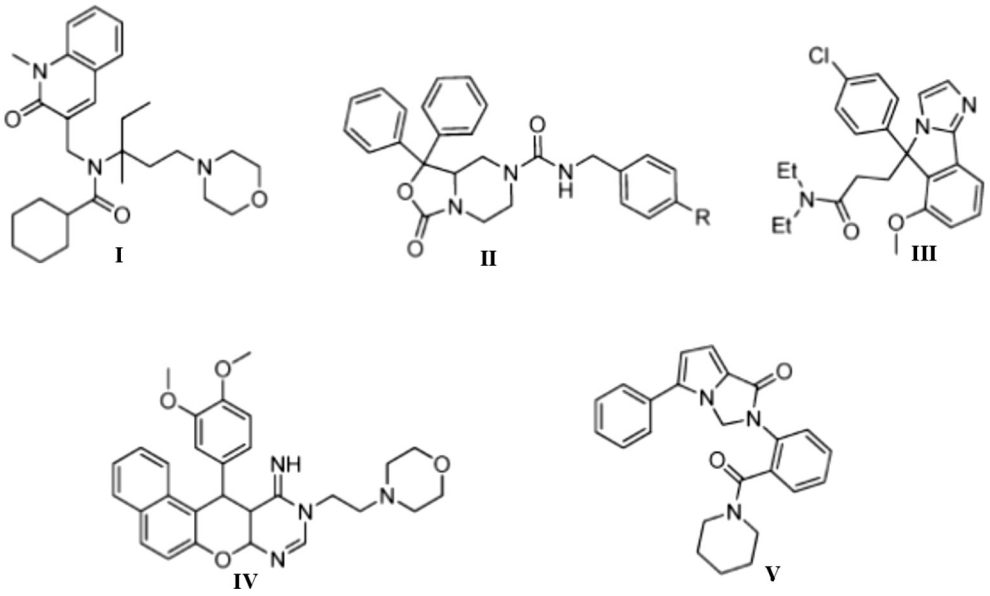

Fig. 1. Examples of potent small-molecules as NPSR antagonists.

The present study provides a strategy to generate a novel class of NPSR antagonists that was built in a chemical diversity around the key pyranopyrimidine moiety depending on modification of the biologically active naphthopyranopyrimidine scaffold. Structure activity relationship study was also displayed hoping to shed light on the possible mechanisms for the behavioral variations between the synthesized series. In this work, we went into the challenge to modulate the lead naphthopyranopyrimidine structure [12] by replacing the naphthalene moiety by the bioactive isosteric chromene core in order to increase the hydrophilic properties of the target molecules hoping for more interactions with the NPSR active site and consequently an increase in the activity. Coumarins (2*H*-chromen-2-ones) are naturally occurring bioactive scaffolds having diverse pharmacological activities particularly neuroprotective properties [14–18]. Our strategy for the choice of the coumarin scaffold in this study relied upon (i) the isosteric characters of the chromene core to the previously used naphthalene moiety which could facilitate the fitting into NPSR binding site, (ii) the presence of two hydrophilic centers (oxygen atom and carbonyl group) in the pyran ring which might increase the probability of H-bond interactions with the binding pocket and hence improve the activity. Based on the impact of ring size variations, molecular orientation and heteroatoms number which are expected to play a role in the interactions and orientation of the new synthesized compounds in NPSR binding site, we have investigated the synthesis of new NPSR antagonists by hybridization of the coumarinyl pyranopyrimidine core with different bioactive moieties like Schiff's bases, and isosteric heterocyclic rings as triazole and tetrazole nuclei hoping to develop more targeted pharmacological profiles and therapies (Fig. 2).

Chemically all the newly synthesized compounds were analyzed using different spectroscopic methods as ^{1}H NMR, ^{13}C NMR, mass and IR. Biologically, the functional coupling of NPSR with Gq and Gs enables the evaluation of the ability of compounds to activate or deactivate the receptor using a functional assay to detect intracellular Ca^{2+} mobilization. In order to determine the mode of interaction between the NPSR antagonists and their binding sites, NPSR homology model was built due to the limited NPS receptor 3D structure information, and modeling stimulation for promising active compounds was performed. In addition, molecular dynamics simulation study was carried out to confirm the stability of the ligand-protein complex and the reliability of the docking studies.

2. Results and discussion

2.1. Chemistry

Michael cycloaddition reaction of the readily available 4-hydroxycoumarin with malononitrile and 3,4-dimethoxybenzaldehyde in ethanol in the presence of piperidine afforded the key intermediate 2-amino-4,5-dihydro-4-(3′,4′-dimethoxyphenyl)-5-oxopyrano[3,2-c] chromene-3-carbonitrile **1** [19]. Treatment of **1** with acetic anhydride under reflux afforded the corresponding benzopyrano [3′,4′:5,6]-pyrano[2,3-*d*]pyrimidine-6,8-dione **2** (Scheme 1).

The enaminonitrile compound **1** reacted with triethylorthoformate to give the corresponding ethoxymethylene amino derivative **3**. The latter compound was used as an intermediate for the preparation of the key pyranopyrimidine compounds **4**, **5a–d and 6**. Thus cyclization of **3** with ammonia solution gave the corresponding 8-amino-7-(3′,4′-dimethoxyphenyl)-6*H*,7*H*-[1]benzopyrano[3′,4′:5,6]pyrano[2,3-*d*]pyrimidine-6-one **4**.

Aminolysis of **3** with different aliphatic primary amines, namely; methylamine, ethylamine, propylamine and/or isopropylamine, yielded the corresponding 9-substituted-8-iminopyrano pyrimidine-6-one derivatives **5a–d**, while hydrazinolysis of **3** in ethanol at room temperature afforded 9-amino-8,9-dihydro-7-(3′,4′-dimethoxyphenyl)-8-imino-6*H*,7*H*-[1]benzopyrano-[3′,4′:5,6]pyrano-[3,2-*d*]pyrimidine-6-one **6** (Scheme 2). The reaction of **6** with aromatic aldehydes in the presence of piperidine drops in refluxing ethanol afforded the Schiff's bases **7a-e** as described in Scheme 3.

Scheme 4 describes the synthesis of triazolopyrimidines **8**–**13** and tetrazolopyrimidine compound **14** using the key intermediate **6**. The reaction of **6** with triethylorthoformate in DMF gave the corresponding 7-(3′,4′-dimethoxyphenyl)-13H,14H-[1] benzopyrano[3′,4′:5,6] pyrano [3,2-e][1,2,4] triazolo [1,5-c]- pyrimidine-13-one **8**. However, its reaction with acetic anhydride afforded the 2-methyl derivative **9**. Triazolone compound **10** was obtained by refluxing **6** with ethyl chloroformate in dry benzene. The reaction of **6** with diethyl oxalate gave the triazoloethylcarboxylate derivative **11** while upon refluxing **6** with excess diethylmalonate, the