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Original article

Towards novel 5-HT$_7$ *versus* 5-HT$_{1A}$ receptor ligands among LCAPs with cyclic amino acid amide fragments: Design, synthesis, and antidepressant properties. Part II

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ABSTRACT

A 26-membered library of novel long-chain arylpiperazines, which contained primary and tertiary amides of cyclic amino acids (proline and 1,2,3,4-tetrahydroisoquinoline-3-carboxamide) in the terminal fragment was synthesized and biologically evaluated for binding affinity for 5-HT$_7$ and 5-HT$_{1A}$ receptors. Docking studies confirmed advantages of Tic-amide over Pro-amide fragment for interaction with 5-HT$_7$ receptors. Selected compounds **32** and **28**, which behaved as 5-HT$_7$Rs antagonist and 5-HT$_{1A}$ partial agonist, respectively, produced antidepressant-like effects in the forced swim test in mice after acute treatment in doses of 10 mg/kg (**32**) and 1.25 mg/kg (**28**). Compound **32** reduced immobility in a manner similar to the selective 5-HT$_7$ antagonist SB-269970.

1. Introduction

Among fourteen types of serotonin (5-HT) receptors, the 5-HT$_7$ receptors (5-HT$_7$Rs) are the latest addition to a subfamily of G-protein-coupled receptors (GPCRs) [1]. It was found that blockade of 5-HT$_7$Rs produced an antidepressant-like effect in animal models of depression [2–4], and may be responsible for improvement of cognitive decline or neurological disorders [5,6]. On the other hand, the activation of 5-HT$_7$Rs is regarded as a potential therapy for treatment of pain [7,8], and the Fragile X syndrome [9]. In view of aforementioned findings, the development of new 5-HT$_7$R agents seems critical for the acquisition of detailed insight into the pharmacological function of 5-HT$_7$Rs.

For decades, long-chain arylpiperazines (LCAPs) have been successfully explored as privileged structures for many subtypes of 5-HTRs [10,11]. Extending SAR studies around this class of compounds, our group modified the amide fragment of LCAPs with *N*-acylated amino acids to optimize potent 5-HT$_{1A}$/5-HT$_{2A}$ receptor ligands [12–14]. Recently, as our interest focused on high-end 5-HTRs ligands, we investigated an influence of the primary amides of cyclic amino acids (pyrrolidine-2-carboxamide – Pro-amide and 1,2,3,4-tetrahydroisoquinoline-3-carboxamide – Tic-amide) on compounds affinity for 5-HT$_7$Rs and selectivity over 5-HT$_{1A}$ sites [15] (Fig. 1).

As an extension of our ongoing efforts aimed at identification of 5-HT$_7$ receptor ligands we designed a new series of LCAPs functionalized with tertiary amides of amino acids in the terminal fragment. Apart from diversification of the amide fragment, structural modifications comprised variation in the length of the alkylene spacer, and introduction of different substituents in *ortho* position of phenylpiperazine (PhP). Herein we present the synthesis of the designed compounds, determination of their affinity

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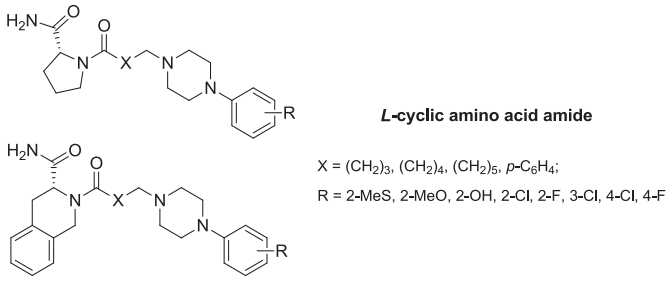

Fig. 1. General structures of LCAP and *L*-Pro and *L*-Tic primary amide derivatives.

for 5-HT$_7$ and 5-HT$_{1A}$ receptors, and investigation of their binding mode for 5-HT$_7$ and 5-HT$_{1A}$Rs, followed with functional *in vitro*, as well as behavioral evaluation in animal model of depression.

2. Results and discussion

2.1. Chemistry

The synthesis of target compounds, grouped in two series **22**–**34** (set **I**) and **35**–**47** (set **II**), was carried out by a multi-step procedure according to Scheme 1. In order to obtain primary amide derivatives **1**–**2**, Boc-protected amino acids: (*S*)–pyrrolidine–2–carboxylic acid (Pro) and (*S*)–tetrahydroisoquinoline-3-carboxylic acid (Tic) were subjected to the amidation reaction with ammonia in the presence of isobutyl chloroformate and *N*-methylmorpholine. Alternatively, tertiary amide derivatives **3**–**6**, were obtained from amino acids and alicyclic secondary amines – piperidine or morpholine, under BOP-promoted coupling reaction. After the removal of Boc group using TFA in DCM, the resulting secondary amines were subsequently acylated with respective ω-bromo-acyl chlorides to afford the corresponding intermediates **7**–**12** and **13**–**21**. These were further reacted with appropriate arylpiperazines in biphasic conditions to give final compounds **22**–**34** and **35**–**47**.

The 1-arylpiperazines were obtained either commercially or were prepared according to the details presented in Supporting Information.

2.2. Molecular modeling

Homology models of 5-HT$_{1A}$ and 5-HT$_7$ receptors were built on crystal structures of 5-HT$_{1B}$ (PDB ID: 4IAR), β_2 (PDB ID: 3P0G), D_3 (PDB ID: 3PBL) and H_1 (PDB ID: 3RZE) receptors. Based on the docking of a structurally diverse set of ligands it was found that their fully coherent binding mode was obtained only in models generated on the 5-HT$_{1B}$ templates. These models were next used for the study of the binding mode of Tic and Pro derivatives for 5-HT$_7$ and 5-HT$_{1A}$Rs. Despite the fact that our previous 5-HT$_{1A}$ and 5-HT$_7$R models were limited only to transmembrane domain and were built on different templates, the marked hot-spot residues were the same [15–17].

2.3. Pharmacology

2.3.1. In vitro evaluation

Radioligand binding assays were employed to determine the affinity and selectivity profiles of the synthesized compounds in competition experiments for human serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$ and 5-HT$_{7B}$ receptors, which were all stably expressed in HEK293 cells. According to the previously published procedures [18], the experiments were carried out using [^{3}H]-8-OH-DPAT (187 Ci/mmol), [^{3}H]-ketanserin (50.3 Ci/mmol), [^{3}H]-LSD (81 Ci/mmol) and [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$ and 5-HT$_{7B}$ receptors, respectively.

2.3.2. Functional in vitro evaluation

The functional activity of the selected compounds **28**, **32**, **35** and **37** on 5-HT$_7$ and 5-HT$_{1A}$ receptors, was determined at Cerep (Le Bois l'Eveque, 86,600 Celle L'Evescault, France), according to the previously published methods [19,20]. Assays were carried out in CHO and HEK-293 cells, which stably expressed the human 5-HT$_7$ and 5-HT$_{1A}$ receptors, respectively.

2.3.3. Behavioral evaluation

Potential antidepressant-like activity of selected compounds with the highest affinities for 5-HT$_{1A}$ (**28**, **35**) and 5-HT$_7$ (**32**, **37**) receptors was evaluated in the forced swim test (FST) in mice [21] and their activity was compared with that of the antidepressant drug imipramine (**28**, **35**) and the selective 5-HT$_7$ antagonist SB-269970 (**32**, **37**). The influence of the effective doses recorded in the FST on spontaneous locomotor activity in mice was studied in order to exclude the possibility of competing behaviors such as general locomotor activity.

2.4. Structure–activity relationship studies

Continuing our studies aimed at verification of structural features responsible for 5-HT$_7$ and 5-HT$_{1A}$Rs affinity and selectivity in a group of LCAPs with cyclic amino acids (Pro- and Tic-amides), we focused our attention on a kind of amide group – primary, tertiary, a length of an alkylene spacer, and a nature of substituent in *ortho* position of phenylpiperazine.

The newly synthesized compounds displayed high-to-moderate affinity for 5-HT$_7$ and 5-HT$_{1A}$ receptors (5–135 nM and 1–66 nM, respectively) (Table 1). Derivatives containing Tic-amides displayed higher 5-HT$_7$Rs affinity than the related Pro-amide compounds (**22** *vs* **27**, and **25** *vs* **31**). On the other hand, a nature of amino acid fragment has not impacted the affinity of compounds for 5-HT$_{1A}$Rs (e.g. **26** *vs* **34**, and **38** *vs* **45**).

Investigation of a binding mode showed that interaction of the Pro- and Tic-moieties with 5-HT$_7$ and 5-HT$_{1A}$ Rs might be driven by different mechanisms. The comparison of the binding modes for structural analogs **22** and **27** (Fig. 2A), showed that the π–π interactions between an aromatic ring of the 1,2,3,4-tetrahydroisoquinoline fragment and Phe3.28 was noticed for 5-HT$_{1A}$, whereas CH–π was detected in 5-HT$_7$ model. Additionally, the Tic-amide fragment created a cation–π interaction with Arg7.36 and Thr2.64 in 5-HT$_7$Rs. Furthermore, Pro- and Tic-amide fragments created H-bond interaction with the Glu7.35, and thus induced an orientation of these fragments to Phe2.38/Arg7.36/Thr2.64 amino acids. Since, the pyrrolidine-2-carboxamide fragment can form only weak (due to a distance) hydrophobic interactions with the above-mentioned amino acids, Pro-derivatives displayed lower affinity for 5-HT$_7$Rs.

In the case of 5-HT$_{1A}$Rs, the Pro-amide moiety created hydrogen bonds with Asn7.39 and Gln2.65, as well as hydrophobic interactions with Tyr2.64, while for Tic-amide fragment an aromatic interaction with Tyr2.64 and Trp78 from EL1 was identified.

Subsequently, following our previous findings that the primary exocyclic amide bond localized in Pro- and Tic-moieties interacted *via* an H-bond with Glu7.35 residue (through NH_2 function) of the 5-HT$_7$Rs, an influence of the H-bond acceptor and hydrophobic properties coming from tertiary amides was evaluated.

It was notable that within compounds **35**–**47** an introduction of the piperidinyl and morpholinyl moieties did not influence the affinity for 5-HT$_7$Rs; this modification decreased 5-HT$_7$/5-HT$_{1A}$