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Research paper

The computer-aided discovery of novel family of the 5-HT$_6$ serotonin receptor ligands among derivatives of 4-benzyl-1,3,5-triazine

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ABSTRACT

The work describes a discovery of new chemical family of potent ligands for the 5-HT$_6$ serotonin receptors. During the search for new histamine H$_4$ receptor antagonists among 1,3,5-triazine derivatives, compound **2** (4-benzyl-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine) was found. Compound **2**, weakly active for the H$_4$ receptor but fitted in 3/4 of pharmacophore features of the 5-HT$_6$R ligand, occurred to be a moderate 5-HT$_6$R agent, useful as a lead structure for further modifications. A series of new derivatives (**3**–**19**) of the lead **2** was synthesized, evaluated in the radioligand binding assay (RBA) and explored in comprehensive molecular modelling, including both pharmacophore- and structure-based approaches with docking to the homology model of 5-HT$_6$R. The most active compounds displayed a potent affinity for the 5-HT$_6$R in the nanomolar range (K$_i$ = 20–30 nM), some of them (**4**, **11** and **19**) were tested in the rat forced swim test that revealed their antidepressant-like effect. SAR-analysis on the basis of both, RBA and docking results, indicated that action on the receptor is related to the hydrophobicity and the size of aromatic moiety substituted by a methylene linker at the position 4 of 1,3,5-triazine.

1. Introduction

The 5-HT$_6$ receptor is the most recently identified member of the serotonin (5-HT) receptor superfamily. The human 5-HT$_6$ receptor (5-HT$_6$R) was discovered by Kohen et al. in 1996 [1], three years later after the first isolation of this receptor from rat striatum by two scientific groups independently [2,3]. The 5-HT$_6$R is distributed in the central nervous system (CNS), especially, is located in brain areas involved in learning and memory processes. Intensive preclinical studies have shown that 5-HT$_6$R antagonists could be a promising drug with cognitive improvement in psychiatric (e.g. schizophrenia, depression) or neurodegenerative diseases (e.g. Alzheimer's disease), and for obesity treatment [4]. Several studies have shown that not only antagonists but also agonists have potency for the treatment of obesity or cognitive disfunctions [5]. Since 1999 when the first selective 5-HT$_6$R antagonists were described, various compounds have been synthesized as potential agents for this protein target. Some of them were successful in the primary pharmacological screening or even have reached clinical studies, e.g. **dimebon** (discontinued after phase III) or **LUAE58054** (idalopirdine), where their utility has been or still is verified [6]. However till now, no 5-HT$_6$ ligand has been accepted as a CNS-drug that could reach pharmaceutical market. Thus, the further search for new chemical families of the 5-HT$_6$R ligands, including compounds with high affinity and good CNS-drugability properties, are a challenge for medicinal chemistry.

More than ten years ago, López-Rodríguez suggested a pharmacophore model for the 5-HT$_6$R antagonists. This model includes a triangle topology with tops of a bulky hydrophobic area (HYD), a positive ionizable nitrogen (PI) and a hydrogen bond acceptor (HBA) as well as a central aromatic fragment (AR, Fig. 1) [7].

For the most potent 5-HT$_6$R ligands, the pharmacophore feature

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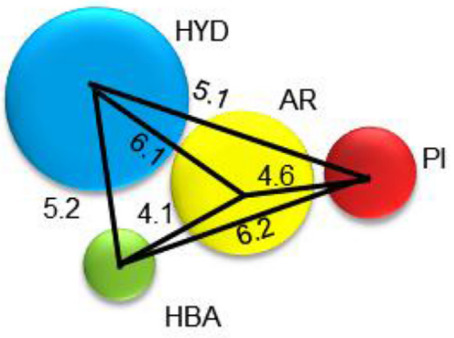

Fig. 1. Pharmacophore model for the 5-HT$_6$R ligands elaborated by López-Rodríguez et al. [7] (distances in Å); PI (red) – positive ionizable atom; HBA (green) – hydrogen bond acceptor, HYD (blue) – hydrophobic site, AR (yellow) – aromatic-hydrophobic ring. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

HYD is frequently represented by fused aromatic rings of a naphthalene or a halogen substituted benzene, an indole or a benzothiophene. A piperazine (un)substituted with methyl seems to be a most profitable moiety containing the feature PI. The feature HBA, occurring in a number of sulfonyl ligands, can also fit in the carbonyl moieties whereas the aromatic heterocyclic groups, e.g. a pyridine, a benzimidazole or a quinolone, form a benefit aromatic feature (AR, Fig. 1) that is desirable for interactions with the 5-HT$_6$R [8].

On the other hand, our previous studies provided a series of 2-amino-4-(4-methylpiperazin-1-yl)-1,3,5-triazine derivatives [9–11] that had been designed as potential histamine H$_4$ receptor ligands. That aim was achieved in the case of derivatives with aryl rings directly substituted at the triazine, e.g. **TR7** (**1**) [9], whereas the compounds with an aromatic moiety separated by the methylene group, e.g. **TR20** (**2**) [11], displayed a weak action on the histamine H$_4$ receptor with K$_i$ values only in micromolar range (Fig. 2).

During an analysis of structural properties of the 2,4,6-trisubstituted 1,3,5-triazines (**1** and **2**) some slight similarity to known selective 5-HT$_6$R ligands, namely **Ro 04–6790** [12], **SB-271046** [13] or **SB-399885** [14] (Fig. 3) can be noted as well as the triazine compounds (Fig. 2) imposed on 3 out of 4 features of the 5-HT$_6$R pharmacophore model of López-Rodríguez.

Furthermore, precise search for structural similarities to the triazine compounds (**1** and **2**) among compounds deposited in ChEMBL v20 database confirmed that no triazine 5-HT$_6$R ligand had been identified so far. The most similar 5-HT$_6$R ligands contained the benzimidazole or the naphthalene core (see Supplementary Table 1S). Taking this into account, we decided to perform modelling studies and evaluate the group of 1,3,5-triazine compounds for their binding ability to this important protein target.

The preliminary *in vitro* studies have shown a very weak

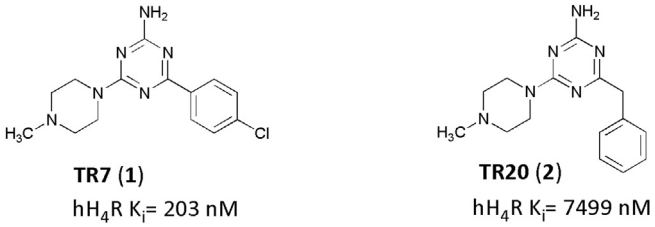

Fig. 2. Structures and the human histamine H$_4$R (hH$_4$R) affinity of **TR7**(**1**) [9] and **TR20**(**2**) [11].

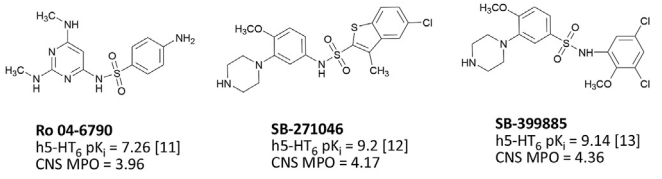

Fig. 3. Structures and the receptor affinity of selected, first identified 5-HT$_6$R ligands. The CNS MPO values were calculated using in-house script (see Experimental section).

micromolar affinity for 5-HT$_6$R in the case of a series of 19 aryltriazine compounds with the aromatic ring directly linked to the triazine core (Modification A, Fig. 4). In contrary, a good 5-HT$_6$R affinity (K$_i$ = 96 nM) was observed for the methylene-spacer compound **2** (Modification B, Fig. 4).

Hence, compound **2** has been selected as a lead structure for further modifications to give a new chemical group of 5-HT$_6$R ligands with therapeutic perspectives. Here, we present the computer-aided design, synthesis and pharmacological evaluation (*in vitro* and *in vivo*) as well as structure-activity relationship (SAR) analysis supported by molecular modelling for the new generation of triazine 5-HT$_6$R agents, including lead **2** and its 17 derivatives (**2**–**19,** Table 1).

2. Results and discussion

2.1. Chemical synthesis

Compounds **2**–**19** (Table 1) have been obtained according to the synthesis route shown in Scheme 1, on the basis of methods described previously [9,10].

In the first step, commercial 1-methylpiperazine dihydrochloride **20** and 1-cyanoguanidine **21** were heated under gradually increasing temperature in butanol to give the 4-methylpiperazin-1-yl biguanide dihydrochloride **22** (Scheme 1a). Carboxylic acid esters **3e**-**5e** and **7e**-**15e** were prepared involving two different methods (Scheme 1a).

Methyl esters **3e** and **7e**-**15e** were obtained by acid-catalyzed esterification of corresponding commercial phenylacetic acids (**3a**, **7a**-**15a**). In the case of esters **4e** and **5e,** the reaction of suitable carboxylic acids (**4a** and **5a**) with iodomethane in the presence of DBU (1,8-diazabicyclo[4.5.0]undec-7-ene) was carried out [15]. In the last step, suitable esters, including commercially available ones

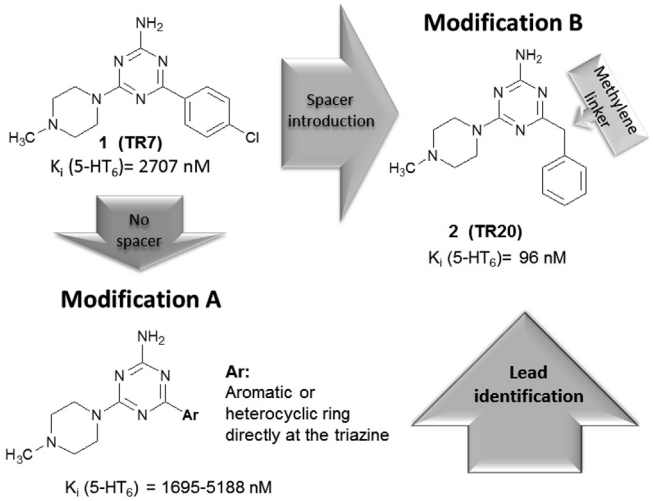

Fig. 4. Identification of a lead structure **2** (**TR20**) for 1,3,5-triazine 5-HT$_6$ ligands.