5-HT2A Receptor Ligands Against Depression

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According to the World Health Organization, depression is a multifactorial disorder that affects around 350 million people worldwide. The most widespread monoamine of the CNS-serotonin (5-HT) is believed to play a vital role in the pathomechanism of this condition, and the importance of the neurotransmitter is elevated by the "serotonin hypothesis", linking the presence of the depression-like symptoms with diminished 5-HT concentration in certain brain regions. Serotonin acts its biological effects via numerous receptors. Out of all seven types of serotonin receptors, the serotonin 2A receptor has been identified as a most promising molecular target valuable for the treatment of mood disorders. Recent medicinal chemistry findings on the structure and function of the serotonin 2A (5-HT2A) receptor facilitated design and discovery of novel anti-depressants.

depression 5-HT2A receptor antidepressant agents

1. Structure of 5-HT$_{2A}$ Receptor

Before the first X-ray structure of a 5-HT$_{2A}$ receptor was developed, information about the structural features of this protein was obtained from in silico simulations. One of the first papers aimed to determine the 3-D structure of subtype 2A of serotonin receptor was published in 1995 [1]. The researchers used data derived from the pharmacophore model of serotonin 2A receptor agonists and antagonists to create a fragmental model depicting the examined structures' binding properties and affinity data. Moreover, a bacteriorhodopsin structure was used to determine a complete model of the three-dimensional (3D) structure belonging to the 5-HT$_{2A}$ receptor.

In 2000, the crystal structure of a Bovine Rhodopsin (PDB ID: 1F88) was determined using X-ray diffraction, and this is considered a starting point in structural studies on GPCRs [2]. In the following years, researchers took advantage of the availability of this first high-resolution GPCR structure and tried to build a homology model based on this template [3]. More recently, these models were replaced by 3D predictions built on the β2-adrenergic receptor template [4][5][6]. Rapid development in this field provided researchers with more three-dimensional experimentally determined protein structures. That gave the opportunity to choose the best template, characterized by the highest level of identity. A recent publication by Jaiteh et al. aimed to examine homology models of 5-HT$_{2A}$ receptors based on distinct X-ray templates. Researchers used 14 structures of β_{1A}R, β_{2A}R, D_3R, D_4R, H_1R, 5-HT$_{1B}$R, 5-HT$_{2B}$R, 5-HT$_{2C}$R, M_1R, M_2R, M_3R, and M_4R, Rhodopsin, CXCR4 chemokine A_2A adenosine, and Cannabinoid 1, characterized by greater and lower identity with a query sequence as molecular modeling templates. Created 3D predictions were submitted into the virtual screening procedure to evaluate whether they can identify known actives among decoys [7]. This procedure contributed to the formulation of new guidelines relevant for GPCR modeling—for templates with >50% identity—, and all should be considered, while those with

>30% identity should be evaluated by retrospective virtual screens [7]. While discussing a 3D protein structure determination, it is essential to mention the computational technique that has recently gained a lot of interest. AlphaFold is a deep-learning-based technique that aims to create a three-dimensional representation of a protein without using previously solved protein structures as templates. First, it was introduced in the Critical Assessment of Protein Structure Prediction competition, where it was applied for the T1008 target structure prediction. It has already been shown that, with the AlphaFold approach, it is possible to build reliable and accurate 3D models [8]. Numerous servers have been created that implement the AlphaFold method for the generation of a three-dimensional representation of a protein. “AlphaFold Protein Structure Database” is an example of a commercially available server that gathers protein structures predicted with the use of this method [8].

When it comes to the experimentally determined receptor structures, the first X-ray representations of the 5-HT_{2A} receptor (PDB ID:6A93; 6A94) [9] were determined by Kimura et al. and deposited in Protein Data Bank in 2018. These structures presented an inactive receptor bound to the atypical antipsychotics: risperidone and zotepine, respectively. More recently, another breakthrough in the field of X-ray protein structure determination was made. Two additional X-ray structures of 5-HT_{2A} receptor complexed with hallucinogenic agonist (PDB ID: 6WGT); inverse agonist (PDB ID: 6WH4) and one cryo-EM derived structure of a receptor bound to 25-CN-NBOH (PDB ID: 6WHA) were obtained by Kim et al. [10]. These structures enabled a better understanding of the structure of this GPCR receptor; moreover, they confirmed several in silico derived hypotheses about the structural features of the protein.

The general structure of the 2A subtype of the serotonin receptor resembles other GPCRs and comprises seven transmembrane helices and intracellular amphipathic helix H8. However, two structural features are believed to be significant for the proper functioning of a receptor. The first one refers to the bottom hydrophobic cleft located in the ligand-binding pocket. The cleft is surrounded by conserved residues, such as $I163^{3.40}$ and $V333^{6.45}$ in the P-I-F motif, and the $W336^{4.68}$ residue, which is considered an “on-off switch” for the receptor. This receptor also contains a side-extended cavity that connects the orthosteric site and the plasma membrane near the bottom hydrophobic cleft and $D155^{3.32}$, stabilized by a hydrogen bond with $Y370^{7.43}$. $D155^{3.32}$ is another example of a highly conserved residue, present among other GPCRs, essential for the ligand binding [8]. Mutations located in the neighborhood of the $D155^{3.32}$ residue lead to the loss of function for almost all 5-HT_{2A} ligands. On the other hand, $S159^{3.36}$ seems to be important for drug binding, as well, since it is involved in the anchoring of the charged terminal amine moiety of 5-HT and other ligands [5]. The previously mentioned side-extended cavity is surrounded by conserved residues located on TM3, TM4, TM5, and the extracellular loop 2. The $G238^{5.42}$ residue located at the entrance is considered to be essential for the formation of the cavity. Moreover, this glycine residue is considered a feature characteristic only for the 5-HT receptors [9].

5-HT_{2A} receptor activation models suggest that it is capable of creating a wide range of ligand-dependent structural responses [10]. This property is also known as “functional selectivity” and is a common feature among many GPCRs. Functional selectivity, understood as protein-mediated activation of $G_{\alpha q/11}$ or pertussis toxin-sensitive $G_{i/o}$ protein, has been considered in terms of this protein’s interactions with hallucinogenic and non-hallucinogenic agonists. Moreover, the functional selectivity of the 5-HT_{2A} receptor also refers to its connections with the β-

arrestin-dependent signaling pathways [11]. Numerous studies have been performed to provide detailed information about this interesting phenomenon, and it turned out that differences in the conformation of binding pocket residues can be identified depending on the type of examined ligand. In silico studies performed by Perez-Aguilar et al. revealed that the second intracellular loop (ICL2) plays a key role in the receptors' interaction with G-protein. Thus, β-arrestins and distinct ligands affect this loop differently. When 5-HT_{2A}R is bound to the hallucinogen (e.g., LSD), ICL2 loop prefers more outward-upward conformations. On the other hand, when this receptor is complexed to the non-hallucinogen drug or present in an unbound form, the unique conformation of a second loop is not that frequently observed. Such conformation may be regulated by the extent of the interaction between $D172^{3.49}$ (from the conserved DRY motif, TM3) and $H183^{3.52}$ (from ICL2) [11]. Recent experimental studies conducted by Kim et al. confirmed that, in the LSD-activated structure of a 5-HT_{2A} receptor, a second extracellular loop forms a lid-like shape that prolongs ligands residence time [10].

Similarly, as in other activated GPCRs, agonist binding leads to a contraction of the extracellular binding pocket and expansion of the intracellular end. That creates additional space for transducers, such as G-proteins or arrestins [10]. Other fragments necessary for the activation have been identified, as well, and are believed to be located in the receptors conserved motifs. Those are an inward shift of residues from the NPxxY motif, rearrangement of $R173^{3.50}$ residue from the E/DRY motif that breaks the ionic lock formed between $R173^{3.50}$ and $E318^{6.30}$. Additionally, modification in the P-I-F motif, involving rotation of the side chain of $W336^{6.48}$ and subsequent movement of the $F332^{6.44}$ side chain, is considered essential for the receptor activation and signal transduction [12]. Kim et al. also cautiously examined the binding poses of another 5-HT_{2A} receptor ligand [10]. Interestingly, a 25-CN NBOH, a selective receptor agonist, showed a unique pose in a receptor's binding pocket. The 2-hydroxyphenyl moiety of a ligand entered a pocket formed between TM3/TM6 and interacted with the indole ring of $W336^{6.48}$. That interfered with a large displacement of the W336's side chain and acted as a pivot for the outward movement of TM6. Moreover, an edge-to-face π–π interaction of a ligand with the previously mentioned residue, hydrogen bond with $S159^{3.36}$, accommodation by the conserved $G369^{7.42}$ are unique for 25CN-NBOH binding. Furthermore, these features can be considered a possible reason for its agonistic selectivity toward the 2A subtype of serotonin receptor [10].

On the other hand, examining the 5-HT_{2A} receptor complexes with commonly used antipsychotics, zotepine and risperidone, enabled a better understanding of the structural changes that occur during the antagonist binding. Both antipsychotics create a salt bridge between $D155^{3.32}$ and a basic nitrogen atom of their molecules. Moreover, their fluorobenzisoxazole ring and benzene ring are located in the bottom hydrophobic cleft, and they interact via CH- π with $S159^{3.36}$. Additional hydrophobic interactions are formed between $I163^{3.40}$, $F243^{5.47}$, $F332^{6.44}$, and those rings. Some other edge-to-edge interactions with $W336^{6.48}$ and $F340^{6.52}$ have been identified as necessary for the ligand binding. Close contacts between ligands and fragments of the P-I-F motif are believed to block the rearrangements of mentioned residues and stabilize the structure in an inactive state.

2. Novel 5-HT_{2A} Ligands as Antidepressant Agents–Agonists Emerge from the Shadows of Antagonists?

Searching the medicinal chemistry literature for the reports of novel ligands of serotonin 5-HT_{2A} receptor with antidepressant-like properties, it will be readily seen that the interest is majorly focused on antagonists of this target. There is a considerable number of scientific papers on newly designed 5-HT_{2A} antagonists with antidepressant potential in the more recent five years. Here are given a few examples of such reports. Kim et al. proposed compounds based on phthalazinone scaffold, acting as 5-HT_{2A} and 5-HT_{2C} antagonists, with an affinity for serotonin transporter [13]. Another research group designed a novel arylpropylamine derivative as an inhibitor of serotonin and noradrenaline reuptake with an antagonist activity toward the 5-HT_{2A} receptor [14]. Evans et al. synthesized thioadatanserin, an analog of adatanserin, and its dialkylated derivatives, antagonists of 5-HT_{2A}, and partial agonists of 5-HT_{1A} receptors [15].

Researchers emphasize the development of 5-HT_{2A} agonists, as an insufficiently investigated, yet promising agents for the development of new therapies of depression. Agonists of the 5-HT_{2A} receptor are suggested to be capable of producing a long-lasting therapeutic effect in depressive disorders by leading to the increase in the neuronal growth in the anterior structures of the brain, such as the prefrontal cortex [16]. Affecting the plasticity of the neural circuits reverses the pathological changes within these structures, thus exerting a more sustained therapeutic effect than just reducing the symptoms of the disorder [17]. However, due to the fact that activating the 5-HT_{2A} receptor is frequently associated with the occurrence of hallucinogenic effects, this field in drug discovery is still underexplored. The reports of novel promising drug candidates against depression in the group of 5-HT_{2A} agonists are very limited. In fact, as a result of searching for articles from the most recent five years on novel agonists of the being discussed receptor in the PubMed database (using Mesh Terms: '5 HT_{2A} agonist' and 'agents, antidepressive' or 'depression'), only one record is returned. The publication by Cameron et al. reveals novel non-hallucinogenic compounds, acting as a potent 5-HT_{2A} agonists, which are analogs of a psychedelic, ibogaine, derived from the plant *Tabernanthe iboga*. In order to elucidate which part of the ibogaine chemical structure is responsible for promoting neural growth, the research group performed function-oriented synthesis. Subsequent deletion of one of the key structural feature of the compound allowed for definition of the pharmacophore model for psychoplastogenic properties of ibogaine. It turned out that the analogs without tetrahydroazepine moiety did not stimulate neuronal growth, but those with removed isoquinuclidine and retained tetrahydroazepine ring remained active. One of the derivatives, ibogainalog (IBG), exhibited comparable psychoplastogenic activity to ibogaine; thus, it was chosen for further optimization [18].

Researchers attempted to design ibogaine analog devoid of hallucinogenic properties. They relied on the fact that the agonist of 5-HT_{2A} receptor, 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), is a potent hallucinogen, but its analog with methoxy substituent shifted from the position five to six does not show such activity. Transferring this structural modification to ibogainalog (IBG) resulted in obtaining the derivative tabernanthalog (TBG), named based on similarity to another alkaloid found in *Tabernanthe iboga*–tabernanthine, with the 5-methoxyindole moiety replaced with 6-methoxyindole fragment. To evaluate the potential hallucinogenic activity of IBG and TBG, the head-twitch response test, a popular test used in the assessment of the activation of 5-HT_{2A} receptor, was performed. The results indicated that IBG displays reduced hallucinogenic properties, while TBG shows a lack of this effect when compared to the 5-MeO-DMT used as a positive control. These findings confirmed the hypothesis that the slight modification of the methoxy substituent position may result in obtaining non-hallucinogenic analogs

of ibogaine. Another advantage of the derivatives deprived of isoquinuclidine fragment over ibogaine is their reduced lipophilicity and, therefore, lower risk of inducing toxic effects, particularly cardiotoxicity. Ibogaine has high tendency to accumulate in the adipose tissue due to its high lipophilicity and, hence, contributes to the toxic effects on the cardiac system through the inhibition of hERG potassium channels [19][20]. Treatment with both IBG and TBG does not lead to arrhythmias and causes significantly fewer malformations and deaths in the zebrafish model comparing to ibogaine [18]. Not without significance is the simplicity of the synthesis of IBG and TBG, which both can be produced in one-step synthesis on a large scale. In contrast, known routes of ibogaine synthesis consist of several steps and result in very low overall yields [21].

Subsequently, Cameron et al. assessed the effect of TBG (as it displayed a better safety profile than IBG) on dendritic growth. They proved that the treatment with TBG increases dendritic arborization of the rat cortical neurons. This effect is suggested to be associated with the activation of 5-HT_{2A} receptor since the pretreatment with ketanserin, a 5-HT_{2A} receptor antagonist, suppresses this response. Additionally, TBG leads to an increase in density of dendritic spines, with the dynamics of spine formation comparable with 2,5-dimethoxy-4-iodoamphetamine (DOI), the hallucinogenic agonist of the 5-HT_{2A} receptor.

As already mentioned, the antidepressant effect of 5-HT_{2A} agonists is believed to emerge from the improvement of neural plasticity mediated by the activation of this receptor. In order to evaluate TBG as a potential antidepressant and to compare its activity with antidepressant, ketamine, a forced swim test was performed. On the first day of the experiment, a pre-test had been performed, and, 24 h after, the tested compounds were administered. Then, the forced swim test was performed 24 h and seven days after injection of the substances. Both TBG and ketamine reduced the time of immobility to a significant extent 24 h after administration, while, after one week, the effect of ketamine seemed more sustained compared with TBG. As expected, the antidepressant effect of TBG was blocked by the treatment with ketanserin, a 5-HT_{2A} antagonist, which confirms the role of 5-HT_{2A} receptor activation in producing the antidepressant-like responses. The effect of TBG on other behaviors related to depressive disorders should be evaluated in further studies [18]. Nevertheless, it may be concluded that these findings will serve as an incentive to give more consideration to the activation 5-HT_{2A} receptor as a new strategy to combat depression.

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