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Application of the bridgehead fragments for the design of conformationally restricted melatonin analogues

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ABSTRACT

Conformationally constrained analogues of the hormone melatonin with a side chain incorporated into the bicyclic bridgehead core were synthesized based on the homology modeling and molecular docking studies performed for the MT_2 melatonin receptor. The methoxy-indole derivative fused with *exo*-N-acetamino-substituted bicyclo[2.2.2]octane was found to possess nanomolar MT_2 receptor affinity.

1. Introduction

Bicyclic bridgehead fragments are used in drug design mainly when these moieties are initially present in the lead compounds (such as atropine, cocaine, and epibatidine). Other attempts to apply these fragments, e.g. as lipophilic groups or as templates, providing a proper space orientation of the substituents, are less common (e.g. [1]). Relative conformational rigidness of the bridgehead bicycles makes them also suitable for the design of conformationally restricted compounds (a classical method for the enhancement of ligand selectivity and/or affinity to a biological target).

For the structures having a rigid core and a flexible side chain, conformational restriction of the latter is usually achieved by introduction of bulky substituents, double bonds, small cycles or by partial or whole incorporation of a flexible fragment into the cycle. In the present work we have chosen the structure of the endogenous hormone melatonin for modification using bicyclic bridgehead moieties as the conformationally constricting elements and suggested to synthesize melatonin analogues with a side chain incorporated into the bicyclic bridgehead cores (Fig. 1).

Melatonin (*N*-acetyl-5-methoxytryptamine) is an endocrine hormone produced by the pineal gland, which plays an important role in regulation of circadian rhythms, in modulation of sleep-wake cycle and in functioning of multiple other biochemical pathways in humans [2]. Physiological effects of melatonin generally result from the activation of G protein-coupled MT_1 and MT_2 receptors (there exists also a melatonin-sensitive form of the enzyme quinone reductase, termed MT_3) [3]. The ligands of melatonin receptors are proposed as agents for the treatment of depression, insomnia, circadian rhythm dysfunction, etc.

In this study we intended to check if the abovementioned conformational restriction, simultaneously providing an introduction of the lipophilic substituent to the C^2 position of melatonin (one of the typical modifications for MT_2 selectivity [4]) could lead to the ligands with affinity and selectivity to MT_2 receptors.

2. Results and discussion

2.1. Molecular docking studies

The choice of the exact structures was initially based on their synthetic availability, and the structures with bicycles containing a six-membered ring fused with an indole fragment were chosen. Based on these structural targets, a molecular modeling study was carried out.

For a long time, the only crystal structure of a G protein-coupled receptor available was bovine rhodopsin. Recently, a number of other GPCRs were crystallized including β_1- and β_2-adrenergic receptors, and the A_{2A} adenosine receptor [5]. In addition, a crystal structure of opsin, a ligand-free form of rhodopsin, in its activated, G protein-coupled form was solved [6]. Since the opsin structure seems to be more related to an active state of other GPCRs, in the present study we used it as a template to build a homology model of the MT_2 melatonin receptor transmembrane domain. The molecular modeling was performed with the Modeller software [7] (the following parameters were used: number of models

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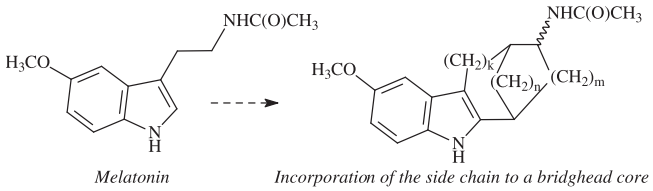

Fig. 1. The general idea of the study.

to generate – 50; library_schedule – autosched.slow; max_var_interactions – 10,000; md_level – refine.very slow). The best model was selected based on the *moldpf* and *DOPE* scoring functions calculated by the Modeller. The general quality of the model was also confirmed by a Ramachandran plot calculated with the Schrödinger suite [8] (Fig. 2a).

The MT_2 receptor model obtained was subjected to refinement with the standard procedure of the Protein Preparation Wizard implemented in the Schrödinger suite. In particular, all hydrogen atoms were added, and partial charges were assigned. Then, a fully automated molecular docking of melatonin as well as methoxyindoles fused with N-acetamino-substituted bicyclo[3.3.1]nonane, bicyclo[3.2.1]octane, bicyclo[2.2.2]octane was carried out with the Glide program of the Schrödinger suite. The receptor grid generation was performed for a box with a center in the centroid of the receptor. The box size was determined automatically as 32 Å and, thus, covered almost the entire receptor (Fig. 2b).

The binding mode of the studied compounds docked to the MT_2 receptor was in excellent agreement with the available data of site-directed mutagenesis, and with the recently published rhodopsin-based models of melatonin receptors [9,10]. As shown in Fig. 3b, the oxygen atom of the ligand methoxy group can form a hydrogen bond with His208 (5.47), and the oxygen atom of the ligand amide moiety is hydrogen bonded to Asn175 (4.60) (the numbers in parentheses correspond to the Ballesteros–Weinstein indexing system). An additional H-bond between the NH-group of amide moiety and the backbone oxygen atom of Ala117 (3.29) was observed for melatonin and compound **3**, but not for **1** and **2**. As analogues to melatonin, the indole core of compounds **1**–**3** was surrounded by Trp264 (6.48), Val124 (3.36) and Leu267 (6.51). The docking results clearly indicated that only the *endo*-isomers were able to interact with MT_2 binding site in the case of bicyclo[3.3.1]nonane and bicyclo[3.2.1]octane derivatives **1** and **2**, while their *exo*-analogues could not provide an energetically favorable binding mode. An opposite situation was observed for the bicyclo [2.2.2]octane derivative **3**, the *exo*-configuration of the bridgehead being preferable in this case (Fig. 3a and b).

Overlay of compounds **1**–**3** and melatonin (Fig. 3) shows that their binding modes are rather similar, and there were no significant differences in their energy scoring functions. But the values of Root Mean Square Deviation (RMSD) calculated for the heteroatoms of melatonin and **1**–**3** inside the receptor binding site were found to be of 0.67 Å for **3**, 1.08 Å for **1** and 0.84 Å for **2**, indicating that the binding mode of **3** is closer to the one of melatonin than the binding modes of **1** and **2**. Thus, the results of molecular modeling allowed us to expect better binding of **3** at the MT_2 receptor than **1** and **2**.

2.2. Chemistry

Synthesis of the compounds **1**–**3** was performed by the indole fragment formation on the bicyclic core using a Fisher reaction. Compound **1** was synthesized from the corresponding oxime **4** obtained in five steps from the Meerwein ester as described in [11].

The oxime **4** was reduced by lithium aluminum hydride to give a mixture of diastereomeric amines **5a,b** (5:1) from which each diastereomer was separated by column chromatography. *N*-acetylation of the *endo*-isomer **5a** led to the desired *endo*-amide **1** (Scheme 1).

To obtain the structure **2** initially methyl *endo*-2-oxo-bicyclo [3.2.1]octan-6-carboxylate was synthesized in four steps as shown at Scheme 2.[1] Formylation of ketal **6** followed by a diazo transfer reaction with tosyl azide gave the corresponding α-diazoketone **8**. Photochemical Wolff rearrangement of the latter [13] led to the isomeric esters **9** and concomitant protection cleavage – to isomeric methyl 2-oxo-bicyclo[3.2.1]octan-6-carboxylate (12:1, *endo*-:*exo*-).

The *endo*-isomer **10** was isolated by column chromatography at 95% purity and its reaction with 4-methoxyphenylhydrazine hydrochloride in glacial acetic acid led to the *endo*-isomer of the indole **11**. While the hydrolysis of the ester group in **11** under alkaline conditions caused epimerization, it was carried out smoothly in the presence of hydrochloric acid to afford the *endo*-acid **12**. The latter was then converted to *endo*-amine **14** in two steps and *N*-acetylation of the amine **14** with acetic anhydride gave a conformationally restricted melatonin analogue **2**.

For the synthesis of the indole derivative fused with bicyclo[2.2.2]octane **3**, a ketal of methyl *endo*-5-oxobicyclo[2.2.2]octane-2-carboxylate **16a** (obtained by reaction of cyclohexenone and methyl acrylate with further protection [14,15]) was epimerized in the reaction with lithium diisopropylamide (Scheme 3).[2]

The mixture of deprotected esters **15** (*endo*-:*exo*- ratio 1:1) was subjected to Fischer reaction conditions and transformed to the isomeric indoles **17a** and **17b**, separated by column chromatography (the isomers configuration was determined by homonuclear ^{1}H double resonance spectroscopy and proved by comparison with the spectrum of indole derivative **17a** directly obtained from **15a**). After the conversion of *exo*-ester **17b** to the corresponding acid **18** and isocyanate **19**, the latter was hydrolyzed by 2 N NaOH at 0–5 °C in THF to the corresponding amine **21**. (Hydrolysis of isocyanate **19** under acidic conditions led to the degradation of the indole system, while an analogous reaction with 3 N NaOH at 40–50 °C in toluene gave disubstituted urea **22** as the only product). Acetylation of the amine **21** led to the desired compound **3**.

2.3. MT_1 and MT_2 affinity results

The synthesized conformationally constrained melatonin analogues **1**–**3** were evaluated for their *in vitro* affinity to the recombinant human MT_1 and MT_2 subtypes expressed in CHO-K1 cells in a radioligand-binding assay using [^{125}I]2-iodomelatonin as a labeled ligand [16,17] according to standard protocols of the MDS Pharma Services (Taipei, Taiwan). K_i values were calculated from IC_{50} values, obtained from competition curves by the method of Cheng and Prusoff. Melatonin and 4-phenyl-2-propionamidotetralin (4P-PDOT, MT_2 antagonist) were tested as the reference compounds in the MT_1 and MT_2 assays correspondingly (melatonin MT_1: IC_{50} 0.34 nM, $K_i \sim 0.18$ nM; 4P-PDOT MT_2: $IC_{50} \sim 0.42$ nM, $K_i \sim 0.22$ nM). It should be mentioned, that CHO-hMT_2 binding affinity of 4P PDOT is very close to that of melatonin [18].

[1] Interestingly, the isomeric methyl *exo*-2-oxo-bicyclo[3.2.1]octan-6-carboxylate can be regioselectively prepared by ring constriction in bicyclo[3.3.1]nonan-2,6-dione by tallium nitrate, that makes the stereoselective synthesis of *exo*-isomer of **2** (described in our previous work [12]) easier than that of **2**.

[2] An attempt to subject a ketal of bicyclo[2.2.2]octane-2,5-dione to a Fisher reaction conditions (in the presence of CH_3COOH, HCl or $ZnCl_2$) led to the protective group cleavage and a formation of a hard to separate mixture, from which the corresponding methoxy-indol derivative fused with bicyclo[2.2.2]octanone was isolated in a very low yield (see Supplementary material).