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Preliminary Communications

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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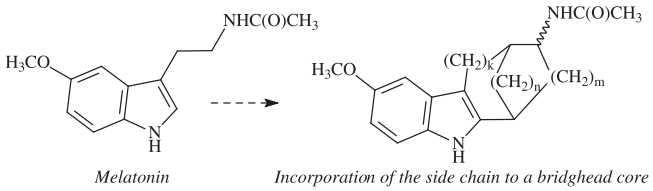


Fig. 1. The general idea of the study.

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]

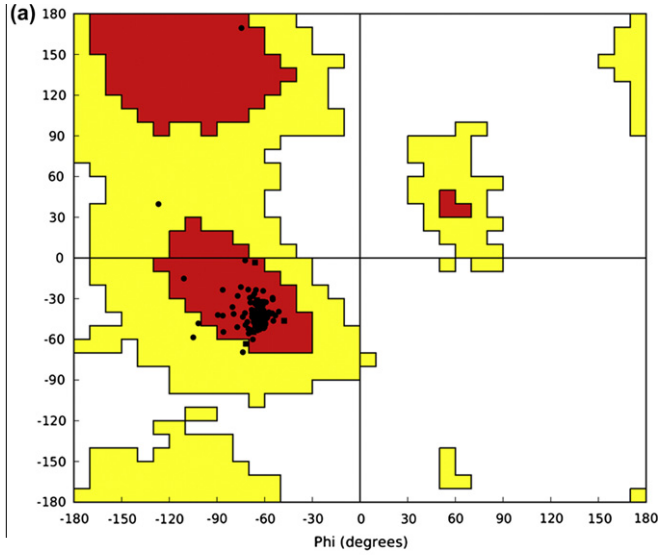
The mixture of deprotected esters **15** (*endo*-:*exo*- ratio 1:1) was subjected to Fischer reaction conditions and transformed to the isomeric indoles **17a** and **17b**, separated by column chromatography (the isomers configuration was determined by homonuclear ^{1}H double resonance spectroscopy 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).



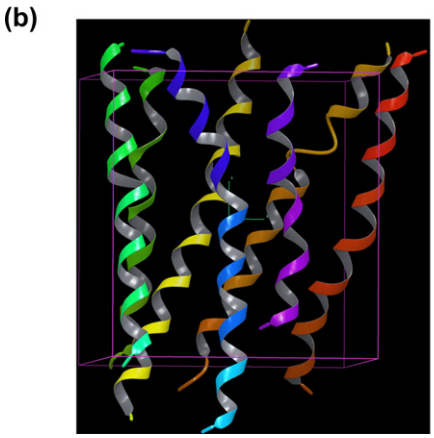
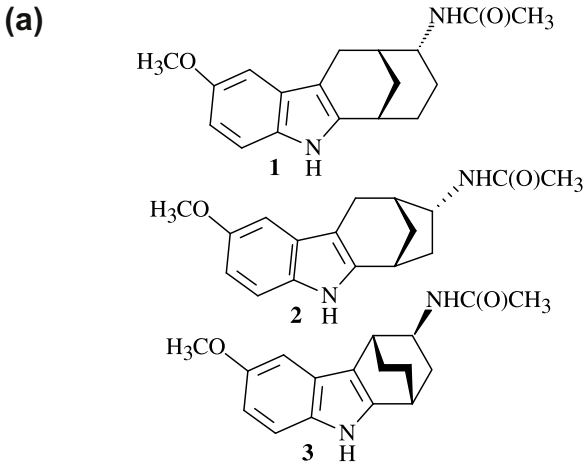


Fig. 2. (a) The Ramachandran plot obtained with the Schrödinger suite for the molecular model of the MT_2 receptor. The most favorable region is colored in red, the allowed regions – in yellow, and the disallowed regions are in white. No amino acid residues were found in the disallowed region. (b) The molecular model of the transmembrane domain of the MT_2 receptor colored by residue positions: TM1 in orange, TM2 in ochre, TM3 in yellow, TM4 in green, TM5 in lime, TM6 in blue, TM7 in purple. For the molecular docking the receptor grid generation was performed for a box covering almost the entire receptor (shown in purple). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



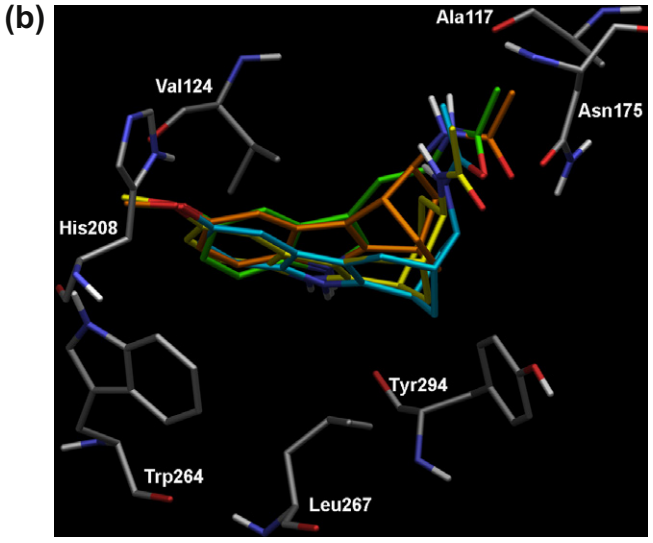


Fig. 3. (a) The newly synthesized bicyclo[3.3.1]nonane, bicyclo[3.2.1]octane, and bicyclo[2.2.2]octane derivatives of melatonin. (b) The superimposition of melatonin and compounds **1**–**3** docked to the MT_2 melatonin receptor. The carbon atoms of melatonin are colored in green, **1** – yellow, **2** – cyan, **3** – orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

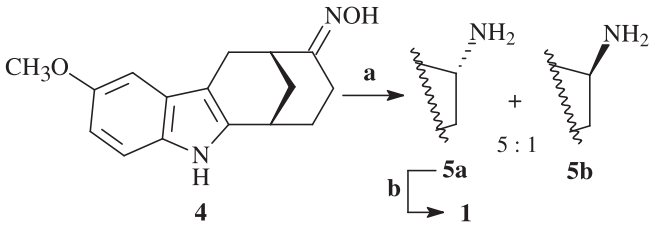
The results indicate that methoxy-indole annelated with the acetamino-substituted bicyclo[2.2.2]octane (**3**) has the highest MT_2 binding affinity (MT_2: $IC_{50} \sim 2.75$ nM, $K_i \sim 1.43$ nM), which is about one order of magnitude lower than that of melatonin, but still in nanomolar range. Taking into account that **3** was tested as racemic mixture, its MT_2 binding affinity is rather high and proves our predictions made on the basis of molecular modeling. Compound **3** demonstrated also a noticeable selectivity to MT_2 subtype (MT_1: $IC_{50} \sim 54.6$ nM, $K_i \sim 28.3$ nM), though it was less then expected: $K_i(MT_1)/K_i(MT_2) \sim 20$.

The affinity of an analogous compound with bicyclo[3.3.1]nonane fragment (**1**) drops to a micromolar range, but it still retains MT_2 selectivity (MT_2: $IC_{50} \sim 1.1$ μM, $K_i \sim 0.57$ μM; MT_1: $IC_{50} > 10$ μM). No significant affinity was observed for bicyclo[3.2.1]octane derivative **2** at both receptors subtypes (MT_1, MT_2: $IC_{50} > 10$ μM). These results confirm the positive effect of the bulk of the C^2-substituent in the indole core on MT_2 selectivity, but indicate that there is no linear dependence between the MT_2 binding affinity and the bridgehead core size among the tested compounds.

The considerable decrease in affinity of bicyclo[3.3.1]nonane and bicyclo[3.2.1]octane derivatives **1** and **2** in comparison with **3** could be partially explained by close location (3.8 Å) of hydrophobic bridge carbon atoms of **1** and **2** from the hydroxyl group of Tyr294 (7.40) in the MT_2 binding site. This proximity might be enough to produce undesired changes in the protein structure or decrease the binding properties of compounds. In contrast the closest carbon atom of bicyclo[2.2.2]octane fragment of **3** is positioned at a distance of 4.6 Å from the Tyr294 (7.40) OH-group.

The drop of affinity of **1** and **2** conforms to the observations, that conformational constriction leading to the movement of melatonin side chain towards the C^2 atom of the indole ring often leads to the decrease of potency [19]. Nevertheless bicyclo[2.2.2]octane analogue **3** with the same type of constriction retains good MT_2 binding affinity. Obviously, additional study is needed to find out if the bridgehead moiety of **3** plays its part in this case.

In summary, bridgehead bicycles were applied for the design of conformationally restricted melatonin analogues. The



Scheme 1. *Reagents and conditions*: (a) $LiAlH_4$, THF, 69% (total yield); (b) Ac_2O, NEt_3, CH_2Cl_2, 72%.

experimentally determined binding affinity of methoxy-indole derivative fused with *exo*-N-acetamino-substituted bicyclo [2.2.2]octane (**3**) confirmed the molecular docking predictions. To our knowledge, this is the first bridgehead melatonin analogue with nanomolar MT_2 affinity. Further molecular modeling and experimental studies are ongoing to evaluate the functional activity of the compounds, to better understand the dependence between MT_2 binding affinity and the bridgehead core size and to extend the application of the bridgehead moieties as conformationally constricting elements to other classes of substrates.

3. Experimental

3.1. General

Flash and column chromatography were performed on silica gel Acros (40–60 μm). Reaction control was carried out by thin-layer chromatography on "Silufol" plates. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz correspondingly and were referenced to residual chloroform (δ 7.26 ppm ^{1}H; δ 77.0 ppm ^{13}C). Elemental analysis was performed on CNH analyzer "Carlo-Erba" ER-20. Electron impact mass spectra were obtained with typical voltage of 70 eV. Infrared spectra (IR) were registered on UR-20 apparatus (thin layer in liquid paraffin) and reported in cm^{-1}. Melting points were measured in block with sealed capillaries.

3.2. Synthesis and characteristics of the key compounds (for the synthesis and characteristics of all other compounds see Supplementary material)

3.2.1. 2-Methoxy-6,7,8,9,10,11-hexahydro-5H-6,10-methanocycloocta[b]indol-9-amine (**5**)

A solution of 0.2 g (0.74 mmol) 2-methoxy-5,6,7,8,10,11-hexahydro-9H-6,10-methanocycloocta[*b*]indol-9-one oxime **4** in 5 mL of dry THF was added dropwise to a stirred suspension of $LiAlH_4$ (0.09 g, 2.22 mmol) in 5 mL of dry THF. The reaction mixture was refluxed for 6 h, then cooled and quenched with water (0.5 mL) and aqueous 2 N NaOH (0.5 mL). The suspension was refluxed for 30 min. The precipitate was filtered, washed with hot THF and was taken up into THF (5 mL) and refluxed for 30 min. This procedure was repeated three times. The combined filtrates were dried over Na_2SO_4 and solvent was removed in vacuum to give a mixture of *endo*-isomer and *exo*-isomer, which was chromatographed on silica gel with increasing amounts of methanol (CH_2Cl_2/methanol/NEt_3 100:1:0.5, then 100:5:0.5). The *endo*-2-methoxy-6,7,8,9,10,11-hexahydro-5H-6,10-methanocycloocta[*b*]indol-9-amine (**5a**) eluted first (0.109 g, white solid, Mp 181–183 °C), followed by the *exo*-2-methoxy-6,7,8,9,10,11-hexahydro-5H-6,10-methanocycloocta[*b*]indol-9-amine (**5b**) (0.022 g, pale yellow oil). Total yield: 69%.

^{1}H NMR of **5a** ($CDCl_3$): δ 1.05 (q, 1H, *J* = 12.4, 5.3), 1.41 (brs, 2H, NH_2), 1.51 (dd, 1H, *J* = 12.9, 3.0), 1.68–1.81 (m, 2H), 1.85 (dt, 1H, *J* = 12.4, 2.8), 2.08 (dd, 1H, *J* = 12.6, 2.3), 2.25 (br d, 1H, *J* = 3.0, $HC^{6(10)}$), 2.75 (dd, 1H, *exo*-HC^{11}, *J* = 16.7, 6.8), 2.89 (d, 1H, *endo*-HC^{11}, *J* = 16.7), 2.94–3.01 (m, 2H), 3.88 (s, 3H, OCH_3), 6.79 (dd, 1H, HC^3, *J* = 8.8, 2.3), 6.97 (d, 1H, HC^1, *J* = 2.3), 7.18 (d, 1H, HC^4, *J* = 8.8), 7.71 (brs, 1H, NH); ^{13}C NMR of **5a** ($CDCl_3$): δ 19.37, 28.62, 28.72, 31.03, 32.90, 34.96, 53.90 (C—NH_2), 55.98 (OCH_3), 100.28, 110.09, 110.49, 111.20, 127.66, 130.65, 137.84, 153.84; IR (mineral oil) 3400 (NH), 3000–3350 (NH_2); Anal. Calcd for $C_{16}H_{20}N_2O$: C, 75.02; H 7.99; N, 10.97. Found: C, 75.00; H, 7.81; N, 10.94.

^{1}H NMR of **5b** ($CDCl_3$): δ 1.29 (m, 1H), 1.41–1.55 (m, 2H), 1.75 (brd, 1H, *J* = 12.63), 1.83–2.06 (m, 1H, NH_2), 2.16 (m, 1H), 2.28 (dt, 1H, *J* = 12.6, 3.0), 2.57 (d, 1H, *endo*-HC^{11}, *J* = 16.4), 3.00 (brs, 1H, $HC^{6(10)}$), 3.06 (dd, 1H, *exo*-HC^{11}, *J* = 16.4, 7.3), 3.15 (brs, 1H, $HC^{6(10)}$), 3.87 (s, 3H, OCH_3), 6.78 (dd, 1H, HC^3, *J* = 8.8, 2.3), 6.94 (d, 1H, HC^1, *J* = 2.3), 7.19 (d, 1H, HC^4, *J* = 8.8), 7.80 (brs, 1H, NH); ^{13}C NMR of **5b** ($CDCl_3$): δ 24.52, 25.89, 26.57, 26.66, 29.32, 34.55, 52.81 (C—NH_2), 55.98 (OCH_3), 100.18, 109.17, 110.45, 111.22, 127.86, 130.76, 137.29, 153.88. IR (mineral oil) 3400 (NH), 3000–3350 (NH_2); Anal. Calcd for $C_{16}H_{20}N_2O$: C, 74.73; H 7.79; N, 10.90. Found: C, 75.00; H, 7.81; N, 10.94.

3.2.2. N-(endo-2-methoxy-6,7,8,9,10,11-hexahydro-5H-6,10-metanocycloocta[b]indol-9-yl)acetamide (**1**)

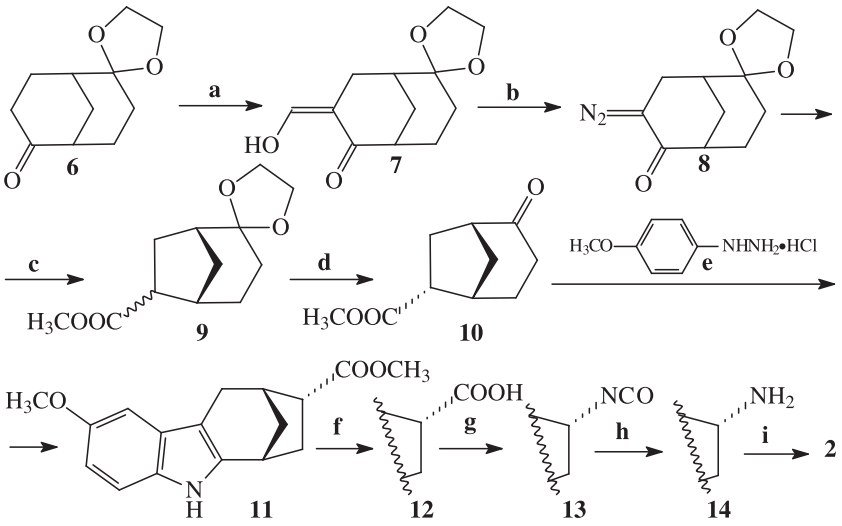
To a solution of 0.13 g **5b** (0.51 mmol) in 5 mL of dry CH_2Cl_2 was added 0.18 mL of triethylamine (1.27 mmol) and 0.072 mL of acetic anhydride (0.76 mmol). The reaction mixture was stirred at room temperature for 1 h, washed with saturated aqueous $NaHCO_3$ (3 mL) and brine (3 mL), dried over Na_2SO_4. After filtration solvent was removed under reduced pressure and the crude product was chromatographed (methanol/CH_2Cl_2, 1:50) to afford 109.0 mg **1** (72% yield) as white solid, mp 149–151°C.

^{1}H NMR ($CDCl_3$): δ 1.14 (q, 1H, *J* = 12.8, 4.9), 1.55 (m, 1H), 1.77–1.90 (m, 2H), 1.96 (dt, 1H, *J* = 12.9, 2.7), 2.03 (s, 3H, CH_3), 2.07 (ddd, 1H, *J* = 12.9, 5.3, 3.0), 2.60 (br s, 1H, $HC^{6(10)}$), 2.65 (d, 1H, *endo*-HC^{11}, *J* = 16.8), 2.84 (dd, 1H, *exo*-HC^{11}, *J* = 16.8, 6.6), 3.01 (t, 1H, $HC^{6(10)}$, *J* = 2.8), 3.89 (s, 3H, OCH_3), 4.10 (m, 1H, HC^9), 5.49 (br d, 1H, NHAc, *J* = 7.6), 6.81 (dd, 1H, HC^3, *J* = 8.6, 2.5), 6.96 (d, 1H, HC^1, *J* = 8.6), 7.21 (d, 1H, HC^4, *J* = 8.6), 7.60 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 20.88, 23.59, 25.16, 28.42, 30.73, 31.13, 32.08, 51.80, 55.93 (C^9), 100.10, 109.59, 110.67, 111.35, 127.47, 130.66, 137.52, 153.94, 169.41 (C═O). IR (mineral oil) 3390 (NH indol), 3300 (NH amide), 1660 (C═O). EI MS (*m/z* (rel. intens.)): 298 $[M]^+$ (100), 281 (3), 254 (24), 238 (46), 224 (16), 210 (47), 198 (73), 183 (18), 167 (34); Anal. Calcd for $C_{18}H_{22}N_2O_2$: C, 72.10; H 7.46; N, 9.25. Found: C, 72.48; H, 7.43; N, 9.40.

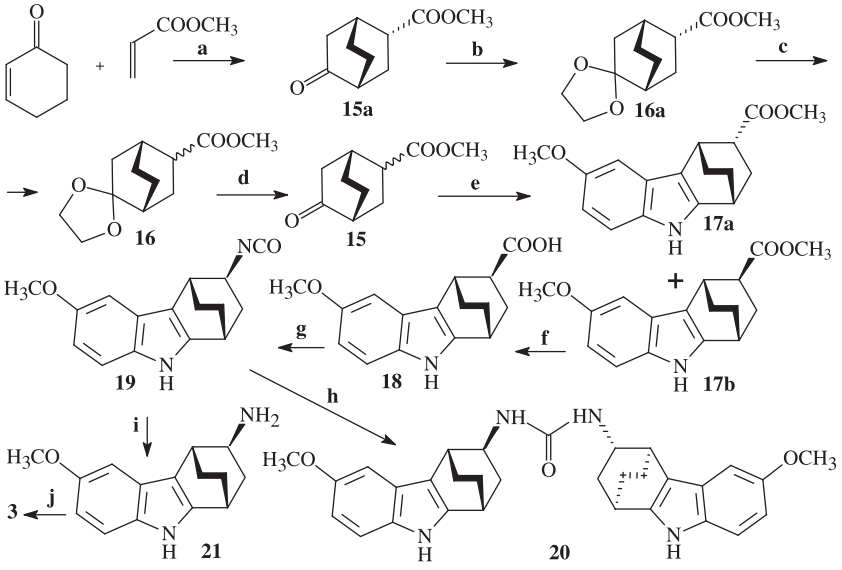
3.2.3. Methyl endo-2-methoxy-5,6,7,8,9,10-hexahydro-6,9-methanocyclohepta[b]indol-8-carboxylate (**11**)

A solution of 0.5 g **10** (2.75 mmol) in 3 ml of glacial acetic acid was added to a suspension of 0.58 g (4-methoxyphenyl)hydrazine hydrochloride (3.29 mmol) in 7 ml of glacial acetic acid at 90–100 °C. The reaction mixture was stirred for 40 min, and then was poured into 25 mL of ice-water and extracted with diethyl ether (3 × 10 mL). The combined organic layers were very carefully washed with aqueous solution of $NaHCO_3$ (5 × 5 mL) and brine. The ether extract was dried over Na_2SO_4, solvent was removed under reduced pressure to afford dark oil. The residue was chromatographed (CH_2Cl_2/benzene 1:1) affording 0.52 g **11** (67% yield) as buff solid, mp 149–151 °C.

^{1}H NMR ($CDCl_3$): δ 2.00 (dt, 1H, *anti*-HC^{11}, *J* = 11.0, 4.5), 2.08 (d, 1H, *syn*-HC^{11}, *J* = 11.0), 2.15 (ddd, 1H, *exo*-HC^7, *J* = 12.9, 11.4, 5.9), 2.42 (ddd, 1H, *endo*-HC^7, *J* = 12.9, 4.3, 2.2), 2.56 (d, 1H, *endo*-HC^{10}, *J* = 16.0), 2.92 (dd, 1H, *exo*-HC^{10}, *J* = 16.0, 4.7), 3.05–3.12 (m, 2H, $HC^{6,9}$), 3.26 (ddd, 1H, H—C—$COOCH_3$, *J* = 11.4, 7.2, 4.3), 3.62 (s, 3H, $COOCH_3$), 3.86 (s, 3H, OCH_3), 6.76 (dd, 1H, HC^3, *J* = 8.6, 2.5), 6.85 (d, 1H, HC^1, *J* = 2.5), 7.16 (d, 1H, HC^4, *J* = 8.6), 7.62 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 25.72, 34.93, 36.51, 38.13 (2C), 47.01.



Scheme 2. *Reagents and conditions*: (a) NaH, HCOOEt, Et_2O, 75%; (b) TsN_3, NEt_3, CH_2Cl_2, 84%; (c) *hv*, CH_3OH, 74%; (d) PTSA, acetone, column chromatography, 80%; (e) CH_3COOH, 67%; (f) HCl, H_2O, 70%; (g) ClCOOEt, NEt_3, CH_2Cl_2, then NaN_3, H_2O, toluene, reflux, 89%; (h) HCl, H_2O, 50%; (i) Ac_2O, NEt_3, CH_2Cl_2, 65%.



Scheme 3. *Reagents and conditions*: (a) LDA, THF, −23 °C, 60%; (b) $HOCH_2CH_2OH$, PTSA, 90%; (c) LDA, THF, −78 °C → −20 °C, 76%; (d) PTSA, acetone, 80%; (e) $CH_3OPhNHNH_2$.HCl, CH_3COOH; column chromatography, 56% (total yield); (f) NaOH, CH_3OH/H_2O, 79%; (g) ClCOOEt, NEt_3, CH_2Cl_2, then NaN_3, H_2O, toluene, reflux, 90%; (h) NaOH, 40–50 °C, $PhCH_3/H_2O$, 71%; (i) NaOH, 0–5 °C, THF/H_2O, 55%; (j) Ac_2O, NEt_3, CH_2Cl_2, 61%.

51.46, 55.93, 100.36, 104.76, 110.12, 111.30, 128.29, 130.56, 142.15, 153.94, 179.18; IR (mineral oil) 3400 (NH),1730 (C=O) cm^{-1}; Anal. Calcd for $C_{17}H_{19}O_3N$: C, 71.66; H 6.70; N, 4.87. Found: C, 71.58; H, 6.67; N, 4.91.

*3.2.4. N-(2-methoxy-5,6,7,8,9,10-hexahydro-6,9-metanocyclohepta[b]indol-8-yl)acetamide (**2**)*

To a solution of 0.15 g **14** (0.62 mmol) in 5 mL of dry CH_2Cl_2 was added 0.22 mL of triethylamine (1.55 mmol) and 0.088 mL of acetic anhydride (0.93 mmol). The reaction mixture was stirred at room temperature for 1 h. The mixture was washed with saturated aqueous $NaHCO_3$ (3 mL), brine (3 mL), dried over Na_2SO_4. After filtration solvent was removed under reduced pressure, and the crude product was chromatographed (methanol/CH_2Cl_2, 1:70) to afford 114.0 mg **2** (65% yield) as white solid, mp 109–112°C.

^{1}H NMR ($CDCl_3$) δ 1.48 (dt, 1H, *endo*-HC^7, *J* = 13.1, 3.1), 1.96 (dt, 1H, HC^{11}, *J* = 11.4, 4.5), 1.99 (s, 3H, CH_3), 2.08 (dd, 1H, HC^{11}, *J* = 11.4, 2.5), 2.50 (ddd, 1H, *exo*-HC^7, *J* = 13.1, 11.2, 5.7), 2.61 (dd, 1H, *endo*-HC^{10}, *J* = 16.0, 1.8), 2.83 (dd, 1H, *exo*-HC^{10}, *J* = 16.4, 4.5), 2.93 (m, 1H, HC^9), 2.97 (t, 1H, HC^6, $J_{6,7\text{-exo}} \approx J_{6,11} \approx 4.7$), 3.85 (s, 3H, OCH_3), 4.72 (dddd, 1H, HC^8, *J* = 11.2, 8.8, 7.4, 3.1), 5.34 (d, 1H, NHCO, *J* = 8.8), 6.79 (dd, 1H, HC^3, *J* = 8.8, 2.6), 6.90 (d, 1H, HC^1, *J* = 2.6), 7.19 (d, 1H, HC^4, *J* = 8.8), 7.79 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 23.25, 23.74, 34.10, 36.61, 37.11, 43.10, 50.65, 56.00, 100.39, 104.73, 110.65, 111.49, 128.02, 130.52, 143.06, 154.18, 169.79; IR (mineral oil) 3400 (NH indol), 3280 (NH), 1650 (C=O); Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.62; H 7.10; N, 9.70. Found: C, 71.83; H, 7.04; N, 9.86.

*3.2.5. Methyl 6-methoxy-2,3,4,9-tetrahydro-1H-1,4-ethanocarbazole-3-carboxylate (**17**)*

To the solution of 1.00 g **15** (5.49 mmol) in 15 mL of glacial acetic acid was added 1.06 g (4-methoxyphenyl)hydrazine hydrochloride (6.07 mmol). The reaction mixture was refluxed for 5 h, cooled

to the room temperature, poured in 50 mL of water and extracted with diethyl ether (3 × 15 mL). The ether extract was carefully washed with saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4. Solvent was removed using rotary evaporator to afford a mixture of *exo*- and *endo*-isomers in 1:1 ratio as determined by NMR. This product mixture was chromatographed on silica gel (benzene/acetone, 100:1). Methyl *exo*-6-methoxy-2,3,4,9-tetrahydro-1H-1,4-ethanocarbazole-3-carboxylate (**17b**) eluted first (0.436 g, yellow oil solidified at cold), followed by the methyl *endo*-6-methoxy-2,3,4,9-tetrahydro-1H-1,4-ethanocarbazole-3-carboxylate (**17a**) (0.397 g, yellow oil). Total yield was 56%.

^{1}H NMR of **17a** ($CDCl_3$): δ 1.39–1.51 (m, 2H), 1.78–1.90 (m, 2H), 1.98–2.03 (m, 2H), 2.93 (ddd, 1H, H—C—$COOCH_3$, *J* = 8.8, 5.3, 2.8), 3.29 (m, 1H, HC^1), 3.49 (s, 3H, $COOCH_3$), 3.87 (s, 3H, OCH_3), 3.91 (m, 1H, HC^4), 6.75 (d, 1H, *J* = 8.7), 7.01 (br s, 1H, HC^5), 7.21 (d, 1H, *J* = 8.7), 7.96 br s, 1H, NH); ^{13}C NMR of **17a** ($CDCl_3$): δ 26.60, 26.82, 29.53, 30.46, 31.45, 44.32, 51.71, 56.01, 99.73, 109.58, 111.70, 112.88, 125.97, 130.38, 141.89, 154.10, 175.13; IR of **17a** (mineral oil) 3390 (NH), 1710 (C=O); MS EI **17a** (*m/z* (rel. intens.)): 285 $[M]^{+\cdot}$ (41), 254 (4), 199 (100), 198 (44), 183 (8), 167 (10), 154 (9), 127 (2).

^{1}H NMR of **17b** ($CDCl_3$): δ 1.29–1.47 (m, 2H, $HC^{10,11}$), 1.68 (ddt, 1H, *endo*-HC^2, *J* = 12.9, 11.2, 3.2), 1.86–1.97 (m, 2H, $HC^{10,11}$), 2.35 (ddd, 1H, *exo*-HC^2, *J* = 12.9, 4.8, 2.5), 2.60 (dddd, 1H, H—C—$COOCH_3$, *J* = 11.2, 5.0, 4.8, 2.3), 3.24 (m, 1H, HC^1), 3.79 (m, 1H, HC^4), 3.81 (s, 3H, $COOCH_3$), 3.90 (s, 3H, OCH_3), 6.80 (dd, 1H, HC^7, *J* = 8.6, 2.5), 7.08 (d, 1H, HC^5, *J* = 2.5), 7.28 (d, 1H, HC^8, *J* = 8.6), 8.00 (brs, 1H, NH); ^{13}C NMR of **17b** ($CDCl_3$): δ 23.60, 26.76, 29.53, 30.25, 31.18, 44.62, 51.93, 55.95, 99.68, 110.01, 111.83, 115.54, 124.97, 130.13, 142.62, 154.21, 175.87; IR of **17b** (mineral oil) 3390 (NH), 1715 (C=O) cm^{-1}; MS EI **17b** (*m/z* (rel. intens.)): 285 $[M]^{+\cdot}$ (37), 254 (3), 199 (100), 198 (43), 183 (8), 167 (9), 154 (8), 127 (3); Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.57; H 6.69; N, 4.96. Found: C, 71.58; H, 6.67; N, 4.91.

*3.2.6. N-(exo-6-methoxy-2,3,4,9-tetrahydro-1H-1,4-ethanocarbazol-3-yl)acetamide (**3**)*

To a solution of 0.070 g **21** (0.289 mmol) in 3 mL of dry CH_2Cl_2 was added 0.100 mL of triethylamine (0.723 mmol) and 0.041 mL of acetic anhydride (0.434 mmol). The reaction mixture was stirred at room temperature for 1 h. The mixture was washed with saturated aqueous $NaHCO_3$ (2 mL), brine (2 mL), dried over Na_2SO_4. Solvent was removed using rotary evaporator. The crude product was chromatographed on silica gel (methanol/CH_2Cl_2, 1:75) to afford 50.0 mg of **3** (yield 61%) as white solid, mp 150–154 °C.

^{1}H NMR ($CDCl_3$): δ 1.24–1.42 (m, 4H), 1.80 (m, 1H), .1.92–2.05 (m, 2H), 2.08 (s, 3H, —CO—CH_3), 3.08 (brs, 1H), 3.52 (brs, 1H), 3.85 (s, 3H, OCH_3), 3.94 (brs, 1H, —CH—NH—), 6.16 (brs, 1H, NHCO), 6.76 (dd, 1H, *J* = 8.8, 2.3, HC^7), 7.03 (d, 1H, *J* = 2.3, HC^5), 7.21 (d, 1H, *J* = 8.8, HC^8), 8.40 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 20.87, 23.64, 27.44, 29.61, 32.37, 35.67, 49.35, 55,93, 99.74, 110.00, 111.90, 114.57, 125.39, 130.41, 142.08, 153.99, 170.25; IR (mineral oil) 3390 (NH indol), 3380–3140 (NH amide), 1650 (C=O); Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.94; H 6.96; N, 9.82. Found: C, 71.83; H, 7.04; N, 9.86.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2011.02.002.

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