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Synthesis and biological testing of conformationally restricted serotonin analogues with bridgehead moieties

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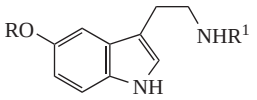
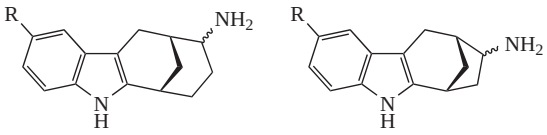
The synthesis of conformationally restricted serotonin analogues based on indol derivatives annelated with bicyclo[3.3.1]nonane and bicyclo[3.2.1]octane moieties is described. The configuration of the amino group in a structure is proved on the basis of X-ray data for *N*-[(9*R*)-2-methoxy-6,7,8,9,10,11-hexahydro-5*H*-6,10-methanocycloocta[*b*]indol-9-yl]acetamide. The *ex vivo* testing results of four products to $5HT_3$ serotonin receptors are presented.

The endocrine hormone melatonin and endogenic neurotransmitter serotonin (5-hydroxytriptamine) have very similar chemical structures, though the compounds regulate very different physiological processes in the human body.



Melatonin: R = Me, R^1 = Ac
Serotonin: R = R^1 = H

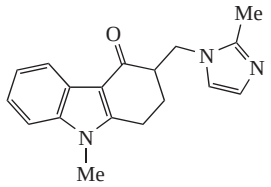
Earlier, we synthesized unusual conformationally rigid melatonin analogues with a side chain incorporated into the bridgehead moiety, such as bicyclo[3.3.1]nonane and bicyclo[3.2.1]octane.[1–4] Since the structures of melatonin and serotonin are close, here we suggested to check if similar indole derivatives annelated with bicyclic carcasses, *i.e.*, structures **I** and **II**, could bind to serotonin receptors.



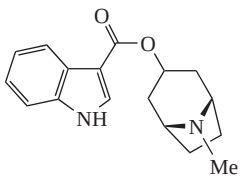
I **II**

Based on the analysis of published data,[5] we believed possible the affinity of the proposed compounds to some serotonin receptor subtypes, mainly to $5HT_3$ subtype.† The known $5HT_3$ antagonists ondansetron and tropisetron are structurally similar to **I** and **II**, which represent an interesting intermediate combination between the structures of both 'setrons'.

We obtained structures **I** and **II** with the hydroxyl group in indole (as in serotonin) or without the substituent (as in 'setrons'). Concerning the configuration of the amino group in a bicycle (*endo*-, *exo*-), we tried to synthesize both isomers because, according to the results of modeling,[7] several amino acid residues occur in the $5HT_3$ binding site (Glu236, Asn128, Glu129, Trp90), which can interact with a tertiary ammonium ion. In some cases, however, only more accessible isomers were obtained.

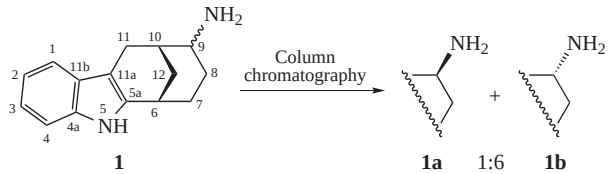


Ondansetron



Tropisetron

For the synthesis of the target compounds, the Fisher indolization at the bridgehead moiety was used. A mixture of diastereomeric amines **1** obtained in six steps from the Meervein ester[3] was separated by column chromatography. The isomer ratio **1a**:**1b** was 1:6 (Scheme 1, for the characteristics of isomers, see Online Supplementary Materials).‡



1 **1a** 1:6 **1b**

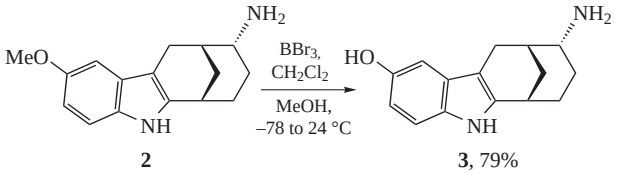
Scheme 1

A hydroxy analogue of **1b**, compound **3**, was synthesized from *endo*-amine **2**[1] with boron tribromide (the synthetic procedure required multiple evaporation of the reaction mixture with methanol for the removal of a BBr_3 excess). Product **3** was isolated in 79% yield (Scheme 2).§

† Affinity of the proposed compounds towards $5HT_3$ receptors is also predicted by the QSAR program 'Microcosm'.[6]

‡ Here and in the following schemes the marked configuration of the compounds is relative (*endo*-, *exo*-) because they represent racemic mixtures.

§ The *exo*-isomer corresponding to compound **3** was not synthesized because of the very low yield of *exo*-analogue of **2**.[1]



2 **3**, 79%

Scheme 2

The *endo*-configuration of the amino group in compounds **1b**, **2** and **3** was attributed on the basis of published data.[8] In this work, we performed an X-ray analysis of the *N*-acetyl derivative of amine **2**, compound **4**,[1] which was successfully crystallized from ethanol (Figure 1),¶ and this investigation unambiguously proved the *endo*-configuration of the amino group in compounds **1b**, **2**–**4**.

Compound **4** crystallizes in achiral space group *Cc* as a hydrate. In crystal molecules are assembled by N(4′)–H···O(3′) hydrogen bonds [N···O 2.846(3) Å] into the chains; the latter are linked into the corrugated layers by N(4′)–H···π [C(4a), C(11b)] interactions (H···C ~2.5 Å). The disordered water molecules are located between the above layers and assembled into the chains by O–H···O hydrogen bonds. To estimate quantitatively the ring conformations in **4**, we have calculated Zefirov–Palyulin (ZP) puckering parameters for these rings by the RICON program.[9] These data show that the cycle C(12)–C(6)–C(5a)–C(11a)–C(11)–C(10), *i.e.*, fused with an indole fragment, is a little flattened and has a conformation intermediate between half-chair and envelope (s = 0.823, θ = 36.4°, σ = 0.64). The conformation of the ring C(12)–C(10)–C(9)–C(8)–C(7)–C(6) in **4** is close to chair with a slight distortion (s = 1.162, θ = 5.4°, σ = 0.34).

The *exo*-amine derivative of indole annelated with bicyclo-[3.2.1]octane **5a** was obtained according to reported procedure,[2] while its *endo*-analogue **5b** was synthesized in four steps, as shown in Scheme 3.

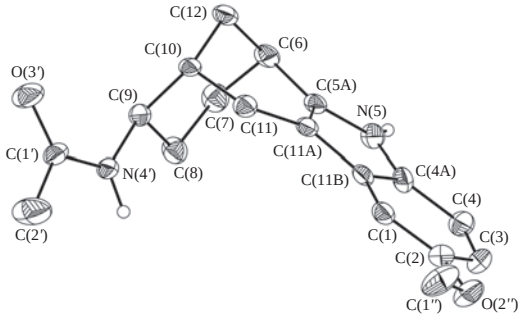
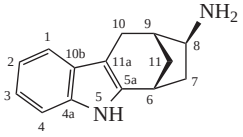


Figure 1 The general view of **4** in representation of atoms by thermal ellipsoids (p = 50%). The C–H hydrogen atoms are omitted for clarity.

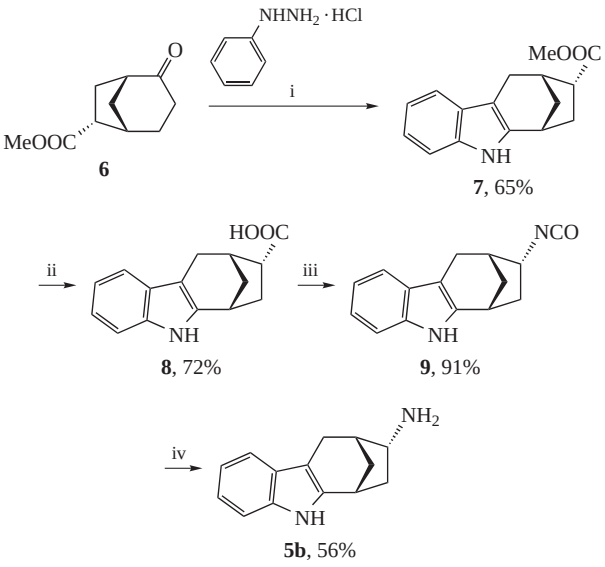
¶ *Crystal data.* Crystals of **4** [$C_{18}H_{24}N_2O_3$ ($C_{18}H_{22}N_2O_2$, H_2O), M = 316.39, from 96% ethanol] are monoclinic, space group *Cc*, at 100 K: a = 11.288(3), b = 24.797(6) and c = 7.5162(12) Å, β = 126.212(4)°, V = 1697.4(7) Å^3, Z = 4 (Z' = 1), d_{calc} = 1.238 g cm^{-3}, μ(MoKα) = 0.85 cm^{-1}. Intensities of 5755 reflections were measured with a Bruker APEX-II CCD diffractometer [λ(MoKα) = 0.71073 Å, 2θ < 54°] and 1856 independent reflections (R_{int} = 0.0479) were used in the further refinement. The structure was solved by a direct method and refined by the full-matrix least-squares technique against F^2 in the anisotropic–isotropic approximation. The refinement converged to wR_2 = 0.1576 and GOF = 0.998 for all independent reflections [R_1 = 0.0604 was calculated against F for 1531 observed reflections with $I > 2\sigma(I)$]. The water molecule is disordered by three positions with s.o.f. equal to 0.49(12), 0.29(15) and 0.22(15). All calculations were performed using SHELXTL PLUS 5.1.

CCDC 847109 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif. For details, see 'Notice to Authors', *Mendeleev Commun.*, Issue 1, 2012.



5a

First, the *endo*-isomer of methyl-2-oxobicyclo[3.2.1]octane-6-carboxylate **6**[1] was subjected to the Fisher reaction with phenylhydrazine hydrochloride in glacial acetic acid to afford corresponding indol derivative **7** with the *endo*-configuration of the ester group in carcass.



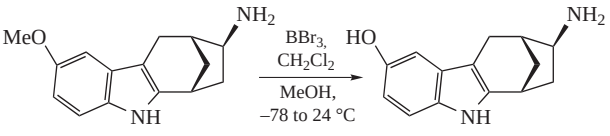
6 **7**, 65%

8, 72% **9**, 91%

5b, 56%

Scheme 3 *Reagents and conditions*: i, MeCOOH; ii, HCl, H_2O; iii, ClCOOEt, NEt_3, THF, –10 °C, then NaN_3, then Δ, toluene; iv, HCl_{aq}, 45–50 °C, then Na_2CO_3.

In the ^{1}H NMR spectrum of compound **7**, resonances of aromatic protons are observed at 7.05–7.39 ppm and a broad singlet of the indole NH proton, at 7.72 ppm. The proton resonance of C^8 (*H*C–COOMe) is displayed at 3.27 ppm (ddd). The presence of the indole core at the bridgehead structure leads to a slight deformation of the latter and, consequently, to a change in some coupling constants in **7** in comparison with **6**, *e.g.*, a coupling constant of the proton *H*C–COOMe and proton at the closest bridgehead carbon in **7** is 4.3 Hz instead of 6.1 Hz in **6** (for all coupling constants of hydrogens in the bridgehead moiety of **7**, see Online Supplementary Materials). The following transformation of ester **7** *via* acid **8** and isocyanate **9** led to amine **5b** (Scheme 3).



10 **11**, 67%

Scheme 4

An analogue of **5b** with a hydroxyl group in the indole core (compound **11**) was obtained from compound **10**[2] using the above procedure with boron tribromide (Scheme 4).

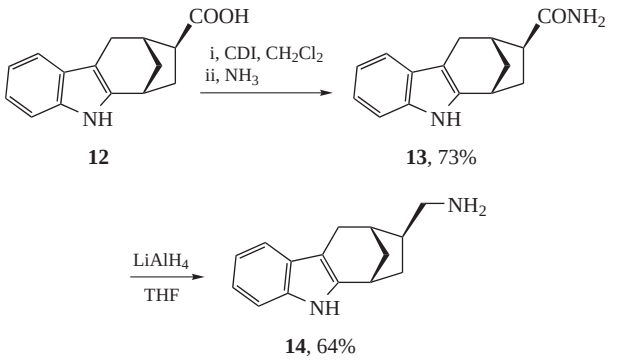
The conversion of amines **1a**, **1b**, **3**, **5a**, **5b** and **11** into the corresponding water-soluble salts was required for the following tests *ex vivo*. However, the treatment of compounds with a hydrogen chloride solution in dry diethyl ether led to resinification. Replacement of the hydrochloride for acetic acid allowed

us to obtain acetates of **1b**, **3**, **5a** and **11**, which were studied in the biotests.

The activity of the compounds (as racemic mixtures) for 5-HT_3 receptors was studied using two experimental procedures based on the measurement of the changes of serotonin-induced (1) positive chronotropic effect in isolated guinea pig atrium preparations[10] and (2) contraction effect of the isolated fragment of guinea pig ileum.[11,††] The 5-HT_3-serotoninergic activity was expressed as a difference between the values of the heart rate (Δ_1% in procedure 1) or as a difference of contractile responses (Δ_2% in procedure 2) obtained in the control and experimental measurements. Ondansetron (1 μM) was used as a control [Δ_1: (–)35±4% and Δ_2: (–)80±2%]. Absolute values Δ_1 and Δ_2 for all tested acetates of **1b**, **3**, **5a** and **11** were found much lower than that of ondansetron, the best result – Δ_2 for compound **5a** – (–)8±3% was one order of magnitude lower than that of the control compounds.

Having accepted that the low activity was a consequence of the low affinity of the tested compounds to 5-HT_3 receptor,[‡‡] we supposed that the reason might be an insufficient distance between the indole core and amino group in their structures, which does not allow the latter to interact with 5-HT_3 receptor as analogues to ondansetron. Therefore, we synthesized two 'elongated' analogues of the structure **5a** – compounds **14** and **16** (the choice of *exo*-isomer was conditioned by the results of biotests).

Compound **14** with a methylene linker between the bridgehead core and the amino group was obtained in two steps from acid **12**[2] *via* amide **13** (Scheme 5).



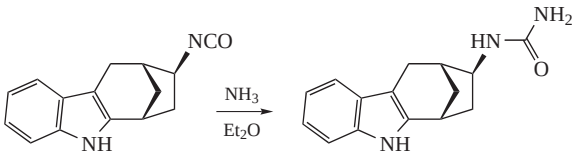
Scheme 5

†† The experiments were performed in five outbred guinea pigs (M/F, 12–16 months, 400–500 g), which were housed and fed in accordance with the Russian Federation Standards of the care and use of experimental animals (R 50258-92, Z 51000.3-96 and 51000.4-96). In the experimental measurement tested compounds (1 μM) were added and incubated for 2 min (procedure 1[10]) or 5 min (procedure 2[11]) before addition of serotonin. In the procedure 1, M-cholinomimetic action of acetylcholine was prevented by addition of atropine sulfate (1 μM). In the procedure 2, 5-HT_2-antagonist ketanserin (0.1 μM) was added during each measurement to avoid the 5-HT_2-mimetic influence of serotonin.

‡‡ Potency of the compound measured in the experiments *ex vivo* depends on both its affinity to the molecular target and ability to penetrate through cell membranes, stability to the action of metabolic enzymes *etc*.

The reduction of amide **13** by lithium aluminum hydride in THF led to the purpose amine. In the ^{1}H NMR spectrum of **14**, the resonances of methylene group protons are displayed as two doublets doublets at 2.65 ppm (^2J 12.4 Hz, ^3J 7.8 Hz) and 2.73 ppm (J 12.4 and 7.0 Hz). Amine **14** was transformed to the corresponding acetate.

Compound **16** with an urea substituent was synthesized by the reaction of isocyanate **15**[2] with ammonia gas in dry diethyl ether (Scheme 6). In the ^{13}C NMR spectrum of monosubstituted urea **16**, the resonance of the carbonyl carbon atom is observed at 158.79 ppm.



15 **16**, 99%

Scheme 6

The physiological activity of compounds **14** and **16** to 5-HT_3 receptors in the tests *in vivo* and *in vitro* and the potency of compounds **1a**, **1b**, **3**, **5a**, **5b** and **11** towards other subtypes of serotonin receptors will be published elsewhere.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2012.03.007.

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