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SYNTHESIS AND PRELIMINARY EVALUATION OF AMINOALKANOL DERIVATIVES OF SELECTED AZATRICYCLOUNDECANE SYSTEM FOR BINDING WITH BETA-ADRENERGIC AND $5HT_{1A}$ AND $5HT_{2A}$ RECEPTORS

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Abstract: A series of aminoalkanol derivatives of 8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$] undec-8-en-1-yl acetate and 1,11-dimethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undecane-3,5,8-trione was prepared. The pharmacological profile of selected compounds was evaluated for affinity to β-adrenoreceptors and serotoninergic receptors ($5HT_{1A}$ and $5HT_{2A}$).

Keywords: 8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-en-1-yl acetate, 1,11-dimethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undecane-3,5,8-trione, β-adrenolytic activity, $5HT_{1A}$ and $5HT_{2A}$ activity

Propranolol, befunolol and pindolol are widely used β-blockers. All these drugs contain 3-isopropyloamino-2-hydroxypropyl group that is associated with their antiarrhythmic and hypotensive activity (1-6).

Pindolol is also reported as a ligand for $5HT_{1A}$ and $5HT_{1B}$ receptors and shows antidepressive and anxiolytic activity (7-10). Many compounds that show a high activity for 5HT receptors contain cyclic imides built into their systems, e.g.: gepirone, tandospirone, NAN 190, BMY 7378 (11-14). With this in mind we have decided to synthesize derivatives of selected azatricycloundecane arrangements, linked with amino-2-hydroxypropyl groups for better activity for β-adrenergic and 5 HT receptor types.

The imides **1** and **2** were synthesized according to the method described previously (15, 16). In the reaction of 3,5-dimethylcyclohex-2-en-1-one and 1*H*-pyrrole-2,5-dione two isomers were obtained: compound **1** and **1a**. The next step was hydrolysis of the mixture of these isomers using anhydrous ethanol with 25% NH_3 (Scheme1). Acetoxy group in the position eight of compound **1a** was converted into carbonyl group to give compound **2** as a result of basic hydrolysis. The imides **1** and **2** were then treated with 2-(chloromethyl)oxirane in the presence of anhydrous K_2CO_3.

In the reaction of imide **1,** only one product **3** was obtained, however, in the reaction of imide **2** a mixture of two N-substituted compounds **10** and **11** was obtained. Then, chromatography column was used to separate these compounds (developing system: chloroform:methanol 100:0.2(0.5) v/v). The structures of purified derivatives were confirmed by ^{1}H NMR and ESI MS spectra.

In the next step, the N-substituted imides: **3**, **10**, **11** were condensed with appropriate amines in a mixture of methanol and water as a solvent. All new derivatives were purified by column chromatography (developing system: chloroform : methanol 50 : 0.5, v/v). Finally, twelve new aminoalkanol derivatives, **4**-**9** for imide **1** (Scheme 2) and **12**-**17** for imide **2** (Scheme 3) were obtained.

All the newly synthesized compounds were converted into their hydrochlorides using a solution of HCl in methanol and recrystallized from methanol/ether.
The pharmacological profile of all compounds was evaluated for their affinities for β-adrenoreceptors, by determining their ability to displace [^{3}H]CGP-12177 from specific binding sites of rat cerebral cortex. The selected compounds were also evaluated for affinity to $5HT_{1A}$ and $5HT_{2A}$ receptors.

EXPERIMENTAL

Melting points were determined in capillaries on an Electrothermal 9100 apparatus and are given uncorrected. Nuclear magnetic resonance spectra of protons (^{1}H NMR) were recorded in $CDCl_3$ on a Bruker AVANCE DMX400 spectrometer operating at 400MHz. The chemical shift values are expressed in ppm (parts per million) relatively to tetramethylsilane used as an internal standard and coupling constants *J* are given in Hz. The ESI MS were recorded on a Mariner Perspective – Biosystem instrument. Column chromatography was performed using 0.05

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– 0.2 mm Kieselgel (70-325 mesh ASTM, Merck). Reactions were monitored by TLC on 0,2 mm thick Kieselgel G plates with 254 nm fluorescent indicator (Merck), eluted with 9.8 : 0.2 or 9.5 : 0.5 chloroform-methanol solvents.

Synthesis of 4-(3-chloro-2-hydroxypropyl)-8,11-dimethyl-3,5-dioxo-4-azatricyclo [5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**3**)

A mixture of 8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**1**) (5 g, 15 mmol), 2-(chloromethyl)oxirane (51 mL) and anhydrous K_2CO_3 (3 g, 22 mmol) was refluxed in a water bath for 96 h. When the reaction was completed, as indicated by TLC, the mixture was filtered and the solvent was evaporated. The oily residue was purified by column chromatography (eluent: chloroform).

Yield: 30%, m.p. 119.4-119.9^{o}C. ESI MS (m/z): 100% = 378.10; 13.3% = 356.10. ^{1}H NMR ($CDCl_3$): 5.83 (s, 1H, H-9); 3.99 (m, 2H, H-2, H-C2'); 3.77-3.71 (m, 1H, H-3'); 3.66-3.61 (m, 1H, H-3'); 3.57-3.49 (m, 2H, H-1'); 3.06 (dd, 1H, H-6, *J* = 8, *J* = 4); 2.81 (s, 1H, H-7); 2.75 (m, 1H, H-10); 2.18 (s, 3H, OAc-1); 2.12 (m, 1H, H-11); 1.82 (s, 3H, CH_3-8); 1.13 (dd, 1H, H-10, *J* = 12, *J* = 4); 0.94 (d, 3H, CH_3-11, *J* = 8). Analysis: calc. 57.39%C, 6.23%H, 3.94%N, for $C_{17}H_{22}NClO_5$ (355.81) found: 57.43%C, 6.12%H, 3.96%N.

General procedure of preparing aminoalkanol derivatives of 8,11-dimethyl-3,5-dioxo-4-azatricyclo-[5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**4-9**)

A mixture of 4-(3-chloro-2-hydroxypropyl)-8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}] undec-8-en-1-yl acetate (**3**) (300 mg, 1 mmol), an appropriate amine (5 mmol) and a solution of methanol/water (29 mL+1 mL) was heated in a water bath at 75^{o}C for 30-40 h. The liquid was evaporated, and the oily residue was purified by column chromatography (chloroform, chloroform : methanol 50 : 0.2(0.5) v/v) to give compounds **4-9**.

4-[2-Hydroxy-3-(isopropylamino)propyl]-8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**4**)

$C_{20}H_{30}O_5N_2$×HCl. M = 378.46×HCl; 75%, m.p. 200-201^{o}C; ^{1}H NMR ($CDCl_3$): 9.54 (s, 1H, HCl); 8.02 (s, 1H, NH); 5.81 (s, 1H, H-9); 5.29 (m, 1H, OH); 4.27 (m, 1H, H-2'); 3.95 (d, 1H, H-2, *J* = 8); 3.61-3.45 (m, 2H, H-1'); 3.37 (m, 1H, H-4'); 3.18-3.15 (m, 1H, H-6); 2.99 (m, 1H, H-3'); 2.78-2.61 (m, 3H, H-7, H-10, H-3'); 2.17 (s, 3H, OAc-1); 2.05 (m, 1H, H-11); 1.75 (s, 3H, CH_3-8); 1.44 (m, 6H, $(CH_3)_2$-4'); 1.11-1.06 (m, 1H, H-10H); 0.88(d, 3H, CH_3-11, *J* = 6.8); ESI MS (m/z): 100% = 379.3.

4-[3-(*tert*-Butylamino)-2-hydroxypropyl]-8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**5**)

$C_{21}H_{32}O_5N_2$×HCl; M = 392.49×HCl; 70%; m.p. 129-130^{o}C; ^{1}H NMR ($CDCl_3$): 9.67 (s, 1H, HCl); 7.08 (m, 1H, NH); 5.87 (s, 1H, H-9); 5.44 (m, 1H, OH); 4.22 (m, 1H, H-2'); 3.67 (s, 1H, H-2); 3.63-3.42 (m, 2H, H-C1'); 3.17 (m, 1H, H-6); 3.05 (m, 1H, H-3'); 2.93 (m, 1H, H-3'); 2.71 (m, 1H, H-7); 2.60 (m, 1H, H-10); 2.11 (s, 3H, OAc-1); 2.04 (m, 1H, H-11); 1.76 (m, 3H, CH_3-8); 1.45 (s, 9H, $(CH_3)_3$); 1.13-1.04 (m, 1H, H-10); 0.88 (d, 3H, CH_3 –11, *J* = 6.8); ESI MS(m/z): 100% = 393.3, 19% = 394.3.

4-[3-(Dimethylamino)-2-hydroxypropyl]-8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**6**)

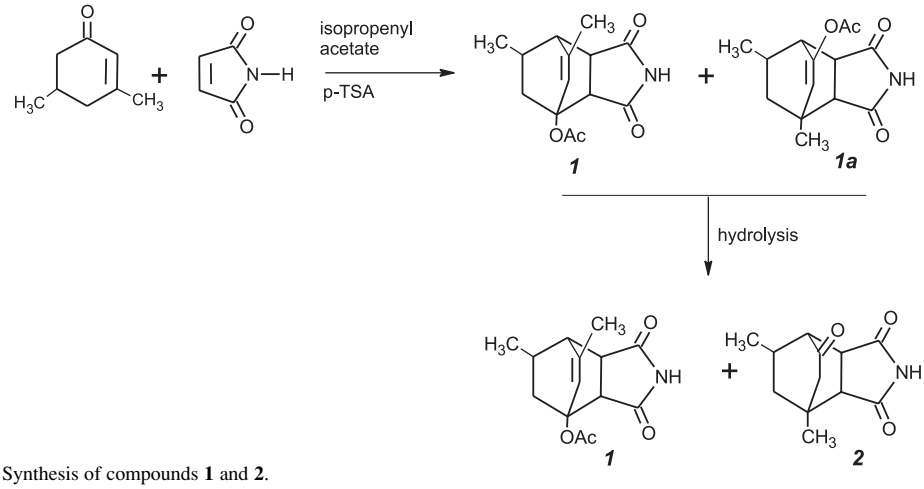
$C_{19}H_{28}O_5N_2$×HCl; M = 365.44×HCl; 63%; m.p. 199.8-201^{o}C; ^{1}H NMR ($CDCl_3$): 11.02 (s, 1H, HCl); 5.78 (d, 1H, H-9, *J* = 6); 5.41 (m, 1H, OH); 4.39 (m, 1H, H-2'); 3.98 (d, 1H, H-2, *J* = 7.6); 3.63-3.41 (m, 2H, H-1'); 3.17 (m, 1H, H-6); 3.06 (m, 2H, H-3'); 2.96 (s, 3H, N-CH_3); 2.89 (s, 3H, N-CH_3); 2.74 (s, 1H, H-7); 2.66 (t, 1H, H-10, *J* = 10.4); 2.12 (s, 3H, OAc-1); 2.06 (m, 1H, H-11); 1.77 (s, 3H, CH_3-8); 1.07 (m, 1H, H-10); 0.88 (d, 3H, CH_3-11, *J* = 6.8); ESI MS (m/z): 100% = 365.1.

4-[3-(Diethylamino)-2-hydroxypropyl]-8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}]undec-8-en-1-yl acetate (**7**)

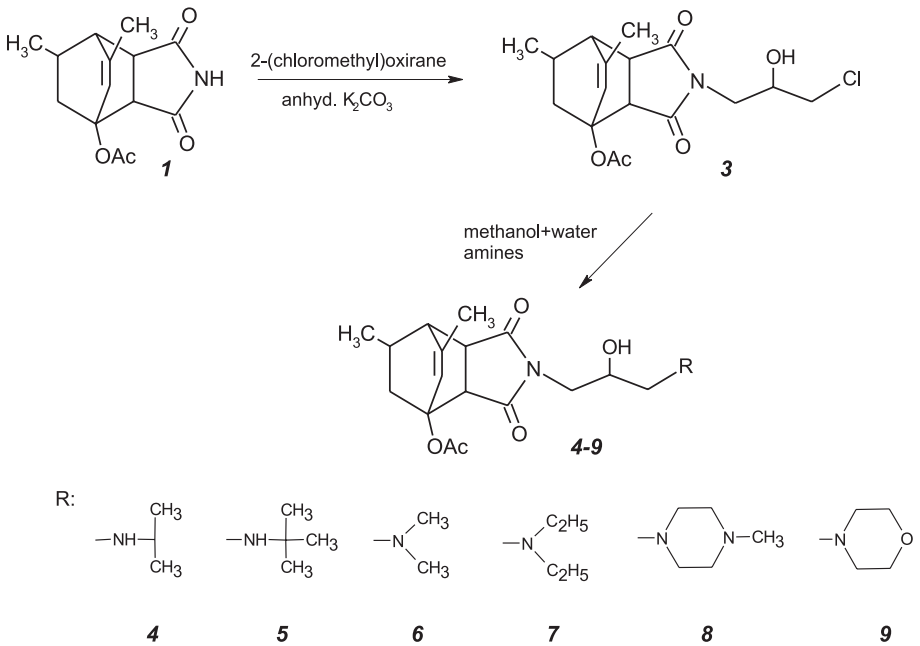
$C_{21}H_{32}O_5N_2$×HCl; M = 392.49×HCl; 59%; m. p. 183-184^{o}C; ^{1}H NMR ($CDCl_3$): 11.42 (m, 1H, HCl); 5.80 (d, 1H, H-9, *J* = 14.8); 5.39-5.33 (m, 1H, OH); 4.42-4.36 (m, 2H, H-2'); 3.91 (t, 1H, H-2, *J* = 8, *J* = 6.8); 3.64-3.58 (m, 1H, H-1'); 3.45-3.36 (m, 1H, H-1'); 3.24-2.89 (m, 7H, H-6, H-3', H-1", H-2"); 2.76 (s, 1H, H-7); 2.69 (m, 1H, H-10); 2.14 (s, 3H, OAc-1); 2.04 (m, 1H, H-11); 1.80 (m, 3H, CH_3-8); 1.43 (m, 6H, H-3", H-4"); 1.11-1.06 (m, 1H, H-10); 0.89 (d, 3H, CH_3-11, *J* = 6.8); ESI MS (m/z): 100% = 393.2.

4-[2-Hydroxy-3-(4-methylpiperazin-1-yl)propyl]-8,11-dimethyl-3,5-dioxo-4-azatricyclo [5.2.2.0^{2,6}]-undec-8-en-1-yl acetate (**8**)

$C_{22}H_{33}O_5N_3$×HCl; M = 419.51×HCl; 64%; m.p. 242-243^{o}C; ^{1}H NMR ($CDCl_3$): 5.76 (s, 1H, H-9); 3.90 (t, 1H, H-2, *J* = 8); 3.84-3.78 (m, 1H, H-2'); 3.56-3.50 (m, 1H, OH); 3.44-3.39 (m, 2H, H-1');



Scheme 1. Synthesis of compounds **1** and **2**.



Scheme 2. Synthesis of compounds **4**-**9**.

3.02-2.97 (m, 1H, H-6); 2.76 (s, 1H, H-7); 2.73-2.62 (m, 3H, H-10, H-3'); 2.43 (m, 4H, H-1", H-2"); 2.29 (m, 4H, H-3", H-4"); 2.28 (s, 3H, N-CH_3); 2.14 (s,3H, OAc-1); 2.04-2.02 (m, 1H, H-11); 1.77 (s, 3H, CH_3-8); 1.11-1.06 (m, 1H, H-10); 0.89 (d, 3H, CH_3-11, J = 7.2); ESI MS (m/z): 100% = 420.3.

4-(2-Hydroxy-3-morpholin-1-ylpropyl)-8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-en-1-yl acetate (**9**)

$C_{21}H_{30}O_6N_2$×HCl; M = 406.47×HCl; 78%; m.p. 222-223°C; ^{1}H NMR ($CDCl_3$): 11.68 (m, 1H, HCl); 5.78 (m, 1H, H-9); 5.27-5.25 (m, 1H, OH); 4.52-4.49 (m, 1H, H-2'); 4.24-4.21 (m, 2H, H-1"); 3.95 (m, 3H, H-2', H-2"); 3.86-3.77 (m, 1H, H-C3'); 3.64-3.54 (m, 2H, H-1'); 3.49-3.39 (m, 1H, H-3'); 3.14 (m, 1H, H-6); 3.07-2.81 (m, 4H, H-3'', H-4"); 2.73 (s ,1H, H-7); 2.64 (t, 1H, H-10, J = 10.8, J = 10.4); 2.12 (s, 3H, OAc-1); 2.04 (m, 1H, H-11); 1.76 (s, 3H, CH_3-8); 1.10-1.05 (m, 1H, H-10); 0.88 (d, 3H, CH_3-11, J = 6.8); ESI MS (m/z): 100% = 407.2, 20.7% = 429.

Synthesis of 4-(3-chloro-2-hydroxypropyl)-1,11-dimethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undecane-3,5,8-tri-

one (**11**) and 1,11-dimethyl-4-(oxiran-2-ylmethyl)-4-azatricyclo [5.2.2.0^{2,6}]undecane-3,5,8-trione (**10**)

A mixture of 1,11-dimethyl-4-azatricyclo-[5.2.2.0^{2,6}]undecane-3,5,8-trione (**2**) (5 g, 23 mmol), 2-(chloromethyl)oxirane (61 mL) and anhydrous K_2CO_3 (3 g, 22 mmol) was refluxed in a water bath for 76 h. When the reaction was complete, as indicated by TLC, the mixture was filtered and the solvent was evaporated. The oily residue was purified by column chromatography (eluent: chloroform).

1,11-Dimethyl-4-(oxiran-2-ylmethyl)-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**10**)

M.p. 115.8-116.6°C, ESI MS (m/z): 100% = 300, 23.6% = 278; ^{1}H NMR ($CDCl_3$): 3.80-3.52 (m, 1H, H-3'); 3.19-3.14 (m, 1H, H-1'); 3.05 (m, 1H, H-2'); 2.73-2.69 (m, 3H, H-2, H-6, H-7); 2.58-2.41 (m, 1H, H-1'); 2.17 (m, 1H, H-11); 2.10-1.92 (m, 3H, H-9, H-10); 1.30 (m, 3H, CH_3-1); 1.13-1.08 (m, 1H, H-10); 0.98 (m, 3H, CH_3-11). Analysis: calc. 64.97%C, 6.91%H, 5.05%N, for $C_{15}H_{19}NO_4$ found 64.60%C, 6.89%H, 4.66%N.

4-(3-Chloro-2-hydroxypropyl)-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**11**)

M.p. 104-105°C, ESI MS (m/z): 100% = 331, 75.7% = 336, 14.9% = 314; ^{1}H NMR ($CDCl_3$): 4.05-3.99 (m, 1H, H-2'); 3.81-3.70 (m,1H, H-1'); 3.60-3.52 (m, 3H, H-1', H-3'); 3.20 (dd, 1H, H-6, *J* = 9.2, *J* = 3.2,); 3.00 (s, 1H, OH); 2.76 (m, 1H, H-2); 2.70 (t, 1H, H-7, *J* = 2.8); 2.50-2.10 (m, 2H, H-9, H-11); 2.00-1.93 (m, 2H, H-9, H-10); 1.33 (s, 3H, CH_3-1); 1.13 (dd, 1H, H-10, *J* = 14, *J* = 4.8); 1.01 (d, 3H, CH_3-11, *J* = 7.2). Analysis: calc. 57.42%C, 6.42 %H, 4.46%N, for $C_{15}H_{20}NO_4Cl$ found 57.11%C, 6.20%H, 4.41%N.

General procedure of preparing aminoalkanol derivatives of 1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]-undecane-3,5,8-trione (**12**-**17**)

A mixture of 4-(3-chloro-2-hydroxypropyl)-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**11**) (330 mg, 1 mmol) or 1,11-dimethyl-4-(oxiran-2-ylmethyl)-4-azatricyclo [5.2.2.0^{2,6}]undecane-3,5,8-trione (**10**) (300 mg, 1 mmol), an appropriate amine (5 mmol) and a solution of methanol/water (29 mL+1 mL) was heated in a water bath at 75°C for 30-40 h. The liquid was evaporated, and the oily residue was purified by column chromatography (chloroform: methanol 50 : 0.5, v/v) to give compounds **12**-**17**.

4-[2-Hydroxy-3-(isopropylamino)propyl]-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**12**)

$C_{18}H_{28}O_4N\times HCl$; M = 371.92; 56%; m.p. 220.7-221.5°C; ^{1}H NMR ($CDCl_3$): 9.48 (s, 1H, HCl); 7.84 (s, 1H, N-H); 5.58 (s, 1H, OH); 4.31 (m, 1H, H-2'); 3.62-3.36 (m, 4H, H-6, H-1', H-4'); 3.05-2.67 (m, 3H, H-2, H-7, H-3'); 2.21-1.90 (m, 4H, H-9, H-10, H-11); 1.46 (m, 6H, $(CH_3)_2$-4'); 1.30 (s, 3H, CH_3-1); 1.09-0.97 (m, 4H, H-10, CH_3-11); ESI MS (m/z): 100% = 355.1, 22.5% = 356.1.

4-[3-(*tert*-Butylamino)-2-hydroxypropyl]-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**13**)

$C_{19}H_{30}O_4N_2\times HCl$; M = 385.95; 45%; m.p. 151-152°C; ^{1}H NMR ($CDCl_3$): 5.56 (m, 1H, OH); 4.25 (m, 1H, H-2'); 3.44-3.37 (m, 1H, H-6); 3.05 (m, 1H, H-3'); 2.90 (d, 1H, H-2, *J* = 9.2); 2.77-2.60 (m, 2H, H-7, H-3'); 2.18 (m, 1H, H-9); 2.07-1.19 (m, 3H, H-9, H-10, H-11); 1.44 (s, 9H, $(CH_3)_3$-4'); 1.07-1.02 (m, 1H, H-10); 0.95 (d, 3H, CH_3-11, *J* = 7.2); ESI MS (m/z): 100%=351.2.

4-[3-(Dimethylamino)-2-hydroxypropyl]-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**14**)

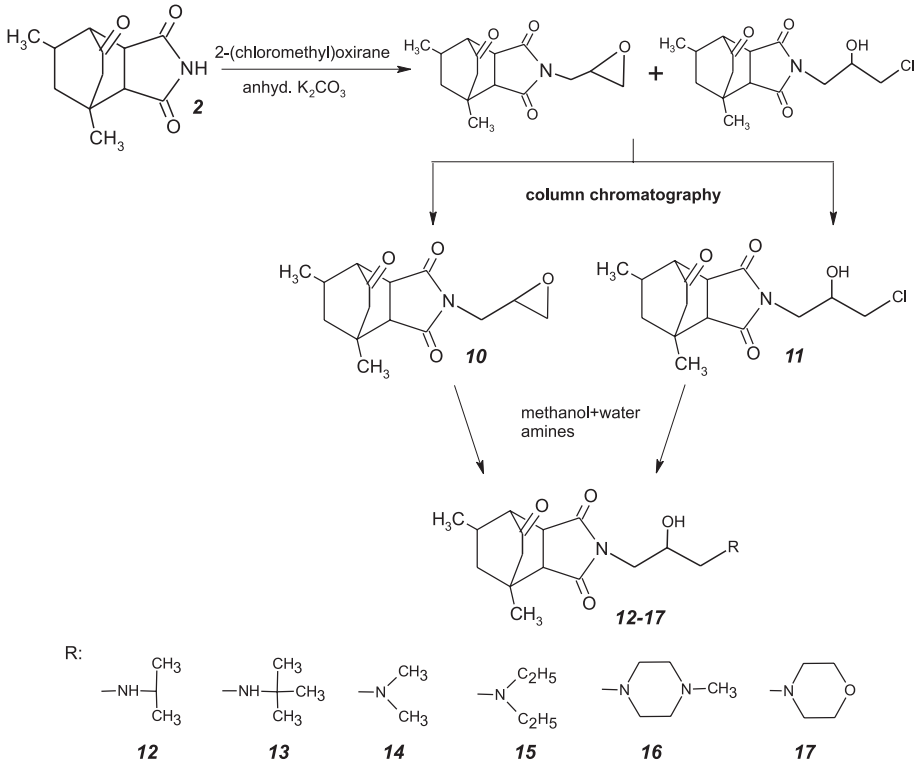
$C_{17}H_{26}O_4N_2$; M = 322.39; 60%; oil; ^{1}H NMR ($CDCl_3$): 4.44 (m,1H, H-2'); 3.69-3.66 (m, 2H, H-1'); 3.59-3.47 (m, 2H, H-3'); 3.35-3.30 (m, 1H, H-6); 3.04-2.82 (m, 7H, $N(CH_3)_2$, OH); 2.69 (m, 2H, H-2, H-7); 2.21-2.15 (m, 2H, H-9, H-11); 2.08-2.00 (m, 2H, H-9, H-10) ; 1.32 (s, 3H, CH_3-1), 1.13-1.10 (m, 1H, H-10); 1.00-0.99 (m, 3H, CH_3-11); ESI MS (m/z): 100% = 323.2.

4-[3-(Diethylamino)-2-hydroxypropyl]-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**15**)

$C_{19}H_{30}O_4N_2$; 350.45; 65%; oil; ^{1}H NMR ($CDCl_3$): 3.80 (m, 1H, H-2'); 3.58-3.52 (m, 2H, H-1'); 3.15 (m, 1H, H-6); 2.71-2.69 (m, 2H, H-2, H-7); 2.62-2.40 (m,5H, OH, H-1", H-2"); 2.29-2.13 (m, 4H, H-9, H-11, H-3'); 1.99-1.90 (m, 2H, H-9, H-10); 1.32 (s, 3H, CH_3-1); 1.10 (dd, 1H, H-10, *J* = 13.6, *J* = 4.8); 0.99 (m, 9H, CH_3-11, H-C3", H-4"); ESI MS (m/z): 100% = 351.2.

4-[2-Hydroxy-3-(4-methylpiperazin-1-yl)propyl]-1,11-dimethyl-4-azatricyclo[5.2.2.0^{2,6}]undecane-3,5,8-trione (**16**)

$C_{20}H_{31}O_4N$; 377.47; 57%; oil; ^{1}H NMR ($CDCl_3$): 3.90-3.80 (m, 1H, H-2'); 3.61-3.48 (m, 2H, H-1'); 3.41-3.37 (m, 1H, H-C3'); 3.14 (dd, 1H, H-6, *J* = 9.2, *J* = 2.8); 2.70 (m,2H, H-2, H-7); 2.63-2.29 (m, 9H, H-1", H-2", H-3", H-4", H-3'); 2.26-2.15 (m, 5H, H-9, H-11, N-CH_3); 1.99-1.89 (m, 2H,



Scheme 3. Synthesis of compounds **12**-**17**.

H-9, H-10); 1.32 (s, 3H, CH_3-1); 1.09 (dd, 1H, H-10, *J* = 14, *J*= 3.6); 0.99 (d, 3H, CH_3-11, *J* = 6.8); ESI MS (m/z): 100%=378.3.

4-(2-Hydroxy-3-morpholin-1-ylpropyl)-,11-dimethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undecane-3,5,8-trione (**17**)

$C_{19}H_{28}O_5N_2$; 364.43; 49%; oil; ^{1}H NMR ($CDCl_3$): 3.92-3.79 (m, 2H, H-2'); 3.67 (m, 4H, H-3", H-4"); 3.63-3.37 (m, 4H, H-1', H-3'); 3.14 (dd, 1H, H-6, *J* = 9.2, *J* = 3.2); 2.70 (m, 2H, H-2, H-7); 2.58 (m, 2H, H-1"); 2.40-2.14 (m, 4H, H-9, H-11, H-2"); 1.99-1.89 (m, 2H, H-9, H-10); 1.31 (s, 3H, CH_3-1); 1.10 (dd, 1H, H-10, *J* = 14, *J* = 4.8); 0.98 (d, 3H, CH_3-11, *J* = 6.8); ESI MS (m/z): 100% = 365.2.

Pharmacology

In vitro – radioligand binding experiments

Adrenoreceptor binding assay

The experiment was carried out on the rat cerebral cortex. [^{3}H]CGP-12177 (48 Ci/mmol) was used. The tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.6), and centrifuged at 1000 × g for 10 min (0-4°C). The supernatant was centrifuged at 20000 × g for 20 min. The cell pellet was resuspended in Tris-HCl buffer and centrifuged again. The final incubation mixture (final volume 300 µL) consisted of 240 µL membrane suspension, 30 µL of [^{3}H]CGP-12177 (0.2 nM) solution and 30 µL of buffer containing from seven to eight concentrations (10^{-11} – 10^{-4} M) of the

Table 1. β-Adrenergic receptor affinity of the compounds

Compound no.	IC_{50} (mM)	K_i (mM)
4	>100	72.8 ± 5.9
5	>100	>100
6	23.1 ± 9,6	10.3 ± 4.3
7	>100	72.4 ± 10
8	>100	~100
9	>100	60.6 ± 9.3
12	>100	>100
13	29.8 ± 0	13.2 ± 0
14	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100

Table 2. 5 HT_{1A} and $5HT_{2A}$ receptors affinity of the compounds.

Compound no.	[^{3}H] 8-hydroxy-DPAT		[^{3}H]-ketanserin	
	IC_{50}	Ki	IC_{50}	Ki
6	465.1 ± 164.0 nM	271.9 ± 96.4 nM	>100 μM	73.7 ± 2.1 μM
9	24.1 ± 2.8 μM	14.1 ± 1.7 mM	>100 mM	58.3 ± 8.9 mM
13	180.9 ± 53.4 μM	105.5 ± 31.2 μM	>100 μM	60.6 ± 10.5 μM

investigated compounds. For measuring unspecific binding, propranolol – 1 μM was applied. The incubation was completed with rapid filtration through glass fiber filters (Whatman GF/C) using a vacuum manifold (Millipore). The filters were then washed 2 times with the assay buffer and placed in scintillation vials with liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA – liquid scintillation counter. All assays were done in duplicates. Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.02 – San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff equation (17).

5-HT_{1A} receptor binding experiments

Radioligand receptor binding studies were conducted on the rat brain. [^{3}H]-8-hydroxy-2-(di-n-propylamino)-tetralin ([^{3}H]-8-OH-DPAT, spec. act. 106 Ci/mmol, NEN Chemicals) was used for labeling 5-HT_{1A} receptors. The membrane preparation and the assay procedure were carried out according to the published procedure (18) with slight modifications. Briefly, the cerebral cortex tissue was homogenized in 20 vol. of 50 mM Tris-HCl buffer (pH 7.7 at 25^{o}C) using Ultra-Turrax® T 25, and was then centrifuged at 32000 × g for 10 min. The supernatant fraction was discarded, and pellet was resuspended in the same volume of Tris-HCl buffer and was then centrifuged. Before the third centrifugation, the samples were incubated at 37^{o}C for 10 min. The final pellet was resuspended in Tris-HCl buffer containing 10 mM pargyline, 4 mM $CaCl_2$ and 0,1% ascorbic acid. One milliliter of the tissue suspension (9 mg of wet weight), 100 ml of 10 mM serotonin (for unspecific binding), 100 mL of [^{3}H]-8-OH-DPAT and 100 mL of the analyzed compound were incubated at 37^{o}C for 15 min. The incubation was followed by a rapid vacuum filtration through Whatman GF/B glass filters, and was then washed 3 times with 5 mL of cold buffer (50 mM Tris-HCl, pH 7.7) using a Brandel cell harvester. The final [^{3}H]-8-OH-DPAT concentration was 1 nM, and the concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-4} M.

5-HT_2 receptor binding experiments

Radioligand receptor binding studies were conducted on the rat brain. [^{3}H]-Ketanserin (spec. act. 60 Ci/mmol, NEN Chemicals) was used for labeling 5-HT_2 receptors. The assay was performed according to the method of Laysen et al. (19) with slight modifications. The cerebral cortex tissue was homogenized in 20 vol. of 50 mM Tris-HCl buffer (pH 7.7 at 25^{o}C) and centrifuged at 32000 × g for 20 min. The resulting pellet was resuspended in the same quantity of the buffer, preincubated at 37^{o}C for 10 min and centrifuged for 20 min. The final pellet was resuspended in 50 vol. of the same buffer. One milliliter of the tissue suspension, 100 mL of 1 mM mianserin (displacer), 100 mL of [^{3}H]-ketanserin and 100 mL of the analyzed compound were incubated at 37^{o}C for 20 min, followed by a rapid vacuum filtration through Whatman GF/B glass filters, and were then washed three times with 5 mL of cold Tris-HCl buffer. The final [^{3}H]-ketanserin concentration was 0.6 nM and the concentrations of analyzed compounds ranged from 10^{-10} to 10^{-4} M.

Radioligand binding studies was performed in the Department of Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University in Kraków.

RESULTS AND DISCUSSION

This work describes synthesis of twelve new aminoalkanol derivatives of 8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-en-1-yl acetate and 1,11-dimethyl-4-azatricyclo[5.2.2.$0^{2,6}$]-undecane-3,5,8-trione.

As shown in Table 1, three (**6**, **9**, and **13**) compounds exhibited modest affinity for β-adrenergic receptors (IC_{50} 23-100 μM, Ki 10-60 μM, respectively). The remaining compounds showed no significant affinity.

In the chemical structure of β-adrenolytic drugs a fragment of 2-alkylaminoethanol is always present and structurally they are all derivatives of propranolol. The following modifications were made to the compounds obtained: phenolic ring was

replaced by the appropriate azatricycloundecane system and also other II° and III° amines were used except of isopropylamine. Modifications that were made influenced the affinity of compounds to β-adrenergic receptors.

However, it was observed that the compounds having the 8,11-dimethyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-en-1-yl acetate system in their structure were demonstrating better affinity to these receptors when compared to derivatives containing 1,11-dimethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undecane-3,5,8-trione system. It may come from their better lipophilicity.

Compounds **6**, **9**, and **13** were also evaluated for affinity to $5HT_{1A}$ and $5HT_{2A}$ receptors. It is known that many β-adrenolytic drugs, e. g. propranolol, pindolol, are used in the treatment of anxiety disorders. They are demonstrating affinity to serotonin 5-HT_{1A} receptors, suppressing their function.

The affinity of the investigated compounds for $5HT_{1A}$ and $5HT_{2A}$ receptors was evaluated on the basis of their ability to displace [^{3}H]-8-OH-DPAT and [^{3}H]-ketanserin, respectively.

The radioligand binding experiments were performed on rat brain using the following structures: the hippocampus for $5HT_{1A}$, and the cortex for $5HT_{2A}$ receptors. The results presented in Table 2 indicate that only compound **6** shows moderate affinity for the $5HT_{1A}$ receptor (IC_{50} – 465 nM, Ki – 271 nM). Compounds **9** i **13** show no significant affinity.

In summary, our results seem to indicate that only compound **6** of the twelve newly synthesized compounds shows moderate affinity for β-adrenergic and $5HT_{1A}$ receptor types.

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