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SYNTHESIS OF OXIRAN-2-YLMETHYL AND OXIRAN-2-YLMETHOXY DERIVATIVES OF SOME 4-AZATRICYCLO[5.2.1.$0^{2,6}$]DEC-8-ENE-3,5-DIONES AS POTENTIAL BETA-ADRENOLYTICS

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Abstract: A series of aminoalkanol derivatives of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione and 4-hydroxy-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione have been prepared. The pharmacological profile of selected compounds was evaluated for their affinities at β-adrenoceptors. The investigated compounds exhibit modest affinity for these receptors.

Keywords: 10-(diphenylmethylene)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione, 4-hydroxy-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione, β-adrenolytic activity, [^{3}H]CGP-12177 binding

Features that are important for adrenergic activity include: an extended aromatic system, a four atom chain separating the aromatic system from a terminal amine, a terminal secondary amine, amine substituents that are bulky or branched and a hydroxyl group on the side chain (1). Numerous compounds containing the 3-amino-2-hydroxypropyl/propoxy group bound to an aryl or heteroaryl system show cardiostimulant, antiarrhythmic and hypotensive activity (2-6). Many members of this class also display considerable affinity for the 5-HT_{1A} and the rodent 5-HT_{1B} receptor subtypes (7-10). Aryloxypropanolamines, e.g. the β-adrenergic antagonist, propranolol, bind at 5-HT_{1A} receptor being its antagonist, whereas pindolol appears as 5-HT_{1A} receptor antagonist and a partial agonist at β_3-adrenoceptors (11). 5-HT_{1A} receptor may be involved in, for example, temperature regulation, sexual activity, appetite control, and, as found most recently, the mechanism of action of a class of anxiolytic agents, i.e. second-generation arylpiperazine anxiolytics (12,13). 5-HT_{2A} serotonin receptors are involved in the actions of hallucinogenic drugs and have been implicated in the pathogenesis and treatment of schizophrenia (14).

This work describes the design and the synthesis of a series of oxiran-2-ylmethyl and oxiran-2-ylmethoxy derivatives of some 4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-diones, obtained by substitution of compounds **1** and **3** at position 4 (Fig.1), with an expected β-adrenolytic activity. This is a continuation of our research in this field (15). Compounds **2g-2i** and **4f** possess also a propyl-(*N*-aryl) substituted piperazine moiety that is believed to give high potency in the treatment of anxiety and depression (16).

The pharmacological profile of selected compounds was evaluated for their affinities at β-adrenoceptors, by determining their ability to displace [^{3}H]CGP-12177 from specific binding sites on rat cerebral cortex. Radioligand binding studies were performed at the Department of Cytobiology and Histochemistry, Collegium Medicum, Jagiellonian University in Cracow (for compounds **4a-b** and **4e-f**) and at the Department of Pharmacology, University of Bonn, Germany (compounds **4c, 4f, 2d**).

EXPERIMENTAL

Chemistry

Melting points were determined in a Kofler's apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker AVANCE DMX400 spectrometer, operating at 400.13 MHz. The chemical shift values are expressed in ppm relative to TMS as an internal standard. Elemental analyses were recorded with a CHN model 2400 Perkin-Elmer analyzer. Flash chromatography was performed on Merck silica gel 60 (200-400 mesh) using chloroform-metanol (19:1, v/v) mixture as eluent. Analytical TLC was carried out on silica gel F_{254} plates of 0.25 mm thickness (Merck).

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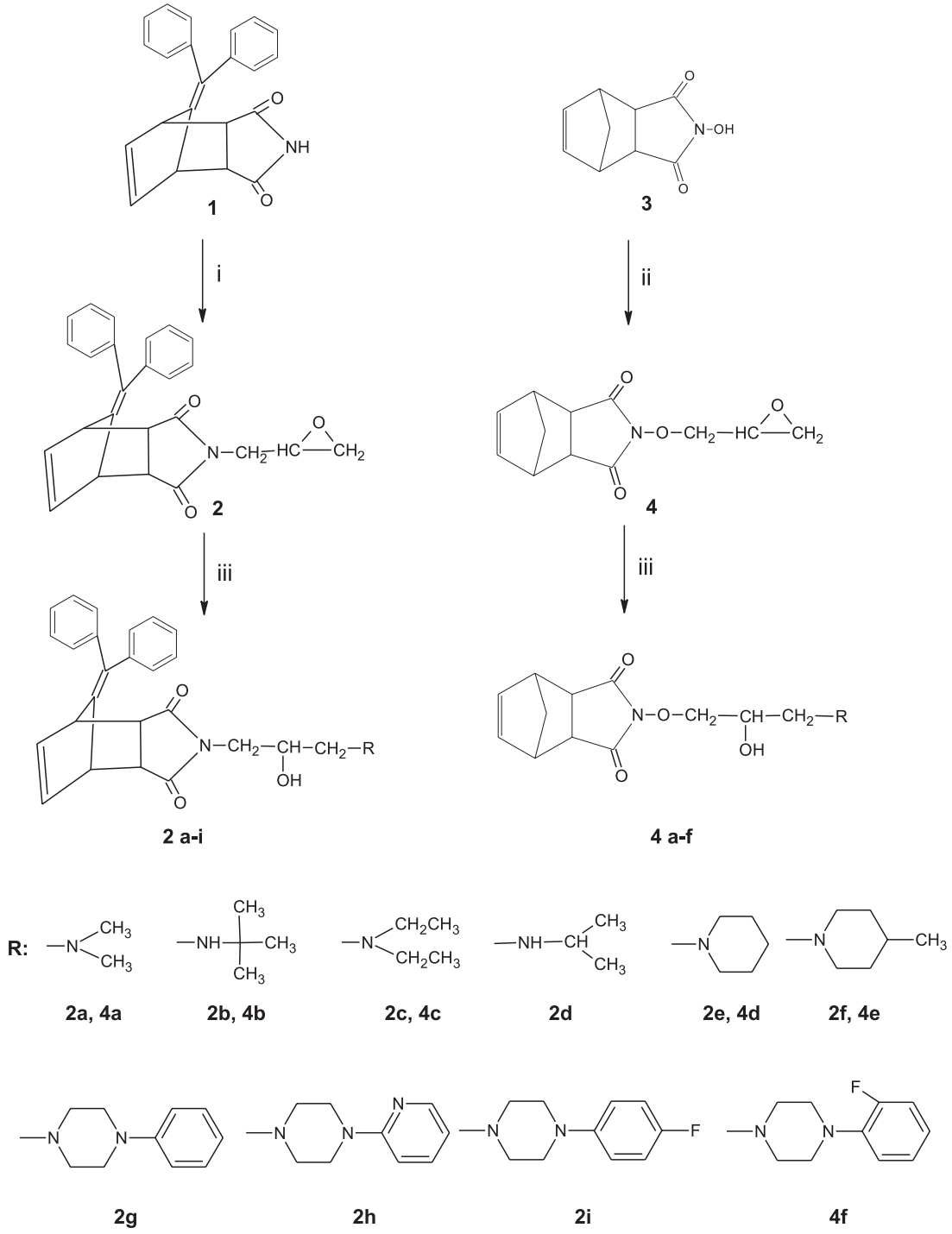

Figure 1. Method for preparation of compounds **2 a-i** and **4 a-f**. (i) Cl-CH_2-CH(O)CH_2, anhydr. K_2CO_3; (ii) Br-CH_2-CH(O)CH_2, anhydr. K_2CO_3; (iii) amine, MeOH, H_2O.

The preparation of 15 new N-substituted derivatives of 10-(diphenylmethylene)-4-(oxiran-2-ylmethyl)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione **2** and 4-(oxiran-2-ylmethoxy)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione **4** was performed. The starting materials were, respectively, 10-(diphenylmethylene)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione **1** (17) and 4-hydroxy-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-

ene-3,5-dione **3** (commercially available). The compounds were subjected to the reaction with 2-(chloromethyl)oxirane or 2-(bromomethyl)oxirane in anhydrous medium to give oxiranes **2** and **4**, which next were reacted with appropriate amines to give the amino alcohols (compounds **2a – 4f**). The ^{1}H NMR spectra of the compounds were in accordance with the proposed structures. For biochemical studies free bases were converted into corresponding hydrochloride salts.

General procedures

10-(Diphenylmethylene)-4-(oxiran-2-ylmethyl)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (**2**)

A mixture of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione **1** (3 g, 0.0092 mole), 2-(chloromethyl)oxirane (25 mL) and anhydrous K_2CO_3 (3 g, 0.022 mole) was refluxed on water bath for 40 h. The solvent was distilled off, then the oily residue was purified by flash chromatography. Yield 65%, m.p. 188–189^{o}C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 2.56–2.58 (m, 1H, O-CH_2), 2.72–2.75 (m, 1H, O-CH_2), 3.05 (s, 1H, CH-O,), 3.47 (s, 2H, CH-C=O), 3.48–3.63 (2×dd, *J* = 5.6 Hz, 2H, N-CH_2), 3.93 (s, 2H, =CH-*CH*), 6.37 (d, *J* = 6.8 Hz, 2H, CH=), 7.05–7.07 (m, 4H, H_{arom}), 7.29–7.33 (m, 6H, H_{arom}), $C_{25}H_{21}NO_3 \cdot H_2O$ (401.47): calcd.: C 74.79, H 5.77, N 3.49; found: C 75.31, H 5.53, N 3.22.

4-(Amino)-2-hydroxypropyl derivatives of 10-(diphenylmethylene)-4-(oxiran-2-ylmethyl)-4-azatricyclo [5.2.1.0^{2,6}]dec-8-ene-3,5-dione (**2a - i)**; general procedure

A mixture of **2** (0.35g, 0.00091 mol), an appropriate amine (0.0046 mol) and water (1 mL) was heated on water bath at 75^{o}C for 50 h. The liquid was distilled off and the oily residue was purified by flash chromatography to give compounds **2a – i.**

2a. Yield 40%, m.p. 224–226^{o}C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 2.18–2.32 (m, 8H, $(CH_3)_2$, CH_2-N), 3.37–3.42 (dd, *J* = 4.4, 4.4, Hz, 1H, N-CH_2), 3.43–3.51 (m, 3H, CH-C=O, CH_2-N), 3.76–3.81 (m, 1H, *CH*-OH), 3.92 (s, 2H, =CH-*CH*), 6.35 (m, 2H, CH=), 7.05–7.07 (d, *J* = 6.8 Hz, 4H, H_{arom}), 7.28–7.32 (m, 6H, H_{arom}), $C_{27}H_{29}ClN_2O_3$ (465.0): calcd.: C 69.74, H 6.29, N 6.03; found: C 69.54, H 6.40, N 6.05.

2b. Yield 45%, m.p. 235–237^{o}C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.07–1.26 (s, 9H, $(CH_3)_3$), 2.54–2.59 (m, 1H, *CH*$_2$-NH), 2.80–2.83 (m, 1H, *CH*$_2$-NH), 3.43–3.48 (dd, *J* = 5.6, 6 Hz, 1H, N-CH_2), 3.49–3.57 (m, 3H, CH-C=O, N-CH_2), 3.89 (s, 2H, =CH-*CH*), 3.95–3.96 (m, 1H, *CH*-OH), 6.33–6.35 (m, 1H, CH=), 6.40–6.42 (m, 1H, CH=), 7.05–7.07 (d, 4H, H_{arom}), 7.27–7.32 (m, 6H, H_{arom}), $C_{29}H_{33}ClN_2O_3 \times ½ H_2O$ (502.06): calcd.: C 69.38, H 6.83, N 5.58; found: C 69.69, H 6.87, N 5.55.

2c. Yield 45%, oil, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.00–1.03 (t, *J* = 7 Hz, 6H, $(CH_3)_2$), 2.28-2.31 (m, 1H, CH_2-N), 2.33 (m, 1H, CH_2-N), 2.43–2.67 (m, 4H, N$(CH_2)_2$), 2.34–2.38 (dd, *J* = 4.4, 4.4 Hz, 1H, N-CH_2), 3.45–3.52 (m, 3H, N-CH_2, CH-C=O), 3.72–3.78 (m, 1H, *CH*-OH), 3.93 (s, 2H, =CH-*CH*), 6.35 (m, 2H, CH=), 7.05–7.07 (m, 4H, H_{arom}), 7.28–7.32 (m, 6H, H_{arom}), $C_{29}H_{32}N_2O_3 \times 3½ H_2O$ (519.66): calcd.: C 67.03, H 6.56, N 5.39; found: C 67.05, H 6.33, N 5.16.

2d. Yield 50%, m.p. 217–219^{o}C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.07–1.12 (d, *J* = 6.4 Hz, 6H, $(CH_3)_2$), 2.49–2.54 (m, 1H, *CH*$_2$-NH), 2.69–2.73 (dd, *J* = 3.6, 3.6 Hz, 1H, *CH*$_2$-NH), 2.82–2.89 (m, 1H, *CH*-$(CH_3)_2$), 3.41–3.52 (m, 4H, CH-C=O, N-CH_2), 3.80–3.82 (m, 1H, *CH*-OH), 3.91 (s, 2H, =CH-*CH*), 6.34–6.38 (m, 2H, CH=), 7.05–7.07 (d, 4H, H_{arom}), 7.28–7.32 (m, 6H, H_{arom}), $C_{28}H_{31}ClN_2O_3 \times \cdot ½ H_2O$ (488.04): calcd.: C 68.91, H 6.61, N 5.74; found: C 68.96, H 6.51, N 5.80.

2e. Yield 50%, m.p. 130–131.5^{o}C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.42–1.43 (m, 2H, $H_{g\ piper}$), 1.54–1.55 (m, 4H, $H_{b\ piper}$), 2.18–2.31 (m, 4H, $H_{a\ piper}$, CH_2-N_{piper}), 2.54 (m, 2H, $H_{a\ piper}$), 3.33-2.37 (dd, *J* = 4.4, 4.4 Hz, 1H, N-CH_2), 3.44–2.51 (m, 3H, CH-C=O, N-CH_2), 3.76– .81 (m, 1H, *CH*-OH), 3.92 (s, 2H, =CH-*CH*), 6.35 (m, 2H, CH=),

Table 1. [^{3}H]CGP-12177 binding data of selected compounds.

Compound × HCl	IC_{50} ± SEM (mM)	K_I ± SEM (μM)
4a	> 100	69.5 ± 6.1
4b	> 100	74.4 ± 3.1
4d	> 100	~ 100
4e	> 100	> 100
4c, 4f, 2d	> 100	> 3.2 each
propranolol	6.5 nM	3 nM

7.05–7.07 (d, J = 6.4 Hz, 4H, H_{arom}), 7.28–7.32 (m, 6H, H_{arom}), $C_{30}H_{32}N_2O_3$ (468.61): calcd.: C 76.89, H 6.88, N 5.89; found: C 76.44, H 6.89, N 5.84.

2f. Yield 45%, m.p. 155–157°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 0.89–0.90 (d, J = 6 Hz, 3H, $CH_{3\ piper}$), 1.16-1.35 (m, 4H, $H_{g\ piper}$, $H_{b\ piper}$), 1.58–1.61 (m, 1H, $H_{b\ piper}$), 1.95–1.98 (t, J = 11 Hz, 1H, $H_{a\ piper}$), 2.21–2.30 (m, 3H, $H_{a\ piper}$), 2.76–2.91 (dd, J = 11.2, 10.8 Hz, 2H, CH_2-N), 3.33–2.37 (dd, J = 4, 4.4 Hz, 1H, N-CH_2), 3.44–3.51 (m, 3H, CH-C=O, N-CH_2), 3.81–3.83 (m, 1H, *CH*-OH), 3.91 (s, 2H, =CH-*CH*), 6.34 (m, 2H, CH=), 7.05–7.07 (d, J = 7.2 Hz, 4H, H_{arom}), 7.27–7.31 (m, 6H, H_{arom}), $C_{31}H_{35}ClN_2O_3 \times 2.5\ H_2O$ (592.15): calcd.: C 66.00, H 7.15, N 4.97; found: C 66.18, H 7.04, N 5.19.

2g. Yield 50%, m.p. 177–179°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 2.33–2.39 (m, 2H, N-CH_2), 2.56–2.57 (m, 2H, CH_2-N), 2.74–2.76 (m, 2H, CH_2-N_{piper}), 3.18 (m, 4H, $(CH_2)_2$-N_{piper}), 3.42–3.55 (m, 4H, CH_2-N_{piper}, CH-C=O), 3.87–3.93 (m, 3H, *CH*-OH, =CH-*CH*), 6.36 (m, 2H, CH=), 6.84–6.92 (s, 3H, $H_{b\ arom}$, $H_{g\ arom}$), 7.06–7.07 (d, J = 6.8 Hz, 4H, H_{arom}), 7.29–7.31 (m, 8H, $H_{a\ arom,}$ H_{arom}), $C_{35}H_{35}N_3O_3$ (545.70): calcd.: C 77.04, H 6.47, N 7.70; found: C 76.68, H 6.81, N 7.63.

2h. Yield 50%, m.p. 69-71°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 2.38–2.40 (d, J = 6.4 Hz, 2H, CH_2-N), 2.53–2.56 (m, 2H, CH_2-N), 2.71–2.74 (m, 2H, CH_2-N_{piper}), 3.40–3.55 (m, 8H, $(CH_2)_3$-N_{piper}, CH-C=O), 3.88–3.93 (m, 3H, *CH*-OH, =CH-*CH*), 6.36 (m, 2H, CH=), 6.61–6.64 (m, 2H, $H_{b\ arom}$, $H_{g\ arom}$), 7.06–7.07 (d, J = 6.4 Hz, 4H, H_{arom}), 7.27–7.33 (m, 6H, H_{arom}), 7.45–7.47 (m, 1H, $H_{b\ arom}$), 8.17–8.18 (m, 1H, $H_{a\ arom}$), $C_{34}H_{36}Cl_2N_4O_3 \times 2\ H_2O$ (619.18): calcd.: C 62.29, H 6.15, N 8.55; found: C 62.14, H 6.16, N 8.45.

2i. Yield 40%, m.p. 228°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 2.33–2.41 (m, 2H, CH_2-N), 2.55–2.57 (m, 2H, CH_2-N), 2.74–2.76 (m, 2H, CH_2-N_{piper}), 3.09 (m, 4H, $(CH_2)_2$-N_{piper}), 3.55–3.41 (m, 4H, CH_2-N_{piper}, CH-C=O), 3.86–3.87 (m, 1H, *CH*-OH), 3.93 (s, 2H, =CH-*CH*), 6.36 (m, 2H, CH=), 6.83–6.87 (m, 2H, $H_{a\ arom}$), 6.92–6.97 (m, 2H, $H_{b\ arom}$), 7.06–7.07 (d, J = 6.4 Hz, 4H, H_{arom}), 7.28–7.32 (m, 6H, H_{arom}), $C_{35}H_{36}Cl_2FN_3O_3 \times ½\ H_2O$ (645.61): calcd.: C 65.11, H 5.78, N 6.51; found: C 65.34, H 5.74, N 6.49.

Synthesis of 4-(oxiran-2-ylmethoxy)-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione (**4**) was published earlier (18).

4-(Amino)-2-hydroxypropoxy derivatives of 4-hydroxy-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione (**4a - f**); general procedure

A mixture of **4** (0.35 g, 0.0015 mol), an appropriate amine (0.0075 mole) and water (1 mL) was left at ambient temperature for 50 h. The liquid was distilled off and the oily residue was purified by flash chromatography to give compounds **4a – f.**

4a. Yield 50%, m.p. 169–171°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.42-1.44 (d, J = 9.2 Hz, 1 H, -CH_2-), 1.68-1.70 (d, J = 9.2 Hz, 1 H, -CH_2-), 2.18-2.24 (m, 7 H, $(CH_3)_2$, CH_2-N), 2.35-2.40 (m, 1H, CH_2-N), 3.13-3.14 (m, 2H, =CH-*CH*), 3.35 (s, 2H, CH-C=O), 3.78–3.82 (m, 2H, CH_2-O-N), 3.93–3.97 (m, 1H, *CH*-OH), 6.08 (s, 2H, CH=), $C_{14}H_{21}N_2O_4Cl \times 1/3\ H_2O$ (322.80): calcd.: C 52.09, H 6.77, N 8.68; found: C 52.33, H 6.55, N 8.67.

Compound **4b** was obtained before by similar method (18).

4c. Yield 60%, m.p. 50–52°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 0.99–1.03 (t, J = 7 Hz, 6 H, $(CH_3)_2$), 1.49-1.51 (d, J = 9.2 Hz, 1 H, -CH_2-), 1.74–1.77 (d, J = 9.2 Hz, 1 H, -CH_2-), 2.47–2.65 (m, 6H, N-$(CH_2)_2$, CH_2-NH), 3.20 (s, 2H, =CH-*CH*), 3.42 (s, 2H, CH-C=O), 3.83–3.92 (m, 2H, CH_2-O-N), 3.99–4.02 (m, 1H, *CH*-OH), 4.38 (s, 1H, *CH*-OH), 6.19 (s, 2H, CH=), $C_{16}H_{25}N_2O_4Cl$ (308.39): calcd.: C 55.73, H 7.30, N 8.13; found: C 56.17, H 7.33, N 8.03.

4d. Yield 50%, m.p. 84–86°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.42–1.43 (m, 2H, -CH_2-), 1.50-1.56 (m, 5H, $H_{b\ piper}$, $H_{g\ piper}$), 1.76–1.78 (d, J = 8.8 Hz, 1 H, $H_{b\ piper}$), 2.34–2.50 (m, 6H, $H_{a\ piper}$, CH_2-N), 3.20–3.21 (m, 2H, =CH-*CH*), 3.43 (s, 2H, CH-C=O), 3.87–3.92 (m, 2H, CH_2-O-N), 4.00–4.01 (m, 1H, *CH*-OH), 6.16 (s, 2H, CH=), $C_{17}H_{24}N_2O_4$ (320.40): calcd.: C 63.73, H 7.55, N 8.75; found: C 63.69, H 7.50, N 8.71.

4e. Yield 56%, m.p. 103–105°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 0.89–0.91 (d, J = 6.4 Hz, 3H, CH_3), 1.13-1.26 (m, 2H, $H_{b\ piper}$), 1.31–1.39 (m, 1H, $H_{g\ piper}$), 1.50–1.52 (d, J = 8.8 Hz, 1H, -CH_2-), 1.58–1.61 (m, 2H, $H_{b\ piper}$), 1.76–1.78 (d, J = 8.8 Hz, 1H, -CH_2-), 1.94–2.00 (t, J = 12.4 Hz, 1H, $H_{a\ piper}$), 2.13–2.20 (t, J = 12.8 Hz, 1H, $H_{a\ piper}$), 2.36–2.44 (m, 2H, $H_{a\ piper}$), 2.78–2.87 (dd, J = 11.2, 11.2 Hz, 2H, CH_2-N_{piper}), 3.18–3.23 (m, 2H, =CH-*CH*), 3.43 (s, 2H, CH-C=O), 3.86–3.92 (m, 2H, CH_2-O-N), 4.00–4.05 (m, 1H, *CH*-OH), 6.16 (s, 2H, CH=), $C_{18}H_{26}N_2O_4 \times 2/3\ H_2O$ (346.44): calcd.: C 62.41, H 7.95, N 8.09; found: C 62.62, H 7.65, N 7.75.

4f. Yield 50%, m.p. 85–87°C, ^{1}H NMR (400.13 MHz, $CDCl_3$): δ (ppm): 1.51–1.52 (d, J = 8.8 Hz, 1H, -CH_2-), 1.77–1.79 (d, J = 8.8 Hz, 1H, -CH_2-), 2.47–2.51 (m, 1H, CH_2-N), 2.56–2.61 (m, 1H, CH_2-N), 2.65–2.69 (m, 2H, CH_2-N_{piper}), 2.74–2.79 (m,

2H, CH_2-N_{piper}), 3.09–3.11 (m, 4H, $(CH_2)_2$-N_{piper}), 3.22–3.24 (m, 2H, =CH-*CH*), 3.44 (s, 2H, CH-C=O), 3.91–3.94 (m, 2H, CH_2-O-N), 3.95–4.09 (m, 1H, *CH*-OH), 6.17 (s, 2H, CH=), 6.92–6.96 (m, 2H, H_{barom}, $H_{g\ arom}$), 6.99–7.07 (m, 2H, $H_{a\ arom,}$ H_{barom}), $C_{22}H_{28}FN_3O_4Cl_2 \times H_2O$ (506.41): calcd.: C 53.88, H 6.17, N 8.57; found: C 53.92, H 5.97, N 8.46.

Pharmacology

***In vitro* studies – radioligand binding experiments**

Adrenoreceptor binding assay (compounds **4a-b** and **4e-d**)

The experiment was carried out on the rat cerebral cortex. [^{3}H]CGP-12177 (48 Ci/mmol) was used. 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^{o}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 buffer containing from seven to eight concentrations (10^{-11}–10^{-4} M) of the investigated compounds. For measuring unspecific binding, propranolol – 1 μM was applied. The incubation was terminated by rapid filtration over 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 (19).

Adrenoceptor binding assay (compounds **4c**, **4f**, **2d**)

Cerebral cortex membranes from male Wistar rats were homogenized (Potter-Elvehjem) in 25 volumes of ice-cold Tris-HCl buffer (Tris 50 mM, pH 7.5; EDTA 5 mM; sucrose 10.27%) and centrifuged at 1000 × *g* for 10 min (4^{o}C). The supernatant was centrifuged at 35,000 × *g* for 20 min (4^{o}C) and recentrifuged (35,000 × *g* for 10 min) twice with 15 mL Tris-HCl buffer (Tris 50 mM, pH 7.5; EDTA 5 mM). The final pellet was resuspended in buffer and frozen at – 80^{o}C.

For saturation binding studies, membranes were incubated with Tris-HCl buffer in a final volume of 0.5 mL containing 110-120 mg protein, 70 nM ICI 118,551 (selective β_2-adrenoceptor antagonist) and eight concentrations (0.01-0.5 nM) of [^{3}H]CGP-12177. For displacement studies, membranes were incubated with Tris-HCl buffer in a final volume of 0.5 mL containing 80-170 mg protein, 70 nM ICI 118,551 and [^{3}H]CGP-12177 at a concentration of 0.07 nM. The incubation (30^{o}C) was terminated after 30 min by rapid filtration though polyethylenimine (0.3%)-pretreated Whatman GF/C filters. Propranolol (10 mM) was used to determine nonspecific binding (15% for [^{3}H]CGP-12177 0.07 nM). Protein concentration was assayed by the method described previously (20). The results are presented in Table 1.

RESULTS

17 new compounds were obtained; among them 7 were tested for β-adrenolytic activity. The investigated compounds exhibit modest affinity for β-adrenergic receptors. For compounds **4a** and **4b** K_i values are below 100 μM.

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