N_1-azinylsulfonyl-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indoles: 5-HT_6 receptor antagonists with pro-cognitive and antidepressant-like properties

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1. Chemistry

Organic transformations were carried out at ambient temperature, unless indicated otherwise. Organic solvents used in this study (Sigma-Aldrich, Chempur) were of reagent grade and were used without purification. All other commercially available reagents were of the highest purity (from Sigma-Aldrich, Fluorochem). All workup and purification procedures were carried out with reagent-grade solvents under ambient atmosphere. Mass spectra were recorded on a UPLC-MS/MS system consisted of a Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C18 column; 2.1 × 100 mm, and 1.7 μm particle size, equipped with Acquity UPLC BEH C18 VanGuard pre-column; 2.1 × 5 mm, and 1.7 μm particle size. The column was maintained at 40°C, and eluted under gradient conditions from 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL min-1. Eluent A: water/formic acid (0.1%, v/v); eluent B: acetonitrile/formic acid (0.1%, v/v). Chromatograms were made using Waters eλ PDA detector. Spectra were analyzed in 200 – 700 nm range with 1.2nm resolution and sampling rate 20 points/s. MS detection settings of Waters TQD mass spectrometer were as follows: source temperature 150 °C, desolvation temperature 350°C, desolvation gas flow rate 600 L h-1, cone gas flow 100 L h-1, capillary potential 3.00 kV, cone potential 40 V. Nitrogen was used for both nebulizing and drying gas. The data were obtained in a scan mode ranging from 50 to 2000 m/z in time 1.0 s intervals. Data acquisition software was MassLynx V 4.1 (Waters). The UPLC/MS purity of all the final compounds was confirmed to be 95% or higher. ^{1}H NMR and ^{13}C NMR spectra were obtained in Varian BB 200 spectrometer using TMS (0.00 ppm) as an internal standard in d_6-DMSO, and were recorded at 300 and 75 MHz, respectively. The *J* values are reported in Hertz (Hz), and the splitting patterns are designated as follows: s (singlet), br. s. (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet).

2. General synthetic procedures

The synthesis of intermediates **6**–**10** and **32**–**33** as well as the synthesis of not commercially available quinoline and isoquinoline sulfonyl chloride was performed according to the procedures reported in literature.[1,2]

2.1. General procedure for the synthesis of final compounds (**11**–**31**)

A mixture of the appropriate 3-(1,2,3,6-tetrahydropyridin-4yl)-1*H*-indol intermediate (0.28 mmol) in CH_2Cl_2 (4 mL), and BTPP (0.31 mmol) was cooled down (ice bath), and arylsulfonyl chloride (0.4 mmol) was added at 0°C in one portion. The reaction mixture was stirred for 2-6 hours under cooling. Then, the solvent was evaporated and the sulfonamides were purified using silica gel column with AcOet/Hex as an eluting system. Final compounds were obtained as hydrochloride salts by treatment of solution of Boc-protected secondary amine in anhydrous ethanol with 1.25 M HCl in MeOH.

2.2. General procedure for the synthesis of final compounds (**37–38**)

Compound **23** (0.25 mmol) was dissolved in acetone (6 ml). Then K_2CO_3 (0.75 mmol) and catalytic amount of KI were added followed by dropwise addition of alkylbromide (0.3 mmol). The reaction was refluxed for 48 h. Inorganic residues were filtered off and organic mixture was concentrated under reduced pressure. The obtained crude product was purified using silica gel with DCM/MeOH as an eluting system. Final compounds were obtained as hydrochloride salts by treatment of solution of Boc-protected secondary amine in anhydrous ethanol with 1.25 M HCl in MeOH.

3. Characterization data for the final compounds

3.1. 3-{[3-(1,2,3,6-Tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**11**)

Yellow solid, 80 mg, LC/MS purity 100%, t_R = 4.96. $C_{22}H_{21}Cl_2N_3O_3S$, MW 462.39. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.65–2.68 (m, 2H), 3.32 (t, *J* = 5.91 Hz, 2H), 3.72–3.74 (m, 2H), 6.28–6.31 (m, 1H), 7.18 (t, *J* = 7.95 Hz, 1H), 7.29 (t, *J* = 7.43 Hz, 1H), 7.58–7.62 (m, 3H), 7.76–7.81 (m, 1H), 7.88 (d, *J* = 7.95 Hz, 1H), 7.98 (d, *J* = 8.47 Hz, 2H), 8.73 (d, *J* = 2.31 Hz, 1H), 9.06 (d, *J* = 2.31 Hz, 1H), 9.21 (br.s., 1H). Monoisotopic Mass for $C_{22}H_{19}N_3O_2S$ 389.12, $[M+H]^+$ 390.1.

3.2. 6-{[3-(1,2,3,6-Tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**12**, **PZ-570**)

Yellow solid, 90 mg, LC/MS purity 100%, t_R = 4.72. $C_{22}H_{21}Cl_2N_3O_3S$, MW 462.39. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.62–2.65 (m, 2H), 3.28 (t, *J* = 6.15 Hz, 2H), 3.72–3.74 (m, 2H), 6.28–6.30 (m, 1H), 7.11–7.16 (m, 1H), 7.20–7.23 (m, 1H), 7.44 (q, *J* = 4.15 Hz, 1H), 7.54–7.56 (m, 2H), 7.87–7.98 (m, 3H), 8.25 (d, *J* = 8.46 Hz, 1H), 8.46 (s, 1H), 8.88 (d, *J* = 4.46, 2.05 Hz, 1H), 9.19 (br.s., 1H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 24.32, 41.25, 55.94, 113.89, 118.45, 121.30, 122.65, 124.25, 125.10, 125.41, 128.00, 128.28, 128.57, 128.95, 129.80, 131.62, 133.06, 134.92, 135.38, 147.71, 154.71. Monoisotopic Mass for $C_{22}H_{19}N_3O_2S$ 389.12, $[M+H]^+$ 390.1. Anal. calcd for . $C_{22}H_{21}Cl_2N_3O_3S$: C: 57.15, H: 4.58, N: 9.09, S: 6.93; Found C: 57.51, H: 4.86, N: 9.19, S: 6.89. Mp for $C_{22}H_{21}Cl_2N_3O_3S$: 235.0–235.3°C.

3.3. 7-{[3-(1,2,3,6-Tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydriochloride (**13**)

Yellow solid, 80 mg, LC/MS purity 99%, t_R = 4.85. $C_{22}H_{21}Cl_2N_3O_3S$, MW 462.39. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.64–2.68 (m, 2H), 3.31 (t, *J* = 5.9 Hz, 2H), 3.70–3.72 (m, 2H), 6.29–6.31 (m, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.58–7.62 (m, 3H), 7.76 (dd, *J* = 8.4, 4.2 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.58 (s, 1H), 9.06 (d, *J* = 4.2 Hz, 1H), 9.20 (br.s., 1H). Monoisotopic Mass for $C_{22}H_{19}N_3O_2S$ 389.12, $[M+H]^+$ 390.1.

3.4. 3-{[3-(1,2,3,6-Tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**14**)

Yellow solid, 80 mg, LC/MS purity 98%, t_R = 4.68. $C_{22}H_{21}Cl_2N_3O_3S$, MW 462.39. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.63–2.67 (m, 2H), 3.30 (t, *J* = 5.9 Hz, 2H), 3.71–3.74 (m, 2H), 6.29–6.32 (m, 1H), 7.19 (t, *J* = 7.9 Hz, 1H), 7.27 (t, *J* = 7.4 Hz, 1H), 7.56–7.62 (m, 3H), 7.88–7.99 (m, 2H), 8.25–8.30 (m, 2H), 8.52 (s, 1H), 9.21 (br.s., 1H), 9.49 (s, 1H). Monoisotopic Mass for $C_{22}H_{19}N_3O_2S$ 389.12, $[M+H]^+$ 390.1.

3.5. 4-{[3-(1,2,3,6-Tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**15**)

Yellow solid, 80 mg, LC/MS purity 100%, t_R = 4.62. $C_{22}H_{21}Cl_2N_3O_3S$, MW 462.39. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.63–2.68 (m, 2H), 3.31 (t, *J* = 5.9 Hz, 2H), 3.70–3.74 (m, 2H), 6.29–6.33 (m, 1H), 7.19 (t, *J* = 7.9 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 7.55–7.62 (m, 3H), 7.85–7.91 (m, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 8.72 (d, *J* = 8.7 Hz, 1H), 9.12 (s, 1H), 9.19 (br.s., 1H), 9.40 (s, 1H). Monoisotopic Mass for $C_{22}H_{19}N_3O_2S$ 389.12, $[M+H]^+$ 390.1.

3.6. 3-{[5-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**16**)

Yellow solid, 100 mg, LC/MS purity 99%, t_R = 5.34. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.54 (t, *J* = 5.7 Hz, 2H), 3.16 (t, *J* = 5.6 Hz, 2H), 3.72–3.75 (m, 2H), 6.28–6.31 (m, 1H), 6.97–7.02 (m, 1H), 7.14–7.20 (m, 2H), 7.69 (dd, *J* = 8.2, 7.0 Hz, 1H), 7.88 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 8.16-8.20 (m, 2H), 8.67 (d, *J* = 2.3 Hz, 1H), 9.15 (br.s., 1H), 9.20 (d, *J* = 2.3 Hz, 1H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.2.

3.7. 5-{[5-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**17**)

Yellow solid, 80 mg, LC/MS purity 99%, t_R = 5.49. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.52 (t, *J* = 5.7 Hz, 2H), 3.17 (t, *J* = 5.6 Hz, 2H), 3.74–3.77 (m, 2H), 6.29–6.31 (m, 1H), 7.01–7.05 (m, 1H), 7.14–7.22 (m, 2H), 7.54 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.86 (dd, *J* = 8.5, 7.0 Hz, 1H), 8.16-8.22 (m, 2H), 8.36 (dd, *J* = 8.5, 1.3 Hz, 1H), 9.01 (dd, *J* = 4.2, 1.7 Hz, 1H), 9.14 (dd,

J = 8.6, 1,7 Hz, 1H), 9.20 (br.s., 1H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.1.

3.8. 7-{[5-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**18**)

Yellow solid, 90 mg, LC/MS purity 99%, t_R = 5.06. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.55–2.60 (m, 2H), 3.14 (t, *J* = 5.6 Hz, 2H), 3.71–3.76 (m, 2H), 6.28–6.31 (m, 1H), 7.00–7.06 (m, 1H), 7.14–7.20 (m, 2H), 7.66 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 8.19 (s, 1H), 8.30 (dd, *J* = 8.4, 1.9 Hz, 1H), 8.56 (s, 1H), 9.04 (dd, *J* = 4.2, 1.9 Hz, 1H), 9.18 (br.s., 1H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 24.29, 41.50, 56.22, 115.46, 119.49, 121.04, 122.13, 122.78, 125.20, 125.99, 127.56, 128.35, 129.53, 131.27, 131.64, 133.73, 137.63, 138.03, 145.30, 152.93. Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.1. Anal. calcd for $C_{22}H_{20}Cl_3N_3O_2S$: C: 53.18, H: 4.06, N: 8.46, S: 6.45; Found C: 53.15, H: 4.43, N: 8.11, S: 6.72. Mp for $C_{22}H_{20}Cl_3N_3O_2S$: 231.5–232.5°C.

3.9. 4-{[5-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**19**)

Yellow solid, 90 mg, LC/MS purity 98%, t_R = 4.99. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.58–2.61 (m, 2H), 3.14 (t, *J* = 5.6 Hz, 2H), 3.72–3.77 (m, 2H), 6.28–6.33 (m, 1H), 7.01–7.06 (m, 1H), 7.85–7.91 (m, 1H), 8.08–8.12 (d, *J* = 8.2 Hz, 1H), 8.70 (d, *J* = 8.5 Hz, 1H), 9.11 (s, 1H), 9.20 (br.s., 1H), 9.40 (s, 1H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.2.

3.10. 3-{[5-Methoxy-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**20**)

Yellow solid, 90 mg, LC/MS purity 99%, t_R = 4.39. $C_{23}H_{23}Cl_2N_3O_3S$, MW 492.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.53 (t, *J* = 5.6 Hz, 2H), 3.17 (t, *J* = 5.6 Hz, 2H), 3.73–3.75 (m, 2H), 3.75 (s, 3H), 6.29–6.31 (m, 1H), 6.97–7.03 (m, 1H), 7.14–7.20 (m, 2H), 7.69 (td, *J* = 7.9, 1.0 Hz, 1H), 7.88 (td, *J* = 8.5, 1.5 Hz, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 8.19 (d, *J* = 8.5 Hz, 2H), 8.69 (d, *J* = 2.3 Hz, 1H), 9.15 (br.s., 1H), 9.25 (d, *J* = 2.3 Hz, 1H). Monoisotopic Mass for $C_{23}H_{21}N_3O_3S$ 419.13, $[M+H]^+$ 420.1.

3.11. 5-{[5-Methoxy-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**21**)

Yellow solid, 80 mg, LC/MS purity 99%, t_R = 4.35. $C_{23}H_{23}Cl_2N_3O_3S$, MW 492.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.54 (t, *J* = 5.65 Hz, 2H), 3.17 (t, *J* = 5.64 Hz, 2H), 3.73–3.75 (m, 2H), 3.76 (s, 3H), 6.28–6.31 (m, 1H), 6.98–7.03 (m, 1H), 7.14–7.20 (m, 2H), 7.50 (dd, *J* = 8.6, 4.2 Hz, 1H), 7.86 (dd, *J* = 8.5, 7.0 Hz, 1H), 8.11–8.20 (m, 2H), 8.36 (dd, *J* = 8.5, 1.3 Hz, 1H), 9.01 (dd, *J* = 4.2, 1.7 Hz, 1H), 9.14 (dd, *J* = 8.6, 1,7 Hz, 1H), 9.20 (br.s., 1H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 24.27, 41.58, 56.13, 104.79, 113.94, 114.20, 114.89, 118.86, 123.00, 125.07, 126.11, 128.13, 129.41, 129.70, 130.20, 134.18, 137.73, 145.97, 157.14. Monoisotopic Mass for $C_{23}H_{21}N_3O_3S$ 419.13, $[M+H]^+$ 420.1. Anal. calcd for $C_{23}H_{23}Cl_2N_3O_3S$: C: 56.10, H: 4.71, N: 8.53, S: 6.51; Found C: 56.27, H: 4.44, N: 8.82, S: 6.53. Mp for $C_{23}H_{23}Cl_2N_3O_3S$: 205.9–206.5°C.

3.12. 6-{[5-Methoxy-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**22**)

Yellow solid, 100 mg, LC/MS purity 97%, t_R = 4.35. $C_{23}H_{23}Cl_2N_3O_3S$, MW 492.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.56 (t, *J* = 5.6 Hz, 2H), 3.18 (t, *J* = 5.6 Hz, 2H), 3.72–3.75 (m, 2H), 3.77 (s, 3H), 6.29–6.31 (m, 1H), 6.99–7.03 (m, 1H), 7.14–7.20 (m, 2H), 7.58 (dd, *J* = 8.3, 4.5 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 8.19-8.24 (m, 2H), 8.29 (dd, *J* = 8.3, 1.7 Hz, 1H), 8.42 (s, 1H), 9.06 (dd, *J* = 4.5, 1.7 Hz, 1H), 9.20 (br.s., 1H). Monoisotopic Mass for $C_{23}H_{21}N_3O_3S$ 419.13, $[M+H]^+$ 420.1.

3.13. 7-{[5-Methoxy-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**23**)

Yellow solid, 110 mg, LC/MS purity 100%, t_R = 4.12. $C_{23}H_{23}Cl_2N_3O_3S$, MW 492.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.74 (t, *J* = 5.7 Hz, 2H), 3.15 (t, *J* = 5.7 Hz, 2H), 3.73–3.74 (m, 2H), 3.77 (s, 3H), 6.28–6.29 (m, 1H), 7.02 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.24 (d, *J* = 2.6 Hz, 1H), 7.72 (q, *J* = 4.0 Hz, 1H), 7.96–8.05 (m, 3H), 8.19 (d, *J* = 8.5 Hz, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 8.62 (s, 1H), 9.05 (dd, *J* = 4.9, 1.5 Hz, 1H), 9.15 (br.s., 1H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 24.30, 41.47, 55.93, 104.82, 113.27, 115.76, 118.79, 122.77, 125.17, 128.03, 129.77, 131.12, 131.45, 136.97, 138.42, 138.24, 140.48, 145.13, 152.76, 157.05. Monoisotopic Mass for $C_{23}H_{21}N_3O_3S$ 419.13, $[M+H]^+$ 420.1. Anal.

calcd for $C_{23}H_{23}Cl_2N_3O_3S$: C: 56.10, H: 4.71, N: 8.53, S: 6.51; Found C: 56.26, H: 4.39, N: 8.22, S: 6.14. Mp for $C_{23}H_{23}Cl_2N_3O_3S$: 206.8–207.5°C.

3.14. 3-{[5-Methoxy-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**24**)

Yellow solid, 80 mg, LC/MS purity 98%, t_R = 4.21. $C_{23}H_{23}Cl_2N_3O_3S$, MW 492.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.57 (t, *J* = 5.6 Hz, 2H), 3.16 (t, *J* = 5.6 Hz, 2H), 3.71–3.74 (m, 2H), 3.78 (s, 3H), 6.28–6.31 (m, 1H), 6.99–7.03 (m, 1H), 7.16–7.20 (m, 2H), 7.90–7.99 (m, 3H), 8.25–8.31 (m, 2H), 8.53 (s, 1H), 9.20 (br.s., 1H), 9.47 (s, 1H). Monoisotopic Mass for $C_{23}H_{21}N_3O_3S$ 419.13, $[M+H]^+$ 420.1.

3.15. 4-{[5-Methoxy-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**25**)

Yellow solid, 90 mg, LC/MS purity 99%, t_R = 4.22. $C_{23}H_{23}Cl_2N_3O_3S$, MW 492.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.56 (t, *J* = 5.6 Hz, 2H), 3.13 (t, *J* = 5.6 Hz, 2H), 3.71–3.74 (m, 2H), 3.77 (s, 3H), 6.28–6.31 (m, 1H), 6.99–7.04 (m, 1H), 7.88–7.92 (m, 1H), 8.08–8.13 (d, *J* = 8.2 Hz, 1H), 8.75 (d, *J* = 8.7 Hz, 1H), 9.05 (s, 1H), 9.20 (br.s., 1H), 9.41 (s, 1H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 24.30, 41.58, 56.08, 104.91, 114.24, 118.82, 121.91, 122.98, 125.56, 127.18, 128.02, 128.68, 129.03, 129.38, 129.71, 130.13, 134.43, 145.33, 156.97, 160.31. Monoisotopic Mass for $C_{23}H_{21}N_3O_3S$ 419.13, $[M+H]^+$ 420.1. Anal. calcd for $C_{23}H_{23}Cl_2N_3O_3S$: C: 56.10, H: 4.71, N: 8.53, S: 6.51; Found C: 55.98, H: 4.38, N: 8.87, S: 6.65. Mp for $C_{23}H_{23}Cl_2N_3O_3S$: 198.8–199.6°C.

3.16. 3-{[6-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**26**)

Yellow solid, 90 mg, LC/MS purity 99%, t_R = 4.80. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.71–2.75 (m, 2H), 3.29–3.31 (m, 2H), 3.72–3.74 (m, 2H), 6.29–6.31 (m, 1H), 7.37 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.77–7.82 (m, 1H), 7.88 (d, *J* = 8.7 Hz, 1H), 7.98 (dd, *J* = 6.9, 1.5 Hz, 1H), 8.07–8.12 (m, 3H), 8.27 (d, *J* = 8.0 Hz, 1H), 9.21 (br.s., 1H), 9.42 (m, 2H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.1.

3.17. 6-{[6-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**27**)

Yellow solid, 80 mg, LC/MS purity 100%, t_R = 5.16. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.62–2.65 (m, 2H), 3.28 (t, *J* = 6.2 Hz, 2H), 3.72–3.74 (m, 2H), 6.28–6.30 (m, 1H), 7.12 (d, *J* = 8.7 Hz, 1H), 7.46–7.54 (m, 3H), 7.88–7.95 (m, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 8.48 (s, 1H), 8.74 (s, 1H), 8.88 (d, *J* = 4.4 Hz, 1H), 9.21 (br.s., 1H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.2.

3.18. 7-{[6-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**28**)

Yellow solid, 100 mg, LC/MS purity 97%, t_R = 4.52. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.72–2.75 (m, 2H), 3.29–3.31 (m, 2H), 3.73–3.75 (m, 2H), 6.28–6.30 (m, 1H), 7.36 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.74 (dd, *J* = 8.1, 4.7 Hz, 1H), 7.87 (d, *J* = 8.5, 1H), 8.07–8.16 (m, 3H), 8.24 (d, *J* = 8.72 Hz, 1H), 8.49 (d, *J* = 8.2 Hz, 1H), 8.74 (s, 1H), 9.08 (d, *J* = 2.6 Hz, 1H), 9.21 (br.s., 1H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.2.

3.19. 3-{[6-Chloro-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**29**)

Yellow solid, 60 mg, LC/MS purity 98%, t_R = 4.34. $C_{22}H_{20}Cl_3N_3O_2S$, MW 496.84. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.72–2.74 (m, 2H), 3.28–3.30 (m, 2H), 3.72–3.75 (m, 2H), 6.29–6.31 (m, 1H), 7.37 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.87 (d, *J* = 8.5 Hz, 1H), 7.93-7.99 (m, 4H), 8.27-8.31 (m, 2H), 8.56 (s, 1H), 9.22 (br.s., 1H), 9.47 (s, 1H). Monoisotopic Mass for $C_{22}H_{18}ClN_3O_2S$ 423.08, $[M+H]^+$ 424.1.

3.20. 3-{[2-Methyl-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**30**)

Yellow solid, 60 mg, LC/MS purity 98%, t_R = 4.84. $C_{23}H_{23}Cl_2N_3O_2S$, MW 476.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.54 (s, 3H), 2.64–2.68 (m, 2H), 3.34 (t, *J* = 5.9 Hz, 2H), 3.71–3.73 (m, 2H), 6.28–6.30 (m, 1H), 7.18 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.58–7.62 (m, 3H), 7.76–7.81

(m, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 8.71 (d, *J* = 2.3 Hz, 1H), 9.06 (d, *J* = 2.3 Hz, 1H), 9.20 (br.s., 1H). Monoisotopic Mass for $C_{23}H_{21}N_3O_2S$ 403.14, $[M+H]^+$ 404.3.

3.21. 7-{[2-Methyl-3-(1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**31**)

Yellow solid, 70 mg, LC/MS purity 97%, t_R = 4.77. $C_{23}H_{23}Cl_2N_3O_2S$, MW 476.42. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.52 (s, 3H), 2.64–2.68 (m, 2H), 3.33 (t, *J* = 5.9 Hz, 2H), 3.71–3.73 (m, 2H), 6.28–6.31 (m, 1H), 7.18 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.58–7.62 (m, 3H), 7.88 (d, *J* = 8.5 Hz, 1H), 8.11 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.56 (s, 1H), 9.05 (d, *J* = 4.2 Hz, 1H), 9.19 (br.s., 1H). Monoisotopic Mass for $C_{23}H_{21}N_3O_2S$ 403.14, $[M+H]^+$ 404.1.

3.22. 7-{[5-Methoxy-3-(piperidin-4-yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**34**)

Yellow solid, 70 mg, LC/MS purity 100%, t_R = 4.87. $C_{23}H_{25}Cl_2N_3O_3S$ MW 494.43. ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.11–1.64 (m, 5H), 2.54–2.64 (m, 3H), 2.99–3.04 (m, 2H), 3.54 (s, 3H), 7.03 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.22 (d, *J* = 2.1 Hz, 1H), 7.71 (dd, *J* = 8.2, 4.9 Hz, 1H), 7.96–8.01 (m, 3H), 8.11 (d, *J* = 8.5 Hz, 1H), 8.45 (dd, *J* = 8.2, 1.5 Hz, 1H), 8.60 (s, 1H), 9.05 (dd, *J* = 4.9, 1.5 Hz, 1H), 9.21 (br.s., 1H). Monoisotopic Mass for $C_{23}H_{23}N_3O_3S$ 421.15, $[M+H]^+$ 422.2.

3.23. 4-{[5-Methoxy-3-(piperidin-4-yl)-1H-indol-1-yl]sulfonyl}isoquinoline dihydrochloride (**35**)

Yellow solid, 90 mg, LC/MS purity 99%, t_R = 4.75. $C_{23}H_{25}Cl_2N_3O_3S$ MW 494.43. ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.11–1.64 (m, 5H), 2.54–2.64 (m, 3H), 2.99–3.04 (m, 2H), 3.54 (s, 3H), 7.03 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.22 (d, *J* = 2.1 Hz, 1H), 7.71 (dd, *J* = 8.2, 4.9 Hz, 1H), 7.97–8.01 (m, 2H), 8.10 (d, *J* = 8.2 Hz, 1H), 8.72 (d, *J* = 8.7 Hz, 1H), 9.06 (s, 1H), 9.22 (br.s., 1H), 9.40 (s, 1H). Monoisotopic Mass for $C_{23}H_{23}N_3O_3S$ 421.15, $[M+H]^+$ 422.2.

3.24. 7-{[5-Methoxy-3-(1-methyl-1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**36**)

Yellow solid, 90 mg, LC/MS purity 99%, t_R = 4.47. $C_{24}H_{25}Cl_2N_3O_3S$, MW 506.44. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.40 (s, 3H), 2.74 (t, *J* = 5.65 Hz, 2H), 3.15 (t, *J* = 5.64 Hz, 2H), 3.73–3.74 (m, 2H), 3.77 (s, 3H), 6.28–6.29 (m, 1H), 7.02 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 7.72

(dd, J = 8.2, 4.9 Hz, 1H), 7.96–8.03 (m, 3H), 8.10 (d, J = 8.5 Hz, 1H), 8.46 (dd, J = 8.2, 1.5 Hz, 1H), 8.61 (s, 1H), 9.07 (dd, J = 4.9, 1.5 Hz, 1H), 9.20 (br.s., 1H). Monoisotopic Mass for $C_{24}H_{23}N_3O_3S$ 433.15, $[M+H]^+$ 434.4.

3.25. 7-{[5-Methoxy-3-(1-propyl-1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**37**)

Yellow solid, 80 mg, LC/MS purity 97%, t_R = 5.03. $C_{26}H_{29}Cl_2N_3O_3S$, MW 534.50. ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.93 (t, J = 7.2 Hz, 3H), 1.60–1.70 (m, 2H), 2.74 (t, J = 5.6 Hz, 2H), 3.15 (t, J = 5.6 Hz, 2H), 3.27 (t, J = 7.2 Hz, 2H), 3.73–3.75 (m, 2H), 3.57 (s, 3H), 6.28–6.29 (m, 1H), 7.01 (dd, J = 8.9, 2.3 Hz, 1H), 7.22 (d, J = 2.3 Hz, 1H), 7.72 (dd, J = 8.2, 4.9 Hz, 1H), 7.96–8.04 (m, 3H), 8.11 (d, J = 8.5 Hz, 1H), 8.46 (dd, J = 8.2, 1.5 Hz, 1H), 8.63 (s, 1H), 9.05 (dd, J = 4.9, 1.5 Hz, 1H), 9.21 (br.s., 1H). Monoisotopic Mass for $C_{26}H_{27}N_3O_3S$ 461.18, $[M+H]^+$ 462.3.

3.26. 7-{[5-Methoxy-3-(1-butyl-1,2,3,6-tetrahydropyridin-4yl)-1H-indol-1-yl]sulfonyl}quinoline dihydrochloride (**38**)

Yellow solid, 80 mg, LC/MS purity 99%, t_R = 5.37. $C_{27}H_{31}Cl_2N_3O_3S$, MW 548.52. ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.91 (t, J = 7.2 Hz, 3H), 1.34-1.47 (m, 2H), 1.62-1.70 (m, 2H), 2.75 (t, J = 5.6 Hz, 2H), 3.16 (t, J = 5.6 Hz, 2H), 3.24 (t, J = 7.2 Hz, 2H), 3.72–3.75 (m, 2H), 3.56 (s, 3H), 6.28–6.30 (m, 1H), 7.01 (dd, J = 8.9, 2.3 Hz, 1H), 7.22 (d, J = 2.3 Hz, 1H), 7.74 (dd, J = 8.2, 4.9 Hz, 1H), 7.96–8.02 (m, 3H), 8.11 (d, J = 8.5 Hz, 1H), 8.47 (dd, J = 8.2, 1.5 Hz, 1H), 8.61 (s, 1H), 9.06 (dd, J = 4.9, 1.5 Hz, 1H), 9.20 (br.s., 1H). Monoisotopic Mass for $C_{27}H_{29}N_3O_3S$ 475.19, $[M+H]^+$ 476.3.

4. *In vitro* pharmacology

4.1. Radioligand binding assay protocol

Radioligand binding assays were employed to determine the affinity and selectivity profiles of the synthesized compounds in competition binding experiments for human serotonin, 5-HT_6, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{7B} and D_{2L} receptors, which were all stably expressed in HEK293 cells. According to the previously published procedures,[3-5] the experiments were carried out using [^{3}H]-LSD (85.2 Ci/mmol), [^{3}H]-8-OH-DPAT (187 Ci/mmol), [^{3}H]-Ketanserin (66.9 Ci/mmol), [^{3}H]-5-CT (39.2 Ci/mmol) and [^{3}H]-Raclopride (74.4 Ci/mmol) for 5-HT_6, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{7B} and D_2, respectively. Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35 000 g for 20 min at 4°C, with incubation for 15 min at 37°C in between. The composition of the assay buffers was as follows: for 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbate; for 5-HT_6R: 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$, for 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbate; for dopamine D_{2L}R: 50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate. All assays were incubated in a total volume of 200 µl in 96-well microtitre plates for 1 h at 37°C, except for 5-HT_{1A}R that was incubated for 1 h at room temperature. The process of equilibration is terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader. Non-specific binding is defined with10 µM of 5-HT in 5-HT_{1A}R and 5-HT_7R binding experiments, whereas10 µM of methiothepine or 1 µM of (+)butaclamol were used in 5-HT_6R and D_{2L} assays, respectively. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation.[6] Results were expressed as means of at least three separate experiments. Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on described protocols (Perkin Elmer). Membrane preparation and general assay procedures for cloned receptors were adjusted to 96-microwell format based on described protocols. The percentage of inhibition for α_1, α_{2C}, H_1, M_1, M_5, 5-HT_{2B}, 5-HT_{2C}, D_3, hERG receptors and SerT for selected compounds **28** and **30** were evaluated at Eurofins Cerep. Experimental conditions for these assays are described online at www.cerep.fr.

4.2. Functional cAMP assay protocol

The antagonist properties of the selected compounds were evaluated in functional cAMP cellular assays, performed at Eurofins Cerep using HEK293 cells, which stably expressed the human 5-HT_6Rs. K_b values were calculated from Cheng-Prusoff equation[6] specific for the analysis of functional inhibition curves: $K_b = IC_{50}/(1+A/EC_{50})$ where A is an agonist concentration, IC_{50} is the concentration of antagonist producing a 50% reduction in the response to agonist, and EC_{50} is the agonist concentration which causes a half of the maximal response. Experimental conditions for these assays are described online at www.cerep.fr.

5. *In vitro* metabolic stability

In vitro biotransformation assays of selected compounds **23** and **25** were performed according to the previously published procedures.[7,8] Test compounds incubation (20 μM) with rat liver microsomes (RLM 0.2 mg/ml) was performed in 100 mM potassium phosphate buffer (PB), pH 7.4, at 37°C for 15 min in a final reaction mixture volume of 150 μl. The reaction was initiated by the addition of an NADPH-regenerating system (NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase in 100 mM PB, pH 7.4) to the incubation mixture. The blank probe without the NADPH – regenerating system was performed in parallel. Subsequently, the samples were incubated at 37°C for 15, 30, 60, 90 min with gentle shaking. Next, an internal standard (levallorphan, 20 μM) was added. The reaction was terminated at different time points with perchloric acid (69-72%, by volume). Thereafter, all samples were centrifuged and the supernatant was analyzed using UPLC/MS in order to determine the quantity of starting material left in solution. All samples were prepared in duplicate. The *in vitro* half-time ($t_{1/2}$) for test compounds was determined from the slope of the linear regression of ln % parent compound remaining versus incubation time. The calculated $t_{1/2}$ was incorporated into the following equation to obtain intrinsic clearance (Cl_{int}) = (volume of incubation [μl]/ protein in the incubation [mg]) × $0.693/t_{1/2}$. RLM, glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase and levallorphan were obtained from Sigma Aldrich (Poznań, Poland).

6. AMES test

AMES test for selected compounds **23** and **25** were performed according to the previously published procedure.[9]

Table 1-SI. Results of Ames test performed on *Salmonella typhimurium* strains TA100, TA1535 and TA98, and TA1537 for selected compounds.

		No of revertants/plate: mean±SD[a]			
Compound	Conc. (μg/ml)	TA100	TA1535	TA98	TA1537
23	16	8±1	8±4	10±3	11±4
	8	10±1	8±3	10±1	8±5
	4	4±2	4±1	9±1	10±3
	0,4	3±2	4±1	13±3	7±1
	0,2	7±3	6±2	10±2	5±2
25	16	10±3	8±3	10±3	11±3
	8	7±2	6±1	9±3	6±2
	4	6±2	5±1	7±4	10±2
	0,4	4±1	3±1	6±1	4±1
	0,2	7±2	2±1	6±2	3±2
Negative control[b]	–	5±2	7±4	7±2	3±2
AS[c]	0,5	50±5	67±7	–	–
NPD[d]	0,25	–	–	25±1	21±4

[a] calculated from three independent experiments
[b] DMSO was used as negative control
[c] AS – sodium azide; used as a positive control in *S. typhimurium* TA100 and TA1535
[d] NPD – 4-nitro-*O*-phenylenediamine; used as a positive control in TA98 and TA1537

7. Interaction with cytochrome P450 (CYP) isoforms

Experiments were carried out on human cDNA-expressed cytochrome P450 isoforms (Supersomes) from Corning (Woburn, MA, USA). The enzymatic activity was assessed on the basis of the rate of CYP isoform-specific metabolic reactions (caffeine 3-N-demethylation, diclofenac 4'-hydroxylation, perazine N-demethylation, bufuralol 1'-hydroxylation and testosterone 6β-hydroxylation) as described previously.[10] The reactions proceeded in the absence and presence of the investigated compounds (**23** or **25**), added *in vitro* at the following concentrations: 0.01, 0.1, 1, 10, 50 and 100 μM. Briefly, the activity of CYP1A2 was studied by measuring the rate of caffeine 3-N-demethylation at a substrate concentration of 1 mM, and Supersomes CYP1A2 of 50 pmol/ml. The activity of CYP2C9 was studied by measuring the rate of diclofenac 4'-hydroxylation, at a substrate concentration of 10 μM, and Supersomes CYP2C9 of 100 pmol/ml. The activity of CYP2C19 was studied by measuring the rate of perazine N-demethylation at a substrate concentration of 250 μM, and Supersomes CYP2C19 of 50 pmol/ml. The activity of CYP2D6 was studied by measuring the rate of bufuralol 1'-hydroxylation, at a substrate concentration of 30 μM, and Supersomes CYP2D6 of 50 pmol/ml. The activity of CYP3A4 was studied by measuring the rate of testosterone 6β-hydroxylation, at a substrate concentration of 100 μM, and Supersomes CYP3A4 of 100 pmol/ml. An incubation time for all reactions was 30 min. Caffeine and its metabolite 3-N-desmethylcaffeine, diclofenac and its metabolite 4'-hydroxydiclofenac, perazine and its metabolite *N*-desmethylperazine, testosterone and its metabolite 6β-hydroxylation were analyzed by HPLC with UV detection. Bufuralol and its metabolite 1'-hydroxybufuralol were analyzed by HPLC with fluorimetric detection. The potency of a compound to inhibit enzyme activity was expressed as IC_{50}.

8. Preliminary pharmacokinetic study

8.1. In vivo study of blood-brain barrier penetration

Experiments were carried out on male Wistar rats, 220 - 250 g (Charles River Laboratory, Germany), having free access to food and tape water. The experiment was carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Kraków. Blood-brain barrier penetration was estimated after intraperitoneal (*ip*) administration of **23** or **25** in a dose of 3 mg/kg. 15 min, 30 min or 90 min after administration rats were decapitated, brains removed and trunk blood was collected. 30% sodium citrate solution was used as an anticoagulant agent. Blood samples were centrifuged (2000 g, 10 min). The obtained blood plasma was separated and stored at -20°C until used for analysis. Brains were stored at -80°C until homogenized and analyzed for the concentration of the compounds. Both compounds were analyzed using LC-MS/MS system described below.

8.2 Plasma and brain sample preparation

Brain samples were thawed on ice and the half of each organ was separated and weighted in a 10 mL polypropylene tube. Samples were homogenized in PBS buffer (100 mg : 500 µL of PBS). 100 µL of homogenate was transferred to a 1.5 mL centrifuge tube and then 10 µL of IS working solution (500 ng/mL) was spiked and mixed for 3 min. Samples were purified by adding MeOH or ACN as a precipitation agent. After mixing (10 min, 1000 x g), the samples were placed in fridge (4°C) for 10 min and then centrifuged (1000 x g, 15min, 4°C). The obtained supernatant was diluted with water (1:1, v/v), transferred to the glass insert and injected into the analytical column. The dry residue was dissolved in 100 µL of a mixture of methanol and water (1:1, v/v), transferred to the insert and injected into the analytical column. Calibration and quality control samples were prepared by spiking 100 µL of homogenate with different levels of studied compounds. The sample purification step was the same as described above. For calibration curves calculation linear regression with weighting (1/x or 1/x2) was used. Samples were prepared by precipitation and linearity range [ng/mL] was 0.5 – 250 for **23** and 1 – 500 for **25**. Plasma samples were thawed in room temperature. 100 µL of sample was transferred to a 1.5 mL centrifuge tube and then 10 µL of IS working solution (500 ng/mL) was added and mixed for 3 min. Further purification steps of plasma samples were analogous to the brain samples. The linearity range [ng/mL] was 0.5 – 250 for **23** and 1 – 500 for **25**.

*8.3. Determination of plasma and brain concentrations of **23** and **25***

8.3.1. Materials and reagents

The internal standard 2-(4-methyl-1-piperazinyl)-4-phenylquinazoline (MPPQ), acetone and formic acid (99%) were provided by Sigma Aldrich (St Louis, MO, USA). HPLC-grade solvents acetonitrile and methanol came from Merck (Darmstadt, Germany), ethyl acetate of analytical grade was from POCH (Gliwice, Poland). Other chemicals (sodium chloride, potassium dihydrogen phosphate, sodium phosphate dibasic dihydrate) were from J.T Baker (Deventer, The Netherlands) or Sigma Aldrich (St Louis, MO, USA).

8.3.2. Instrumentation

LC-MS/MS method for each analyte was developed by the application of UHPLC Ultimate 3000 (Dionex) coupled to triple quadrupole mass spectrometer TSQ Quantum Ultra (Thermo Scientific, MA, USA) equipped with HESI II probe. Data acquisition and quantitation were performed using Xcalibur software.

8.3.3. Chromatographic conditions

The separation of studied substances was performed on a Hypersil GOLD C18 (Thermo Scientific) analytical column 150x4.6 mm with a 3µm particle size, which was maintained at 30°C. The mobile phase consisted of 0.1% formic acid in acetonitrile (eluent A) and 0.1% formic acid in water (eluent B). The injection volume of sample was 10 µL and the autosampler temperature was set at 10°C. The analytes were eluted under isocratic condition with the flow rate of solvents set at 800 µL/min. The mobile phase composition was 35% of eluent A and 65% of eluent B (v/v). The retention time t_r [min] was 4.12 for **23** and 4.26 for **25**.

8.3.4. MS/MS conditions

For MS/MS detection a TSQ Quantum Ultra (Thermo Scientific) with heated electrospray ionization source (HESI) operating in positive ion mode was employed. Mass spectrometer parameters such as spray voltage, vaporizer temperature, tube lens, sheath gas (nitrogen) and auxiliary gas (nitrogen) pressure were optimized for each compound. The temperature of transfer capillary was set at 350°C. Chromatograms were obtained using selected reaction monitoring (SRM) scan type. Optimum value of collision energy were found for each analyte. Argon was used as the collision gas. The MS/MS optimized conditions were shown in the Table.

Table 2-SI. Mass spectrometer parameters for each studied compound **23** and **25**

Analyte	MRM Transition (m/z)	CE [V]	Vaporizer Temperature [^{o}C]	Spray Voltage [V]	Sheath gas	Auxiliary gas	Tube Lens [V]
23	420.1→199.0 420.1→391.0	23 13	450	3500	45	10	120
25	420.1→199.0	20	450	4500	45	10	100
MPPQ	305.1→248.0	21		IS			120

9. *In vivo* pharmacology

9.1.Novel object recognition protocol

The test session comprising of two trials separated by an inter-trial interval (ITI) of 1 h was carried out on the next day. During the first trial (familiarization, T1) two identical objects (A1 and A2) were presented in the opposite corners of the open field, approximately 10 cm from the walls. During the second trial (recognition, T2) one of the A objects was replaced by a novel object B, so that the animals were presented with the A=familiar and B=novel objects. Both trials lasted for 3 min and the animals were returned to their home cages after T1. The objects used were the glass beakers filled with the gravel and the plastic bottles filled with the sand. The heights of the objects were comparable (~12 cm) and the objects were heavy enough not to be displaced by the animals. The sequence of presentations and the location of the objects was randomly assigned to each rat.

The animals explored the objects by looking, licking, sniffing or touching the object while sniffing, but not when leaning against, standing or sitting on the object. Any rat exploring the two objects for less than 5 s within 3 min of T1 or T2 was eliminated from the study. Exploration time of the objects and the distance traveled were measured using the Any-maze® video tracking system. Based on exploration time (E) of two objects during T2, discrimination index (DI) was calculated according to the formula: $DI = (E_B–E_A)/(E_A+A_B)$.

Phencyclidine (PCP), used to attenuate learning, was administered at the dose of 5 mg/kg (*i.p.*) 45 min before familiarization phase (T1). The compounds were administrated *i.p.* 1 hour and 15 min before T1.

9.1.1. Animal

The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee for Animal Experiments, Institute of Pharmacology. Male Sprague–Dawley rats (Charles River, Germany) weighing ~250 g at the arrival were housed in the standard laboratory cages, under standard colony A/C controlled conditions: room temperature 21 ± 2°C, humidity (40–50 %), 12-hr light/dark cycle (lights on: 06:00) with ad libitum access to food and water. Rats were allowed to acclimatize for at least 7 days before the start of the experimental procedure. During this week animals were handled for at least 3 times. Behavioral testing was carried out during the light phase of the light/dark cycle. At least 1 h before the start of the experiment, rats were transferred to the experimental room for acclimation. Rats were tested in a dimly lit (25 lx) “open field” apparatus made of a dull gray plastic

(66 × 56 × 30 cm). After each measurement, the floor was cleaned and dried. The procedure lasting for 2 days consisted of the habituation to the test arena (without any objects) for 5 min.

9.1.2. Statistics

Data on exploratory preference were analyzed using three-way mixed-design ANOVAs with ketamine and the respective drug treatment as between-subject factors and object as a repeated measure; DI data were analyzed by two-way ANOVAs, and distance travelled was analyzed using mixed-design ANOVAs with ketamine and the respective drug treatment as between-subject factors and trial as a repeated measure.

9.2. Modified forced swim test in rats

The experiment was carried out according to the method of Porsolt *et al*. modified by Detke *et al*. (1995).[11,12] On the first day of an experiment, the animals were gently individually placed in Plexiglas cylinders (50 cm high, 18 cm in diameter) containing 30 cm of water maintained at 23–25 °C for 15 min. On removal from water, the rats were placed for 30 min in a Plexiglas box under a 60-W bulb to dry. On the following day (24 h later), the rats were re-placed in the cylinder and the total duration of immobility, swimming, and climbing was recorded during the whole 5-min test period. The swimming behavior entailed active swimming motions, e.g., moving horizontally around in the cylinder. Climbing activity consisted of upward directed movements of the forepaws along the side of the swim chamber, and immobility was assigned when no additional activity was observed other than that necessary to keep the rat's head above the water.[6]Fresh water was used for each animal. Compounds were given *i.p*. 60 min before the test.

9.3. Open field test in rats

The experiment was performed using Motor Monitor System (Campden Instruments, Ltd., UK) consisted of two Smart Frame Open Field (OF) stations (40 × 40 × 38 cm) with 16 × 16 beams, located in sound attenuating chambers and connected to PC software by control chassis. Individual vehicle- or drug-injected animals were gently placed in the centre of the station. An automated Motor Monitor System the total distance covered by a rat for 5 min.

9.4. Vogel conflict drinking test

Table 3-SI. Effects of compounds **23**, **25** and SB-742457 in the Vogel conflict drinking test in rats.

Compound	Dose [mg/kg]	Number of „licks"/5min	Number of shocks accepted/5min
23	-	132.9 ± 31.3	6.1 ± 1.5
	0.1	106.4 ± 11.2	4.8 ± 0.5
	0.3	99.5 ± 17.5	4.6 ± 0.8
	1.0	105.0 ± 17.5	4.7 ± 0.8
		F(3,27)=0.51950 ns	F(3,27)=0.52333 ns
35	-	127.4 ± 23.1	5.6 ± 1.1
	0.3	105.4 ± 7.3	4.9 ± 0.3
	1.0	155.4 ± 23.5	6.9 ± 1.1
	3.0	115.0 ± 14.0	5.1 ± 0.6
		F(3,26)=1.3009 ns	F(3,26)=1.0082 ns
SB-742457	-	109.7 ± 16.5	5.0 ± 0.8
	1.0	160.5 ± 18.7	7.4 ± 0.8
	3.0	203.9 ± 32.4*	9.1 ± 1.5*
	10.0	110.6 ± 12.1	5.1 ± 0.6
		F(3,25)=4.4177 ns	F(3,25)=3.7914 ns

Data showed as mean ± standard error of mean (SEM), N = 6-8, *p<0.05 (one way ANOVA followed by Bonferroni's post-hoc test)
ns – non-significant

The testing procedure was based on a method of Vogel *et al.*[13] as a conditional model where a noxious stimulus is applied. Anxiety Monitoring System Vogel test produced by TSE Systems (Germany) was used. It was consisted of polycarbonate cages (dimensions 26.5 × 15 × 42 cm), equipped with a grid floor made from stainless steel bars and drinking bottles containing tap water. Experimental chambers were connected to PC software by control chassis and electric shocks' generator. On the first day of the experiment, the rats were adapted to the test chambers and drink water from the bottle spout for 10 min. Afterwards, the rats were returned to their home cages and were given 30 min free access to water followed by a 24 h water deprivation period. The adaptation session and water deprivation protocols were repeated on the second day of the experiment. On the

third day the rats were placed again in the test chambers 60 min after the compounds administration and were given free access to the drinking tube. Recording data started immediately after the first lick and rats were punished with an electric shock (0.5 mA, lasting 1 s) delivered to the metal drinking tube every 20 licks. The number of licks and the number of shocks received during a 5 min experimental session were recorded automatically.

9.5. Animals

The experiments were performed on male Wistar rats (230–260 g). The animals were housed in polycarbonate Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm) in an environmentally controlled room (ambient temperature 21 ± 2 °C; relative humidity 50–60 %; 12:12 light/dark cycle, lights on at 8:00), in groups of four rats. Standard laboratory food (LSM-B) and filtered water were freely available. Animals were assigned randomly to treatment groups. All the experiments were performed by three observers unaware of the treatment applied between 9:00 and 14:00 on separate groups of animals. All animals were used only once. All the experimental procedures were approved by the First Local Ethical Committee on Animal Testing at the Jagiellonian University in Krakow.

9.6. Data analysis

All of the results are shown as the means ± SEM. The data were evaluated by an analysis of variance (ANOVA): one-way ANOVA followed by Bonferroni's multiple comparison test; $p < 0.05$ was considered significant.

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