Supporting Information

Dopamine/Serotonin Receptor Ligands, Part 16[1]: Expanding Dibenz[*d,g*]azecines to 11- and 12-membered Homologues - Interaction with Dopamine D_1-D_5 Receptors

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The supporting Information contains detailed experimental procedures for the pharmacological investigations (radioligand binding studies and functional Ca-assay), the synthesis and analytical data (NMR, GC/MS) of the substances. Purity of the substances is in the "Purity Appendix".

Experimental procedures:

General methods: Melting points are uncorrected and were measured in open capillary tubes, using a Gallenkamp melting point apparatus. ^{1}H and ^{13}C-NMR spectral data were obtained from a Bruker Advance 250 spectrometer (250 MHz) and Advance 400 spectrometer (400 MHz), respectively. Elemental analysis were performed on a Hereaus Vario EL apparatus. TLC was performed on silica gel F254 plates (Merck). MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific).

7-Methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d,g*]azacycloundecene-12-ol (5): Evaporation of the solvent yielded a white foam with >99% GC/MS purity. Yield: 76%. Mp.: 119 - 121°C, ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.9 – 2.1 (mc, 2H, 9); 2.1 - 2.2 (mc, 2H, 8); 2.34 (s, 3H, N-Me); 2.58 (mc, 2 H, 5); 2.66 (mc, 2H, 6); 2.7 (t, *J* = 6.2, 2H, 10); 4.41 (s, br, 1H, 15); 6.58 – 6.63 (dd, *J* = 2.5, 8.3, 1H, 13); 6.75 – 6.77 (d, *J* = 2.5, 1H, 11); 6.82 – 6.85 (d, *J* = 8.5, 1H, 14); 6.95 – 7.3 (m, 4H, 1,2,3,4). GC/MS m/z: 281 (30%); 207 (35%); 195 (22%); 176 (15%); 165 (11%); 146 (10%); 133 (13%) 115 (12%); 84 (18%); 70 (36%); 58 (47%) ; 44 (100%), 32 (95%). Anal. ($C_{19}H_{23}NO$) C,H. N: calcd, 4.98; found, 4.47.

12-Methoxy-7-methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d,g*]azacycloundecene (6): Recrystallization from methanol/diethyl ether Mp.: 78 - 79°C, ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.7 – 1.8 (qin, *J* = 6.4, 2 H, 9); 1.9 - 2.0 (t, *J* = 6.4, 2H, 8); 2.1 (s, 3H, N-Me); 2.3 (mc, 2H, 5); 2.4 (mc, 2H, 6); 2.7 (t, *J* = 6.4, 2H, 10); 3.7 (s, 3H, O-Me); 4.5 (s, br, 1H, 15); 6.49 – 6.54 (dd, *J* = 2.7, 8.4, 1H, 13); 6.67 – 6.68 (d, *J* = 2.7, 1H, 11); 6.70 – 6.74 (d, *J* = 8.4, 1H, 14); 6.92 – 7.2 (m, 4H, 1,2,3,4). HCl salt: Mp.: 198°C, ^{1}H NMR: 250 MHz ($CDCl_3$) 2.2 – 2.6 (mc, 2H); 2.7 (d, *J* = 5, N-Me); 2.8 – 3.4 (mc, 8H); 3.73 (s, 3H, O-Me); 3.86 – 4.17 (mc, *J* = 15, 2H, 15); 6.65 – 6.72 (m, 2H, 11,13); 7.03 d, *J* = 8, 1H, 14) 7.1-7.4 (m, 4H, 1,2,3,4). GC/MS m/z: 295 (32%); 209 (30%); 190 (23%); 178 (20%); 165 (20%); 160 (16%); 146 (11%); 133 (10%) 115 (19%); 84 (20%); 70 (43%); 58 (59%). Anal. ($C_{20}H_{25}NO$) C,H,N.

7-Methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d,g*]azacycloundecene-3-ol x HCl (7):.extracted with ether at pH 9, aqueous phase saturated with K-Na-tartrate. White foam. Yield: 68% Mp.: 137 - 138°C, ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.7 – 1.9 (quin, *J* = 6, 2H, 9); 2.0 - 2.09 (t, *J* = 6, 2H, 8); 2.09 (s, 3H, N-Me); 2.25 – 2.28 (mc, 2 H, 5); 2.41 – 2.45 (mc, 2H, 6); 2.7 (t, *J* = 6, 2H, 10); 4.44 (s, br, 1H, 15); 6.43 – 6.50 (d, *J* = 2.8, 1H, 4) 6.54 – 6.6 (dd, *J* = 2.8, 8, 1H, 2); 6.95 – 7.3 (m, 4H, 11, 12, 13, 14). GC/MS m/z: 281 (70%); 235 (10%); 221 (15%); 209 (25%); 195 (52%); 176 (21%); 160 (33%) 152 (17%); 130 (31%); 115 (30%); 91 (12%) ; 84 (32%); 70 (84%); 58 (100%). Anal. ($C_{19}H_{23}NO$) C,H,N.

3-Methoxy-7-methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d,g*]azacycloundecene x HCl (8): Mp.: 143 - 148°C, ^{1}H NMR: 250 MHz ($CDCl_3$): δ 2.2 – 2.6 (mc, 2H, 9); 2.2 – 2.6 (mc, 2H); 2.7 (d, *J* = 5, N-Me); 2.8 – 3.4 (mc, 8H); 3.76 (s, 3H, O-Me); 3.86 – 4.17 (mc, *J* = 15, 2H, 15); 6.66 – 6.70 (d, *J* = 2.7, 1H, 4); 6.78 – 6.85 (dd, *J* = 2.7, 8.3, 1H, 2) 7.1-7.2 (m, 4H, 11, 12, 13, 14) 7.28 – 7.33 (d, *J* = 8.3, 1H, 1). GC/MS m/z: 295 (87%); 209 (60%); 190 (30%); 178 (38%); 165 (42%); 160 (40%); 146 (19%); 130 (50% ;) 115 (40%); 84 (35%); 70 (95%); 58 (100%). Anal. ($C_{20}H_{25}NO$ x HCl x H_2O) C,H,N.

8-Methyl-5,6,7,8,9,10,11,16-octahydrodibenzo[*e,h*]azacyclododecene-3-ol x HCl (9): After evaporation of the ether, the residual free base solidified slowly. It was converted into the HCl salt with diethyl ether and ethereal HCl to yield a white powder with Mp.: 215°C. Yield: 54% ^{1}H NMR: 250 MHz ($CDCl_3$): (base): δ 1.48 – 1.51 (mc, 4H, 6, 10); 2.03 – 2.07 (mc, 4H, 7, 9); 2.17 (s, 3H, N-CH_3); 2.65 – 2.75 (mc, 4H, 5, 11); 4.06, (s, 2H, 16); 6.47 – 6.52 (dd, *J* = 2.6, 8.2, 1H, 2); 6.61 (d, *J* = 2.6, 1H, 4); 6.86 – 6.90 (d, *J* = 8.2, 1H, 1); 7.01 – 7.14 (m, 4H, 12, 13, 14, 15). GC-MS: (base) m/z: 295 (89%); 235 (47%); 223 (10%); 209 (14%); 195 (40%) ; 147 (14%); 130 (55%) ; 115 (23%) ; 91 (20%); 70 (100%). Anal. ($C_{20}H_{25}NO$ x HCl x 1.5 H_2O): C,H,N.

3-Methoxy-8-methyl-5,6,7,8,9,10,11,16-octahydrodibenzo[*e,h*]azacyclododecene x HCl (10) After removal of the solvent, the resulting oil was dissolved in 3 mL of isopropanol and 5 drops of concentrated. HCl acid. Ether was added until the turbidity maintained. The resulting fine crystalline product was washed with additional ether and dried. Mp.:182°C. Yield: 67% ^{1}H NMR: 250 MHz (MeOH d_4): δ 1.85 –2.00 (mc, 4H, 10, 6); 2.6 – 2.8 (mc, 2H, aliphatic); 2.69 (s, 3H, N-CH_3); 3.02 – 3.25 (mc, 6H, aliphatic); 3.63 – 3.69 (d, *J* = 15, 1H, 16); 3.76, (s, 3H, O-Me); 4.33 – 4.40 (d, *J* = 15, 1H, 16); 6.73 – 6.78 (dd, *J* = 3, 7, 1H, 2); 6.8 (d, *J* = 3, 1H, 4); 7.13 – 7.17 (d, *J* = 7, 1H, 1); 7.17 – 7.30 (m, 4H, 12, 13, 14, 15). GC-MS: (base) m/z: 309 (88%); 249 (43%); 237 (12%); 223 (16%); 209 (42%); 190 (13%) ; 178 (41%); 165 (25%); 147 (17%); 130 (55%) ; 115 (28%) ; 70 (100%). Anal. ($C_{21}H_{27}NO$ x HCl x 1.25 H_2O): C,H,N.

12-Methoxy-7-methyl-5,6,8,9,10,14b-hexahydroisoquino[1,2-*a*][2]benzazepnium iodide: (13) Evaporation of the solvent yielded a yellow foam that did not crystallize. Yield: 82% Mp.:145 – 148°C. ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.8 – 2.9 (mc, 4H, aliphatic); 3.0 – 3.5 (mc, 6H, aliphatic, with singlets at 3.37 and 3.50 for the N-Me); 3.7 – 4.2 (mc, 5H, aliphatic, with singlets at 3.75 and 3.81 for the O-Me); 4.3 – 4.8 (mc 1H, aliphatic); 6.0 – 7.8 (mc, 8H, aromatic and methin H).

12-Hydroxy-7-methyl-5,6,8,9,10,14b-hexahydroisoquino[1,2-*a*][2]benzazepnium bromide: (14) Evaporation of the solvent yielded a yellow foam that crystallized from isopropanol/etylacetate. Yield: 70% Mp.: 276°C. ^{1}H NMR: 250 MHz (DMSO d_6): δ 1.8 – 2.8 (mc, 3H, aliphatic); 3.0 – 3.3 (mc, 4H, aliphatic, with singlets at 3.05 and 3.16 for the N-Me); 3.45 – 4.3 (mc, 6H, aliphatic); 6.0 – 7.5 (mc, 8H, aromatic and methin H); 9.86 – 9.93 (d, J = 15, 1H, OH).

3-Methoxy-7-methyl-5,6,8,9,10,14b-hexahydroisoquino[1,2-*a*][2]benzazepinium iodide (16): Yellow powder**:** Yield: 86% Mp.: 252°C. ^{1}H NMR: 250 MHz (DMSO d_6): δ 1.9 – 2.8 (mc, 4H, aliphatic); 2.9 – 3.3 (mc, 5H, aliphatic, with singlets at 3.02 and 3.18 for the N-Me); 3.3 – 4.2 (mc, 7H, aliphatic, with singlets at 3.73 and 3.79 for the O-Me); 6.0 – 7.5 (mc, 8H, aromatic and methin H).

3-Hydroxy-7-methyl-5,6,8,9,10,14b-hexahydroisoquino[1,2-*a*][2]benzazepinium bromide (17): White, grainy powder from toluene/acetone. Yield: 79%. Mp.: 230 – 235°C. ^{1}H NMR: 250 MHz (DMSO d_6): δ 1.9 – 2.8 (mc, 3H, aliphatic); 2.9 – 3.45 (mc, 4H, aliphatic, with singlets at 3.00 and 3.17 for the N-Me); 3.45 – 4.3 (mc, 6H, aliphatic); 5.9 – 7.5 (mc, 8H, aromatic and methin H); 9.7 (s, 1H, OH).

2-(3-Hydroxypropyl)-*N*-[3-(3-methoxyphenyl)propyl]benzamide (20): A solution of 43 mmol of benzoxepinone (7.11g) and 43 mmol of 3-methoxy-phenylpropylamine (7.1g) in 10 mL of toluene was refluxed for 30h under nitrogen. The mixture was diluted with additional 20 mL of toluene and extracted wit 2.5 N H_2SO_4 to remove the amine. Removal of the solvent resulted in a tan oil which was decolorized by boiling with activated charcoal in methanol. After filtration and evaporation of the solvent yielded a yellow oil that crystallized after addition of ether and scratching with a glass rod. Yield: 48% Mp.: 69 – 70°C. ^{1}H NMR: 200 MHz ($CDCl_3$): δ 163 – 2.02 (m, J = 7.5, 4H, –CH_2-C<u>H</u>$_2$-CH_2-); 2.66 – 2.73 (t, J = 7.5, 2H, ar–C<u>H</u>$_2$-CH_2-CH_2-); 2.82 – 2.89 (t, J = 6.7, 2H, ar–C<u>H</u>$_2$-CH_2-CH_2-); 3.79 (s, 3H, O-Me); 6.00 (s, 1H, NH or OH); 6.71 – 6.81 mc, 3H, aromatic); 7.15 – 7.33 (mc, 5H, aromatic). GC/MS m/z: 327 (35%); 296 (15%); 206 (15%); 193 (46%); 174 (50%); 162 (77%); 148 (72%); 131 (100%); 117 (82%); 103 (26%); 91 (65%) ;77 (32%); 65 (14%).

3-Methoxy-6,7,9,10,11,15b-hexahydro-5*H*-[2]benzazepino[1,2-*a*][2]benzazepine x HCl (21): A solution of 10 mmol **20** was refluxed under nitrogen in a mixture of 65 mL acetonitrile and 7 mL of phosphorylchloride for 24h. After removal of the solvents, the residual $POCl_3$ was repeatedly leached out with petroleum ether(40-60°C). The residue was dissolved in 200 mL of methanol and within 30 min 2.5 g of $NaBH_4$ was added at rt. After the addition, the solution was refluxed for 1h, the solvents removed under vacuum, taken up with 100 mL of water and extracted with diethyl ether. The organic layer was decolorized by boiling with activated charcoal in methanol. After filtration and evaporation, the clear oil was dissolved in 5 mL of isopropanol and 7 drops of concentrated. HCl acid. Overnight large white crystals formed. The total yield was 13%. Mp.: 214°C. ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.6 – 2.0 (mc, 4H, –CH_2-C<u>H</u>$_2$-CH_2-); 2.6 – 3.3 (mc, 8H, aliphatic); 3.79 (s, 3H, O-Me); 5.5 (s, 1H, methin C-H); 6.69 – 7.0 (mc, 3H, aromatic); 7.10 – 7.26 (m, 4H, aromatic). HCl salt Mp.: 214°C ^{1}H NMR: proofed by cosy and dept: 250 MHz (MeOH d_4): δ 1.8 – 1.9 (mc, 3H, 6, 10); 2.2 – 2.4 (m, 1H, 6); 2.6 – 2.9 (mc, 1H, 11); 3.0 – 3.2 (dd, J = 5, 13, 1H, 5); 3.3 – 3.45 (d, J = 13, 1H, 5); 3.47 – 3.52 (t, J = 6 2H, 9); 3.58 – 3.69 (mc, 2H, 7); 3.77 (s, 3H, O-Me); 5.9 (s, 1H, 15b); 6.48 (d, J = 8.6 1H, 1); 6.62 – 6.69 (dd, J = 3, 8.6, 1H, 2); 6.94 (d, J = 2.7, 1H, 4); 7.4 – 7.55 (m, 4H, 12, 13, 14, 15). GC/MS m/z: 293 (45%); 292 (40%); 264 (11%); 202 (100%); 189 (25%); 172 (23%); 159 (17%) 147 (9%); 131 (5%); 115 (10%); 91 (8%). Anal. ($C_{20}H_{23}NO$ x HCl x 2.25 H_2O) C, N. H: calcd, 7.76; found, 6.93.

3-Methoxy-8-methyl-6,7,9,10,11,15b-hexahydro-5*H*-[2]benzazepino[1,2-*a*][2]benzazepinium iodide (22) The tan oil did not crystallize. Decolorized by boiling with activated charcoal in chloroform. Removal of the solvent yielded a white foam with Mp.: 138°C. ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.9 – 2.3 (mc, 4H, aliphatic); 2.7 – 3.1 (mc, 2H, aliphatic); 3.24 and 3.27 (two s, 3H, N-Me); 3.4 – 4.2 (mc, 9H, aliphatic, with singlets at 3.78 and 3.83 for the O-Me); 6.6 – 7.8 (mc, 8H, aromatic and methin H 15b).

3-Hydroxy-8-methyl-6,7,9,10,11,15b-hexahydro-5*H*-[2]benzazepino[1,2-*a*][2]benzazepinium bromide (23) The resulting oil did not crystallize. Recrystallization from acetone / ethanol yielded after decolorization with activated charcoal in white crystals with Mp.: >300°C. ^{1}H NMR: 250 MHz (MeOH d_4): δ 1.9 – 2.4 (mc, 4H, aliphatic); 2.5 – 2.7 (mc, 1H, aliphatic); 2.8 – 3.1 (mc, 5H, two s, 3H, N-Me at 2.97 and 3.0); 3.2 – 3.3 (mc, 2H, aliphatic); 3.6 – 4.1 (mc, 3H, aliphatic); 6.14 (s, 1H, methin H 15b); 6.45 – 7.0 (mc, 3H, 1, 2, 4); 7.15 – 7.5 (mc, 4H, 12, 13, 14, 15).

Ethyl 12-methoxy-5,6,8,9,10,15-hexahydro-7*H*-dibenzo[*d*,*g*]azacycloundecine-7-carboxylate (24): Oily compound that solidified to a glass after several days in the refrigerator. Yield: 102%* Mp.: 93.5°C. ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.1 – 1.6 (mc, 5H, 9, a); 2.70 (t, J = 7.6, 2H, 5); 3.1 – 3.3 (mc, 4H, 6,8); 3.5 (mc, 2H,10); 3.78 (s, 3H, O-Me); 3.97 (s, 2H, 15);4.13 – 4.22 (quart, J = 7, 2H, b); 6.70 – 6.77 (mc, 2H, 11, 13); 7.0 – 7.3 (mc, 5H, 1, 2, 3, 4, 14). GC/MS m/z: 353 (30%); 324 (17%); 280 (5%); 248 (13%); 220 (39%); 209 (38%); 178 (15%); 165 (15%) 147 (10%); 135 (5%); 116 (16%); 104 (100%); 91 (10%). *After this reaction the calculated yield is often more than 100% probably due to boron containing impurities that are neither detectable by GC-MS, TLC or NMR.

6,7,8,9,10,15-Hexahydro-5*H*-dibenzo[*d*,*g*]azacycloundecin-12-ol x HCl (25): The HCl salt was extracted as an ionpair with dichloromethane. White powder. Mp.: 227°C (starting decomp.) - 257°C. ^{1}H NMR: 250 MHz (MeOH d_4): δ 1.75 – 1.95 (mc, 2H, 9); 2.6 – 2.75 (mc, 4H, 8, 10); 2.77 – 2.82 (t, J = 6, 2H, 5); 2.91 – 2.97 (t, J = 6, 2H, 6); 3.4 (s, br, 1H, OH, exchangeable after D_2O addition); 4.07 (s, 2H, 15); 6.48 – 6.52 (dd, J = 2.5, 8.3, 1H, 13); 6.58 (d, J = 2.5, 1H, 11); 6.94 (d, J = 8.3, 1H, 14); 7.0 – 7.2 (mc, 4H, 1, 2, 3, 4). GC/MS (base) m/z: 267 (42%); 235 (16%); 221 (13%); 209 (20%); 195 (37%); 178 (15%); 162 (100%); 146 (30%) 132 (27%); 115 (22%); 104 (27%); 91 (15%); 56 (60). Anal. ($C_{18}H_{21}NO$ x HCl x 1/3 CH_2Cl_2) C,H,N;

***N*-[2-(3-Chloro-4-methoxyphenyl)ethyl]-2-(3-hydroxypropyl)benzamide (27):** Recrystallization from toluene, washed with diethyl ether. Yield: 57% Mp.:91.5 – 92.5°C. ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.86 – 1.96 (mc, 2H, -CH_2-C<u>H</u>$_2$-

CH_2-); 2.80 – 2.90 (mc, 4H, 2x ar-C$\underline{H_2}$-CH_2); 3.48 (t, *J* = 5.2, 2H, -C$\underline{H_2}$-OH); 3.64 – 3.72 (q, *J* = 6, 2H, -C$\underline{H_2}$-NH); 3.90, (s, 3H, O-Me); 5.38 (t br, 1H, N$\underline{H}$); 6.90 (d, *J* = 8.3, 1H, 4); 7.11 – 7.17 (dd, *J* = 2.2, 8.3; 1H, 6); 7.2 – 7.45 (m, 5H, 3', 4', 5', 6', 2).

2-[2-(3-Chloro-4-methoxyphenyl)ethyl]-2,3,4,5-tetrahydro-1*H*-2-benzazepine (28): A solution of 10 mmol **CM67HA** was refluxed under nitrogen in a mixture of 100 mL acetonitrile and 50 mL of phosphorylchloride for 80h. After removal of the solvents, the residual $POCl_3$ was repeatedly leached out with petroleum ether (40-60°C). The residue was dissolved in 100 mL of methanol and within 30 min 2 g of $NaBH_4$ was added at rt. After the addition, the solution was refluxed for 1h, the solvents removed under vacuum, taken up with 100 mL of water and extracted with diethyl ether. After filtration and evaporation, the yellow oil was purified by column chromatography with $ChCl_3$/MeOH 9:1 as eluent. Mp:127°C; The HCl salt was prepared from ether with ethereal HCl. Mp was 200°C. ^{1}H NMR: 250 MHz ($CDCl_3$): (base): δ 1.73 – 1.82 (mc, 2H, -CH_2-C$\underline{H_2}$-CH_2-); 2.56 – 2.62 (mc, 2H, ar-CH_2-C$\underline{H_2}$-N); 2.75 – 2.81 (mc, 2H, ar-C$\underline{H_2}$-CH_2-N); 2.92 – 2.96 (mc, 2H, ar-C$\underline{H_2}$-CH_2-CH_2-N); 3.21 – 3.25 (mc, 2H, ar-CH_2-CH_2-C$\underline{H_2}$-N); 3.79 (s, 3H, O-Me); 4.03 (s, 2H, ar-C$\underline{H_2}$-ar); 6.82 – 6.85 (d, *J* = 8.3, 1H, 5'); 7.00 – 7.04 (dd, *J* = 2.1, 8.3, 1H, 6'); 7.16 – 7.21 (mc, 5H, 2', 6, 7, 8, 9). structure proofed by cosy spectroscopy. GC-MS: (base) m/z: 315 (<1%); 160 (100%); 117 (50%); 105 (17%); 91 (9%); 77 (9%); 42 (10%). Anal. ($C_{19}H_{21}ClNO$ x HCl): C,H,N.

3-[2-({[2-(3-Chloro-4-methoxyphenyl)ethyl]amino}carbonyl)phenyl]propyl ethyl carbonate (29) To a stirred solution of 5.2 g (15 mmol) **27** in a mixture of 20 mL chloroform and 40 mL pyridine there was added in 30 min a solution of 30 mmol ethylchloroformate in 20 mL chloroform. After the addition, the solution was stirred for additional 30 min and the solvents removed under vacuum. The residue was dissolved in 100 mL chloroform and washed with 2N HCl (2x 30 mL) and 2N NaOH (2x 30 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The resulting yellow oil crystallized overnight in the refrigerator. The solids were removed by filtration, recrystallized twice from diethyl ether to obtain 5.3 g of . 84% yield. Mp.:84°C. ^{1}H NMR: 250 MHz ($CDCl_3$): δ 1.20 – 1.32 (t, *J* = 7.1; 3H, -O-CH_2-C$\underline{H_3}$); 1.93 – 2.0 (mc, 2H, -CH_2-C$\underline{H_2}$-CH_2-); 2.80 – 2.90 (mc, 4H, 2x ar-C$\underline{H_2}$-CH_2); 3.6 – 3.7(q, *J* = 6, 2H, -C$\underline{H_2}$-NH); 3.90, (s, 3H, O-Me); 4.09 – 4.21 (mc, 4H, 2x -C$\underline{H_2}$-O-C=O); 5.38 (t br, 1H, N$\underline{H}$); 6.87 (d, *J* = 8.3, 1H, 4); 7.18 – 7.21 (dd, *J* = 2.1, 8.3; 1H, 6); 7.2 – 7.32 (m, 5H, 3', 4', 5', 6', 2).

3-Chloro-2-methoxy-5,6,8,9,10,14b-hexahydroisoquino[1,2-a][2]benzazepine (30): A solution of 4.4 g (10,4 mmol) of the protected benzamide **29** in 150 mL of a 2/1 mixture of acetonitrile and $POCl_3$ was refluxed for 4 days under nitrogen. The solvents were removed under a hard vacuum, and the remaining residue portioned between 170 mL of 2 N HCl and 30 mL of ethylacetate, and the aqueous layer washed again with additional 20 mL of ethylacetate. The desired dihydroisoquinolinium salt was extracted as an ion pair from the acidic aqueous layer with $CHCl_3$ (5 x 40 mL). After evaporation of the pooled $CHCl_3$ layers, the residue (2.44 g) was stirred in 70 mL of 20 % KOH solution in aqueous ethanol (70% EtOH, 30 % H_2O) at r. t. for 12 h. The solvents were concentrated to about 10 mL *in vacuo*, maintaining the temperature below 40°C. To this residue 160 mL of 2N HCl were added and extracted with $CHCl_3$ (5 x 30 mL). After drying (Na_2SO_4)and evaporating to dryness, the remaining oil was dissolved in 17 mL of $POCl_3$ and stirred for 15 min. at 60°C. The mixture was cooled to r. t. and 100 mL of petroleum ether (40 – 60) were added with vigorous stirring. The oil was allowed to sit, and the upper layer, containing $POCl_3$ and petroleum ether was decanted off and discarded. This procedure was repeated, until no more $POCl_3$ was detectable by smell. To the remaining residue, dissolved in 85 mL of methanol there was added portionwise with cooling 3 g of $NaBH_4$. After the reaction has subsided, the solution was refluxed for 1 h, evaporated to dryness and taken up with 150 mL of water. Extraction with diethyl ether (5 x 40 mL), drying (Na_2SO_4) and evaporating yielded 1.3 g of the crude **30** as free base. The HCl salt was obtained by dissolving the base in 10 mL of diethyl ether and the addition of ethereal HCl. The precipitated HCl salt was recrystallized from isopropanol. The total yield from **29** to **30** was 39%. Mp.: 202°C (HCl salt); ^{1}H NMR: 250 MHz ($CDCl_3$): (base): 1.9 – 2.2 (mc, 2H, 9); 2.6 – 3.0 (mc, 5H, aliphatic); 3.0 – 3.2 (mc, 1H, aliphatic); 3.25 – 3.40 (mc, 1H, aliphatic); 3.50 – 3.92 (mc, 4H, aliphatic + O-Me); 6.1 – 6.9 (mc, 3H, aromatic and methin H); 7.2 – 7.5 (mc, 4H, aromatic). GC-MS: (base) m/z: 313 (32%); 312 (46%); 284 (10%); 224 (32%); 222 (100%); 168 (17%); 115 (19%); 102 (10%); 91 (24%). Anal. ($C_{19}H_{20}ClNO$ x HCl x ½ H_2O) C,H,N.

3-Chloro-2-methoxy-7-methyl-5,6,8,9,10,14b-hexahydroisoquino[1,2-*a*][2]benzazepinium iodide (31) White powder, Yield: 72%. Mp.: 292°C. ^{1}H NMR: 250 MHz (DMSO d_6): δ 1.9 – 2.1 (mc, 2H, 9); 2.60 – 2.65 (mc, 2H, aliphatic); 2.9 – 3.0 (mc, 2H, aliphatic); 3.19 (s, 3H, N-Me); 3.4 – 3.55 (mc, 4H, aliphatic); 3.71 (s, 3H, O-Me); 5.7 – 7.6 (mc, 7H, aromatic and methin H 14b).

2-Methoxy-7-methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d*,*g*]azacycloundecene (32): After evaporation of the solvent, the oily residue solidified. Mp.: 72 – 75°C. ^{1}H NMR: 250 MHz ($CDCl_3$): (base): δ 1.93 – 1.98 (quin, *J* = 6, 2H, 9); 2.13 – 2.17 (t, *J* = 6 2H, 8); 2.24 (s, 3H, N-Me); 2.38 – 2.41 (mc, 2H, 5); 2.54 – 2.58 (mc, 2H, 6), 2.86 – 2.91 (t, *J* = 6, 2H, 10); 3.88 (s, 3H, O-Me); 4.73 (s br, 2H, 15); 6.75 – 6.79 (dd, *J* = 2.7, 8.2, 1H, 3); 6.89 (d, *J* = 2.7, 1H, 1); 6.96 – 7.3 (mc with d at 7.05, *J* = 8.2, 5H, 4, 11, 12, 13, 14). GC/MS m/z: 295 (21%); 223 (17%) ; 209 (22%); 190 (9%); 178 (19%); 165 (24%); 160 (15%); 115 (20%); 84 (20%); 70 (68%); 58 (83%) ; 44 (100%). Anal. ($C_{20}H_{25}NO$) C,H,N.

3-Chloro-2-hydroxy-7-methyl-5,6,8,9,10,14b-hexahydroisoquino[1,2-*a*][2]benzazepinium iodide (33): Yellow, hygroscopic powder, recrystallized from acetone/diethyl ether Mp.: 277°C. ^{1}H NMR: 250 MHz (DMSO d_6): δ 1.9 – 2.1 (mc, 2H, 9); 2.50 – 2.58 (mc, 2H, aliphatic); 3.10 (s, 3H, N-Me); 3.3 – 3.58 (mc, 4H, aliphatic); 3.8 – 4.3 (mc, 2H, aliphatic); 5.9 – 7.52 (mc, 7H, aromatic and methin H 14b).

7-Methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d*,*g*]azacycloundecene-2-ol (34): Extracted at pH 9 with diethyl ether, evaporation yielded a white foam. Yield: 93%. Mp.: 70 – 72°C. ^{1}H NMR: 250 MHz ($CDCl_3$): (base): δ 1.92 (mc, 2H, 9); 2.08 – 2.21 (mc, 5H, N-Me, 8); 2.37 (mc, 2H, 5); 2.51 (mc, 2H, 6) ; 2,80 – 2.85 (t, *J* = 6.5, 2H, 10) ; 4.54 (s br, 2H, 15); 6.62 – 6.66 (dd, *J* = 2.6, 8.0, 1H, 3); 6.71 (d, *J* = 2.6, 1H, 1); 6.89 – 6.92 (d, *J* = 8.0, 1H, 4); 6.93 – 7.21 (m, 4H, 11, 12, 13, 14). GC-MS: (base) m/z: 281 (43%); 238 (9%); 221 (8%); 209 (29%); 195 (46%); 178 (17%); 165 (24%) ; 160 (28%) ; 152 (11%); 129 (19%); 115 (25%); 91 (19%) ; 84 (33%); 70 (100%). Anal. ($C_{19}H_{23}NO$) C,H,N.

1,3-Dichloro-2-methoxy-7-methyl-6,7,8,9,10,15-hexahydro-5*H*-dibenzo[*d*,*g*]azacycloundecene x HCl (35): The mixture of HCl salts was repeatedly recrystallized from boiling methanol, yielding in pure **35** with mp.:291 – 294°C (HCl salt). The mp. of the base was 68-69.5°C. ^{1}H NMR: 250 MHz (MeOH d_4): (base): δ 1.88 – 2.00 (mc, 2H, 9); 2.08 – 2.20 (mc,

5H, N-Me, 8); 2.34 (mc, 2H, 5); 2.48 (mc, 2H, 6); 2,79 – 2.87 (mc, 2H, 10); 5.1 (s br, 2H, 15); 6.63 – 6.66 (d, *J* = 7.6, 1H, 11 or 14); 6.95 – 7.01 (dt, *J* = 1.5, 7.4, 1H, 12 or 13); 6.99 (s, 1H, 4); 7.08 – 7.14 (t, *J* = 7.4, 1H, 12 or 13); 7.20 – 7.23 (d, *J* = 7.4, 1H, 11 or 14). GC-MS: (base) m/z: 349 (19%); 314 (5%); (263 (6%); 235 (47%); 242 (9%); 228 (12%) ; 207 (9%); 195 (40%) ; 178 (14%) ; 160 (25%) ;139 (10%); 130 (17%) ; 115 (18%); 84 (65%); 70 (100%). Anal. base ($C_{19}H_{21}Cl_sNO$): C,H,N.

Experimental Procedures for the Pharmacological Investigations:

Dopamine Receptor Ligand Activity:

i) Cell culture and receptor density

Human D_1, D_{2L}, D_3, $D_{4.4}$ and D_5 receptors were stably expressed in Chinese hamster ovary (CHO) cells or human embryonic kidney cells (HEK293). D_1, D_{2L}, D_3, and D_5 are expressed in HEK cells and D_{2L}, D_3, and $D_{4.4}$ receptors are expressed in CHO cells, respectively. The density of receptors, measured with [^{3}H]-SCH 23390 was 6087 fmol/mg protein for D_5 receptor expressed in HEK293 cells. For D_1 it was 3139 fmol/mg protein. The densities of receptors measured with [^{3}H]-spiperone, were 186.53 fmol/mg protein for the D_2 receptor expressed in HEK cells, 6043 fmol/mg for the D_4 receptor and 14474 fmol/mg for D_3 receptor, both expressed in CHO cells. Cells were grown at 37°C under a humidified atmosphere of 5% CO_2: 95% air in HAM/F12-medium (Sigma-Aldrich) for CHO cells and Dulbecco's modified Eagles Medium Nutrient mixture F-12 Ham for HEK293 cells, each supplemented with 10% fetal bovine serum, 1 mM *L*-glutamine and 0.2 µg/mL of G 418 (all by Sigma-Aldrich).

ii) Preparation of Whole-Cell-Suspension[2]

Human D_2, D_3, D_4 and D_5 receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85% confluency, the medium was removed and the cells were incubated with 3 mL trypsine-EDTA-solution (Sigma-Aldrich) to remove the cells from the culture dish. After incubation, cells were suspended in 3-6 mL added medium in order to stop the effect of trypsine-EDTA-solution. The resulting suspension was centrifuged (1800-2400 rot/min, 4°C, 4 min.), the pellet resuspended in 10 mL PBS (ice-cooled, calcium- and magnesium-free), pelleted, and this procedure was repeated. The resulting pellet was then resuspended in 12 mL of buffer (5 mM magnesium chloride, 50 mM TRIS-HCl, pH=7.4) and the resulting suspension was directly used for the radioligand binding assay.

iii) Radioligand Binding Assay

The binding studies were performed following the protocol previously described but in 96- well format.[2] The assays with the whole-cell-suspension were carried out in triplicate in a volume of 550 µL (final concentration): TRIS-Mg^{2+}-buffer (345 µL), [^{3}H]-ligand (50 µL), whole-cell-suspension (100 µL) and appropriate drugs (55 µL). Non-specific binding was determined using fluphenazine (100 µM) in D_5 test and haloperidol (10 µM) in D_2, D_3 and D_4 tests. The incubation was initiated by addition of the radioligand. It was carried out in 96 deep well plates (Greiner bio-one, Frickenhausen) using a Thermocycler (Thermocycler comfort, Eppendorf, Wessling) at 27°C. The incubation was terminated after 90 min by rapid filtration with a PerkinElmer Mach III Harvester™ using a PerkinElmer Filtermat A, previously treated with a 0,25% polyethyleneimine-solution (Sigma-Aldrich) and washed once with water. The filtermat was dried for 3 min with 400 watt using a microwave (MW 21, Clatronic, Kempen). The dry filtermat was placed in a filter plate (Omni filter plates, PerkinElmer Life Sciences) and each field of the filtermat moistened with 50 µL Microscint 20™ scintillation cocktail. The radioactivity retained on the filters was counted using a Top Count NXT™ microplate scintillation counter (Packard, Ct., USA). For determining the K_i values at least two independent experiments each in triplicate were performed.

The competition binding data were analyzed with GraphPad Prism™ software using nonlinear least squares fit. For calculating the mean, standard deviation and standard error of the mean the software Microsoft Excel™ was used. K_i values were calculated from IC_{50} values applying the equation of Cheng and Prusoff.[3]

Functional assay measuring intracellular Ca^{2+} with a fluorescence microplate reader[4;5]

i) Cell Culture

Human D_{2L} and D_5 receptors were stably expressed in human embryonic kidney cells (HEK293) and cultured as mentioned above.

ii) Preparation of Whole-Cell-Suspension

Human D_2 and D_5 receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85-90% confluence. The medium was removed and cells rinsed twice with 6 mL Krebs-HEPES buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 11.7 mM D-Glucose, 1.3 mM $CaCl_2$, 10 mM HEPES, pH 7.4) each time. After two washes, cells were loaded with 3 µL of a 0.5 M Oregon Green 488 BAPTA-1/AM-solution (Molecular Probes, Eugene, OR) (in DMSO) in 6 mL of the same buffer containing 3 µL of a 20% Pluronic F-127-solution (Sigma Aldrich) (in DMSO) for 45 min at 37°C. After 35 min incubation, the culture dish was rapped slightly in order to remove all cells from the dish for further incubation. Then 5 mL of Krebs-HEPES buffer were added and cells were suspended. The resulting suspension was separated in 10 vials (1.5 mL) and centrifuged (10 000 rot/min, 10 sec), the pellets were resuspended in 1 mL Krebs-HEPES buffer twice per five pellets and centrifuged again. The pellets were resuspended in

16 mL (for screening of antagonistic activity) or 18 mL (for screening of agonistic activity) Krebs-HEPES buffer. And plated into 96-well plates (OptiPlate HTRF-96, Packard, Meriden, CT; Cellstar, Tissue Culture Plate, 96W, Greiner bio-one, Frickenhausen) Microplates were kept at 37°C under a saturated humidity atmosphere including 5% CO_2 for 30 min.

iii) Calcium Assay[5]

Screening for agonistic and antagonistic activity was performed using a NOVOstar microplate reader (BMG LabTechnologies) with a pipettor system. Agonistic activity was tested after 30 min incubation of the plated cell suspension by injecting 20 µL buffer alone, standard agonist, or test compounds, respectively, dissolved in buffer sequentially into separate wells. Screening of compounds for antagonist activity or dose response curves in presence of an antagonist were performed by preincubating the cells with 20 µL of the solutions of compounds (final concentrations: 100 µM, 50 µM, 10 µM, 5 µM, 1 µM, 500 nM, 100 nM, 50 nM, 10 nM, 1 nM, 0,1 nM) at 37°C 30 min prior to injection of 20µL standard agonist. Final concentration of test compounds for screening of agonist or antagonist activity was 10 µM, respectively. Quinpirole was used as standard agonist for hD_2 receptors and SKF 38393 for hD_5 receptors (final concentration: 1 µM).

Fluorescence intensity was measured at 520 nm (bandwidth 25nm) for 30 s at 0.4 s intervals. Excitation wavelength was 485 nm (bandwidth 20 nm). IC_{50} values were obtained by determination of the maximum fluorescence intensity of each data set and non-linear regression with sigmoidal dose-response equation using a four parameter logistic equation on GraphPadPrism™ 3.0. K_i values were then calculated to account for different agonist concentrations and EC_{50} values applying the modified *Cheng-Prusoff* equation:

$$K_i = \frac{IC_{50}}{1 + \frac{L}{EC_{50}}}$$

L: concentration of standard agonist (M); EC_{50}: effective concentration 50% of the standard agonists (M); IC50: inhibitory concentration 50% of test compounds at the given experimental conditions, i.e., standard agonist concentration.

Appendix (Purity)

Dopamine/Serotonin Receptor Ligands, Part 16[1]: Expanding Dibenz[*d,g*]azecines to 11- and 12-membered Homologues - Interaction with Dopamine D_1-D_5 Receptors

*Christoph Enzensperger, Franziska K. U. Müller, Bärbel Schmalwasser, Petra Wiecha, Heidi Traber and Jochen Lehmann**

Compound		C	H	N
(5)	found	80.92	8.50	4.47
	calc. for $C_{19}H_{23}NO$	81.10	8.24	4.98
(6)	found	81.47	8.86	4.37
	calc. for $C_{20}H_{25}NO$	81.31	8.53	4.74
(7)	found	81.19	8.30	4.80
	calc. for $C_{19}H_{23}NO$	81.10	8.24	4.98
(8)	found	68.78	8.07	3.73
	calc. for $C_{20}H_{25}NOxHClxH_2O$	68.65	8.07	4.00
(9)	found	66.79	7.93	3.75
	calc. for $C_{20}H_{25}NO$ x HCl x 1.5 H_2O	66.93	8.14	3.90
(10)	found	68.55	8.33	3.64
	calc. for $C_{21}H_{27}NO$ x HCl x 1.25 H_2O	68.46	8.34	3.80
(21)	found	64.99	6.93	3.63
	calc. for $C_{20}H_{20}N_2$ 2.25 H_2O	64.85	7.76	3.78
(25)	found	66.18	6.89	4.06
	calc. for $C_{18}H_{21}NO$ x HCl x 1/3 CH_2Cl_2	66.30	6.88	4.2
(28)	found	64.95	6.70	3.93
	calc. for $C_{19}H_{22}ClNO$ x HCl	64.78	6.58	3.98
(30)	found	63.21	6.22	3.67
	calc. for $C_{19}H_{20}ClNO$ x HCl x ½ H_2O	63.52	6.17	3.90
(32)	found	80.93	8.46	4.75
	calc. for $C_{20}H_{25}NO$	81.31	8.53	4.74

(34)	found	80.62	8.42	4.83
	calc. for $C_{19}H_{23}NO$	81.10	8.24	4.98
(35)	found	64.75	6.43	4.07
	calc. for $C_{19}H_{21}Cl_2NO$	65.15	6.04	4.00

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