Synthesis and 5-HT\textsubscript{2A} Radioligand Receptor Binding Assays of DOMCl and DOMOM, Two Novel 5-HT\textsubscript{2A} Receptor Ligands

A synthesis of two new active substances, DOMCl (1-(4-chloromethyl-2,5-dimethoxyphenyl)-2-propanamine; 2) and DOMOM (1-(2,5-dimethoxy-4-methoxymethylphenyl)-2-propanamine; 3), was developed. Unexpectedly, the Blanc reaction permitted successful synthesis of 2,5-dimethoxyphenylpropylamine derivatives having a substituted methyl group in position 4 since solvation of the reactant occurs during the reaction. Afterwards, their affinities towards the 5-HT\textsubscript{2A} receptor were examined in 5-HT\textsubscript{2A} radioligand receptor binding assays. The study of these substances is of considerable interest because they were predicted, by preliminary molecular modeling studies based on mescalin units, to be potential new hallucinogens that should be added to the list of substances prohibited by law. It was assumed that DOMCl would be 82 times more potent as a hallucinogen than mescalin, and DOMOM would be 94 times more potent. The 5-HT\textsubscript{2A} radioligand receptor binding studies showed that the affinities of DOMCl and DOMOM for the 5-HT\textsubscript{2A} receptor are less than expected but are nevertheless 1.6 and 8.7 times higher, respectively, than that of mescalin. Therefore, scheduling these substances as potential drugs of abuse might be considered.

Keywords: 5HT\textsubscript{2A} receptor; 5-HT\textsubscript{2A} radioligand receptor binding assay; DOMCl; DOMOM; Synthesis

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Table 1. Correlation of mescalin units, calculated by molecular modeling, and $K_i$ values of 5-HT$_{2A}$ radioligand receptor binding experiments.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mescalin units</th>
<th>$K_i$ (nM) ± SEM of $[^{3}H]$ketanserin HCl and rat prefrontal cortices</th>
<th>$K$ value of mescalin related to $K_i$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mescalin</td>
<td>1 [23]</td>
<td>17,850 ± 1,899</td>
<td>1</td>
</tr>
<tr>
<td>DOMCl</td>
<td>82 [15]</td>
<td>11,420 ± 690</td>
<td>1.6</td>
</tr>
<tr>
<td>DOMOM</td>
<td>94 [15]</td>
<td>2,057 ± 273</td>
<td>8.7</td>
</tr>
</tbody>
</table>

Figure 1. Scheme of synthesis of DOMCl (2) and DOMOM (3).

over, the resulting benzyl halide 2 (DOMCl) is reactive and unstable against bases. Therefore it is essential to perform the synthesis in one step (Figure 1). The problems mentioned can be avoided using the Blanc reaction, if it is possible to find a dissolver for 2,5-dimethoxyamphetamine 1 [17], which forms a hydrochloride salt under these working conditions, and is therefore insoluble in the known solvents for this reaction. A solvent which is polar enough to dissolve the educt as hydrochloride is unsuitable in this Blanc reaction. Although it was not to be expected that a Blanc reaction would be applicable here in the first place, this reaction was successful on using chlorobenzene as dissolver. The intermediate hydroxymethyl cation causes a solution of the reaction mixture. At the end of the reaction, compound 2 precipitates without any further step. Thus, the Blanc reaction is also suitable for polar substances.

For the synthesis of 3 (DOMOM) the fact was used that the chlorine atom in 2 can be easily substituted by a nucleophilic reactant. Thus it was possible to form 3 by Williamson’s ether synthesis.

5-HT$_{2A}$ Radioligand receptor binding assays

The 5-HT$_{2A}$ radioligand receptor binding assays resulted in $K_i$ values of 17.850 nM ± 1899 nM (SEM) for mescalin, 11.420 nM ± 690 nM (SEM) for DOMCl (Figure 2), and 2.057 nM ± 273 nM (SEM) for DOMOM (Figure 3). Thus, the affinity of DOMCl towards the 5-HT$_{2A}$ receptor is 1.6 times higher than the affinity of mescaline, and DOMOM has a 8.7 times higher affinity towards the 5-HT$_{2A}$ receptor than mescaline (Table 1).

Figure 2. Typical competition isotherms of DOMCl-HCl. Data of a representative experiment is shown, using rat frontal cortex membranes, 250 µM – 500 nM DOMCl-HCl, and 1–1.5 nM $[^{3}H]$ketanserin-HCl. The assay was performed on MultiSreen® filter plates as described in Experimental Section.

Figure 3. Typical competition isotherm of DOMOM-HCl. Data of a representative experiment is shown, using rat frontal cortex membranes, 50 µM – 100 nM DOMOM-HCl, and 1–1.5 nM $[^{3}H]$ketanserin-HCl. The assay was performed on MultiSreen® filter plates as described in Experimental Section.
Yield 0.85 g (70.8 %), decomposition at 180–185 °C.– IR (KBr), ˜ ν(C=O) = 2933 (ν N=O–H), 2958 (ν N–H), 1602, 1509 (ν C=O), 1214 (ν C=O; 1041 (ν C=O). – 1H-NMR (CDCl3, δ ppm): 6.9 (s, 3-H), 6.7 (s, 6'-H); 3.7 (s, 7'-H3/8'-H3); 3.2 (m, 2-H); 2.74 (dd, 1-H, J=13.0 Hz, J=5.9 Hz); 2.51 (dd, 1-H, J=13.0 Hz, J=8.1 Hz); 1.43 (br s, 2H, NH); 1.11 (d, 3-H3, J=6.4 Hz). – MS: (EI), m/z (%) = 239.0 (5, M+); 197.1 (5); 196.0 (75); 181.0 (5); 163.9 (20); 134.1 (10); 104.9 (5); 91.0 (10); 77.0 (5); 44.0 (100). Purity (HPLC) > 98 %.

1-(2,5-Dimethoxy-4-methoxymethylphenyl)-2-propanamine (DOMOM)

To a solution of NaOH, prepared by addition of sodium metal (0.2 g, 8.7 mmol) and methanol (30 mL), was added 2 HCl (1.5 g, 5.4 mmol). The reaction mixture was stirred under reflux for 5 h. Then the solvent was completely removed in vacuo. The residue was dissolved in diethyl ether. The ether phase was extracted with small portions of 0.1 N hydrochloric acid (10 mL each) until the water phase turned to yellow. The yellow fraction was not added to the others. The water phases were united and evaporated in vacuo. The precipitate was washed several times with ether until the oil completely crystallized. The precipitate of 3 was removed by filtration.

Yield 0.8 g (24 %), mp: 106–109 °C.– IR (KBr), ν/cm–1 = 2933 (ν N=O–H), 2958 (ν N–H), 1602, 1509 (ν C=O); 1214 (ν C=O; 1041 (ν C=O), – 1H-NMR (CDCl3, δ ppm): 6.9 (s, 3-H), 6.7 (s, 6'-H); 3.7 (s/s, 7'-H3/8'-H3); 3.2 (m, 2-H); 2.74 (dd, 1-H, J=13.0 Hz, J=5.9 Hz); 2.51 (dd, 1-H, J=13.0 Hz, J=8.1 Hz); 1.43 (br s, 2H, NH); 1.11 (d, 3-H3, J=6.4 Hz). – MS: (EI), m/z (%) = 239.0 (5, M+); 197.1 (5); 196.0 (75); 181.0 (5); 163.9 (20); 134.1 (10); 104.9 (5); 91.0 (10); 77.0 (5); 44.0 (100). Purity (HPLC) > 98 %.
tion at 1,000 g for 30 min. The pellet was rehomogenized in the same mixture of sucrose and HEPES (1:5 v/w), using an Ultra-Turrax® homogenizer at 8,000 rpm for 10 s, and centrifuged as mentioned above. Combined supernatants were centrifuged at 39,000 g for 15 min. The supernatants were discarded and the pellets rehomogenized (Ultra-Turrax®) in buffer I (1:30 v/w) and centrifuged as above. The resulting pellets were rehomogenized (Ultra-Turrax®) in buffer II (1:30 v/v) and centrifuged at 39,000 g for 15 min, the pellets rehomogenized (Ultra-Turrax®) in buffer I (1:30 v/w) and centrifuged as above. The resulting pellets were rehomogenized (Ultra-Turrax®) in buffer II (1:30 w/v) and were preincubated at 37 °C for 20 min to destroy endogenous serotonin. The membrane homogenates were centrifuged at 39,000 g for 15 min, the pellets rehomogenized (Ultra-Turrax®) and were incubated at 37 °C for 20 min to destroy endogenous serotonin. The membrane homogenates were centrifuged at 39,000 g for 15 min, the pellets rehomogenized (Ultra-Turrax®) in buffer I, and the suspensions combined, diluted 1:4 (original wet weight/volume) with buffer I, and frozen in 2 mL aliquots at −70 °C. One aliquot per filter plate was used for the assays, after the membranes had been washed with buffer I by centrifugation and then rehomogenized (Ultra-Turrax®) with assay buffer to a final membrane concentration of 2.5 mg original wet weight/well, corresponding to 70 µg protein/well.

Radioligand binding assays

Each well of a MultiScreen® MAFB filter plate was pretreated for 2 h with 100 µL of 0.5 % polyethyleneimine (PEI) in assay buffer, and subsequently aspirated with the Vacuum Manifold® filtration system. All experiments were performed in triplicate in total volume of 200 µL/well. Mianserin-HCl (50 µM) was used to determine nonspecific binding. Competition experiments were performed using 10 different concentrations, of the test substance and approximately 1.5 nM [3H]ketanserin-HCl. At this concentration 65 % of total binding (1,100 cpm) was specific. 100 µL of membrane suspension (70 µg of protein) was added last. After incubation at 37 °C for 30 min, the filter plate was subsequently aspirated on the Vacuum Manifold® and washed 3 times with 200 µL/well of buffer I. After blotting dry the plastic underdrain of the plate with paper towels, it was carefully removed and the plate was blotted dry once more. Then the plate was dried at 50 °C for 2 h. Afterwards, it was snapped into a 1450–106 Millipore Cassette® including support frame that was sealed before with Multi Screen Tape® at the bottom. 30 µL of BetaPlate Scint™ scintillation cocktail was added to each well and the cassette was then also sealed with the tape at the top. After 10 h of equilibration time, the plate was counted in a MicroBeta® PLUS scintillation counter. In order to determine the applied concentration of [3H]ketanserin-HCl per experiment, the same volume as pipetted onto the filter plate was pipetted with the same tip of the pipette into a scintillation vial with 10 mL of Ultima Gold® scintillation cocktail. This was measured after 12 h of equilibration time in a 1219 Rackbeta® port frame that was sealed before.

Data analysis

Data analysis was performed with PRISM® Software, Version 2.0 (GraphPad Software, Inc., San Diego, CA). Kᵢ was found from previous saturation experiments to be 2.0 ± 0.2 nM as described in ref. [16]. Kᵢ values were calculated according to the equation of Chang and Prusoff [20] as: \[ Kᵢ = \frac{IC₅₀}{(1 + (conc_Radioligand/Kᵢ))}. \] IC₅₀ Values were determined with PRISM® one-site competition nonlinear regression: \[ y = (bottom + (top – bottom))/(1 + 10^{xLogEC₅₀}), \] Hill coefficient equals unity. Kᵢ values of six individual experiments for DOMCI and five individual experiments for DOMOM were calculated and averaged.

References