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Synthesis and 5-HT_{2A} Radioligand Receptor Binding Assays of DOMCI and DOMOM, Two Novel 5-HT_{2A} Receptor Ligands

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A synthesis of two new active substances, DOMCI (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_{2A} receptor were examined in 5-HT_{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 mescaline units, to be potential new hallucinogens that should be added to the list of substances prohibited by law. It was assumed that DOMCI would be 82 times more potent as a hallucinogen than mescaline, and DOMOM would be 94 times more potent. The 5-HT_{2A} radioligand receptor binding studies showed that the affinities of DOMCI and DOMOM for the 5-HT_{2A} receptor are less than expected but are nevertheless 1.6 and 8.7 times higher, respectively, than that of mescaline. Therefore, scheduling these substances as potential drugs of abuse might be considered.

Keywords: 5HT_{2A} receptor; 5-HT_{2A} radioligand receptor binding assay; DOMCI; DOMOM; Synthesis

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Introduction

The 5-hydroxytryptamine 2 (5-HT₂, serotonin 2) receptor family comprises the 5HT_{2A}, 5HT_{2B}, and 5HT_{2C} receptor, and is one of seven 5-HT receptor families (5-HT₁ – 5-HT₇) [1]. Each of these 5-HT₂ receptors has been cloned and shown to be a G-protein linked single molecule that mediates its effect through activation of phospholipase C which causes hydrolysis of phosphoinositides to inositol triphosphate and diacylglycerol. 5-HT₂ receptors are important for mediating many physiological functions, including platelet aggregation, modulation of mood, anxiety, and feeding behavior.

A large number of psychopharmaceuticals, e.g. anxiolytics, atypical antipsychotic drugs, antidepressants, and hallucinogens, mediate their actions at least partially via interactions with various 5-HT₂ receptors. Most psychedelic drugs are potent agonists at 5-HT_{2A} and 5-HT_{2C} receptors, and their binding affinity to these receptors is strongly correlated with their human potency as hallucinogens [2–14]. Most of these substances are prohibited by law as drugs of abuse in cases where their

structure is known. New synthetic drugs of abuse, which are often called “designer drugs”, are frequently produced by making minor modifications to the known illegal drugs of abuse to obtain substances that have similar effects but are not yet prohibited by law. To identify these potential new drugs of abuse and to quickly make possible their classification as controlled substances, we calculated their potential hallucinogenic potency by molecular modeling studies [15]. DOMCI (1-(4-chloromethyl-2,5-dimethoxyphenyl)-2-propanamine; **2**) and DOMOM (1-(2,5-Dimethoxy-4-methoxymethylphenyl)-2-propanamine; **3**) (see Figure 1) are two of the calculated molecules that were predicted to have high activities as hallucinogens. We developed a synthesis for DOMCI and DOMOM, and then determined their 5-HT_{2A} receptor binding affinities. Therefore, a 5-HT_{2A} radioligand receptor binding assay on 96-well Multi-Screen[®] filter plates was developed and validated in a former publication [16].

Results and discussion

Chemistry

In order to synthesize a dimethoxyamphetamine with a chloromethyl group in position 4, it is necessary to avoid reactions with the highly reactive amino function. More-

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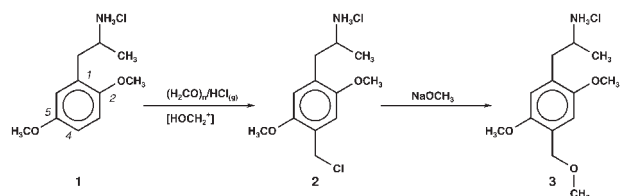


Figure 1. Scheme of synthesis of DOMCI (2) and DOMOM (3).

over, the resulting benzyl halide **2** (DOMCI) 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 $17.850 \text{ nM} \pm 1899 \text{ nM (SEM)}$ for mescaline, $11.420 \text{ nM} \pm 690 \text{ nM (SEM)}$ for DOMCI (Figure 2), and $2.057 \text{ nM} \pm 273 \text{ nM (SEM)}$ for DOMOM (Figure 3). Thus, the affinity of DOMCI 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).

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

Substance	Mescaline units	K_i (nM) \pm SEM of [³ H]ketanserin HCl and rat prefrontal cortices	K_i value of mescaline related to K_i value
Mescaline	1 [23]	$17,850 \pm 1,899$	1
DOMCI	82 [15]	$11,420 \pm 690$	1.6
DOMOM	94 [15]	$2,057 \pm 273$	8.7

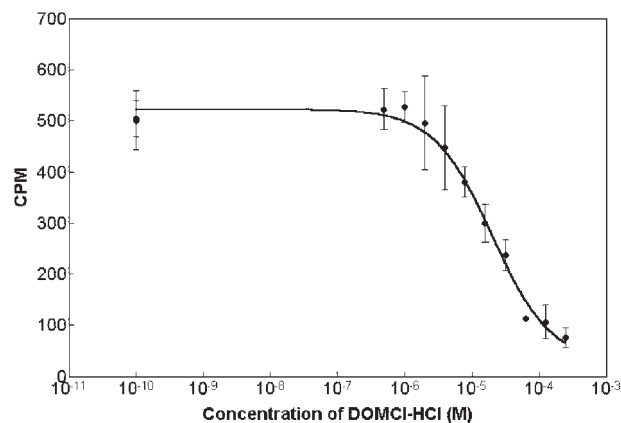


Figure 2. Typical competition isotherms of DOMCI-HCl. Data of a representative experiment is shown, using rat frontal cortex membranes, $250 \mu\text{M} - 500 \text{ nM}$ DOMCI-HCl, and $1 - 1.5 \text{ nM}$ [³H]ketanserin-HCl. The assay was performed on MultiSreen® filter plates as described in *Experimental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

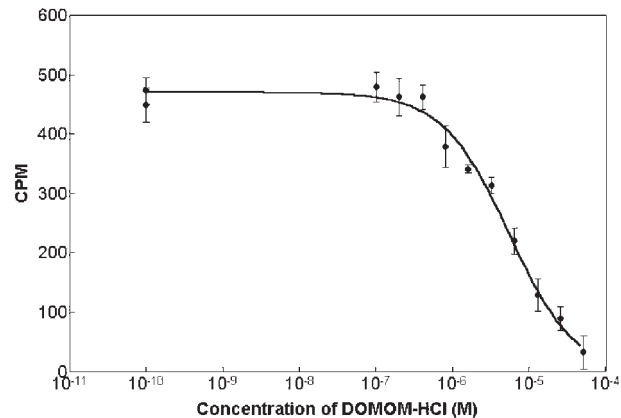


Figure 3. Typical competition isotherm of DOMOM-HCl. Data of a representative experiment is shown, using rat frontal cortex membranes, $50 \mu\text{M} - 100 \text{ nM}$ DOMOM-HCl, and $1 - 1.5 \text{ nM}$ [³H]ketanserin-HCl. The assay was performed on MultiSreen® filter plates as described in *Experimental Section*.

Conclusion

Using a modified Blanc reaction made it possible to synthesize 2,5-dimethoxyphenylpropanamine derivatives which have a substituted methyl group in position 4.

In preliminary studies the hallucinogenic potency of DOMCI and DOMOM was calculated by molecular modeling. It was calculated in mescaline units, based on mescaline as a reference substance, which has a mescaline unit of one [18]. The mescaline unit is defined as quotient of the effective dose of mescaline and the effective dose of the investigated substance, calculated as free base [18]. DOMCI was calculated to have 82 mescaline units, and DOMOM was calculated to have 94 mescaline units. This means that the substances were expected to be 82 and 94 times more potent, respectively, as hallucinogens than mescaline. The results of the 5-HT_{2A} radioligand and receptor binding assays showed that the affinities towards the 5-HT_{2A} receptor are much lower than expected (see Table 1). With these results the models might now be improved.

Experimental section

Chemistry

Materials and equipment

Products from Merck of p.a. grade were used as anhydrous solvents. – Melting points: Melting point apparatus from Galenkamp, uncorrected. – IR: IR-Spectrometer FTIR IFS 48 (Bruker). – MS: Mass spectrometer TSQ 70 (Finnigan). – ¹H-NMR (250 MHz) AC 250-spectrometer (Bruker), tetramethylsilane as internal standard, δ in ppm.

2,5-Dimethoxyamphetamine 1

This compound was prepared as described in ref. [17].

1-(4-Chloromethyl-2,5-dimethoxyphenyl)-2-propanamine 2 (DOMCI)

A mixture of **1** (1 g, 4.3 mmol) as hydrochloride and chlorobenzene (50 mL), paraformaldehyde (0.5 g), and of concentrated hydrochloric acid (4 drops) was stirred well and saturated with a constant stream of hydrochloric gas. Any contact with the reaction mixture or the gas that streams out of the apparatus is to be avoided, because paraformaldehyde and HCl gas react to dichlorodimethyl ether which is carcinogenic. The temperature should not exceed 20 °C. After **1** was completely dissolved, HCl gas was bubbled through the solution for one more hour. Then the gas stream was stopped and after 30 min of stirring precipitated **2** was removed by filtration and washed several times with ether. The white solid was recrystallized from ethanol.

Yield 0.85 g (70.8 %), decomposition at 180–185 °C. – IR (KBr), $\bar{\nu}/\text{cm}^{-1}$ = 2880 (v N⁺-H); 2734 (v OCH₃); 2001 (v N⁺-H); 1607, 1511, 1494 (v C=C); 1466, 1404 (v C-H); 1222 (v C_{ar}-O); 1037 (v C_{ar}-O). – ¹H-NMR (DMSO-d₆, δ [ppm]): 8.17 (br s, 3H, NH); 7.1 (s, 3'-H); 6.8 (s, 6'-H); 4.7 (s, 9'-H₂); 3.80/3.75 (s/s, 7'-H₃/8'-H₃); 3.7 (m, 2-H); 2.95 (dd, ²J = 13.2 Hz, ³J = 5.6, 1-H); 2.74 (dd, ²J = 13.1 Hz, ³J = 8.4 Hz, 1-H); 1.12 (d, J = 6.3 Hz, 3-H₃). – MS: (EI), *m/z* (%) = 246.1 (5, M+1+2); 244.1 (10, M+1); 202.0

(20); 200.0 (45); 164.0 (55); 135.1 (15); 134.1 (20); 91.0 (25); 77.0 (15); 44.1 (100). Purity (HPLC) > 98 %.

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

To a solution of NaOCH₃, 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 entirely dried in vacuo. The residue was stirred under diethyl 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), $\bar{\nu}/\text{cm}^{-1}$ = 2933 (v N⁺-H); 2058 (v N⁺-H); 1602, 1509 (v C=C); 1214 (v C_{ar}-O); 1041 (v C_{ar}-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, ²J = 13.0 Hz, ³J = 5.9 Hz); 2.51 (dd, 1-H, ²J = 13.0 Hz, ³J = 8.1 Hz); 1.43 (br s, 2-H, 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 %.

5-HT_{2A} radioligand receptor binding assays

Materials and equipment

Frozen, unstripped rat brains were purchased from Pel-Freez Biologicals (Rogers, AR, USA). The Ultra-Turrax[®] homogenizer was from IKA (Staufen, Germany). [³H]Ketanserin-HCl (66.4 Ci/mmol) was obtained from NEN Life Science Products, Inc. (Boston, MA, USA). Ultima Gold[®] scintillation cocktail and Quenched Standards for Tritium in Ultima Gold[®] (No. 52) were from Packard Instrument Company (Meriden, CT, USA). Multi-Screen[®] MAFB filter plates, the Vacuum Manifold[®] filtration system, and MultiScreen Tape[®] were from Millipore GmbH (Eschborn, Germany). The 1450-106 Millipore Cassette[®] including support frame as well as Betaplate Scint[®] scintillation cocktail, the Microbeta[®] PLUS microplate scintillation counter, and the LKB 1219 Rackbeta[®] liquid scintillation counter were from Wallac (Freiburg, Germany). Mianserin-HCl was purchased from RBI (Natick, MA, USA). All other chemicals were of A.C.S. grade, obtained from Sigma Aldrich Chemie (Deisenhofen, Germany). Mescaline was from Degkwitz (Freiburg, Germany). DOMCI and DOMOM were synthesized as described above.

Buffer composition

Buffer I: 50 mM HEPES, 10 mM MgCl₂, and 0.5 mM EDTA, pH 7.4 at 4 °C.

Buffer II: 50 mM HEPES, 10 mM MgCl₂, and 0.5 mM EDTA, pH 7.4 at 37 °C.

Assay buffer: 50 mM HEPES, 10 mM MgCl₂, 0.5 mM EDTA, and 0.1 % ascorbic acid, pH 7.4 at 37 °C.

Tissue preparation

Rat prefrontal cortex membranes were used as they have a high density of 5-HT_{2A} receptors [19]. The membranes were prepared as described in ref. [16]. In short, 50 rat brains were first placed in ice-cold (4 °C) 0.32 M aqueous sucrose solution and left to thaw. Prefrontal cortices were collected and placed immediately in a 10-fold volume of ice-cold 0.32 M sucrose and 10 mM HEPES (pH 7.4). The tissue was homogenized with a Potter glass tube and a motor-driven Teflon pestle. Nuclei, cell debris, erythrocytes, and myelin were removed by centrifuga-

tion at 1,000 g for 30 min. The pellet was rehomogenized in the same mixture of sucrose and HEPES (1:5 w/v), 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 w/v) 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®) 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® liquid scintillation counter that had been standardized with Quenched Standards for Tritium in Ultima Gold® before.

Data analysis

Data analysis was performed with PRISM® Software, Version 2.0 (GraphPad Software, Inc., San Diego, CA). K_D was found from pervious saturation experiments to be 2.0 ± 0.2 nM as described in ref. [16]. K_i values were calculated according to the equation of Chang and Prusoff [20] as: $K_i = IC_{50}/(1 + (conc_{Radioligand}/K_D))$. IC_{50} values were determined with PRISM® one-site competition nonlinear regression: $[y = bottom + (top - bottom)/(1 + 10^{*-LogEC50})]$, Hill coefficient equals unity]. K_i values of six individual experiments for DOMCI and five individual experiments for DOMOM were calculated and averaged.

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