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Monoamine receptor interaction profiles of 4-thio-substituted phenethylamines (2C-T drugs)

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ABSTRACT

Background: 4-Thio-substituted phenethylamines (2C-T drugs) are potent psychedelics with poorly defined pharmacological properties. Because of their psychedelic effects, 2C-T drugs are sometimes sold as new psychoactive substances (NPSs). The aim of the present study was to characterize the monoamine receptor and transporter interaction profiles of a series of 2C-T drugs.

Methods: We determined the binding affinities of 2C-T drugs at monoamine receptors and transporters in human cells that were transfected with the respective receptors or transporters. We also investigated the functional activation of serotonergic 5-hydroxytryptamine 2A (5-HT_{2A}) and 5-HT_{2B} receptors, activation of human trace amine-associated receptor 1 ($TAAR_1$), and inhibition of monoamine uptake transporters.

Results: 2C-T drugs had high affinity for 5-HT_{2A} and 5-HT_{2C} receptors (1–54 nM and 40–350 nM, respectively). With activation potencies of 1–53 nM and 44–370 nM, the drugs were potent 5-HT_{2A} receptor and 5-HT_{2B} receptor, respectively, partial agonists. An exception to this were the benzylthiophenethylamines, which did not potently activate the 5-HT_{2B} receptor (EC_{50} > 3000 nM). Furthermore, the compounds bound to serotonergic 5-HT_{1A} and adrenergic receptors. The compounds had high affinity for the rat $TAAR_1$ (5–68 nM) and interacted with the mouse but not human $TAAR_1$. The 2C-T drugs did not potently interact with monoamine transporters (K_i > 4000 nM).

Conclusion: The receptor binding profile of 2C-T drugs predicts psychedelic effects that are mediated by potent 5-HT_2 receptor interactions.

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1. Introduction

Substituted phenethylamines are a class of drugs that includes several potent psychedelics that exert their effects through interactions with the serotonergic 5-hydroxytryptamine 2 (5-HT_2) receptor site (Glennon et al., 1982, 1984; Nelson et al., 1999; Nichols, 2004; Titeler et al., 1988). Many psychedelic phenethylamines were first synthesized by Alexander Shulgin during the 1970s and 1980s and were described in the book *PiHKAL: A Chemical Love Story* (Shulgin and Shulgin, 1995). 2C drugs are a subfamily of substituted phenethylamines, consisting of 2,5-dimethoxy-4-substituted phenethylamines. The term 2C refers to the two carbon atoms between the benzene ring and amino group (Shulgin and Shulgin, 1995). Originally proposed as psychotropic agents for psychotherapy (Shulgin and Shulgin, 1995; Shulgin and Carter, 1975), 2C drugs are now popular among recreational drug users because of their psychedelic and entactogenic properties (de Boer and Bosman, 2004;

Abbreviations: 4-bromo-2,5-dimethoxyphenethylamine, 2C-B; 2,5-dimethoxy-4-methylthiophenethylamine, 2C-T-1; 2,5-dimethoxy-4-(β-methallyl)thiophenethylamine, 2C-T-3; 2,5-dimethoxy-4-isopropylthiophenethylamine, 2C-T-4; 2,5-dimethoxy-4-propylthiophenethylamine, 2C-T-7; 2,5-dimethoxy-4-allylthiophenethylamine, 2C-T-16; 2,5-dimethoxy-4-*n*-butylthiophenethylamine, 2C-T-19; 2,5-dimethoxy-4-(2,2-difluoroethylthio)phenethylamine, 2C-T-21.5; 2,5-dimethoxy-4-(2,2,2-trifluoroethylthio)phenethylamine, 2C-T-22; 2,5-dimethoxy-4-isobutylthiophenethylamine, 2C-T-25; 2,5-dimethoxy-4-benzylthiophenethylamine, 2C-T-27; 2,5-dimethoxy-4-(3-fluoropropylthio)phenethylamine, 2C-T-28; 2,5-dimethoxy-4-(4-fluorobutylthio)phenethylamine, 2C-T-30; 2,5-dimethoxy-4-(4-trifluoromethylbenzylthio)phenethylamine, 2C-T-31; 2,5-dimethoxy-4-(3-methoxybenzylthio)phenethylamine, 2C-T-33; 5-hydroxytryptamine (serotonin), 5-HT; dopamine, DA; dopamine transporter, DAT; fluorescence imaging plate reader, FLIPR; high-performance liquid chromatography, HPLC; lysergic acid diethylamide, LSD; norepinephrine, NE; norepinephrine transporter, NET; new psychoactive substance, NPS; serotonin transporter, SERT; trace amine-associated receptor 1, $TAAR_1$.

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Gonzalez et al., 2015). Today, the Internet appears to be the main source for both acquiring information on and purchasing 2C drugs and other NPSs (Brandt et al., 2014; Orsolini et al., 2017; Schifano et al., 2005). Although classic 2C drugs are considered physiologically safe, several incidences, including sympathomimetic toxicity, psychosis, and death, have been documented (Bosak et al., 2013; Curtis et al., 2003; Huang and Bai, 2011; Miyajima et al., 2008; Stoller et al., 2017). Additionally, several 2C fatalities have been reported in the media (Dean et al., 2013). Moreover, newly emerged highly potent phenethylamine hallucinogens, including *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-substituted ("NBOMe") phenethylamines, were found to be unexpectedly toxic and recently associated with several fatalities (Nichols, 2016; Nikolaou et al., 2015; Poklis et al., 2014; Rose et al., 2013; Suzuki et al., 2015). We previously reported the receptor and transporter interaction profiles of 2C drugs compared with their NBOMe analogs (Rickli et al., 2015). The sulfur-containing 2C drugs (2C-T-2, 2C-T-4, and 2C-T-7) that were included in the study proved to be potent agonists at 5-HT_2 receptors (Rickli et al., 2015). Several other compounds of the 2C-T series have been described (Shulgin and Shulgin, 1995; Trachsel, 2003), but little information is available regarding their interactions with monoamine receptors and transporters. On Internet drug discussion websites such as bluelight.org, the most commonly discussed 2C-T drugs are 2C-T-2, 2C-T-4, 2C-T-7, and 2C-T-21. Other compounds of the series are only sporadically mentioned and their use does currently not seem to be widespread. However, NPSs constantly emerge and it is possible that several other 2C-T compounds will appear on the drug market in the future.

In the present study, we determined and compared the monoamine receptor and transporter affinities of 14 compounds of the 2C-T series (Fig. 1). The numbering of the compounds of the 2C-T series describes the sequence of construction and has no structural relationship (Shulgin and Shulgin, 1995). 2C-T-3 was first named 2C-T-20; however, because its amphetamine analog 2,5-dimethoxy-4-(beta-methallylthio)amphetamine was originally named Aleph-3, 2C-T-20 was later renamed 2C-T-3 to maintain consistency between the 2C-T and Aleph series (Shulgin and Shulgin, 1995). The unusual number of 2C-T-21.5 is based on the fact that with its difluoroethylthio substitution, 2C-T-21.5 lies between the mono-fluorinated 2C-T-21 and tri-fluorinated 2C-T-22 (Shulgin and Shulgin, 1995).

2. Material and methods

2.1. Drugs

The 2C-T drugs were synthesized as hydrochlorides as described previously (Shulgin and Shulgin, 1995; Trachsel, 2003) and provided by ReseaChem GmbH. High-performance liquid chromatography (HPLC) purity was >98.5%. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) hydrochloride, D-methamphetamine hydrochloride, and lysergic acid diethylamide (LSD) were purchased from Lipomed (Arlesheim, Switzerland), with high-performance liquid chromatography (HPLC) purity > 98.5%.

2.2. 5-HT_{1A} and 5-HT_{2A} receptor radioligand binding assays

For membrane preparations, HEK 293 cells that were transiently transfected with the 5-HT_{1A} or 5-HT_{2A} receptor were released from the culture flasks using trypsin/ethylenediaminetetraacetic acid (EDTA), harvested, washed twice with ice-cold phosphate-buffered saline (PBS; without Ca^{2+} and Mg^{2+}), pelleted at 1000 rotations per minute (rpm) for 5 min at 4 °C, frozen, and stored at −80 °C. Frozen pellets were suspended in 20 ml HEPES-NaOH (20 mM, pH 7.4) that contained 10 mM EDTA and homogenized with a Polytron (PT 6000, Kinematica, Luzern, Switzerland) at 14,000 rpm for 20 s. The homogenates were centrifuged at 48,000 × g for 30 min at 4 °C. Subsequently, the supernatants were removed and discarded, and the pellets were resuspended in 20 ml HEPES-NaOH (20 mM, pH 7.4) that contained 0.1 mM EDTA using the Polytron (20 s at 14,000 rpm). This procedure was repeated, and the final pellets were resuspended in HEPES-NaOH that contained 0.1 mM EDTA and homogenized using the Polytron. Typically, aliquots of 2 ml membrane portions were stored at −80 °C. With each new membrane batch, the dissociation constant (K_d) was determined by a saturation curve.

For the competitive binding assays, [^{3}H]-8-OH-DPAT and [^{3}H]-ketanserin were used as 5-HT_{1A} and 5-HT_{2A} receptor radioligands, respectively, at concentrations equal or close to the K_d values. Specific binding of the radioligands to the target receptors was defined as the difference between total binding (binding buffer alone) and nonspecific binding that was determined in the presence of 10 μM pindolol (for the 5-HT_{1A} receptor radioligand) or 10 μM spiperone (for the 5-HT_{2A} receptor radioligand). The compounds were tested at a broad range of concentrations (30 pM–30 μM) in duplicate. The test compounds were diluted in binding assay buffer at pH 7.4 (50 mM Tris/HCl, 10 mM $MgCl_2$, and 1 mM EGTA), and dilution curves were constructed in assay microplates (Greiner, 96-well, U-bottom, PS). Radioligand (50 μl) and the membrane suspension (100 μl) were added to the assay plates to a final volume of 200 μl in each well and incubated and shaken for 30 min at room temperature. Incubations were terminated by rapid filtration through Unifilter-96 plates (Packard Instrument Company, PerkinElmer, Schwerzenbach, Switzerland) and GF/C glass filters (PerkinElmer) that were presoaked for a minimum of 1 h in 0.3% polyethylenimine and washed three times with ice-cold washing buffer (50 mM Tris/HCl, pH 7.4). After the addition of Microscint 40 (45 μl/well, PerkinElmer), the Unifilter-96 plates were sealed. After 1 h, radioactivity was counted using a TopCount Microplate Scintillation Counter (Packard Instrument Company). IC_{50} values were determined by calculating nonlinear regression curves for a one-site model using at least three independent 10-point concentration-response curves, run in duplicate, for each compound. K_i (affinity) values, which correspond to the dissociation constants, were determined using the Cheng-Prusoff equation: $K_i = IC_{50} / (1 + \text{radioligand concentration} / K_d)$. K_i values are presented as means ± SD (in μM).

2.3. 5-HT_{2C} receptor radioligand binding assay

For membrane preparations, HEK 293 cells that were transiently transfected with the 5-HT_{2C} receptor were released from the culture flasks using trypsin/EDTA, harvested, washed twice with ice-cold PBS (without Ca^{2+} and Mg^{2+}), pelleted at 1000 rpm for 5 min at 4 °C, frozen, and stored at −80 °C. Frozen pellets were suspended in 20 ml HEPES/NaOH (20 mM, pH 7.4) that contained 10 mM EDTA and homogenized with a Polytron (PT 6000, Kinematica) at 14,000 rpm for 20 s. The homogenates were centrifuged at 48,000 × *g* for 30 min at 4 °C. Subsequently, the supernatants were removed and discarded, and the pellets were resuspended in 20 ml HEPES-NaOH (20 mM, pH 7.4) that contained 0.1 mM EDTA using the Polytron (20 s at 14,000 rpm). This procedure was repeated, and the final pellets were resuspended in HEPES/NaOH that contained 0.1 mM EDTA and homogenized using the Polytron. Typically, 2 ml aliquots of membrane portions were stored at −80 °C. With each new membrane batch, the dissociation constant (K_d) was determined by a saturation curve.

For the competitive binding assay, [^{3}H]-mesulergine was used as the 5-HT_{2C} receptor radioligand at a concentration equal or close to the K_d value. Specific binding of the radioligand to the target