Pharmacologic Activity of Substituted Tryptamines at 5-Hydroxytryptamine (5-HT)$_{2A}$ Receptor (5-HT$_{2A}$R), 5-HT$_{2C}$R, 5-HT$_{1A}$R, and Serotonin Transporter

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ABSTRACT

Novel psychoactive substances, including synthetic substituted tryptamines, represent a potential public health threat. Additionally, some substituted tryptamines are being studied under medical guidance as potential treatments of psychiatric disorders. Characterizing the basic pharmacology of substituted tryptamines will aid in understanding differences in potential for harm or therapeutic use. Using human embryonic kidney cells stably expressing 5-hydroxytryptamine (5-HT)$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors (5-HT$_{1A}$R, 5-HT$_{2A}$R, and 5HT$_{2C}$R, respectively) or the serotonin transporter (SERT), we measured affinities, potencies and efficacies of 21 substituted tryptamines. With the exception of two 4-acetoxy compounds, substituted tryptamines exhibited affinities and potencies less than one micromolar at the 5-HT$_{2A}$R, the primary target for psychedelic effects. In comparison, half or more exhibited low affinities/potencies at 5-HT$_{2C}$R, 5-HT$_{1A}$R, and SERT. Sorting by the ratio of 5-HT$_{2A}$ to 5-HT$_{2C}$, 5-HT$_{1A}$, or SERT affinity revealed chemical determinants of selectivity. We found that although 4-substituted compounds exhibited affinities that ranged across a factor of 100, they largely exhibited high selectivity for 5-HT$_{2A}$Rs versus 5-HT$_{1A}$Rs and 5-HT$_{2C}$Rs. 5-substituted compounds exhibited high affinities for 5-HT$_{1A}$Rs, low affinities for 5-HT$_{2C}$Rs, and a range of affinities for 5-HT$_{2A}$Rs, resulting in selectivity for 5-HT$_{2A}$Rs versus 5-HT$_{2C}$Rs but not versus 5-HT$_{1A}$Rs. Additionally, a number of psychedelics bound to SERT, with non–ring-substituted tryptamines most consistently exhibiting binding. Interestingly, substituted tryptamines and known psychedelic standards exhibited a broad range of efficacies, which were lower as a class at 5-HT$_{2A}$Rs compared with 5-HT$_{2C}$Rs and 5-HT$_{1A}$Rs. Conversely, coupling efficiency/amplification ratio was highest at 5-HT$_{2A}$Rs in comparison with 5-HT$_{2C}$Rs and 5-HT$_{1A}$Rs.

SIGNIFICANCE STATEMENT

Synthetic substituted tryptamines represent both potential public health threats and potential treatments of psychiatric disorders. The substituted tryptamines tested differed in affinities, potencies, and efficacies at 5-hydroxytryptamine (5-HT)$_{2A}$, 5-HT$_{2C}$, and 5HT$_{1A}$ receptors and the serotonin transporter (SERT). Several compounds were highly selective for and coupled very efficiently downstream of 5-HT$_{2A}$ versus 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors, and some bound SERT. This basic pharmacology of substituted tryptamines helps us understand the pharmacologic basis of their potential for harm and as therapeutic agents.

Introduction

Novel psychoactive substances represent a major public health threat, and their number is rapidly increasing, with hundreds detected since 2005 (Liechti, 2015). Among novel psychoactive substances, synthetic substituted tryptamines are of interest to the Drug Enforcement Administration. The current panel of 21 structurally related designer substituted tryptamines was selected by the Drug Enforcement Administration for pharmacologic evaluation.

Designer substituted tryptamines are part of a large group of indole-containing compounds; naturally occurring indoles are found in bacteria, fungi, plants, and animals and/or are used as drugs in clinical practice (Kaushik et al., 2013). *N,N*-dimethyltryptamine (DMT) is derived from leaves of the *Psychotria viridis* bush (Grob et al., 1996; Callaway et al., 2005) and psilocybin and psilocin [4-phosphoryloxy-DMT and 4-hydroxy (4-OH)-DMT, respectively] from mushrooms of the genus *Psilocybe* (Van Court et al., 2022). Many substituted tryptamines are psychoactive, and some exhibit psychedelic properties.

These known psychedelic-substituted tryptamines exert their effects via activation of Gq-coupled 5-hydroxytryptamine (5-HT)$_{2A}$ receptors (5-HT$_{2A}$Rs) (Roth et al., 1984; Roth et al., 1986; Kim et al., 2020) and possibly Gi-coupled 5-HT$_{1A}$ receptors (5-HT$_{1A}$Rs) (Krebs-Thomson et al., 2006; Pokorny et al.,

2016). 5-HT$_{2A}$Rs are localized predominantly in cortex, claustrum, and ventral striatum (Pompeiano et al., 1994), whereas 5-HT$_{1A}$Rs are expressed on 5-HT-releasing neurons in the dorsal raphe nucleus, playing a role in feedback control of 5-HT release (Pompeiano et al., 1992). Many known substituted tryptamines are also 5-HT$_{2C}$ receptor (5-HT$_{2C}$R) agonists and either 5-HT (serotonin) transporter (SERT) substrates or inhibitors—additional features that likely influence their psychoactive properties and potential toxicities (Blough et al., 2014).

Other designer tryptamines, with substitutions at either the 4 or 5 position or at the methylated positions of DMT (see Fig. 1), are novel psychoactive substances that are mostly poorly characterized and little studied (Gatch et al., 2011; Blough et al., 2014; Gatch et al., 2020). We previously characterized *N,N*-diisopropyltryptamine (DiPT), 5-*N,N*-diethyl-5-methoxytryptamine (5-MeO-DET), and 5-methoxy-α-methyltryptamine (5-MeO-AMT) for affinity (inhibitory constant K$_i$) and/or potency (EC$_{50}$) at 5-HT$_{1A}$R and 5-HT$_{2A}$R and in monoamine transporter binding assays, as well as for their ability to substitute for the discriminative stimulus properties of DMT, lysergic acid diethylamide tartrate (LSD), 2,5-dimethoxy-α,4-dimethyl-benzene ethanamine (DOM), 3,4-ethylenedioxy-methamphetamine (MDMA), and cocaine (Gatch et al., 2011). We found that properties of these drugs were consistent with those of other classic psychedelics such as DMT. A larger group of substituted tryptamines remain understudied. *N,N*-diethyltryptamine (DET), *N,N*-dipropyltryptamine (DPT), 4-OH-*N,N*-diethyltryptamine (4-OH-DET), 4-OH-*N,N*-diisopropyltryptamine (4-OH-DiPT), 4-OH-N-methyl-N-ethyltryptamine (4-OH-MET), 4-OH-*N*-methyl-N-isopropyltryptamine (4-OH-MiPT), 4-OH-*N*-methyl-N-propyltryptamine (4-OH-MPT), 4-methoxy-*N*-methyl-N-isopropyltryptamine (4-MeO-MiPT), 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DiPT), and 5-methoxy-*N*-methyl-N-isopropyltryptamine (5-MeO-MiPT) were first described by Alexander Shulgin (Shulgin and Shulgin, 1997). 4-Acetoxy-*N,N*-dimethyltryptamine (4-AcO-DMT) was reported as an easier-to-synthesize alternative to psilocybin and psilocin that, as a prodrug, is deacetylated to psilocin (Nichols and Frescas, 1999). 4-Acetoxy-*N,N*-diethyltryptamine (4-AcO-DET) was first synthesized by Albert Hoffman in 1958 (Klein et al., 2020). The synthesis and/or use of others such as 4-acetoxy-*N,N*-diisopropyltryptamine (4-AcO-DiPT), 4-acetoxy-N-methyl-N-ethyltryptamine (4-AcO-MET), 4-acetoxy-*N*-methyl-N-isopropyltryptamine (4-AcO-MiPT), 4-methoxy-*N,N*-diisopropyltryptamine (4-MeO-DiPT), *N,N*-diallyl-5-methoxytryptamine (5-MeO-DALT), and 5-methoxy-*N,N*-dipropyltryptamine (5-MeO-DPT) have been described in psychedelic forums.

Despite their importance with respect to illicit use and potential toxicity, affinities for these substituted tryptamines at key 5-HT receptors and SERT are largely unknown. Analysis of 436 samples submitted by recreational users and purported to be tryptamines included many of the poorly characterized substituted tryptamines described above (Palma-Conesa et al., 2017). For a large subset, EC$_{50}$ values at some 5-HT receptors, including 5-HT$_{2A}$R, and at monoamine transporters have been reported using calcium mobilization assays as receptor activation readout (Blough et al., 2014; Klein et al., 2018; Klein et al., 2020). 4-OH-DMT, 4-AcO-DMT, 4-OH-MET, 4-OH-DET, 4-AcO-DET, 5-MeO-MIPT, 4-AcO-MIPT, 4-OH-DIPT, and 4-AcO-DIPT substituted for the discriminative stimulus effects of DOM, with ED$_{50}$ values ranging across an order of magnitude (Gatch et al., 2020). Some substituted tryptamines have been associated with fatalities (Tanaka et al., 2006; Malaca et al., 2020). The toxic potential is increased with coadministration of monoamine oxidase inhibitors (Malcolm and Thomas, 2022).
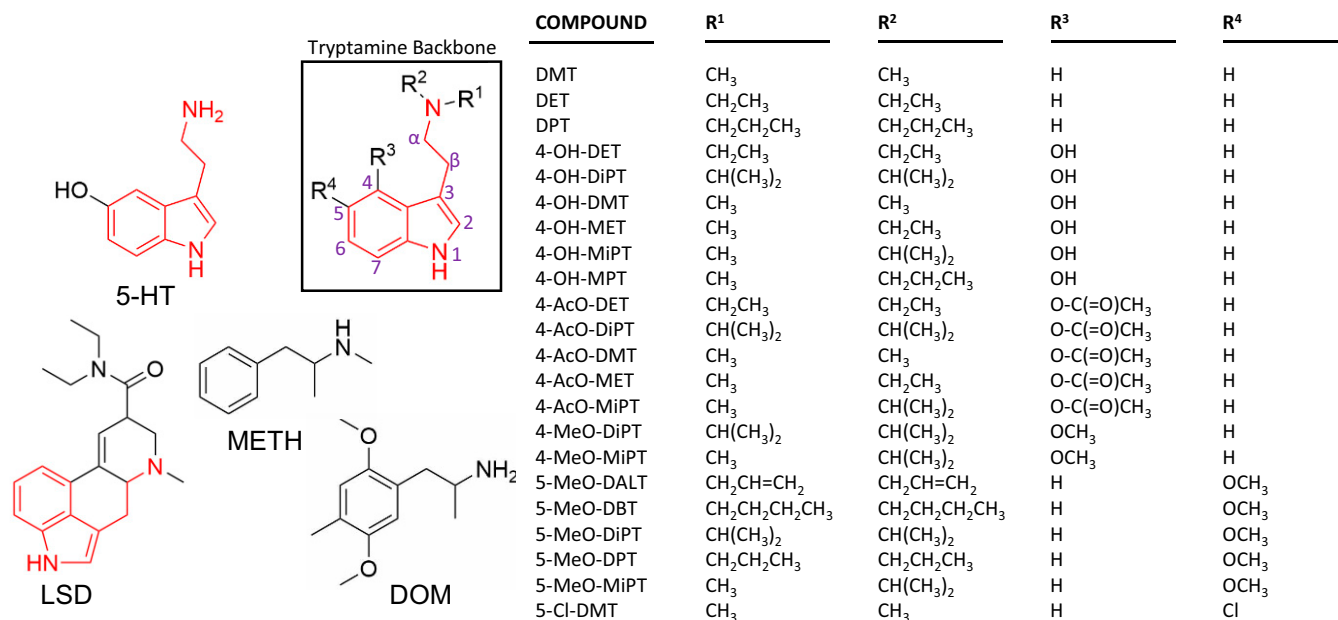
Finally, interest in therapeutic potential of psychedelics has intensified in recent years. Evidence suggests that psilocybin (prodrug for psilocin, 4-OH-DMT) exhibits rapid and sustained antidepressant effects (Carhart-Harris et al., 2016; Griffiths et al., 2016; Nutt et al., 2020; Carhart-Harris et al., 2021) and is being studied with respect to efficacy in treating psychiatric disorders such as PTSD and substance use disorders (Abbas et al., 2021). A fuller characterization of substituted tryptamines may help to identify chemical determinants of pharmacologic selectivity. To achieve these goals, we characterized the affinities, potencies and efficacies of a large panel of substituted tryptamines at 5-HT$_{1A}$R, 5-HT$_{2A}$R, 5-HT$_{2C}$R, and SERT.

Materials and Methods

Drugs and Chemicals. DMT, DET, DPT, 4-OH-DET, 4-OH-DiPT, 4-OH-DMT, 4-OH-MET, 4-OH-MiPT, 4-OH-MPT, 4-AcO-DET, 4-AcO-DiPT, 4-AcO-DMT, 4-AcO-MET, 4-AcO-MiPT, 4-MeO-DiPT, 4-MeO-MiPT, 5-MeO-DALT, 5-methoxy-*N,N*-dibutyltryptamine (5-MeO-DBT), 5-MeO-DiPT, 5-MeO-DPT, 5-MeO-MiPT, 5-chloro-*N,N*-dimethyltryptamine (5-Cl-DMT) and 5-HT were purchased from Cayman Chemicals (Ann Arbor, MI).

(−)DOM, (−)cocaine, and S(+)methamphetamine (METH) and LSD were provided by the National Institute on Drug Abuse Drug Supply Program (Rockville, MD). [Propyl-2,3-ring-1,2,3-3H]-8-Hydroxy-DPAT ([^{3}H]8-OH-DPAT), [^{125}I]RTI-55, [^{3}H]5-HT and [^{35}S]GTPγS were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). The inositol monophosphate (IP-1) Elisa kit was purchased from Cisbio (Bedford, MA). Other reagents were purchased from Sigma (St. Louis, MO).

ABBREVIATIONS: 4-AcO-DET, 4-acetoxy-*N, N*-diethyltryptamine (HCl); 4-AcO-DiPT, 4-acetoxy-*N, N*-diisopropyltryptamine, ipracetin (acetate); 4-AcO-DMT, 4-acetoxy-*N, N*-dimethyltryptamine (HCl or fumarate); 4-AcO-MET, 4-acetoxy-N-methyl-N-ethyltryptamine (HCl); 4-AcO-MiPT, 4-acetoxy-*N*-methyl-N-isopropyltryptamine (HCl); 5-Cl-DMT, 5-chloro-*N, N*-dimethyltryptamine (HCl); DET, *N, N*-diethyltryptamine (HCl); DMT, *N, N*-dimethyltryptamine (succinate or fumarate); DOM, 2, 5-dimethoxy-α, 4-dimethyl-benzene ethanamine (HCl); DPT, *N, N*-dipropyltryptamine (HCl); HEK, human embryonic kidney; hSERT, human serotonin transporter; 5-HT, 5-hydroxytryptamine, serotonin; 5-HT$_{1A}$R, 5-HT$_{1A}$ receptor; 5-HT$_{2A}$R, 5-HT$_{2A}$ receptor; 5-HT$_{2C}$R, 5-HT$_{2C}$ receptor; IP-1, inositol monophosphate; K$_i$, inhibitory constant; LSD, lysergic acid diethylamide tartrate; MDMA, 3, 4-ethylenedioxy-methamphetamine; 5-MeO-DALT, *N, N*-diallyl-5-methoxytryptamine (no salt); 5-MeO-DBT, 5-methoxy-*N, N*-dibutyltryptamine (no salt); 4-MeO-DiPT, 4-methoxy-*N, N*-diisopropyltryptamine (HCl); 5-MeO-DiPT, 5-methoxy-*N, N*-diisopropyltryptamine, Foxy (no salt); 5-MeO-DPT, 5-methoxy-*N, N*-dipropyltryptamine (no salt); 4-MeO-MiPT, 4-methoxy-*N*-methyl-N-isopropyltryptamine (HCl); 5-MeO-MiPT, 5-methoxy-*N*-methyl-N-isopropyltryptamine (no salt); METH, methamphetamine (HCl); 4-OH, 4-hydroxy; 4-OH-DET, 4-hydroxy-*N, N*-diethyltryptamine (no salt); 4-OH-DiPT, 4-hydroxy-*N, N*-diisopropyltryptamine (HCl); 4-OH-DMT, 4-hydroxy-*N, N*-dimethyltryptamine, psilocin (no salt); 4-OH-MET, 4-hydroxy-*N*-methyl-N-ethyltryptamine, metocin (no salt); 4-OH-MiPT, 4-hydroxy-*N*-methyl-N-isopropyltryptamine (no salt); 4-OH-MPT, 4-hydroxy-*N*-methyl-N-propyltryptamine; SERT, serotonin transporter.

COMPOUND	R[1]	R[2]	R[3]	R[4]
DMT	CH_3	CH_3	H	H
DET	CH_2CH_3	CH_2CH_3	H	H
DPT	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	H	H
4-OH-DET	CH_2CH_3	CH_2CH_3	OH	H
4-OH-DiPT	$CH(CH_3)_2$	$CH(CH_3)_2$	OH	H
4-OH-DMT	CH_3	CH_3	OH	H
4-OH-MET	CH_3	CH_2CH_3	OH	H
4-OH-MiPT	CH_3	$CH(CH_3)_2$	OH	H
4-OH-MPT	CH_3	$CH_2CH_2CH_3$	OH	H
4-AcO-DET	CH_2CH_3	CH_2CH_3	$O-C(=O)CH_3$	H
4-AcO-DiPT	$CH(CH_3)_2$	$CH(CH_3)_2$	$O-C(=O)CH_3$	H
4-AcO-DMT	CH_3	CH_3	$O-C(=O)CH_3$	H
4-AcO-MET	CH_3	CH_2CH_3	$O-C(=O)CH_3$	H
4-AcO-MiPT	CH_3	$CH(CH_3)_2$	$O-C(=O)CH_3$	H
4-MeO-DiPT	$CH(CH_3)_2$	$CH(CH_3)_2$	OCH_3	H
4-MeO-MiPT	CH_3	$CH(CH_3)_2$	OCH_3	H
5-MeO-DALT	$CH_2CH=CH_2$	$CH_2CH=CH_2$	H	OCH_3
5-MeO-DBT	$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$	H	OCH_3
5-MeO-DiPT	$CH(CH_3)_2$	$CH(CH_3)_2$	H	OCH_3
5-MeO-DPT	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	H	OCH_3
5-MeO-MiPT	CH_3	$CH(CH_3)_2$	H	OCH_3
5-Cl-DMT	CH_3	CH_3	H	Cl

Fig. 1. Structures of tested substituted tryptamines and standards. The tryptamine backbone is highlighted in red. Structures of test compounds 5-HT, LSD, METH and DOM are shown. For each of the compounds tested the R1-R4 substitution is shown in the table on the right.

Radioligand Binding Assays. The radioligands for each receptor assay were chosen on the basis of exhibiting high affinity for the receptor and being commercially available. Individual receptor assays were optimized by assessing the best incubation time, temperature, and protein amount for each assay.

5-HT$_{1A}$R: [^{3}H]8-OH-DPAT Radioligand Binding. Human embryonic kidney (HEK) cells expressing the human 5-HT$_{1A}$R (HEK-5-HT$_{1A}$, passage numbers 14–16, 18–19, and 21–22) were used. The methods for transfection of HEK cells, cell membrane preparation, and [^{3}H]8-OH-DPAT agonist binding have been described previously (Eshleman et al., 1999). The density and affinity of [^{3}H]8-OH-DPAT binding sites were 1670 fmol/mg protein and 5.0 nM, respectively. Briefly, the binding reaction mixture contained test compound, cell homogenate (0.05 mg of protein), and [^{3}H]8-OH-DPAT (0.4–0.6 nM final concentration) in a final volume of 1 ml (assay buffer: 25 mM Tris-HCl, pH 7.4, containing 1mM ascorbic acid and 10 μM pargyline) and was incubated at 25°C for 1 hour. Nonspecific binding was determined with 1 μM dihydroergotamine. The reaction was terminated by filtration through polyethylenimine-soaked "A" filtermats on a Tomtec 96-well cell harvester (Tomtec, Hamden, CT), and radioactivity was counted on a Perkin Elmer (Boston, MA) MicroBeta scintillation counter.

5-HT$_{1A}$R: [^{35}S]GTPγS Binding. The method for [^{35}S]GTPγS binding has been described (Gatch et al., 2011). In brief, cell membranes (0.040–0.075 mg protein) were preincubated (10 minutes, room temperature) with test compound in duplicate in assay buffer (20 mM HEPES, pH 7.4, 10 mM MgCl$_2$, 100 mM NaCl, and 0.2 mM dithiothreitol). The reaction was initiated by addition of GDP (3 μM) and [^{35}S]GTPγS (~150,000 cpm, 1350 Ci/mmol) in a final volume of 1 ml. The reaction was incubated for 1 hour at 25°C and terminated as described above. Agonist efficacy is expressed relative to that of 100 nM 5-HT, which was determined for each experiment.

5-HT$_{2A}$R and 5-HT$_{2C}$R: [^{3}H]5-HT Binding. [^{3}H]5-HT binding to 5-HT$_{2A}$R and 5-HT$_{2C}$R was tested in HEK-293 cells expressing either the human 5-HT$_{2A}$R (hHEK-5-HT$_{2A}$ cells, passage numbers 9, 11 to 12, and 18 to 19) or the human 5-HT$_{2C}$R (HEK-5-HT$_{2C}$ cells, passage numbers 8, 11 to 12, and 15 to 16) as previously described (Eshleman et al., 2020).

For h5-HT$_{2A}$R and h5-HT$_{2C}$R, the density and affinity of [^{3}H]5HT binding sites were 612 and 900 fmol/mg protein and 27 and 10 nM,

respectively. Briefly, the binding reaction mixture contained test compound, cell homogenate, and [^{3}H]5-HT (1.5–7 nM final concentration) in a final volume of 250 μl (assay buffer: 50 mM Tris-HCl, pH 7.4, containing 5 mM ascorbic acid, 5mM CaCl$_2$, and 10 μM pargyline). The assay was incubated for 45 minutes at 37°C and terminated as described above. Nonspecific binding was determined with 10 μM 5-HT.

5-HT$_{2A}$R and 5-HT$_{2C}$R: Inositol Monophosphate Formation. Activation of 5-HT$_{2A}$R (passage numbers 9, 11, and 13–19) and 5-HT$_{2C}$R (passage numbers 6–8, 10–12, 14–16, and 18) was tested by measuring the accumulation of inositol monophosphate using the Cisbio IP-1 Elisa kit as described previously (Gatch et al., 2011; Eshleman et al., 2014). Briefly, cells were plated at a density of 400,000 cells per well in 24-well plates. The next day, cells were starved with Dulbecco's Modified Eagle's Medium (DMEM) for 1 hour, medium was removed, and stimulation buffer was added. After 10 minutes incubation, agonists were added and plates were incubated for 60 minutes at 37°C in a humidified 5% CO$_2$ incubator. Cells were lysed, and 50 μl aliquots of the lysates were added to the IP-1 plate. The assay was conducted according to kit instructions. Stimulated IP-1 formation was normalized to the maximal effect of 5-HT, which was determined in each assay.

SERT: Inhibition of [^{125}I]RTI-55 Binding to and [^{3}H]5-HT Uptake by Human SERT in Clonal Cells. The methods for characterizing radioligand binding and functional uptake assays have been described previously (Eshleman et al., 2018) using human embryonic kidney (HEK-293) cells expressing the human SERT (HEK-hSERT, passage numbers 6–12, 14, 16, 20–21, and 25–27). The density and affinity of [^{125}I]RTI-55 binding sites were 0.85 pmol/mg protein and 0.98 nM for SERT. Binding assays were conducted with a total particulate membrane preparation and incubated at room temperature for 90 minutes. The uptake assay was conducted in duplicate and initiated by the addition [^{3}H]5-HT, (8–12 nM final concentration) to intact detached cells and incubated at 25°C for 10 minutes.

Data Analysis. For competition binding assay results, data were normalized to the specific binding in the absence of drug. Three or more independent competition experiments were conducted with duplicate determinations. The number of independent experiments were determined using an acceptable error set at ≤35% of the mean. GraphPad Prism (La Jolla, CA) was used to analyze the ensuing data,
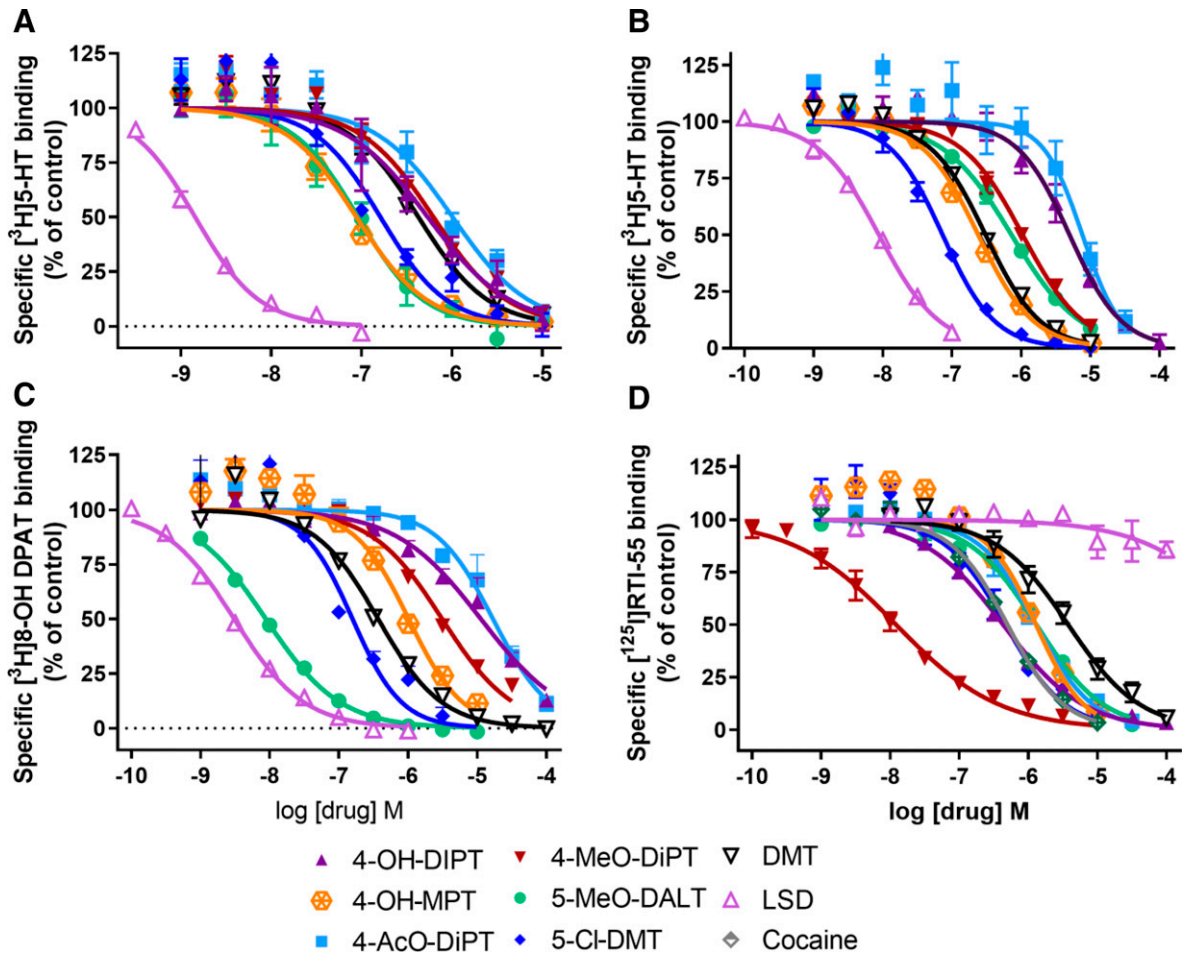
Fig. 2. Concentration-response curves of substituted tryptamines in radioligand binding assays at 5-HT$_{2A}$R (A), 5-HT$_{2C}$R (B), 5-HT$_{1A}$R (C), and SERT (D). Data shown for substituted tryptamines are the means ± S.E.M. of three experiments (5-HT$_{1A}$R) or three to five experiments (5-HT$_{2A}$R, 5-HT$_{2C}$R, and SERT) conducted in duplicate.

with IC$_{50}$ values converted to K$_i$ values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). For signal transduction assays, GraphPad Prism was used to calculate EC$_{50}$ values using data expressed as percent 5-HT stimulation for 5-HT$_{1A}$R–stimulated [^{35}S]GTPγS binding and 5-HT$_{2A}$R– and 5-HT$_{2C}$R–mediated IP-1 formation and for percent total specific [^{3}H]5HT uptake for transporters. Differences in affinities, potencies, or efficacies were assessed by one-way ANOVA using the logarithms of the K$_i$ or EC$_{50}$ values for test compounds and standards followed by Dunnett's multiple comparison test with statistical significance set at $P < 0.05$. A Grubbs test was used to determine whether data should be excluded ($P < 0.05$, two sided). Paired comparisons of group coupling efficiencies (amplification ratio) at 5-HT$_{2A}$Rs versus 5-HT$_{2C}$Rs and 5-HT$_{1A}$Rs were made using a paired t test. GraphPad Prism was used to calculate the Spearman correlation coefficient for the affinities and potencies at each 5-HT receptor using the logarithms of the K$_i$ and EC$_{50}$ values. MATLAB (MathWorks, Natick, MA) was used to generate heat maps and scatter plots. To determine the amplification ratio for each tryptamine and reference compound, the ratio of the binding affinity (K$_i$) to the functional potency (EC$_{50}$) for each tryptamine relative to that ratio for 5-HT was calculated (Strange, 2008).

Results

5-HT$_{2A}$ Receptor. The 5-HT$_{2A}$R is the primary target for most hallucinogens (Nichols, 2018). The affinity of the indolealkylamine hallucinogen DMT for the [^{3}H]5-HT binding site

on 5-HT$_{2A}$R was 347 nM, significantly lower than the affinities of the standard compounds 5-HT (19.5 nM), the ergoline hallucinogen LSD (1.26 nM), and the phenethylamine hallucinogen DOM (27.3 nM; Figs. 2A and 4A; Table 1). To begin to characterize the effect of chemical substitution of tryptamines, we compared the substituted tryptamines that we screened to the simplest psychedelic tryptamine, DMT. DET (530 nM) and DPT (374 nM), with diethyl or dipropyl N-substitutions, had similar affinities as DMT. Many substituted tryptamines had significantly higher affinities than DMT, including 4-OH-DMT, 4-OH-MET, 4-OH-MPT, 4-AcO-DMT, 4-AcO-MET, 5-MeO-DALT, 5-MeO-DPT, and 5-MeO-MiPT. All of the 4-OH and 5-MeO substituted compounds had similar or higher affinity than DMT. In comparison, only two compounds had considerably lower affinity: 4-AcO-DiPT and 4-AcO-MiPT; both are likely prodrugs, with 4-AcO converted to 4-OH in the body (Nichols and Frescas, 1999). The rank order of affinity for DMT analogs was 4-OH-DMT (79 nM), 4-AcO-DMT (93 nM), 5-Cl-DMT (134 nM), and DMT (347 nM).

In the 5-HT$_{2A}$R IP1 functional assay, DMT had significantly lower potency (527 nM) than 5-HT, LSD, and DOM (Figs. 3A and 4A; Table 1). In addition, DMT was a partial agonist in this assay with only 38% stimulation compared with 5-HT. DET and DPT had similar potencies as DMT, and DPT had

TABLE 1
Affinities, potencies, and efficacies of substituted tryptamines at the 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_{1A}$ receptors

Drug	5-HT$_{2A}$R		5-HT$_{2C}$R		5-HT$_{1A}$R	
	Inhibition of [^{3}H]5-HT Binding K$_i$ (nM) ± S.E.M. (n)	Stimulation of IP-1 Formation EC$_{50}$ (nM) ± S.E.M. (n) % Max Effect	Inhibition of [^{3}H]5-HT Binding K$_i$ (nM) ± S.E.M. (n)	Stimulation of IP-1 Formation EC$_{50}$ (nM) ± S.E.M. (n) % Max Effect	Inhibition of [^{3}H]8-OH-DPAT Binding K$_i$ (nM) ± S.E.M. (n)	[^{35}S]GTPγS Binding EC$_{50}$ (nM) ± S.E.M. (n) % Max Effect
DMT	347 ± 47 (22)	527 ± 45 (20) / 38.4% ± 1.8%	234 ± 16 (22)	104 ± 11 (20) / 97.6% ± 2.1%	356 ± 34 (17)	210 ± 31 (18) / 97.1% ± 2.4%
DET	530 ± 120 (4)	612 ± 97 (3) / 46.1% ± 6.7%	970 ± 280 (3)[a]	660 ± 210 (4)[a] / 106.4% ± 4.5%	370 ± 25 (3)	138 ± 25 (4) / 97.9% ± 5.6%
DPT	374 ± 97 (3)	943 ± 88 (4) / 85.2% ± 5.1%[a]	807 ± 86 (3)[a]	444 ± 55 (3)[b] / 93.2% ± 3.6%	186 ± 31 (3)	274 ± 55 (3) / 98.5% ± 7.5%
4-OH-DET	269 ± 79 (5)	296 ± 59 (3) / 80.2% ± 5.2%[a]	388 ± 45 (3)	151 ± 33 (3) / 83.0% ± 5.0%	1840 ± 350 (3)[a]	1030 ± 250 (5)[b] / 80.3% ± 4.0%[c]
4-OH-DiPT	430 ± 140 (3)	334 ± 55 (3) / 104.4% ± 2.8%[a]	3800 ± 870 (3)[a]	1080 ± 130 (3)[a] / 103.73% ± 0.77%	8400 ± 2000 (3)[a]	3900 ± 1100 (4)[a] / 36.1% ± 9.9%[a]
4-OH-DMT	79 ± 23 (4)[a]	69 ± 22 (3)[a] / 48.3% ± 6.9%	84 ± 28 (3)[d]	9.1 ± 1.2 (3)[a] / 85.6% ± 3.2%	374 ± 35 (3)	130 ± 11 (3) / 96% ± 13%
4-OH-MET	46.3 ± 4.9 (3)[a]	87 ± 22 (5)[a] / 54.1% ± 3.0%[b]	153 ± 43 (3)	34.6 ± 8.7 (4) / 100.7% ± 4.9%	950 ± 210 (3)[c]	1390 ± 490 (5)[d] / 89.8% ± 5.8%
4-OH-MiPT	113 ± 31 (4)	306 ± 11 (3) / 74.2% ± 1.8%[a]	750 ± 110 (3)[d]	261 ± 45 (3) / 98.4% ± 4.4%	5870 ± 430 (6)[a]	2590 ± 640 (3)[a] / 82.7% ± 2.0%
4-OH-MPT	71.0 ± 5.9 (4)[a]	63.8 ± 9.4 (4)[a] / 53.4% ± 3.6%[c]	203 ± 25 (5)	66.0 ± 3.5 / 99.5% ± 1.5%	910 ± 120 (3)[b]	490 ± 170 (3) / 90.4% ± 9.3%
4-AcO-DET	248 ± 26 (3)	309 ± 97 (4) / 79.1% ± 4.7%[a]	1510 ± 360 (3)[a]	1010 ± 390 (3)[a] / 93.9% ± 9.9%	4570 ± 690 (3)[a]	4500 ± 1400 (4)[a] / 53.9% ± 2.1%[a]
4-AcO-DiPT	850 ± 280 (5)[c]	559 ± 97 (3) / 106.6% ± 1.8%[a]	5000 ± 1100 (4)[a]	1410 ± 310 (3)[a] / 92.8% ± 4.8%	12900 ± 2000 (3)[a]	>100 μM (4)[d] / 14.3% ± 6.7%[a]
4-AcO-DMT	93 ± 16 (3)[b]	109 ± 15 (5)[a] / 39.5% ± 2.6%	224 ± 15 (3)	144.7 ± 3.3 (3) / 91.8% ± 7.4%	202 ± 62 (3)	673 ± 77 (3) / 63.4% ± 2.4%[d]
4-AcO-MET	86 ± 15 (3)[b]	201 ± 39 (5)[c] / 40.1% ± 1.6%	460 ± 120 (3)	91 ± 19 (4) / 89.7% ± 2.2%	1210 ± 210 (3)[a]	3080 ± 700 (3)[a] / 82.5% ± 2.8%
4-AcO-MiPT	800 ± 100 (4)[c]	445 ± 69 (3) / 69.2% ± 1.4%[a]	2230 ± 220 (4)[a]	539 ± 81 (4)[d] / 87.2% ± 6.1%	11,000 ± 1200 (4)[a]	8200 ± 2100 (3)[a] / 58.3% ± 1.7%[a]
4-MeO-DiPT	500 ± 44 (3)	870 ± 110 (4) / 92.1% ± 2.6%[a]	833 ± 67 (3)[a]	179 ± 56 (4) / 85.4% ± 7.8%	2830 ± 300 (3)[a]	1930 ± 590 (4)[a] / 112.6% ± 7.4%
4-MeO-MiPT	178 ± 24 (3)	376 ± 69 (3) / 63.2% ± 6.9%[a]	510 ± 100 (3)[c]	120 ± 12 (3) / 82.4% ± 7.9%	731 ± 42 (4)	1490 ± 140 (3)[d] / 98.8% ± 3.5%
5-MeO-DALT	48 ± 15 (4)[a]	89.6 ± 3.2 (4)[a] / 97.2% ± 4.5%[a]	573 ± 48 (3)[b]	299 ± 83 (5) / 99.2% ± 3.8%	7.95 ± 0.52 (3)[a]	3.4 ± 1.2 (4)[a] / 102.3% ± 3.3%
5-MeO-DBT	562 ± 57 (5)	620 ± 220 (4) / 17.5% ± 3.0%[a]	3130 ± 430 (5)[a]	2400 ± 300 (4)[d] / 75.6% ± 2.8%[d]	337 ± 28 (3)	267 ± 86 (3) / 106.2% ± 8.2%
5-MeO-DiPT	162 ± 32 (4)	84 ± 20 (3)[a] / 99.7% ± 2.7%[a]	1740 ± 440 (3)[a]	326 ± 92 (4)[c] / 96.6% ± 3.6%	149.3 ± 7.6 (3)	56 ± 20 (5)[b] / 93.8% ± 6.7%
5-MeO-DPT	66 ± 12 (4)[a]	102 ± 11 (4)[a] / 81.4% ± 1.8%[a]	1290 ± 320 (3)[a]	810 ± 100 (3)[a] / 95.7% ± 4.0%	14.5 ± 3.5 (3)[a]	5.41 ± 0.50 (3)[a] / 94.8% ± 6.0%
5-MeO-MiPT	113 ± 31 (4)[b]	290 ± 62 (3) / 89.14% ± 0.70%[a]	790 ± 120 (3)	179 ± 56 (6) / 101.2% ± 6.5%	143 ± 27 (8)[d]	610 ± 210 (8) / 109.12% ± 0.55%
5-Cl-DMT	134 ± 21 (3)	310 ± 100 (3) / 45.1% ± 7.1%	55.4 ± 1.9 (3)[a]	21.8 ± 3.7 (3)[b] / 81.2% ± 4.8%	33.4 ± 6.9 (3)[a]	41.7 ± 1.7 (3)[c] / 94.3% ± 2.8%

TABLE 1 continued

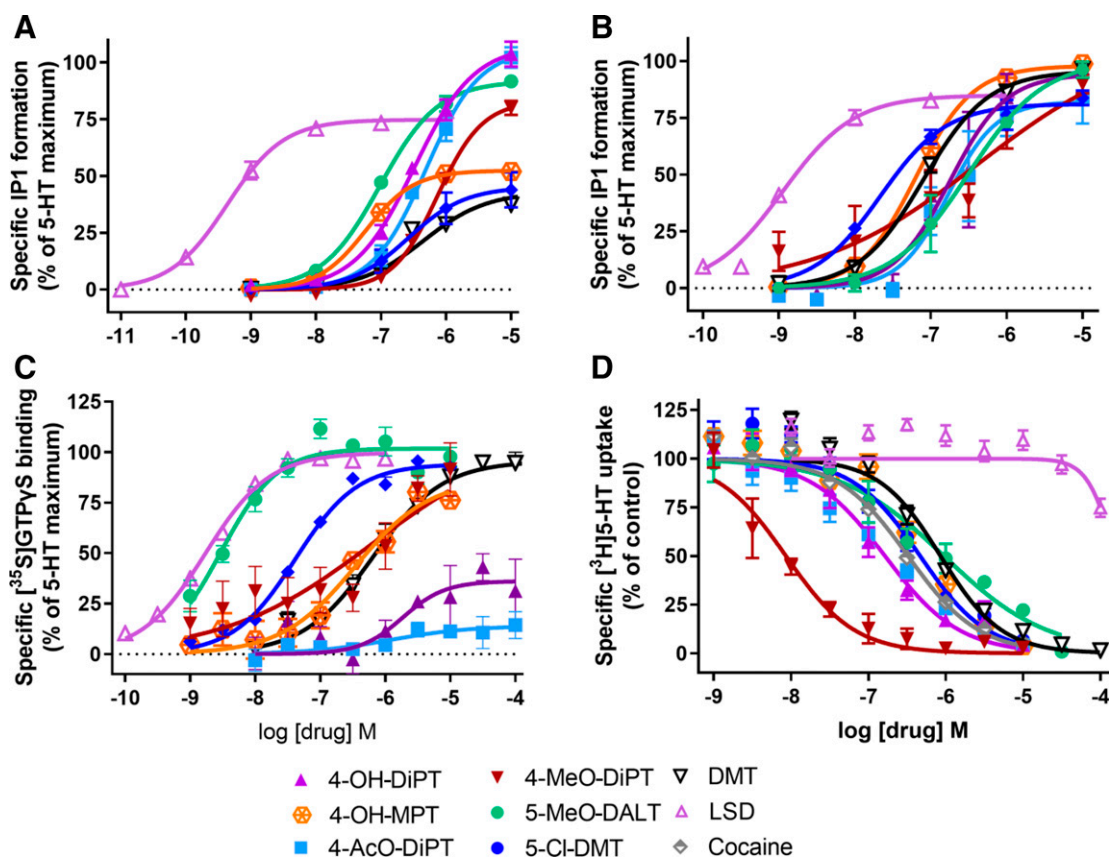|	5-HT$_{2A}$R		5-HT$_{2C}$R		5-HT$_{1A}$R	
Drug	Inhibition of [^{3}H]5-HT Binding K$_i$ (nM) ± S.E.M. (n)	Stimulation of IP-1 Formation EC$_{50}$ (nM) ± S.E.M. (n) % Max Effect	Inhibition of [^{3}H]5-HT Binding K$_i$ (nM) ± S.E.M. (n)	Stimulation of IP-1 Formation EC$_{50}$ (nM) ± S.E.M. (n) % Max Effect	Inhibition of [^{3}H]8-OH-DPAT Binding K$_i$ (nM) ± S.E.M. (n)	[^{35}S]GTPγS Binding EC$_{50}$ (nM) ± S.E.M. (n) % Max Effect
Standards						
5-HT	19.5 ± 1.5 (24)[a]	33.3 ± 3.0 (25)[a] 101.9% ± 1.1%[a]	5.84 ± 0.46 (23)[a]	1.20 ± 0.15 (27)[a] 98.3% ± 1.4%	3.26 ± 0.47 (20)[a]	2.52 ± 0.36 (34)[a] 103.4% ± 1.5%
LSD	1.26 ± 0.19 (25)[a]	0.77 ± 0.12 (25)[a] 74.8% ± 1.9%[a]	6.52 ± 0.72 (22)[a]	1.02 ± 0.15 (27)[a] 86.0% ± 2.0%[b]	2.97 ± 0.35 (22)[a]	2.17 ± 0.36 (31)[a] 98.2% ± 2.0%
DOM	27.3 ± 3.1 (12)[a]	45.8 ± 8.1 (11)[a] 97.0% ± 2.5%[a]	46.1 ± 5.6 (12)[a]	24.4 ± 5.0 (10)[a] 97.4% ± 2.0%	8200 ± 1600 (9)[a]	10,800 ± 3200 (8)[a] 76.2% ± 2.9%[d]

(n), number of experiments conducted in duplicate.
[a]$P < 0.0001$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[b]$P < 0.01$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[c]$P < 0.05$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[d]$P < 0.001$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.

higher efficacy. Most substituted tryptamines had higher potencies (lower EC$_{50}$ values) than DMT, including 4-OH-DMT, 4-OH-MET, 4-OH-MPT, 4-AcO-DMT, 4-AcO-MET, 5-MeO-DALT, 5-MeO-DiPT, and 5-MeO-DPT, whereas no compounds had significantly lower potency. Most substituted tryptamines had higher efficacies at the 5-HT$_{2A}$R than DMT, including DPT, 4-OH-DET, 4-OH-DiPT, 4-OH-MET, 4-OH-MIPT, 4-OH-MPT, 4-AcO-DET, 4-AcO-DiPT, 4-AcO-MiPT, 4-MeO-DiPT, 4-MeO-MiPT, 5-MeO-DPT, and 5-MeO-MiPT. Several compounds were full or nearly full agonists at 5-HT$_{2A}$R, including DPT, 4-OH-DET, 4-OH-DiPT, 4-AcO-DiPT, 4-MeO-DiPT, 5-MeO-DALT, 5-MeO-DiPT, 5-MeO-DPT, and 5-MeO-MiPT. The rank order of potency for DMT analogs was 4-OH-DMT (69 nM), 4-AcO-DMT (109 nM), 5-Cl-DMT (310 nM), and DMT (540 nM). A handful of compounds exhibited efficacies comparable to DMT, including DET, 4-OH-DMT, 4-AcO-DMT, 4-AcO-MET, and 5-Cl-DMT. 5-MeO-DBT was the only compound that had lower efficacy than DMT and exhibited the lowest efficacy at 5-HT$_{2A}$R: 17.5%.

5-HT$_{2C}$ Receptor. The 5-HT$_{2C}$R, a modulator of dopamine neuronal function and a therapeutic target of obesity treatments (Wold et al., 2019), is an additional target for many psychedelics. The affinity of DMT, the least-substituted psychedelic tryptamine, for the [^{3}H]5-HT binding site on 5-HT$_{2C}$R was 234 nM, significantly lower than the affinities of the standard compounds 5-HT, LSD, and DOM (Figs. 2B and 4B; Table 1). DET and DPT had lower affinities than DMT. Two compounds had significantly higher affinities than DMT: 4-OH-DMT and 5-Cl-DMT. Among the 4-OH and 4-AcO substituted analogs, 4-OH-DiPT, 4-OH-MiPT, 4-AcO-DET, 4-AcO-DiPT, and 4-AcO-MiPT had lower affinities. All of the 4-MeO and 5-MeO substituted compounds had lower affinities than DMT. The rank order of affinity for DMT analogs was 5-Cl-DMT (55.4 nM), 4-OH-DMT (84 nM), 4-AcO-DMT (224 nM), and DMT (234 nM).

In the 5-HT$_{2C}$R IP1 functional assay, DMT was a full agonist with potency (104 nM) that was significantly lower than the potencies of 5-HT, LSD, and DOM (Figs. 3B and 4B; Table 1). Similar to their binding affinities, DET and DPT had lower potencies than DMT but were full agonists. Only two compounds had significantly higher potency than DMT: 4-OH-DMT and 5-Cl-DMT. Several compounds had lower potencies (higher EC$_{50}$ values) than DMT, including 4-OH-DiPT, 4-AcO-DET, 4-AcO-DiPT, 4-AcO-MiPT, 5-MeO-DBT, 5-MeO-DiPT, and 5-MeO-DPT. All of the substituted tryptamines were full or nearly full 5-HT$_{2C}$R agonists, with 5-MeO-DBT exhibiting the lowest efficacy, at 75.6%. The rank order of potency for DMT analogs was 4-OH-DMT (9.1 nM), 5-Cl-DMT (21.8 nM), DMT (104 nM), and 4-AcO-DMT (144.7 nM).

5-HT$_{1A}$ Receptor. The 5HT$_{1A}$R is an additional target for many classic psychedelic drugs, and 5-HT$_{1A}$R agonists decrease or increase 5-HT$_{2A}$R–mediated function (Darmani et al., 1990; Reissig et al., 2005). The affinity of the least-substituted psychedelic tryptamine DMT for the [^{3}H]8-OH-DPAT binding site on 5-HT$_{1A}$R was 356 nM, significantly lower than the affinities of 5-HT and LSD and significantly higher than the affinity of DOM (Figs. 2C and 4C; Table 1). DET and DPT had similar affinities as DMT. Five of the six 4-OH substituted compounds had lower affinities than DMT, with only 4-OH-DMT having similar affinity. Likewise, four of five 4-AcO substituted compounds had lower affinities than DMT; only 4-AcO-DMT had similar affinity. Four compounds had higher affinities than DMT: 5-MeO-DALT (8.0 nM),

Fig. 3. Agonist activity of substituted tryptamines at recombinant 5-HT$_{2A}$R (A), 5-HT$_{2C}$R (B), 5-HT$_{1A}$R (C), and inhibition of [^{3}H]5-HT uptake at SERT (D). All receptor data, conducted in duplicate, were normalized to the maximal effect of 5-HT, which was measured on each experimental day. (A) 5-HT$_{2A}$R IP-1 assay. $n = 3–4$ independent experiments. (B) 5-HT$_{2C}$R IP-1 assay. $n = 3–5$ independent experiments. (C) 5-HT$_{1A}$R [^{35}S]GTPγS binding. $n = 3$ to 4 independent experiments. (D) Inhibition of [^{3}H]5-HT uptake by SERT. $n = 4$ to 5 independent experiments conducted with duplicate determinations. Data shown are the means ± S.E.M.

5-MeO-DPT (14.5 nM), 5-MeO-MiPT (143 nM), and 5-Cl-DMT (33.4 nM). The rank order of affinity for DMT analogs was 5-Cl-DMT (33.4 nM), 4-AcO-DMT (202 nM), DMT (356 nM), and 4-OH-DMT (374 nM).

In the 5-HT$_{1A}$R [^{35}S]GTPγS functional binding assay, DMT was a full agonist with a 210-nM potency, which was significantly lower than the potencies of 5-HT and LSD and significantly higher than the potency of DOM (Figs. 3C and 4C). Consistent with their binding affinities, DET and DPT had similar potencies as DMT and were full agonists. Among the 4-OH substituted compounds, all had lower potencies than DMT except for 4-OH-DMT and 4-OH-MPT, which had similar potencies. Among the 4-AcO and 4-MeO substituted compounds, all but one had lower potency than DMT. 4-AcO-DMT had similar potency to DMT. All 4-OH substituted compounds were full agonists except 4-OH-DiPT, which was a partial agonist at the 5-HT$_{1A}$R. Among the 5-MeO substituted compounds, all had higher potency than DMT except for 5-MeO-DBT and 5-MeO-MiPT, which had similar potency. 5-MeO-DALT and 5-MeO-DPT had very high potencies (3.4 nM and 5.41 nM, respectively), and 5-Cl-DMT and 5-MeO-DiPT also had high potencies (41.7 nM and 56 nM, respectively). All 5-MeO substituted compounds were full 5-HT$_{1A}$R agonists. The rank order of potency for DMT analogs was 5-Cl-DMT (41.7 nM), 4-OH-DMT (130 nM), DMT (210 nM), and 4-AcO-DMT (673 nM).

To better visualize patterns of affinity and potency, we sorted compounds by 5-HT receptor affinities and function (Fig. 4). We

found that 5-MeO substituted tryptamines exhibited higher affinity and potency for 5-HT$_{1A}$R and lower affinity and potency for 5-HT$_{2C}$R. 5-MeO-DALT and 5-MeO-DPT stood out due to their particularly high affinity and potency for 5-HT$_{1A}$R. Both 4- and 5-substituted tryptamines exhibit a broad range of affinity and potency for 5-HT$_{2A}$R, although of the compounds tested only 4-OH-MET, 4-OH-MPT and 5-MeO-DALT exhibited similar 5-HT$_{2A}$ affinity and potency as 4-OH-DMT (psilocin).

Serotonin Transporter. The SERT regulates the free 5-HT in the synaptic space and is an additional target for some hallucinogens. Chemicals that bind to SERT can block reuptake and/or act as substrates at SERT. We have shown previously that the ratio of the K$_i$ versus [^{125}I]RTI-55 to the IC$_{50}$ versus [^{3}H]5-HT at SERT indicates whether a compound acts as a substrate or a reuptake inhibitor, with high ratios indicating that a chemical acts as a substrate (Eshleman et al., 2017). As a result, we characterized the substituted tryptamines in this study with respect to the K$_i$ versus [^{125}I]RTI-55 and IC$_{50}$ versus [^{3}H]5-HT at SERT and calculated the ratio. DMT had very low affinity for the [^{125}I]RTI-55 binding site on SERT (4560 nM), lower than the affinity of the standard compound cocaine but higher affinity than METH, LSD, DOM, and MDMA (Figs. 2D and 5A; Table 2). LSD had no measurable affinity for SERT. A few compounds exhibited moderate or high affinity for the [^{125}I]RTI-55 binding site on SERT. The di-ethyl and di-propyl substitutions exhibited significantly higher affinity for SERT. Among the 4-OH
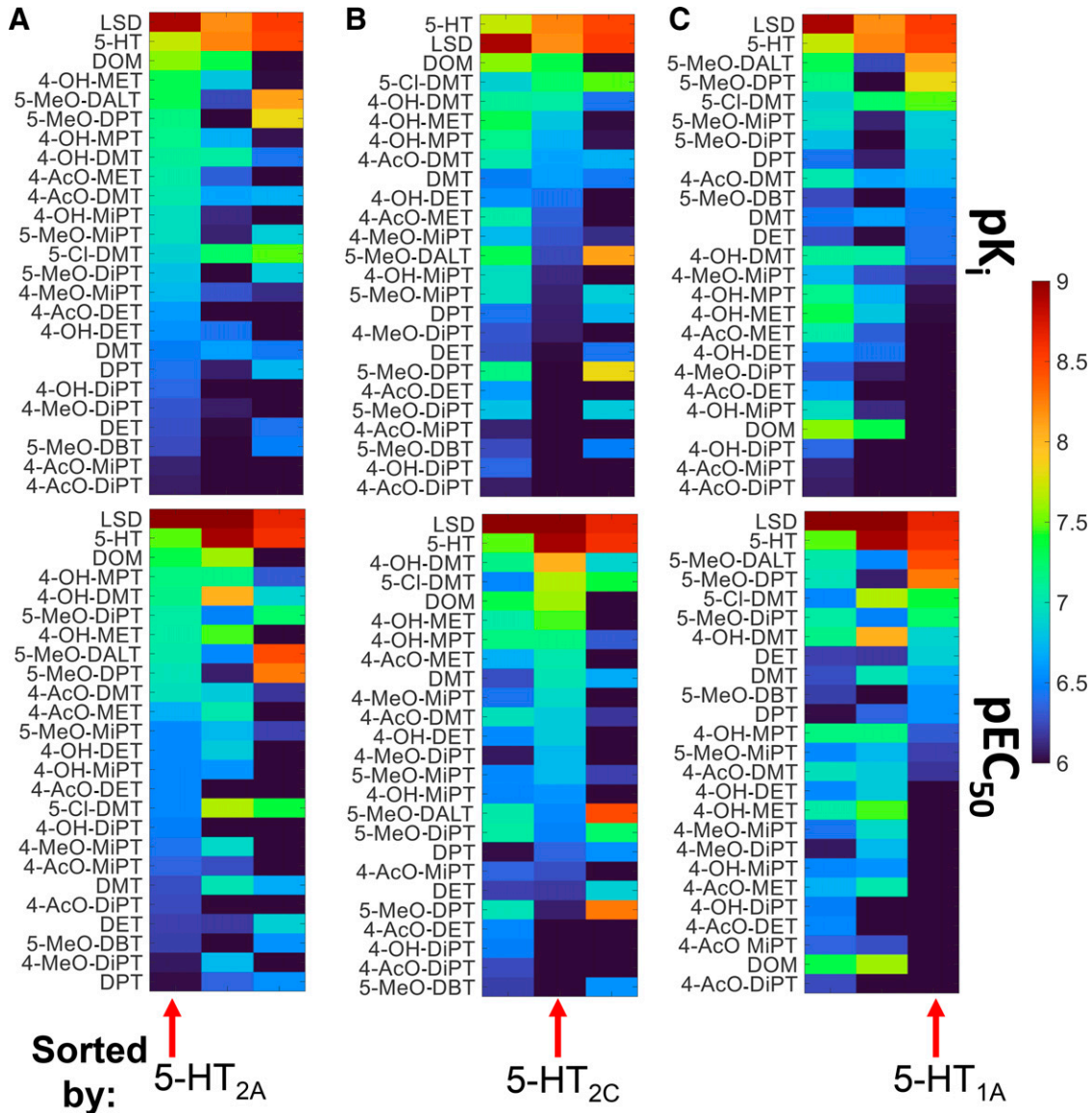
Fig. 4. Substituted tryptamines sorted by pK_i and pEC_{50} for 5-HT$_{2A}$R (A), 5-HT$_{2C}$R (B), and 5-HT$_{1A}$R (C) to show ordered relative affinities (top row) and relative potencies (bottom row) for all compounds to emphasize any chemical bases for affinity and potency. (A) Both 4- and 5-substituted tryptamines exhibit a broad range of affinities and potencies for 5-HT$_{2A}$Rs depending on the N,N substitutions (left panel). (B) 5-MeO and N,N DiPT –substituted tryptamines exhibit the lowest affinities and potencies for 5-HT$_{2C}$Rs (middle panel). (C) 5-MeO- and N,N-substituted tryptamines exhibit highest affinities and potencies for 5-HT$_{1A}$Rs (right panel).

substituted compounds, all had higher affinities than DMT except for 4-OH-DMT and 4-OH-MET, which had similar affinities. 4-AcO-DiPT had higher affinity, whereas the other 4-AcO substituted compounds had similar or lower affinities than DMT. Of note, the 4-MeO substituted compounds 4-MeO-DiPT and 4-MeO-MiPT had high affinities for SERT, the highest of all compounds tested, including the standards. The rank order of affinity for DMT analogs was 5-Cl-DMT (830 nM), 4-OH-DMT (3650 nM), DMT (4560 nM), and 4-AcO-DMT (8000 nM).

In the [3H]5-HT functional assay, DMT had 6-fold higher potency for inhibiting 5-HT uptake (712 nM) than its affinity in the binding assay, which suggests that it may be a substrate for SERT as opposed to a reuptake inhibitor (Eshleman et al., 2017). This potency was lower than that of cocaine and MDMA but significantly higher than the potencies of METH, LSD, and DOM (Figs. 3D and 5A). Consistent with the

binding results, 4-MeO-DiPT and 4-MeO-MiPT had the highest

potencies of all compounds tested. A larger number of compounds inhibited SERT [3H]5-HT uptake compared with the blockade of the [125I]RTI-55 binding site. Sorted pK_i [-log(K_i)] and pIC_{50} [-log(IC$_{50}$)] heat maps (Fig. 5, A and B) revealed that the di-isopropyl substitution was associated with the highest SERT affinity and potency (Fig. 5, A and B). Sorting substituted tryptamines by the ratio of [125I]RTI-55 K_i to [3H]5-HT IC$_{50}$ at SERT showed that DMT, which has previously been shown to cause 5-HT release (Rickli et al., 2016), had the highest ratio, indicating that it was the most likely to act as a substrate (Fig. 5C). A handful of other compounds exhibited ratios of approximately 5 or greater. Interestingly, 4-MeO-MiPT and 4-MeO-DiPT exhibited ratios close to 1 despite very strong affinity values at SERT, suggesting that they may be

TABLE 2
Affinities and potencies of substituted tryptamines for hSERT

Drug	HEK-hSERT Inhibition of [^{125}I]RTI-55 Binding K_i, nM ± S.E.M. (n)	HEK-hSERT Inhibition of [^{3}H]5-HT Uptake IC_{50} nM ± S.E.M. (n)	HEK-hSERT Ratio [^{125}I]RTI-55 Binding/ [^{3}H]5-HT Uptake
DMT	4560 ± 350 (21)	712 ± 99 (22)	6.40
DET	1200 ± 170 (3)[a]	254 ± 29 (4)[b]	4.72
DPT	480 ± 34 (4)[c]	172 ± 35 (5)[c]	2.79
4-OH-DET	1411 ± 99 (3)[d]	383 ± 41 (4)	3.68
4-OH-DiPT	419 ± 37 (3)[c]	163 ± 52 (4)[a]	2.57
4-OH-DMT	3650 ± 270 (3)	1140 ± 210 (3)	3.20
4-OH-MET	2310 ± 130 (3)	830 ± 170 (3)	2.78
4-OH-MIPT	483 ± 20 (4)[c]	373 ± 16 (3)	1.29
4-OH-MPT	1180 ± 240 (4)[c]	575 ± 72 (4)	2.05
4-AcO-DET	13100 ± 2600 (5)[a]	2600 ± 900 (3)[c]	5.04
4-AcO-DIPT	1180 ± 100 (3)[a]	237 ± 77 (4)[a]	4.98
4-AcO-DMT	8000 ± 1200 (3)	8300 ± 1300 (3)[c]	0.96
4-AcO-MET	17,500 ± 1800 (4)[c]	3240 ± 310 (6)[c]	5.40
4-AcO-MiPT	10,400 ± 1200 (4)[b]	5800 ± 1200 (5)[c]	1.79
4-MeO-DiPT	12.1 ± 3.1 (5)[c]	11.1 ± 2.7 (4)[c]	1.09
4-MeO-MiPT	38.0 ± 3.8 (3)[c]	53 ± 13 (3)[c]	0.72
5-MeO-DALT	1270 ± 89 (4)[a]	930 ± 110 (4)	1.37
5-MeO-DBT	1180 ± 240	2120 ± 190 (4)[c]	0.56
5-MeO-DiPT	874 ± 71 (3)[c]	239 ± 39 (3)[b]	3.66
5-MeO-DPT	1070 ± 260 (3)[c]	910 ± 190 (4)	1.18
5-MeO-MiPT	4040 ± 500 (3)	2680 ± 460 (3)[c]	1.51
5-Cl-DMT	830 ± 140 (3)[c]	394 ± 66 (5)	2.11
Standards			
Cocaine	590 ± 50 (38)[c]	349 ± 30 (25)[a]	1.69
METH	124,600 ± 1400[c]	59,500 ± 1300 (4)[c]	2.09
LSD	>100,000 (11)[c]	>100,000 (10)[c]	1
DOM	48,500 ± 3400 (7)[c]	53,100 ± 8000 (9)[c]	0.91
MDMA	14700 ± 2900 (3)[e]	109 ± 17 (5)[e]	135

(n), number of experiments conducted in duplicate.
[a]$P < 0.001$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[b]$P < 0.05$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[c]$P < 0.0001$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[d]$P < 0.01$, one-way ANOVA followed by Dunnett's multiple comparison test compared with DMT.
[e]Data from Eshleman et al. (2017).

SERT reuptake inhibitors. None of the tryptamines tested exhibited the very high ratios that are characteristic of stronger 5-HT releasers like MDMA (Eshleman et al., 2017).

Receptor Selectivity, Efficacy, and Efficiency. The selectivity of substituted tryptamines at key 5-HT receptors and SERT is a critical determinant of the effects and toxicities associated with these drugs. To better characterize the chemical determinants of selectivity, we generated sorted heat maps corresponding to the ratio of the affinities for two 5-HT receptors (Fig. 6, A–C) or between 5-HT$_{2A}$R and SERT (Fig. 6, D and E). 5-MeO compounds exhibit high selectivity for 5-HT$_{2A}$R versus 5-HT$_{2C}$R but not versus 5-HT$_{1A}$R. A number of substituted tryptamines also exhibit poor selectivity for 5-HT$_{2A}$R versus SERT, including most prominently non–N,N-substituted compounds and N,N-DiPT compounds. Two compounds, 4-MeO-MiPT and 4-MeO-DiPT, stood out due to their 10- to 100-fold selectivity for SERT over 5-HT$_{2A}$R. A scatter map of 5-HT$_{2A}$R selectivity ratios that includes a heat map representation of SERT selectivity ratio as well (Fig. 7) shows that a number of compounds that are highly selective for 5-HT$_{2A}$R versus 5-HT$_{1A}$R and 5-HT$_{2C}$R are not as selective versus SERT (e.g., 4-OH-DiPT), whereas others are also selective versus SERT (e.g., 4-OH-MiPT and 4-OH-MET).

Another critical factor in mediating the psychedelic potential of the tryptamines tested is their efficacy at 5-HT$_{2A}$R, which we compared with 5-HT$_{1A}$R and 5-HT$_{2C}$R (Fig. 8; Table 1). Interestingly, although the tryptamines tested were largely full or near full agonists at 5-HT$_{1A}$R and 5-HT$_{2C}$R, they exhibited a broad and more evenly distributed range of efficacies at 5-HT$_{2A}$R. This includes well known psychedelics such as DMT, which is a weak partial agonist at 5-HT$_{2A}$R (40%) and a full agonist at 5-HT$_{1A}$R and 5-HT$_{2C}$R, and LSD, which is also a partial agonist at 5-HT$_{2A}$R (74.8%) and 5-HT$_{2C}$R (86%) and full agonist at 5-HT$_{1A}$R. With the exception of 5-MeO-DBT, which was a very weak partial agonist at 5-HT$_{2A}$R (17.5%), 5-MeO compounds were full agonists at 5-HT$_{1A}$R, 5-HT$_{2A}$R, and 5-HT$_{2C}$R. The 4-AcO compounds tested were partial agonists at 5-HT$_{1A}$R.

We noted that the relationship between binding affinity and functional EC_{50}s via Gq appeared to vary considerably by compound and receptor. To quantify further, we calculated the amplification ratio (a measure of coupling efficiency) for each tryptamine and reference compound relative to 5-HT as the ratio of the K_i (vs. [^{3}H]5-HT or [^{3}H]8-OH-DPAT) to the EC_{50} for each tryptamine relative to that ratio with 5-HT (Strange, 2008). Ratios greater than one signify greater Gq- or Gi-coupling than 5-HT relative to receptor binding, and ratios less than one indicate less Gq- or Gi-coupling than 5-HT relative to receptor binding. Notably, two prototypical classic psychedelics, LSD and 4-OH-DMT (psilocin), exhibited high ratios across 5-HT$_{1A}$R, 5-HT$_{2A}$R, and 5-HT$_{2C}$R, suggesting that they are broadly efficient actuators of downstream signaling after binding (Fig. 9). 5-MeO-DiPT also stood out as exhibiting a similar pattern of broadly high amplification ratios relative to 5-HT. As a class, the tryptamines tested exhibited a higher average amplification ratio at 5-HT$_{2A}$R relative to 5-HT (1.44), a
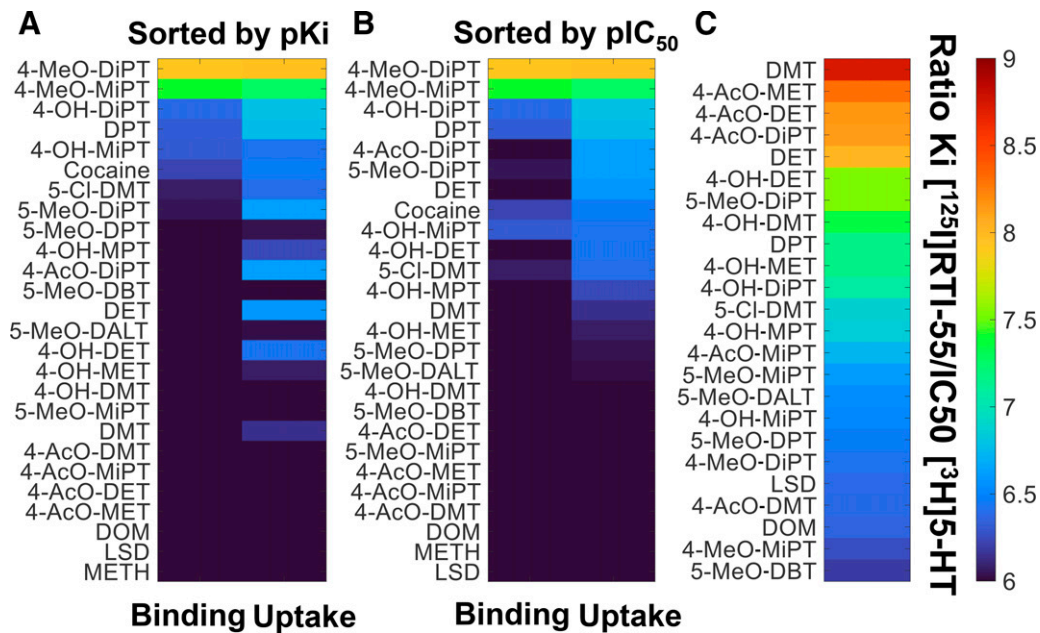
Fig. 5. Substituted tryptamines sorted by pK_i and pIC_{50} at SERT to show relative affinity and potency. (A) Substituted tryptamines were sorted by pK_i of [^{125}I]RTI-55 binding or (B) sorted by pIC_{50} of [^{3}H]5-HT uptake at SERT. pK_i or pIC_{50} of the partner assay ([^{3}H]5-HT uptake for (A) and [^{125}I]RTI-55 binding for (B) were included alongside the sorted column. Of the tryptamines tested, 4-MeO-substituted tryptamines exhibit the highest binding at the RTI-55 and 5-HT uptake sites on SERT. Other 4-substituted *N,N*-diisopropyl and non–ring-substituted *N,N*-diethyl tryptamines predominantly exhibit binding to the [^{3}H]5-HT site of SERT. C) Substituted tryptamines were sorted by the ratio of K_i vs. [^{125}I]RTI-55 and IC_{50} vs. [^{3}H]5-HT at SERT. Of the tryptamines tested, 4-AcO-substituted compounds and those with only *N,N*-substitutes exhibited the highest ratios of K_i at the [^{125}I]RTI-55 binding site on SERT vs. IC_{50} competing against ^{3}H]5-HT. Compounds with higher ratios are more substrate-like at SERT.

lower relative ratio at 5-HT$_{2C}$R (0.68; group difference was significant by paired t test, P value = 0.001), and 5-HT$_{1A}$R (1.09; group difference was significant by paired t test, P value = 0.011). Notably, some chemicals with the low amplification ratios at 5-HT$_{2A}$R are well known psychedelic drugs such as DPT (Pottie et al., 2020) and 4-OH-MiPT (Repke et al., 1985).

Discussion

In these studies, we pharmacologically characterized a large group of substituted tryptamines along with the nontryptamine standards LSD and DOM and tryptamine standards 5-HT, DMT, and 4-OH-DMT (psilocin). We found that all are agonists or partial agonists at 5-HT$_{2A}$Rs, consistent with potential psychedelic activity. We also noted that the pattern of substitutions affected affinity, potency, efficacy, and selectivity for 5-HT$_{2A}$R versus 5-HT$_{1A}$R, 5-HT$_{2C}$R, and SERT. As a result, the chemicals tested are likely to exhibit differential psychoactive properties and toxicities.

With respect to affinity and potency at 5-HT$_{2A}$R, *N,N*-substituted tryptamines with substitutions at both the 4 and 5 position largely tended to exhibit higher affinity and potency for 5-HT$_{2A}$R than non–4- and 5-substituted tryptamines such as DMT, DET, and DPT. But, both 4- and 5-substituted compounds exhibited a broad range of 5-HT$_{2A}$R affinities. with neither substitution appearing to clearly confer higher 5-HT$_{2A}$R affinity or potency. However, 5-MeO–substituted tryptamines exhibited high 5-HT$_{1A}$R but low 5-HT$_{2C}$R affinity and potency. Not surprisingly, we found that *N,N*-substituted tryptamines and those with 5-MeO substitutions exhibit low 5-HT$_{2A}$R/5-HT$_{1A}$R selectivity but high 5-HT$_{2A}$R/5-HT$_{2C}$R selectivity. Two

substituted tryptamines stood out for 5-HT$_{1A}$R > 5-HT$_{2A}$R > 5-HT$_{2C}$R selectivity: 5-MeO-DALT and 5-MeO-DPT.

Notably, a sizable subset of the tryptamines tested exhibited some binding to SERT, with 4-MeO-MiPT and 4-MeO-DiPT having the highest affinities by far. A handful of these compounds exhibited a SERT binding pattern that suggests they could cause some 5-HT release. There is previous evidence that DMT, DPT, MiPT, and DiPT are 5-HT releasers whereas larger compounds such as 5-MeO-DiPT are 5-HT reuptake inhibitors (Cozzi et al., 2009; Blough et al., 2014; Rickli et al., 2016). Of the compounds with SERT binding, DiPT-substituted compounds and tryptamines with no 4- or 5- substitution tended to lack selectivity for 5-HT$_{2A}$R over SERT. 4-MeO-MiPT and 4-MeO-DiPT both exhibited a pattern of SERT activity consistent with an ability to inhibit 5-HT reuptake via SERT. Given the very high affinity of these compounds for SERT, the increased 5-HT that would result from acute ingestion may compete with these chemicals at 5-HT$_{2A}$R, blunting or altering 5-HT$_{2A}$R–mediated effects, especially at low-to-moderate doses. Our examination of selectivity patterns for 5-HT$_{2A}$R versus 5-HT$_{1A}$R and 5-HT$_{2C}$R did not reveal any clear relationship between G-protein–coupled receptor selectivity for 5-HT$_{2A}$R over 5-HT$_{1A}$R or 5-HT$_{2C}$R and SERT activity.

As a class, these substituted tryptamines exhibited significantly higher mean affinity at 5-HT$_{2A}$R versus 5-HT$_{1A}$R and 5-HT$_{2C}$R but lower efficacy at 5-HT$_{2A}$R versus 5-HT$_{2C}$R. As a group, the compounds screened are relatively unimpressive in terms of efficacy at 5-HT$_{2A}$R, with most of them exhibiting partial agonist activity. In contrast, at 5-HT$_{2C}$R all of the substituted tryptamines tested are full or near full agonists. At 5-HT$_{1A}$R, most of the compounds were full or near full agonists, with a handful of largely AcO compounds exhibiting
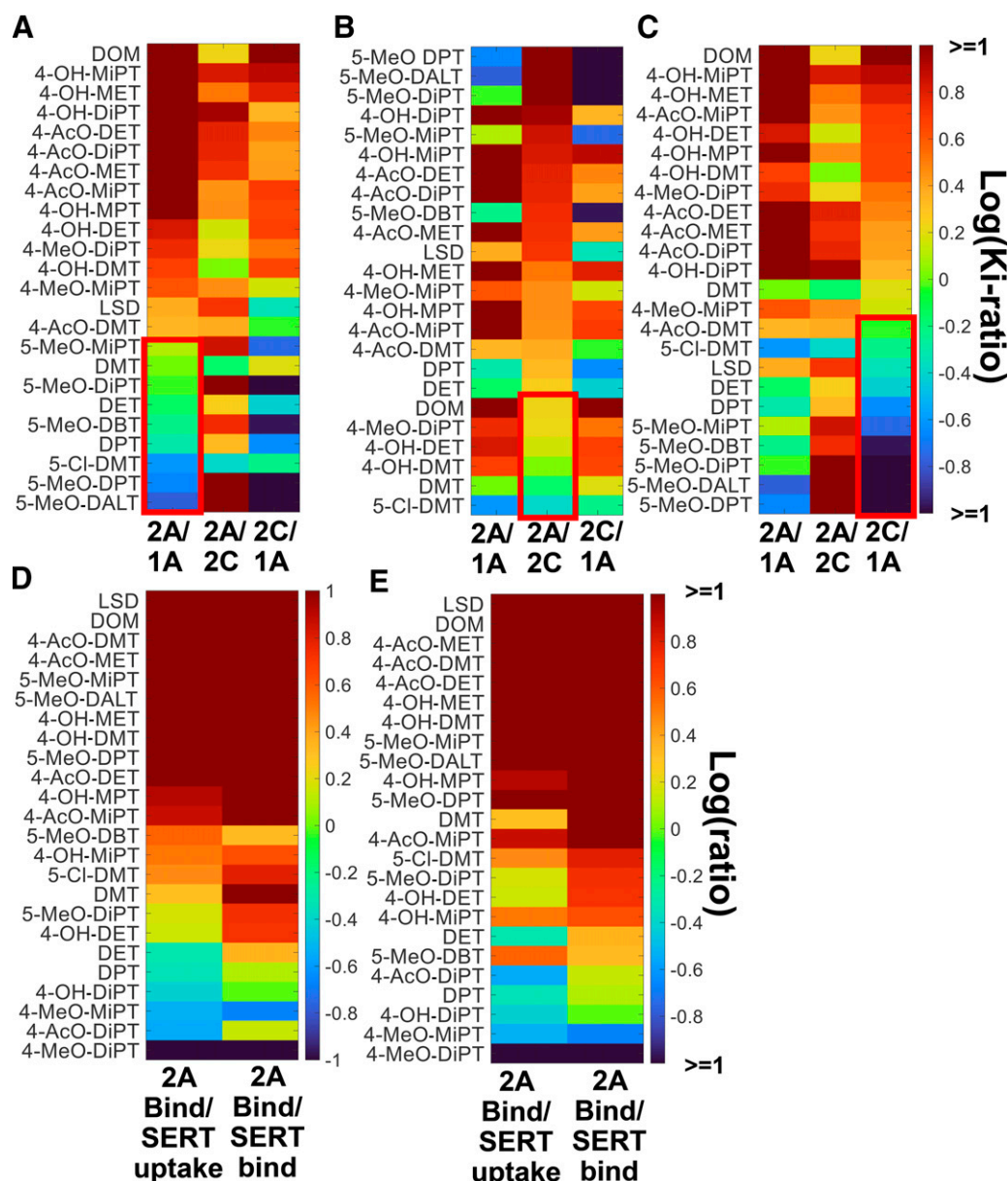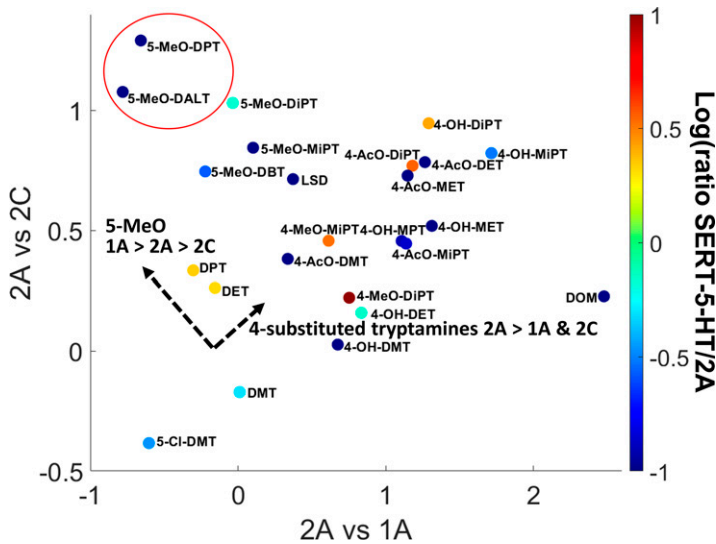
Fig. 6. Substituted tryptamine receptor selectivity. Areas enclosed in red rectangles highlight compounds with low selectivity for 5-HT$_{2A}$R or that prefer 5-HT$_{1A}$R or 5-HT$_{2C}$R. Substituted tryptamines were sorted by receptor-receptor selectivity ratio (A) 2A/1A ratios, (B) 2A/2C ratios, and (C) 2C/1A ratios. Of the tryptamines tested, 5-MeO-substituted compounds and those with only *N,N*-substitutions tend to exhibit moderate selectivity for 5-HT$_{2A}$R vs. 5-HT$_{2C}$R but limited selectivity for 5-HT$_{2A}$R or 5-HT$_{2C}$R vs. 5-HT$_{1A}$R or even preferentially bind 5-HT$_{1A}$ receptors. (D) Substituted tryptamines were sorted by 5-HT$_{2A}$R binding affinity/[³H]5-HT SERT uptake IC$_{50}$ or (E) 5-HT$_{2A}$R binding affinity/K$_i$ at SERT vs. [¹²⁵I]RTI-55 binding. Of the tryptamines tested, DiPT-substituted compounds and those with only *N,N*-substitutions tended to exhibit decreased selectivity for 5-HT$_{2A}$R receptors over SERT. The two 4-MeO compounds in particular strongly preferred SERT over 5-HT$_{2A}$R receptors.

partial agonist activity. This suggests that high G-protein–mediated efficacy is not a critical feature of psychedelics. Notably, the tryptamines tested as a class exhibited a higher mean amplification ratio at 5-HT$_{2A}$R relative to 5-HT than at 5-HT$_{2C}$R. A number of well known psychedelics such as tryptamines like 4-OH-DMT, 5-MeO-DiPT, and the reference psychedelic LSD exhibited particularly high amplification ratios at 5-HT$_{2A}$R, 5-HT$_{1A}$R, and 5-HT$_{2C}$R. A high amplification ratio relative to 5-HT at 5-HT$_{2A}$R and perhaps other closely related 5-HT receptors may be a key feature of some psychedelics, with LSD and psilocin representing exemplar chemicals in this respect. Together, these data suggest that the activity "ceiling" (efficacy) is not as important as the activity "flux" (amplification ratio) in mediating the unique receptor-based activities of psychedelic chemicals. The overall picture is likely more complex, as some known psychedelics like DPT, 5-MeO-MiPT, and 4-OH-MiPT exhibit low amplification ratios relative to 5-HT at 5-HT$_{2A}$R. One possible explanation for this is that these compounds exhibit highly biased and/or highly amplified signaling via arrestin-mediated pathways, and there is evidence to support this notion for DPT (Pottie et al., 2020). Alternatively, 5-HT$_{2A}$R selectivity may be a factor, as our results show that although 4-OH-MiPT has a low amplification ratio, it is among the most selective (vs. 5-HT$_{1A}$R and 5-HT$_{2C}$R; see Fig. 7) of the substituted tryptamines tested.

Notably, these results highlight potential avenues to better understand the rare but severe somatic toxicities, possibly due

Fig. 7. Integrating patterns of selectivity of substituted tryptamines. A number of tryptamines that are selective for 5-HT$_{2A}$R receptors are also either nonselective for 5-HT$_{2A}$R vs. SERT or prefer SERT (see compounds marked with warmer colors to indicate those that do not prefer 5-HT$_{2A}$R vs. SERT). The *N,N*-substituted tryptamines that were tested (DMT, DET, and DPT) are all relatively nonselective for 5-HT$_{2A}$R vs. SERT. There are several compounds, typically 4-substituted, that exhibit selectivity for 5-HT$_{2A}$R vs. 5-HT$_{1A}$ and 5-HT$_{2C}$R and vs. SERT. The 5-MeO compounds tested prefer 5-HT$_{2A}$R relative to SERT.

to serotonin syndrome, occasionally associated with psychedelic use (Malcolm and Thomas, 2022). Because the effects in humans of many of the substituted tryptamines characterized in these studies are little known, understanding their pharmacologic activity patterns and comparing to better known psychedelics is particularly important with respect to understanding what those effects might be. Many psychedelics, including LSD and psilocybin, have been associated with hyperthermia in 2%–4% of a large sample of thousands of calls to US poison centers (Friedman and Hirsch, 1971; Leonard et al., 2018). Despite this clear link to hyperthermia, nonbehavioral fatalities with LSD and psilocybin are almost nonexistent; we could find only one decades-old case report of a nonbehavioral fatality attributed to LSD in which the circumstances were not described (Fysh et al., 1985; Nichols and Grob, 2018). Despite their relative recency, a number of fatalities have been associated with non-tryptamine N-benzyl-dimethoxy phenethylamines (NBOMes) (Hill et al., 2013; Kueppers and Cooke, 2015; Shanks et al., 2015) and the substituted tryptamine 5-MeO-DiPT (Tanaka

et al., 2006). The NBOMes have ~1000-fold higher affinities for 5-HT$_{2A}$R compared with SERT (Eshleman et al., 2018). In contrast, similar to 5-MeO-DiPT, a number of the substituted tryptamines in this study exhibit comparable affinity for 5-HT$_{2A}$R and SERT or prefer SERT. Also of note, many of the substituted tryptamines screened lack selectivity for 5-HT$_{2A}$R versus 5-HT$_{1A}$R, a feature that may mitigate hyperthermia, as 5-HT$_{1A}$R agonists centrally decrease body temperature (Lesch et al., 1990; Hedlund et al., 2004; Voronova, 2021).

One potential mechanism for psychedelic-mediated hyperthermia is 5-HT$_{2A}$R–mediated skeletal muscle contraction (Guillet-Deniau et al., 1997; Wappler et al., 1997; Hajduch et al., 1999). The prominent SERT activity of several substituted tryptamines that were characterized in this study also highlights a potential role for substituted tryptamine-induced serotonin syndrome in mediating psychedelic-induced hyperthermia. 5-MeO-DiPT exhibits balanced affinity for 5-HT$_{2A}$R and SERT, suggesting the possibility that synergistic toxicities could confer additional risk (Malcolm and Thomas, 2022). We noted a number of features
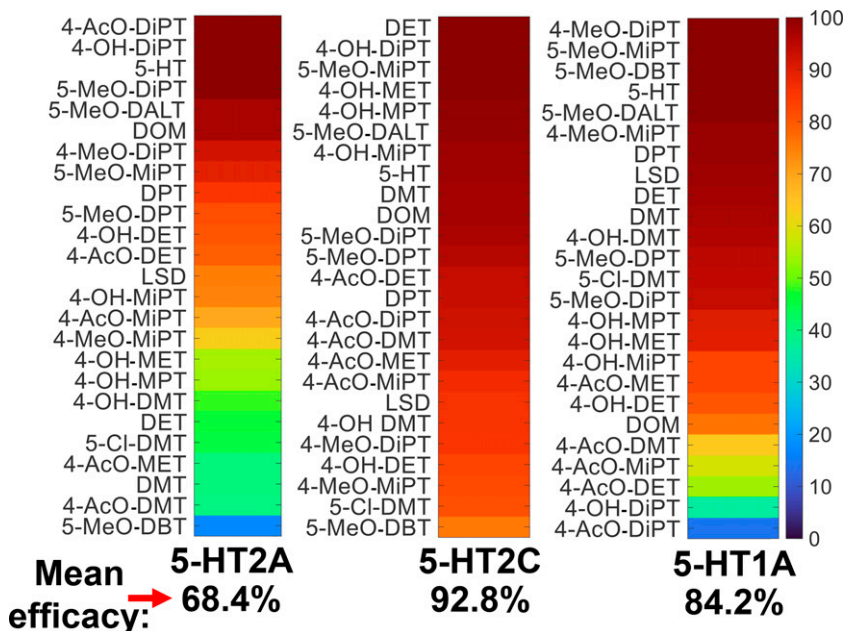
Fig. 8. Receptor efficacy. With the exception of most of the 4-AcO compounds, most tryptamines tested are full or near full agonists at 5-HT$_{1A}$R. All tryptamines tested were full agonists at 5-HT$_{2C}$R. Many of the tryptamines tested were partial agonists at 5-HT$_{2A}$R, including a sizable number that were weak partial agonists with less than 50% efficacy. At the bottom of each heat map is the mean efficacy of the substituted tryptamines tested at each receptor.

Fig. 9. Coupling efficiency of substituted tryptamines compared with 5-HT. To assess G-protein coupling efficiency, or amplification ratio, after binding of each chemical to receptor, we calculated a ratio of the K_i to EC_{50} relative to that ratio with 5-HT. Many but not all known psychedelics exhibit higher coupling efficiency than 5-HT at $5\text{-HT}_{2A}R$ but lower coupling efficiency at $5\text{-HT}_{2C}R$. The mean amplification ratio was calculated as the average of individual amplification ratios. As a class, the compounds tested exhibit the highest average ratio at $5\text{-HT}_{2A}R$, higher than for $5\text{-HT}_{1A}R$ and $5\text{-HT}_{2C}R$. At the bottom of each heat map is the mean amplification ratio of the substituted tryptamines tested at each receptor.
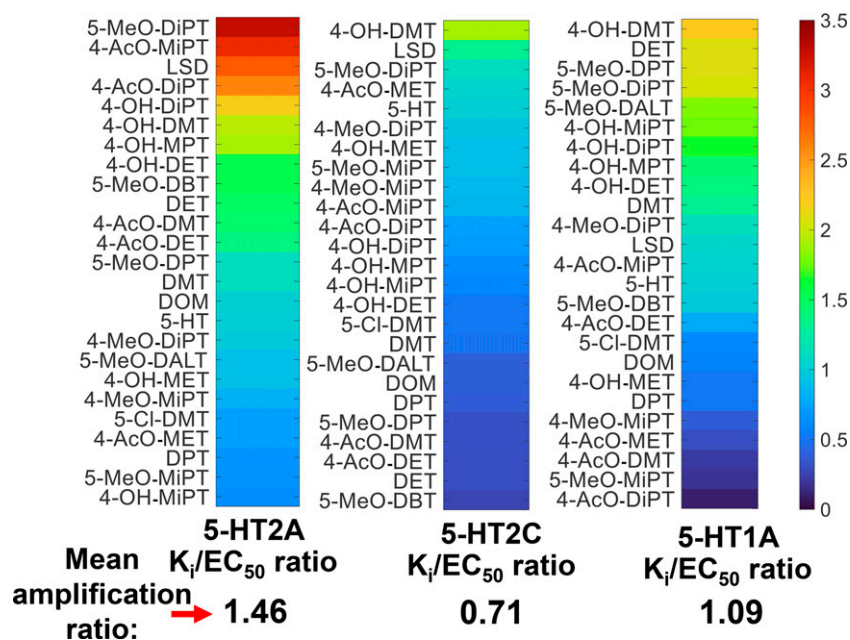
that may influence the toxicities and other effects of substituted tryptamines in humans, including a range of selectivities for $5\text{-HT}_{2A}R$ versus other targets and a range of efficacies and amplification factors at $5\text{-HT}_{2A}R$. Given the diverse pharmacology of these substituted tryptamines, their wide use and frequent association with toxicity in the community, and their more recently noted therapeutic potential (Carhart-Harris et al., 2016; Griffiths et al., 2016; Nutt et al., 2020; Carhart-Harris et al., 2021), further preclinical or clinical research aimed at differentiating the in vivo effects and pharmacology of substituted tryptamines is warranted.

Authorship Contributions

Participated in research design: Eshleman, Janowsky.

Conducted experiments: Eshleman, Swanson, Bloom, Wolfrum, Schmachtenberg.

Performed data analysis: Kozell, Eshleman, Swanson, Bloom, Wolfrum, Schmachtenberg, Abbas.

Wrote or contributed to the writing of the manuscript: Kozell, Eshleman, Olson, Janowsky, Abbas.

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