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Serotonin 1A receptors modulate serotonin 2A receptor-mediated behavioral effects of 5-methoxy-*N,N*-dimethyltryptamine analogs in mice

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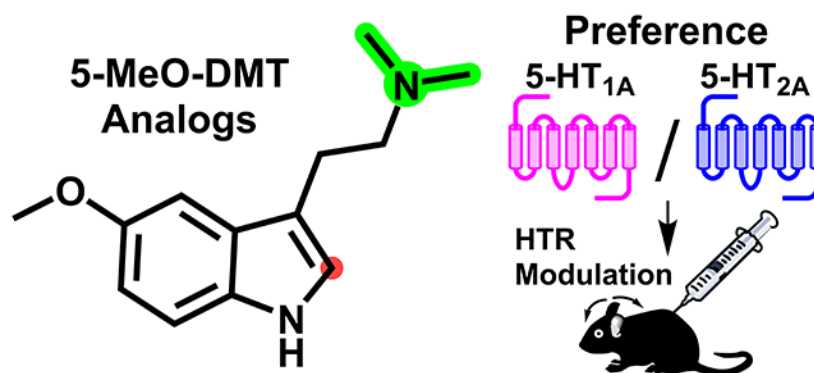
Conflict of Interest

A.R.C. is the CEO of CaaMTech Inc. and owns patents related to psychedelic tryptamines and derivatives thereof. All other authors report no competing financial or other conflicts of interest with respect to the current work.

Abstract

5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) analogs are used as recreational drugs, but they are also being developed as potential medicines, warranting further investigation into their pharmacology. Here, we investigated the neuropharmacology of 5-MeO-DMT and several of its *N*-alkyl, *N*-allyl, and 2-methyl analogs, with three major aims: 1) to determine *in vitro* receptor profiles for the compounds, 2) to characterize *in vitro* functional activities at serotonin (5-HT) 2A receptors (5-HT_{2A}) and 1A receptors (5-HT_{1A}), and 3) to examine the influence of 5-HT_{1A} on 5-HT_{2A}-mediated psychedelic-like effects in the mouse head twitch response (HTR) model. *In vitro* receptor binding and functional assays showed that all 5-MeO-DMT analogs bind with high affinity and activate multiple targets (e.g., 5-HT receptor subtypes, alpha adrenergic receptors), including potent effects at 5-HT_{2A} and 5-HT_{1A}. In C57Bl/6J mice, subcutaneous injection of the analogs induced HTRs with varying potencies (ED₅₀ range = 0.2 – 1.8 mg/kg) and maximal effects (E_{max} range = 20 – 60 HTRs/ 30 min), while inducing hypothermia and hypolocomotion at higher doses (ED₅₀ range = 3.2 – 20.6 mg/kg). 5-HT_{2A} antagonist pretreatment blocked drug-induced HTRs, whereas 5-HT_{1A} antagonist pretreatment enhanced HTRs. In general, *N,N*-dialkyl and *N*-isopropyl derivatives displayed HTR activity, while the *N*-methyl, *N*-ethyl, and 2-methyl analogs did not. Importantly, blockade of 5-HT_{1A} unmasked latent HTR activity for the *N*-ethyl analog and markedly increased maximal responses for other HTR-active compounds (40 – 90 HTRs/ 30 min), supporting the notion that 5-HT_{1A} agonist activity can dampen 5-HT_{2A}-mediated HTRs. Suppression of 5-HT_{2A}-mediated HTRs by 5-HT_{1A} only occurred after high 5-MeO-DMT doses, suggesting involvement of other receptors in modulating psychedelic-like effects. Overall, our findings provide key information about the receptor target profiles for 5-MeO-DMT analogs, the structure-activity relationships for inducing psychedelic-like effects, and the critical role of 5-HT_{1A} agonism in modulating acute psychoactive effects of 5-HT_{2A} agonists.

Graphical Abstract



Keywords

5-MeO-DMT; tryptamines; head twitch response; mice; 5-HT_{1A}; 5-HT_{2A}

5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) is a naturally-occurring psychedelic tryptamine found in certain species of fungi, plants, and animals.^{1–5} 5-MeO-DMT is being investigated as a potential therapeutic agent in the treatment of depression and anxiety, in

part due to its shorter duration of subjective effects when compared to other psychedelic compounds, like 4-phosphoryloxy-*N,N*-dimethyltryptamine (psilocybin).^{6–8} In addition to 5-MeO-DMT, there is interest in the development of 5-MeO-DMT analogs that may have advantages for clinical use, such as better oral bioavailability, and may serve as useful tools in pharmacological research.^{9–14}

5-MeO-DMT induces complex behavioral effects in humans and animals, presumably due to its non-selective serotonin (5-HT) receptor activation profile, including stimulation of 5-HT 2A receptors (5-HT_{2A}) and 5-HT 1A receptors (5-HT_{1A}).^{15–18} The subjective effects of 5-MeO-DMT in humans are reportedly quite different from those produced by its structural analog, *N,N*-dimethyltryptamine (DMT), and other serotonergic psychedelics, often associated with less visual effects, “enhanced ego loss”, “white light”, and “a feeling of oneness with others”.^{9, 19, 20} It is generally accepted that 5-MeO-DMT produces its subjective effects in humans and psychedelic-like effects in rodents via agonist actions at 5-HT_{2A},^{21–26} but other 5-HT receptors (e.g., 5-HT_{1A}, 5-HT_{2C}) can modulate this activity.^{18, 26–34} Analogs of 5-MeO-DMT which display high selectivity for 5-HT_{1A} agonism have been discovered,^{35–37} and two recent studies characterized the preclinical pharmacology of such derivatives, including 5-methoxy-*N,N*-tetramethylenetryptamine (5-MeO-PyrT).^{33, 34} Beyond pharmacologically relevant activity at 5-HT_{2A} and 5-HT_{1A}, there is evidence that 5-MeO-DMT and related analogs can interact with other 5-HT receptors, monoamine transporters, as well as dopaminergic, adrenergic, and sigma receptors.^{1, 33, 38–43} Importantly, the receptor target profiles for many 5-MeO-DMT analogs have not been well-characterized.

5-MeO-DMT and its chemical analogs are sold on recreational drug markets worldwide as new psychoactive substances (NPS), and the psychoactive effects for many of the compounds were first described in “Tryptamines I Have Known and Loved” (TiHKAL).^{9, 44–53} 5-MeO-DMT is a Schedule I controlled substance in the United States,¹ but 5-MeO-DMT and many of its analogs are not controlled internationally. In the United States, 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DiPT, or “foxy methoxy”) was placed into emergency Schedule I control in 2003 after appearing on the NPS market, and was permanently scheduled in 2004.^{54, 55} The recreational use of 5-MeO-DMT and related tryptamines is associated with a number of minor and transient medical consequences (e.g., hypertension),^{56–60} but rare fatal overdoses have been reported after ingestion of 5-MeO-DMT, 5-MeO-DiPT, and 5-methoxy-*N,N*-diallyltryptamine (5-MeO-DALT).^{61–64} The precise risks posed by tryptamines are often complicated by misrepresentation of psychedelic NPS sold online.⁶⁵ Therefore, a more complete understanding of the comparative pharmacology of 5-MeO-DMT analogs is important to better assess their relative potencies and safety.

Here we examined structure-activity relationships (SARs) for 5-MeO-DMT analogs varying in steric bulk at the amine group or with addition of a methyl group at the 2-position on the indole ring. Specifically, we compared the *in vitro* receptor profiles and acute *in vivo* effects of 5-methoxytryptamine (5-MeO-T), 5-methoxy-*N*-methyltryptamine (5-MeO-NMT), 5-MeO-DMT, 5-methoxy-2-methyl-*N,N*-dimethyltryptamine (5-MeO-2-Me-DMT), 5-methoxy-2-methyl-*N,N,N*-trimethyltryptammonium (5-MeO-2-Me-TMT), 5-methoxy-*N*-

ethyltryptamine (5-MeO-NET), 5-methoxy-*N*-methyl-*N*-ethyltryptamine (5-MeO-MET), 5-methoxy-*N,N*-diethyltryptamine (5-MeO-DET), 5-methoxy-*N*-isopropyltryptamine (5-MeO-NiPT), 5-methoxy-*N*-methyl-*N*-isopropyltryptamine (5-MeO-MiPT), 5-MeO-DiPT, 5-methoxy-*N*-methyl-*N*-allyltryptamine (5-MeO-MALT), 5-MeO-DALT, and 5-methoxy-*N,N*-dipropyltryptamine (5-MeO-DPT; see Figure 1 for structures). Our investigation focused on receptor binding affinities across multiple targets, functional activities at 5-HT_{2A} and 5-HT_{1A} subtypes, and dose-related effects of acute drug administration in mice (i.e., head twitch response or HTR, body temperature, and locomotor activity). Based on existing evidence for the regulation of 5-HT_{2A}-mediated behavioral effects by 5-HT_{1A} activity, we surmised that the preference for functional activity at these two receptor subtypes would dictate potency and/or efficacy for inducing psychedelic-like effects *in vivo*. Our results highlight important differences across the compounds with respect to *in vitro* receptor target profiles, propensity to induce psychedelic-like HTRs, and to induce 5-HT_{1A}-mediated effects. We examined the effects of pretreatment with receptor-selective antagonists at 5-HT_{2A} and 5-HT_{1A} to delineate the influence of these receptor sites in modulating HTRs in mice. Overall, our results bolster and extend evidence for an important role of 5-HT_{1A} in dampening the psychedelic-like effects induced by 5-HT_{2A} activation, but other receptors may also have modulatory roles. Insights from the present study have implications for the development of optimized 5-MeO-DMT analogs for various therapeutic indications.

RESULTS and DISCUSSION

Pharmacological profiling of 5-MeO-DMT analogs *in vitro*

5-HT receptor pharmacological activities: Compounds were first assessed using a receptor binding screen to determine potentially relevant pharmacological targets of interest.⁶⁶ The initial screen tested the ability of a fixed 10 micromolar concentration of each compound to compete for radioligand binding across a number of receptors and protein targets (see Materials and Methods for a full list). Any receptor or target where a given compound displayed 50% or greater inhibition of radioligand binding was further advanced for full concentration response testing and determination of inhibition affinity constants (K_i). Results revealed that all of the compounds displayed comparable target profiles across most of the 5-HT receptor subtypes assessed (Table S1), showing inhibition of radioligand binding at 5-HT₁, 5-HT₂, 5-HT₅, 5-HT₆, and 5-HT₇ subtypes. Of note, the only compound to compete for binding at 5-HT₃ was the quaternary ammonium analog, 5-MeO-2-Me-TMT, similar to the related compound 5-hydroxy-*N,N,N*-trimethyltryptammonium (bufotenidine).^{67, 68}

Following up the 10 micromolar binding screen, inhibition constants for the identified targets were determined in transfected cell lines.⁶⁶ The inhibition constants for the compounds at human 5-HT receptors are listed in Figure S1. The inhibition constants at 5-HT receptors for most compounds were generally in the low to mid nanomolar range (1 – 500 nM), with a few exceptions. 5-MeO-2-Me-TMT was the only compound demonstrating 5-HT₃ affinity, with an observed affinity constant (K_i) of ~2,600 nanomolar. Additionally, with exception of 5-HT₆, 5-MeO-2-Me-DMT displayed 4 – 27-fold weaker K_i constants at all other 5-HT receptors when compared to 5-MeO-DMT, showing that

addition of a 2-methyl ring-substitution or *N,N,N*-trimethyl group is unfavorable for binding at serotonergic sites. Affinities at 5-HT_{2C} revealed that increasing steric bulk at the *N*-alkyl/*N*-allyl group, beyond methyl-ethyl or methyl-allyl, reduced affinity to above 1 micromolar, with no *N,N*-dialkyl compounds except 5-MeO-DMT, displaying low nanomolar affinity at this receptor. Relative to 5-MeO-DMT, all compounds with *N*-isopropyl substitution (5-MeO-NiPT, 5-MeO-MiPT, 5-MeO-DiPT) plus other *N,N*-dialkyl analogs (5-MeO-DET, 5-MeO-DPT) displayed slightly weaker binding affinities across 5-HT receptors.

Next, functional activities of the 5-MeO-DMT analogs were assessed at identified 5-HT receptors and other targets using fixed concentration screens (3 or 10 μ M) in the Tango assay, to determine effects relative to control standard agonists (listed in Table S2).⁶⁶ All of the compounds, except for the 2-methyl derivatives, displayed agonist-like activity at 5-HT₁, 5-HT₅, 5-HT₆, and 5-HT₇ subtypes, exhibiting a trend for larger *N*-alkyl groups (e.g., DET, iPTs, DPT) with or without the 2-methyl addition to diminish functional activity and corroborating findings from earlier binding screens (Figure S2). Notably, only compounds with *N*-methyl or *N*-ethyl substitutions (i.e., DMT or MET) displayed any agonist-like activity at 5-HT₄ receptors. Of particular interest to the present study is functional activity at 5-HT_{1A}, given its supported role in the pharmacological effects of 5-MeO-DMT and some of its analogs.^{18, 26, 34, 69–71} Here, the screening data show that all of the analogs tested, except the 2-methyl compounds, displayed agonist-like efficacy in a 5-HT_{1A} functional screen using the Tango assay (Figure S2).⁶⁶ Previous work from members of our group showed that many of the analogs examined here are agonists at 5-HT_{1A} (EC_{50} = ~100 – 900 nM, E_{max} = 40 – 95% 5-HT).⁴⁰ In our prior study, the *N*-isopropyl, *N*-methyl-*N*-isopropyl, and the *N,N*-diisopropyl compounds did not exhibit agonist activity up to 10 micromolar. Our current data support this observation, as these three compounds displayed the lowest 5-HT_{1A} functional values relative to 5-HT (56 – 73% vs 85 – 103% for all other analogs) and slightly reduced 5-HT_{1A} affinity relative to the other compounds in the series (Figure S1).

Using a BRET-based functional assay platform, with 5-HT as the reference agonist, the compounds were further assessed for potencies and efficacies measuring G protein dissociation or arrestin recruitment at 5-HT_{1A} ($G_{\alpha 0B}$), mouse 5-HT_{1A} (m5-HT_{1A}, $G_{\alpha 0B}$) 5-HT_{2A} ($G_{\alpha q}$, β arrestin 2), mouse 5-HT_{2A} (m5-HT_{2A}, $G_{\alpha q}$), 5-HT_{2B} ($G_{\alpha q}$), and 5-HT_{2C} ($G_{\alpha q}$) in transfected cells. Heatmaps of the potencies and relative efficacies of the compounds can be found in Figure 2A–B, while the corresponding concentration-response curves can be found in Figure 2C–F or Figure S3. At 5-HT_{1A}, all of the compounds except the 2-methyl derivatives displayed low nanomolar potencies (EC_{50} = ~1 – 40 nM) for $G_{\alpha 0B}$ dissociation with high efficacy (E_{max} = 99 – 115%), corroborating the present Tango data (Figure S2) and published results.^{33, 34, 40} Using cells expressing the mouse 5-HT_{1A} variant (m5-HT_{1A}), nearly identical potencies (EC_{50} = ~1 – 74 nM, ~0.5 – 2 fold vs. the human receptor) and efficacies (E_{max} = 85 – 107%) were observed across the series. Similarly, all compounds except the quaternary ammonium, displayed low nanomolar potencies (EC_{50} = 2 – 80 nM) and agonist efficacy (E_{max} = 80 – 104%) for $G_{\alpha q}$ dissociation at 5-HT_{2A}. For the same signal transduction pathway at m5-HT_{2A}, the series displayed slightly right-shifted potencies (EC_{50} = 7 – 132 nM) and comparable maximal efficacies relative to 5-HT (E_{max} = 90 – 104%), with the 2-methyl compounds exhibiting weak or no functional activity.

Regarding β arrestin 2 recruitment at h5-HT_{2A}, the compounds also had low nanomolar potencies (EC_{50} = 6 – 85 nM), with the 2-Me-TMT analog being the exception (EC_{50} = 6,450 nM). Notably, many of the compounds did not reach > 80 % the efficacy of 5-HT (i.e., NMT, DMT, 2-Me-DMT, 2-Me-TMT, MET, DET, MALT, DPT analogs), suggesting these compounds are not fully efficacious for β arrestin 2 recruitment at h5-HT_{2A}. Outside of the 2-Me-TMT analog, potencies and efficacies at 5-HT_{2B} (EC_{50} = 1 – 221 nM, E_{max} = 61 – 103% 5-HT) and 5-HT_{2C} (EC_{50} = 0.1 – 160 nM, E_{max} = 76 – 115% 5-HT) were more variable across the series compared to effects at 5-HT_{2A}. As an example, 5-MeO-NiPT, 5-MeO-MiPT, 5-MeO-DET, and 5-MeO-2-Me-DMT were ~72 – 316-fold less potent for activation of 5-HT_{2B} vs. 5-MeO-T, which was the most potent compound in this regard. At 5-HT_{2C}, the series had nearly identical relative signal transduction efficacies, but compounds with either a 2-methyl substitution or amine/allyl group bulkier than methyl-ethyl or methyl-allyl displayed reduced potencies (EC_{50} = 19 – 160 nM) vs. other compounds tested (EC_{50} = 0.1 – 10 nM). Collectively, the data indicate that most of the compounds are potent 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} agonists with subtle differences in functional activities based on bulkiness at the 2-position or *N*-alkyl/allyl group as well as the specific receptor isoform studied.

Interestingly, some 5-MeO-DMT analogs displayed reduced potencies at m5-HT_{2A} compared to h5-HT_{2A}, consistent with prior evidence for species differences in drug potency at 5-HT_{2A}.^{72–75} Here we found the 2-Me-DMT analog was 23-fold more potent at the human vs. the mouse 5-HT_{2A} receptor, whereas the rest of the analogs were 2 – 6-fold more potent at the human receptor variant. Previous studies showed that mutating a single serine residue (i.e., S242 in human 5-HT_{2A}) to an alanine (i.e., A242 in m5-HT_{2A}) significantly alters *in vitro* drug potencies at 5-HT_{2A}.^{72–75} One published data set investigating 4-substituted tryptamines showed that psilocin and norpsilocin display small ~2-fold differences in 5-HT_{2A} activation potency, but not efficacy, between human and mouse receptor variants *in vitro*.⁷⁶ Recently, mice with the A242S mutation, effectively “humanizing” their 5-HT_{2A}, have been developed for laboratory use (see <https://doi.org/10.1101/2023.09.25.559347>). Therefore, it will be interesting to utilize these mutant “humanized” 5-HT_{2A} mice to further understand the impact of the A242S mutation in the preclinical pharmacology of psychedelics. The potencies for psychedelics to induce HTR in C57Bl/6J mice are highly correlated with the typical human doses that produce psychedelic subjective effects,⁷⁷ so the translational value of A242S vs. wild-type mice could be an important future consideration in psychedelic drug development.

The potencies and efficacies of the 5-MeO-DMT analogs were also determined at human 5-HT₂ subtypes using an orthogonal $G_{\alpha q}$ calcium mobilization assay.⁶⁶ Potency and efficacy values (relative to 5-HT) can be found in Figure S4, while concentration response curves for 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors are shown in Figure S5, S6, and S7 respectively. At 5-HT_{2A}, most of the compounds displayed low nanomolar potencies (0.7 – 19 nM) and full agonist efficacies (91 – 113% 5-HT) similar to results from the BRET-based functional assessments. The 2-methyl compounds were the exception, with reduced potencies (77 and 5,636 nM) and/or efficacies at 5-HT_{2A} (71% for 5-MeO-2-Me-DMT) relative to the other compounds. Functional potencies of the compounds at 5-HT_{2B} were generally similar to potencies observed at 5-HT_{2A} across the series, with slightly reduced potencies for 5-HT_{2B}

in some cases (2 – 179 nM), more variability in $E_{\max}$ (73 – 99% 5-HT), and the same reduction in functional activity seen with 2-methyl substitutions (EC_{50} = 110 and 8,139 nM, $E_{\max}$ = 50 and 76%). Notably, functional potencies of the compounds at 5-HT_{2C} (52 – 3,664 nM) were reduced relative to 5-HT_{2A} and 5-HT_{2B}, except for the two compounds with a methyl or no substitution at the amine (i.e., 5-MeO-T and 5-MeO-NMT, EC_{50} = 1.5 & 12.8 nM respectively), corroborating our data from the BRET assay. The quaternary ammonium 2-methyl analog did not have measurable activity at 5-HT_{2C} up to 5,000 nanomolar. Across all assay platforms, the 5-MeO-DMT analogs were agonists at 5-HT_{1A} and 5-HT₂ subtypes, with high potencies at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2B}, but lower potencies at 5-HT_{2C} in most cases.

It is worth comparing the present SAR findings with two recent studies that examined the preclinical pharmacology of 5-MeO-DMT analogs.^{33, 34} In general, our findings are consistent with the prior work, especially with regard to drug activities at 5-HT_{1A} and 5-HT_{2A}. Our binding and functional screening results (Figure 2A, Figure S1 – S3) agree with Puigseslloses et al.³³ who showed that 5-MeO-iPTs (i.e., 5-MeO-NiPT, 5-MeO-MiPT, 5-methoxy-*N*-ethyl-*N*-isopropyltryptamine or 5-MeO-EiPT, and 5-MeO-DiPT) have slightly reduced 5-HT_{1A} affinities and potencies vs. other *N*-alkyl substitutions, while all compounds have similar high potencies for $G_{\alpha q}$ agonist activities at 5-HT_{2A}. Consistent with the results of Warren et al.³⁴, we found low nanomolar potencies at 5-HT_{1A} and 5-HT_{2A} across 5-MeO-DMT analogs, and a reduced 5-HT_{1A} potency for 5-methoxy-*N*-propyl-*N*-isopropyltryptamine (5-MeO-PriPT). Agonist efficacies at human 5-HT_{1A} reported previously (95–120%) are nearly identical to those reported here (99–115%), while agonist efficacies at human 5-HT_{2A} reported previously are slightly lower (75–89%) compared to those observed in the present $G_{\alpha q}$ BRET and calcium mobilization assays (93–104% and 91–117% respectively). One notable compound tested by both Warren et al. and Puigseslloses et al. was 5-MeO-PyrT, which exhibits potent 5-HT_{1A} agonism and is highly selective for 5-HT_{1A} over 5-HT_{2A}. While 5-MeO-PyrT was not tested in our experiments, we did include several compounds with 5-HT_{1A}-preferring properties (e.g., 5-MeO-NET). Collectively, our *in vitro* results confirm and extend existing knowledge about the pharmacology of 5-MeO-DMT analogs by including multiple functional assessments for 5-HT_{1A} and 5-HT_{2A} activities, measuring relative activities across 5-HT₂ receptors, investigating additional structural derivatives (2-methyl derivatives, 5-MeO-T, 5-MeO-NMT, and 5-MeO-NET), and characterizing potential non-5-HT receptor sites of action.

Potential off target pharmacological profiles at non-5-HT receptors: In addition to the 5-HT receptors, most of the test compounds exhibited competition for binding (i.e., >50%) in primary binding screens (Table S1) for alpha adrenergic receptors (alpha_{2A}, alpha_{2B}, alpha_{2C}), with a few compounds competing for binding at dopaminergic receptors (D₃, D₄), histaminergic receptors (H₁, H₃), sigma receptors, the kappa opioid receptor (KOR) and the serotonin transporter (SERT). Interestingly, 5-MeO-NiPT was the only compound to display exclusive affinity at 5-HT receptors at 10 micromolar. K_i constants for non-5-HT receptors are listed in Figure S8. In general, most of the binding affinities for non-5-HT receptors were > 1 micromolar, suggesting that they would only be relevant after high doses of 5-MeO-DMT analogs. Sub micromolar affinities (range = ~100 – 900

nM) were observed for α_{2A} (5-MeO-DMT, 5-MeO-2-Me-DMT, 5-MeO-MET, 5-MeO-DET, 5-MeO-MALT, 5-MeO-DALT), α_{2B} (5-MeO-2-Me-DMT, 5-MeO-DALT), α_{2C} (5-MeO-MET, 5-MeO-DALT), D_3 (5-MeO-DALT), D_4 (5-MeO-NMT), sigma 1 (5-MeO-DALT, 5-MeO-DPT), sigma 2 (5-MeO-DET, 5-MeO-MiPT, 5-MeO-DiPT, 5-MeO-DALT, 5-MeO-DPT), SERT (5-MeO-DiPT), and KOR (5-MeO-DALT).

5-MeO-DMT analogs were also screened for functional activities at non-5-HT targets identified in the binding screen using Tango, monoamine transporter, or G α i-cAMP functional assays.⁶⁶ All of the results are shown in Figure S9 and Figure S10. Results revealed that none of the compounds had agonist-like activity at α adrenergic receptors, D_1 -like dopamine receptors, or histamine receptors. Because six of the 5-MeO-DMT analogs displayed ~250 – 850 nanomolar affinities for α_{2A} adrenergic receptors, these compounds may act as antagonists at these receptors or be G protein-biased ligands. By contrast, several compounds had agonist-like activity at D_2 , D_3 , and D_4 receptors. 5-MeO-DiPT and 5-MeO-DALT displayed weak agonist-like activity at the μ -opioid (MOR), delta-opioid (DOR), and KOR, though 5-MeO-DALT displayed more substantial activity at the KOR (~76% of salvinorin A at 1 μ M), which was preferential for G protein-dependent cAMP vs. arrestin signaling.

The compounds additionally had selective activity at SERT (~30 – 85% fluoxetine at 30 μ M) vs. the dopamine transporter (DAT) and the norepinephrine transporter (NET). Results from the present screening assays are consistent with more in-depth examinations published previously, where various tryptamines had effects on SERT uptake and release.^{40–42} In the Blough et al. study,⁴⁰ analogs with longer or bulkier *N*-alkyl groups had the highest potencies for inhibition of SERT uptake, while only small *N*-monoalkyl compounds (5-MeO-NMT and 5-MeO-NET) served as substrates capable of inducing SERT-mediated 5-HT release. More recently, Puigseslloses et al. studied the effects of 5-MeO-DMT analogs in SERT uptake inhibition and release assays, showing that many compounds were weak uptake blockers, while 5-MeO-DMT, 5-MeO-MET, 5-MeO-DET, and 5-MeO-PyrT exhibited partial substrate activities.³³ The transporter data agree that 5-MeO-tryptamines, with the exception of the α -methyl derivative,⁴¹ display selective actions at SERT over other monoamine transporters that could contribute to *in vivo* effects of the drugs as compared to other serotonergic psychedelics.

Several of the compounds displayed agonist-like activity in the Tango assay at the melatonin receptor-1 (MT₁), while only 5-MeO-T and 5-MeO-2-Me-TMT displayed > 20% activity at the melatonin receptor-2 (MT₂) (Figure S10). It was shown almost 40 years ago that 5-MeO-T and 5-MeO-DMT are agonists at retinal melatonin receptors.⁷⁸ A subsequent study demonstrated partial agonist activity of 5-MeO-DMT at both MT₁ and MT₂ (EC₅₀ = ~100 – 250 nM, E_{max} = 28 – 62%) for G protein dependent signaling.⁷⁹ Together, the results agree that 5-MeO-tryptamines may have agonist-like effects at melatonin receptors *in vivo* which could affect circadian rhythms and other associated processes.⁸⁰ The exact consequences of these actions are unclear, but previous studies show that melatonin receptor agonists reduce the psychedelic-like effects of 2,5-dimethoxy-4-iodoamphetamine (DOI) and that endogenous melatonin may play a role in the circadian and diurnal variations in sensitivity to HTRs.^{81–84} Dual targeting of both serotonergic and melatonergic receptors has

been previously employed for designing novel drugs to treat depression (i.e., agomelatine), and thus may be a useful approach for psychedelic-assisted therapies which seek to address multiple symptoms of certain psychiatric disorders (e.g., depression and circadian rhythm dysfunction).⁸⁵ Melatonin itself is reportedly a weak antagonist at 5-HT₂ receptors.⁸⁶ This finding, combined with evidence for the dual targeting of serotonin and melatonin receptors by 5-MeO-DMT, further supports the potential utility of designing tryptamine compounds optimized for multiple therapeutic purposes.

Taken together, our pharmacological screening data demonstrate that the primary high-affinity receptor targets for 5-MeO-DMT analogs are 5-HT receptors. Importantly, the target profiles of 5-MeO-DMT and the analogs shown here are similar to what has been previously reported for a few of the same compounds.^{1, 38, 39} We found that the bulkier compounds in the series had reduced affinities and potencies at 5-HT_{2C} relative to 5-HT_{2A} and 5-HT_{1A}, supporting existing work which examined a small subset of 5-MeO-DMT analogs.^{41, 42} Finally, the present results extend current knowledge about the pharmacological target profiles of various analogs of 5-MeO-DMT, which includes potentially relevant activities across alpha adrenergic, dopamine, opioid, and melatonin receptors in addition to SERT. These findings will aid in further understanding reported differences in their pharmacological effects in rodents and psychoactive effects in humans.⁹

Competition of 5-MeO-DMT analogs for 5-HT_{1A} and 5-HT_{2A} radioligand binding in mouse brain:

As a means to examine the receptor binding of the compounds in a native tissue preparation, binding affinities of 5-MeO-DMT analogs were further assessed at 5-HT_{1A} and 5-HT_{2A} in postmortem brain tissue from C57BL/6J mice (m5-HT_{1A} and m5-HT_{2A} respectively). K_i constants and concentration-effect curves are shown in Figure S11 and Figure S12. K_i constants of the compounds at m5-HT_{1A} for [³H]8-OH-DPAT agonist binding (1 – 179 nM, Figure S11) were nearly identical to values obtained in cells transfected with human 5-HT_{1A}, using the antagonist radioligand [³H]WAY100635 (5 – 269 nM, Figure S11). Similarly, the K_i constants of the series at m5-HT_{2A} receptors for [³H]M100907 binding (84 – 1,337 nM, Figure S12) were also similar to values obtained at the human 5-HT_{2A} expressed in cells, using the antagonist radioligand [³H]ketanserin (79 – 540 nM, Figure S1). Importantly, the control compounds for [³H]8-OH-DPAT binding (5-HT and WAY100635) and [³H]M100907 binding (DOI and M100907) displayed expected low nanomolar affinities (0.3 – 7.9 nM). The mouse brain competition binding data agree with the present and previously published results from assays using human receptors, showing that 5-MeO-DMT analogs compete for binding at 5-HT_{1A} and 5-HT_{2A}.³³

Relationships between pharmacological parameters at 5-HT_{1A} and 5-HT_{2A} receptors in vitro:

Given the contributions of 5-HT_{1A} and 5-HT_{2A} in the effects of 5-MeO-DMT and its analogs^{18, 26, 33, 34, 69–71}, the relationships between *in vitro* receptor affinities and functional activities for the series at these receptors were examined using Spearman correlation analyses. At 5-HT_{2A}, drug affinities for both the human and mouse receptors were highly correlated ($r = 0.7143$, $p = 0.0079$, Figure 3A). Similarly, the affinities of the compounds at m5-HT_{1A} and human 5-HT_{1A} were also highly correlated ($r = 0.9636$, $p < 0.0001$, Figure 3B). The affinities of the drugs at m5-HT_{1A} ($r = 0.8462$, $p = 0.0005$

& $r = 0.8790$, $p < 0.0001$) and 5-HT_{1A} ($r = 0.9394$, $p = 0.0002$ & $r = 0.9760$, $p < 0.0001$) were positively correlated with 5-HT_{1A} and m5-HT_{1A} potencies in BRET functional assays, but not relative $E_{\max}$ at this receptor. Potencies for BRET dissociation at human 5-HT_{1A} were also highly correlated with potencies for BRET dissociation at m5-HT_{1A} ($r = 0.8680$, $p < 0.0001$). Interestingly, the 5-HT_{2A} potencies of the compounds in BRET assays were positively correlated with pharmacological parameters at both 5-HT_{2A} and 5-HT_{1A}. Specifically, 5-HT_{2A} BRET potencies were significantly correlated with 5-HT_{2A} affinities ($r = 0.8297$, $p = 0.0008$), 5-HT_{2A} β arrestin 2 potencies ($r = 0.8297$, $p = 0.0008$), 5-HT_{2A} calcium mobilization potencies ($r = 0.7692$, $p = 0.0031$), m5-HT_{2A} BRET potencies ($r = 0.9121$, $p < 0.0001$, Figure 3C), 5-HT_{1A} affinities ($r = 0.8667$, $p = 0.0022$), m5-HT_{1A} affinities ($r = 0.7253$, $p = 0.0067$), m5-HT_{1A} BRET potencies ($r = 0.6920$, $p = 0.0110$), and 5-HT_{1A} BRET potencies ($r = 0.8077$, $p = 0.0014$). These findings support the observation that many of the compounds in the series display similar rank orders of potencies at 5-HT_{1A} and 5-HT_{2A} across measures, despite a slight preference for most of the compounds for *in vitro* 5-HT_{1A} activity. The 5-HT_{1A}/5-HT_{2A} potency ratios from BRET functional assays further support that many of the compounds were 5-HT_{1A} preferring *in vitro* (Figure 3D), as only a few compounds were 5-HT_{2A}-preferring (both 2-methyl compounds, 5-MeO-MiPT, 5-MeO-DiPT) or somewhat balanced (EC_{50} ratio = 0.6 – 1.4; 5-MeO-NiPT, 5-MeO-MALT). The 5-HT_{1A}/5-HT_{2A} potency ratios were also positively correlated with 5-HT_{1A} ($r = 0.7818$, $p = 0.0105$, Figure 3E) and m5-HT_{1A} affinities ($r = 0.6538$, $p = 0.0182$, Figure 3F) as well as 5-HT_{1A} BRET potencies ($r = 0.7527$, $p = 0.0042$, Figure 3G) and m5-HT_{1A} potencies ($r = 0.7200$, $p = 0.0070$).

Assessment of the role of 5-HT_{1A} and 5-HT_{2A} receptors on effects of 5-MeO-DMT analogs in mice

Acute effects of 5-MeO-DMT analogs in mice: We next characterized dose-response relationships (0.03 – 30 mg/kg s.c.) for acute effects of the drugs on HTR counts, body temperature change (°C), and locomotor activity (distance traveled cm) in male C57BL/6J mice. Calculated potency (ED_{50}) values and maximum effect ($E_{\max}$) values for each compound for all three measures can be found in Figure 4, whereas mean effects for each dose and information about statistical comparisons can be found in Table S3 and Materials and Methods.

Dose-response curves for total HTR events displayed a typical biphasic inverted U shape, as shown in Figure 5A – D.^{77, 87} Potencies and maximal effects for stimulation of HTRs were variable across the compounds (ED_{50} range = 0.25 – 1.47 mg/kg s.c., $E_{\max}$ range = 19 – 65 mean events/30 min) with a rank order of potencies of 5-MeO-MET $\geq$ 5-MeO-DPT $\geq$ 5-MeO-MALT $\geq$ 5-MeO-DMT > 5-MeO-MiPT > 5-MeO-DET > 5-MeO-NiPT $\geq$ 5-MeO-DiPT $\geq$ 5-MeO-DALT. The time-course for HTR-active compounds revealed that dose-related peak effects occurred within ~5 – 15 min post administration and mostly returned to vehicle baseline by the end of the 30 min session (Figure S15). At the highest drug doses tested, effects of HTR-active drugs were rapid and returned to baseline within ~10 – 15 mins. Importantly, analogs with either a 2-methyl substitution or *N*-alkyl group smaller than *N,N*-dimethyl (i.e., 5-MeO-T, 5-MeO-NMT, 5-MeO-NET) were inactive for

stimulation of HTR at doses up to 30 mg/kg, and in some cases, these compounds even reduced total HTRs below vehicle levels (Figure S16, Figure 4, Table S3).

The only *N*-monoalkyl compound to induce HTR was 5-MeO-NiPT. The results with 5-MeO-NiPT are notable because the other *N*-monoalkyl compounds did not induce HTR, even though they displayed potent and efficacious stimulation of 5-HT_{2A} *in vitro*. The lack of HTRs associated with *N*-monoalkyl analogs of 5-MeO-DMT is reminiscent of the findings with the 4-position ring-substituted *N*-monoalkyl tryptamine, norpsilocin vs. related analogs.^{76, 87, 88} Our data with 5-MeO-NiPT corroborate HTR activity of other *N*-monoalkyl 5-MeO-tryptamine compounds with bulky *N*-substitutions³³ and might be relevant to the *in vivo* effects of related tryptamines, since 5-MeO-NiPT is an *N*-dealkylated metabolite of 5-MeO-MiPT and 5-MeO-DiPT.^{61, 89, 90} 5-MeO-NiPT is also an interesting example of differences in potency and maximal effects for inducing HTR in this series. 5-MeO-NiPT displayed slightly higher potency vs. 5-MeO-DiPT and lower potency vs. 5-MeO-MiPT, but had 2 – 3-fold lower maximal counts in both cases. 5-MeO-DMT, 5-MeO-MET, 5-MeO-DET, 5-MeO-NiPT, 5-MeO-DPT, and 5-MeO-DALT all had similar maximal HTR counts (19 – 26 HTR/30 min), which were ~ 2 – 3-fold lower than the other analogs that were HTR active (35 – 65 HTR/30 min). These differences were also reflected in the observed maximal rates of HTR from time-course plots (Figure S15 – 16), which show that 5-MeO-MALT, 5-MeO-MiPT, and 5-MeO-DiPT had the highest HTR rates across the sessions (~9 – 14 HTR events/5 min, Figure S17) compared to HTR-inactive (~1 – 2 HTR events/5 min) and other HTR-active compounds (~4 – 6 HTR events/5 min). Demonstrating the potential importance of the relationship between 5-HT_{1A} and 5-HT_{2A} activities, the 5-HT_{1A}/5-HT_{2A} potency ratios of the most active compounds to induce HTRs were 5-HT_{2A}-preferring or balanced (Figure 3D). Taken together, the present HTR data are consistent with the reported psychedelic subjective effects of these compounds in humans,⁹ which can be reliably predicted by HTR activity in C57BL/6J mice.⁷⁷ At present, it is unclear if the differences observed in maximal HTR counts in mice might translate to differences in psychoactive effects in humans, but such differences are likely related to the unique pharmacological profile and functional activities for each individual drug (i.e., interactions at 5-HT_{1A} or other non-5-HT_{2A} receptor sites).

We also monitored body temperature change and locomotor activity in the dose-response experiments, since previous reports indicate that 5-MeO-DMT and related analogs can induce 5-HT syndrome-like behaviors in rodents, characterized by forepaw treading, Straub tail, flat body posture, hypolocomotion.^{1, 33, 91, 92} Potency and maximal effect values for each compound are found in Figure 4B, while dose-response curves are depicted in Figure S18 and Figure S19. All compounds produced dose-related hypothermia (ED₅₀ = 2.80 – 18.48 mg/kg s.c.) and hypolocomotion (ED₅₀ = 0.98 – 18.21 mg/kg s.c.), coinciding with the descending limb of the HTR dose-response curves. For most compounds, the potencies for inducing hypothermia and hypolocomotion were ~7 – 33-fold lower than the potencies for inducing HTRs. Interestingly, some compounds induced biphasic effects on locomotor activity, with locomotor stimulation at low doses and depression at high doses (e.g., 5-MeO-MET, 5-MeO-DiPT, 5-MeO-DALT). Previous studies show that 5-MeO-DALT, 5-MeO-DET, and 5-methoxy- α -methyltryptamine (5-MeO-AMT) can produce biphasic effects on locomotor activity over the first 30–60 min after drug administration.^{93, 94} Consistent with

hypolocomotor effects, 5-MeO-DMT and related analogs are reported to reduce locomotor activity as well as exploratory behaviors via 5-HT_{1A} agonist-like effects in rats and mice.^{26, 33, 34, 39, 69, 70} The summed *in vivo* findings agree that 5-MeO-DMT and its analogs dose-dependently reduce locomotor activity and body temperature, but under some circumstances certain analogs may produce biphasic responses on motor activity.

Maximal temperature reductions across the series were 4.0 – 6.3 °C (vs. ~0.5 for vehicle), while maximal hypolocomotor effects were between 295 – 1,207 cm traveled across the session (vs. ~2,000 cm for vehicle). The hypothermic and hypolocomotor effects of the 5-MeO-DMT analogs shown here are consistent with previous results shown by others in CD-1 mice,³³ and agree with similar effects of 4-position ring-substituted tryptamine psychedelics in mice.^{28, 87, 95} One prior study showed that 5-MeO-DiPT produces hypothermia and hypolocomotion after 20 mg/kg in rats,⁹⁶ whereas another study found that 2 – 20 mg/kg of 5-MeO-DMT induces hyperthermia in CYP2D6-humanized mice.⁹⁷ The limited findings available suggest there may be species (rat vs. mouse) and/or strain differences (C57BL/6J vs. CYP2D6-humanized) in the thermoregulatory effects of 5-MeO-tryptamines. Despite some discordant results reported across studies, most results agree that body temperature changes induced by tryptamines are related to agonist actions at 5-HT_{1A}, which are known to influence the pharmacological effects of 5-MeO-DMT and may impact psychedelic-like activity.^{27–30, 32–34} Regarding translatability of thermic responses in rodents to those in humans, there is little evidence that classical psychedelics induce hypothermia in humans, even in overdose situations,⁹⁸ despite a demonstrated role of 5-HT_{1A} activity in the subjective effects of psilocybin and DMT.^{29, 30} On the other hand, administration of selective 5-HT_{1A} agonists can induce hypothermia in humans,^{99–104,105} but this effect may only apply to full agonists, as partial 5-HT_{1A} agonists do not always lower body temperature.¹⁰⁶

As noted already, the hypothermic and hypolocomotive effects of HTR-active compounds seemed to directly correspond with the descending limb of the inverted U-shaped HTR dose-response curves (Figure 5). Close inspection of the time-course for HTR induction by 5-MeO-DMT analogs supports this notion, showing that at high doses where hypothermia or hypolocomotion were present, the duration of HTRs was compressed to the first ~5 – 15 min of the session vs. ~25 – 30 min for doses on the ascending limb of the HTR dose-response curves (Figure S15).

Jefferson et al. recently reported that 5-MeO-DMT (5 – 40 mg/kg i.p.) has a shorter duration of action for increasing the HTR (< 5 min) relative to 1 mg/kg i.p. psilocybin (~10 min) in C57BL/6J mice,¹⁰⁷ which is consistent with the data shown here and may be related to the recruitment of hypothermic and hypolocomotive effects at high drug doses. Using an approximation of the potency of 5-MeO-DMT to increase HTRs in the Jefferson et al. study (ED₅₀ = ~7.5 mg/kg), which is similar to other studies utilizing i.p. drug administration,^{26, 108} the potency after i.p. injection is reduced compared to the potency reported here after s.c. injection (ED₅₀ = 0.33 mg/kg). Another recent study using Swiss CD-1 mice and measuring HTR for 10 min post drug administration showed that several 5-MeO-DMT analogs administered i.p. had reduced HTR potencies (ED₅₀ range = ~1 – 10.5 mg/kg) and efficacies (E_{max} range = ~8 – 38 events/10 min) relative to those observed presently with s.c.

drug administration (Figure 4A, Figure 5).³³ One notable discrepancy from the Puigseslloses et al. study is data for 5-MeO-DiPT, which we found to be 6 times more efficacious for inducing the HTR after s.c. vs. i.p. administration ($E_{\max}$ = 65 vs. 11 HTRs). It is important to mention that the only strain of mice where increases in HTRs are known to predict potencies for psychedelic subjective effects in humans is the C57BL/6J mouse.⁷⁷ Therefore, strain differences in the predictive validity of the HTR paradigm may be present when using CD-1 (also called ICR mice) vs. C57BL/6J mice. Canal and Morgan previously reported on the variability in the HTR induced by 2,5-dimethoxy-4-iodoamphetamine (DOI) across various mouse strains, showing CD-1/ICR mice have reduced HTR counts vs. C57BL/6J mice (8 – 25 vs. 30 – 68 HTR events/10 min).¹⁰⁹ Further, another recent study administering s.c. 5-MeO-MET to C57BL/6J mice reported a lack of HTR for this compound alone, but only measured the behavior for 10 min post drug administration similar to the Puigseslloses et al. study.^{33, 34} In our experiments, 5-MeO-MET induced peak HTR counts with 1 mg/kg s.c. at ~10–15 min post injection, with elevated HTR counts persisting through the end of the session (Figure S15). Therefore, studies using shorter observation periods of only 10 mins may miss differences in HTR events critical for determining the potential of a compound to produce psychedelic-like effects. Overall, the discrepancies in HTR counts between various studies are likely due to several factors including: 1) mouse strain differences in the propensity to express HTRs, 2) length of the HTR observation period (i.e., 10 vs. 30 min), and 3) potential variability and greater first-pass hepatic metabolism with the i.p. route vs. the s.c. route^{110, 111}. The rapid degradation and metabolism of 5-MeO-DMT can influence its pharmacological effects,^{97, 112–115} which convergent data suggest may be less relevant with s.c. drug administration.⁹ Future experiments comparing the pharmacokinetics and pharmacodynamic effects of 5-MeO-DMT analogs across various routes of administration would thus be informative.

Many other behavioral effects of 5-MeO-DMT have been described in various animal species, and these effects are discussed by Ermakova et al. in a comprehensive review.¹ 5-MeO-DMT and some of its analogs have been previously tested in drug discrimination experiments utilizing various training drugs (e.g., lysergic acid diethylamide or LSD and 2,5-dimethoxy-4-methylamphetamine or DOM).¹¹⁶ Results suggest a complex stimulus cue for 5-MeO-DMT, primarily mediated by 5-HT_{1A}, but also functionally relevant interactions at 5-HT₂ subtypes.^{18, 117} In a study by Winter et al., the training dose of 5-MeO-DMT was 3 mg/kg, consistent with the maximal dose inducing HTR in our present dose-response study, and consistent with findings that show drug discrimination and HTR potencies of psychedelics are often positively correlated.^{18, 77}

Relationships between *in vitro* 5-HT_{1A} or 5-HT_{2A} and mouse potencies: The relationships between *in vitro* receptor activities and *in vivo* effects in mice (excluding the HTR inactive compounds due to inability to calculate ED₅₀ values) were assessed by computing Spearman rank order correlations. HTR potencies were positively correlated with potencies for hypolocomotor effects ($r = 0.9038$, $p = 0.0016$, Figure 6A), hypothermic effects ($r = 0.9000$, $p = 0.0020$, Figure 6B), m5-HT_{2A} BRET EC₅₀ ($r = 0.8000$, $p = 0.0138$, Figure 6C) or $E_{\max}$ values ($r = 0.8500$, $p = 0.0061$), and 5-HT_{2A} β arrestin 2 EC₅₀ ($r = 0.7000$, $p = 0.0433$) or $E_{\max}$ values ($r = 0.7333$, $p = 0.0311$). HTR potencies were not

significantly correlated with any other *in vivo* or *in vitro* measures. For mouse behavioral endpoints, the correlation findings demonstrate that potencies for HTR, hypothermic, and motor suppressive effects follow a comparable rank order across compounds, despite ~7 – 33-fold higher potency values for inducing HTRs vs. other effects.

HTR maximal rates were positively correlated with HTR $E_{\max}$ values ($r = 0.9725$, $p < 0.0001$, Figure 6D) and 5-HT_{2A} β arrestin 2 EC₅₀ values ($r = 0.5604$, $p = 0.0499$), but were not correlated with potency or maximal efficacies of the compounds in other 5-HT_{2A} functional assays. It has been previously shown that potency and threshold efficacy (i.e. > 70%) for 5-HT_{2A}-mediated G_{αq} activation is critically involved in mediating the HTR in mice.^{88, 108, 118, 119} The present results suggest that the rank order of potencies or maximal efficacies for G_{αq} activation are not strongly related to the rank orders of HTR maximal effects or potencies for this series of compounds. This disconnect may be related to the potent and efficacious activities of compounds with the smallest amine substitution pattern, which did not increase the HTR in mice and may have brain permeability limitations similar to related 4-substituted tryptamines.⁸⁸ Additionally, this could also be related in part to the polypharmacology of the series, necessitating more complex multivariate approaches to assess relationships among pharmacological parameters.³¹

Lastly, hypolocomotor and hypothermia potencies were highly correlated with one another ($r = 0.8722$, $p = 0.0004$, Figure 6E) and with h5-HT_{1A} affinities ($r = 0.9500$ & 0.8303 , $p = 0.0004$ & 0.0047), m5-HT_{1A} affinities ($r = 0.7496$ & 0.6593 , $p = 0.0067$ & 0.0169), h5-HT_{2A} affinities ($r = 0.6760$ & 0.6319 , $p = 0.0188$ & 0.0236), h5-HT_{2A} BRET potencies ($r = 0.8581$ & 0.7363 , $p = 0.0007$ & 0.0056), h5-HT_{2A} calcium mobilization potencies ($r = 0.7356$ & 0.7143 , $p = 0.0083$ & 0.0079), h5-HT_{2A} β arrestin 2 potencies ($r = 0.8021$ & 0.5659 , $p = 0.0026$ & 0.0473), and m5-HT_{2A} BRET potencies ($r = 0.8722$ & 0.7473 , $p = 0.0004$ & 0.0046). Hypolocomotor potencies were also correlated with h5-HT_{1A} potencies in BRET functional assays ($r = 0.7110$, $p = 0.0119$, Figure 6F), but not for maximal relative efficacies ($r = 0.0930$, $p = 0.7711$). The correlation results show that rank orders for 5-HT_{1A} pharmacological parameters across the series are consistent, highlighting translatability of results from the receptor to the behavioral/physiological levels of analyses. The results also suggest that the rank orders of pharmacological parameters related to 5-HT_{2A} activities are similar to those at 5-HT_{1A}, despite higher affinities and potencies observed *in vitro* for 5-HT_{1A} vs. 5-HT_{2A} activities and vice versa *in vivo*.

Effects of 5-HT_{2A} and 5-HT_{1A} antagonist pretreatment on acute effects of 5-MeO-DMT analogs:

In follow up mouse experiments, we sought to examine the roles of 5-HT_{2A} and 5-HT_{1A} in the *in vivo* effects produced by 5-MeO-DMT analogs. To this end, mice were pretreated with either a 5-HT_{2A} (M100907, 0.01 mg/kg s.c.) or 5-HT_{1A} (WAY-100635, 3 mg/kg s.c.) antagonist prior to agonist administration, followed by measurement of the same endpoints from dose-response studies.

It was previously shown that 5-MeO-DMT produces dose-related increases in HTR that are not present in 5-HT_{2A} knock out mice or in mice pretreated with the non-selective 5-HT_{2A} antagonist ketanserin.^{26, 33} These findings are consistent with a large number of studies implicating 5-HT_{2A} as a main target responsible for psychedelic-like effects (i.e., increases

in HTR in mice, psychedelic-like discriminative stimulus effects in rats, and psychedelic subjective effects in humans) of serotonergic psychedelics.^{21, 22, 24, 77, 87, 116} On the other hand, findings from rodent studies and *in vitro* experiments support a role for 5-HT_{1A} in the effects of 5-MeO-DMT and related analogs, in addition to relevant activities at 5-HT_{2A}.^{18, 26, 33, 34, 120} Therefore, we surmised that both receptors would play a role in the effects of 5-MeO-DMT analogs in our experiments.

To investigate the role of 5-HT_{2A}, we tested the HTR-active compounds for their ability to induce the HTR when mice were pretreated with M100907 (0.01 mg/kg s.c.) to block 5-HT_{2A}. The HTRs induced by 5-MeO-DMT analogs at near maximal doses, as determined in dose-response studies, were blocked in animals pretreated with M100907, without exception (Figure 7, Table S4, Table S5). In some cases, in the M100907/vehicle and M100907/agonist conditions, a significant reduction of the HTR vs. the vehicle/vehicle group was observed, showing that blockade of 5-HT_{2A} can reduce basal HTRs in addition to psychedelic-induced HTRs. Importantly, administration of M100907 at 0.01 mg/kg had negligible effects on locomotor activity, suggesting no generalized motor impairment (Figure S20, Figure S21, Figure S22, Table S4, Table S5).⁸⁷ Some of the 5-MeO-DMT analogs also slightly increased locomotor activity relative to the vehicle/vehicle and other groups (Figure S22, Table S4, Table S5). Given the extra habituation time in the test chambers afforded by antagonist experiments (i.e., 30 min pretreatment time plus 30 min experiment time) and the known influence of habituation on motor effects of psychedelics,¹²¹ 5-MeO-DMT analogs may interfere with normal habituation behavior to produce mild locomotor stimulation above vehicle control levels. As noted already, some compounds also exhibited mild locomotor stimulation at selected doses in the dose-response studies (Figure S19, Table S3).

Next, we tested effects of 5-HT_{1A} blockade by administering WAY100635 (3 mg/kg s.c.) 30 min prior to administration of a high dose of each DMT analog (i.e., doses that induced hypothermia and hypolocomotor effects in dose-response studies) and measured body temperature, locomotor activity, and HTRs. We previously showed that high-dose effects of related psychedelic tryptamines are mediated in part by actions of the drugs at 5-HT_{1A} receptors.^{28, 87} First, at the doses administered, the compounds all produced robust hypothermia with some accompanying hypolocomotor effects (Figure 8, Table S6 – S7, Figure S23 – 26). The hypothermic effects were partially to completely reversed by WAY100635, supporting a role for 5-HT_{1A} receptors in mediating body temperature decreases. Hypolocomotor effects were partially or fully reversed by blockade of 5-HT_{1A}, with several compounds (i.e. 5-MeO-DET, 5-MeO-NiPT, 5-MeO-MiPT, 5-MeO-DiPT, 5-MeO-DALT, 5-MeO-DPT) displaying the opposite hyperlocomotor effects when combined with WAY100635 pretreatment. Importantly, WAY100635 pretreatment had no effect of its own on body temperature or motor activity measures relative to saline vehicle controls.

High-dose administration of 5-MeO-DMT and its analogs did not alter HTR levels in saline-pretreated mice, consistent with these doses falling on the descending limb of the HTR dose-response curves (Figure 5). Unexpectedly, when these same ineffective drug doses were given 30 min after 5-HT_{1A} blockade, the 5-MeO-DMT analogs induced robust HTRs (Figure 9, Table S6 - S7). The magnitude of HTR activity in the presence of 5-HT_{1A} blockade was 2 – 8-fold greater than the maximal counts observed in dose-response studies

(Figure 4). In the case of 5-MeO-NET, this compound did not increase the HTR above baseline levels in our initial dose-response evaluation, but under the conditions of 5-HT_{1A} blockade, this compound produced psychedelic-like effects (Figure 9B), though other HTR-inactive compounds did not (Figure S26, Table S6 – S7). Similar to the results shown here, a prior study showed that lysergic acid morpholide (LSM-775) lacks HTR activity when administered alone despite online reports of its mild psychoactive effects, but once 5-HT_{1A} activity of the drug was blocked, its HTR activity was revealed.²⁷ We have previously shown that 5-HT_{2A}-mediated HTRs of other psychedelic tryptamines can be masked by their 5-HT_{1A} activities at high drug doses.^{28, 87} Similar to the present work, Puigseslloses et al. recently reported that 5-HT_{1A} blockade in CD-1 mice enhances HTR activity for 5-MeO-DMT analogs.³³ In the Puigseslloses et al. study, 5-MeO-PyrT displayed little or no HTR activity until blockade of 5-HT_{1A}, analogous to our findings with 5-MeO-NET and prior findings with LSM-775.^{27, 33} Collectively, these results highlight the importance of further investigating the effects of HTR-inactive compounds, as the polypharmacology of many psychedelics may alter the expression of the HTR in mice, and perhaps subjective psychedelic effects in humans.^{122, 123}

Of note, 5-MeO-T and 5-MeO-NMT failed to increase HTRs under any conditions, but induced 5-HT_{1A}-mediated hypothermia and hypolocomotion. Based on the 5-HT_{2A} functional potencies of these compounds *in vitro*, HTR activity would be expected. However, the 5-HT_{1A}/5-HT_{2A} BRET potency ratios show that these compounds and 5-MeO-NET have similar 5-HT_{1A} preference, yet WAY100635 pretreatment revealed latent HTRs only for 5-MeO-NET (Figure 9B, Figure S26). Thus, factors unrelated of 5-HT_{1A} and 5-HT_{2A} activities must be involved in the lack of HTRs associated with 5-MeO-T and 5-MeO-NMT. These factors could include differences in target profiles at other 5-HT receptors, effects at non-5-HT targets (e.g. melatonin and dopamine receptors, Figure S9 - 10), and potential pharmacokinetic differences due to reduced brain permeability or enhanced drug metabolism.⁸⁸

Similar to recent findings with 5-MeO-PyrT and LSM-775,^{27, 33} we found that 5-MeO-NET failed to produce detectable increases in HTRs without first blocking 5-HT_{1A} activity. As noted above, this compound displays a preference for 5-HT_{1A} vs. 5-HT_{2A} activity in functional assays (Figure 2A, Figure 3D). In a recent study by Warren et al., a 4-fluoro analog of 5-MeO-PyrT (4F-5-MeO-PyrT) did not produce detectable HTRs even with 5-HT_{1A} antagonist pretreatment, but this result is likely attributable to the profound selectivity at 5-HT_{1A} vs. 5-HT_{2A} (>800-fold) for 4-fluoro additions and cyclization of the amine moiety.³⁴ 4F-5-MeO-PyrT produced antidepressant-like effects in social defeat stress and sucrose preference paradigms, suggesting selective 5-HT_{1A} activity may be a viable avenue for design of potential antidepressants.³⁴ However, 5-MeO-PyrT is highly 5-HT_{1A} selective over 5-HT_{2A} (~40 – 500-fold)³⁴ and produces undesirable acute effects in humans based on anecdotal accounts.⁹ Our study specifically focused on hybrid 5-HT_{1A}/5-HT_{2A} ligands with balanced to moderate (i.e. < 10-fold) selectivity at 5-HT_{1A} vs. 5-HT_{2A}.

After observing the suppressed HTR activity at high doses across the series, we further tested the ability of 5-HT_{1A} blockade with WAY100635 (3 mg/kg sc. 15 min pretreatment) to influence the dose-response effects of 5-MeO-DMT (0.03 – 30 mg/kg sc.). Specifically,

we tested whether WAY100635 pretreatment could reduce hypothermic and hypolocomotor effects, while enhancing HTRs. Data from our experiments revealed that relative to the dose-response curve for 5-MeO-DMT alone, the curve in the presence of WAY100635 showed reduced maximal effects for hypothermia (3 – 30 mg/kg) and enhanced HTR counts at the highest doses tested ($E_{\max}$ = 132 currently vs. 23 previously observed HTR counts), with no hypolocomotor effects observed (Figure 10A – B, Figure 5A, Table S2, Table S8, Figure S27). WAY100635 pretreatment also enhanced the rate of HTR observed across the session vs. 5-MeO-DMT alone (max rate = ~30 vs. 6 HTR counts/5 min; Figure 10C, Figure S15A). The potency of 5-MeO-DMT with WAY100635 pretreatment (ED_{50} = 7.34 mg/kg) was ~23 times weaker vs. 5-MeO-DMT alone (ED_{50} = 0.33 mg/kg), but this effect was driven by the increased HTR maximum at the two highest doses tested. Our data indicates that the maximum 5-HT_{2A}-mediated HTR count of tryptamines like 5-MeO-DMT may be masked or reduced by 5-HT_{1A} agonism. It is unclear whether higher doses of 5-MeO-DMT and WAY100635 could induce even greater maximal HTRs, since the dose-response curve for the drug combination (i.e., see Figure 10B) did not reach a clear plateau. Regardless, our findings are intriguing and support the suppressive influence of 5-HT_{1A} on the expression of 5-HT_{2A}-mediated behaviors at high drug doses.

It is noteworthy that WAY100635 pretreatment prior to 5-MeO-DMT administration only enhanced HTRs at doses > 3 mg/kg. This observation is intriguing because 1 mg/kg 5-MeO-DMT alone produced maximal HTR counts, and it seems likely that there would be activation of both 5-HT_{1A} and 5-HT_{2A} at this dose. Given that the acute 5-HT syndrome-like effects displayed by 5-MeO-DMT analogs and other psychedelic tryptamines appear to correspond with the descending limb of the HTR dose-response curves (Figure 5),^{28, 87} the data suggest that differential receptor occupancies may be required for expression of 5-HT_{2A}- (i.e., HTRs) vs. 5-HT_{1A}-mediated effects (i.e., hypothermia and hypolocomotion). Stated more simply, it seems feasible that low fractional occupancy of total 5-HT_{2A} sites is sufficient to evoke robust HTRs, whereas high fractional occupancy of 5-HT_{1A} sites might be required to observe hypothermia, hypolocomotion, and suppression of HTR. With regard to 4-substituted tryptamines, these compounds display weaker *in vitro* pharmacological activities at 5-HT_{1A} compared to 5-MeO-DMT, yet these compounds also exhibit 5-HT_{1A}-mediated hypothermia and hypolocomotion at high doses that coincide with the descending limb of their HTR curves.^{28, 87, 88, 124} Another possibility is that activities at other receptors - such as alpha adrenergic receptors, other 5-HT receptor subtypes (e.g. 5-HT₇, 5-HT_{2C}), dopamine receptors, melatonin receptors, or SERT (Figure S8 – 10) - could play a role in modulating the influence of 5-HT_{1A} agonism on the 5-HT_{2A}-mediated HTR.^{81, 82, 109, 125–127} For example, alpha_{2A} receptors have been shown to modulate 5-HT_{1A}-mediated attenuation of DOI-induced HTR in mice.¹²⁵ Our target screening data show that most 5-MeO-DMT analogs have nanomolar affinities at alpha_{2A} receptors (Figure S8), but the compounds lack any agonist-like activity in the Tango screening assay at this receptor (Figure S9), suggesting they may be antagonists or G protein-biased. Future studies should examine the potential role of various receptor subtypes in modulating the inhibitory effects of 5-HT_{1A} on 5-HT_{2A}-mediated HTRs.

Our results suggest that it might be possible to leverage non-5-HT_{2A} activities of psychedelic drugs for increasing or decreasing psychedelic-like effects, and perhaps

designing non-psychoactive 5-HT_{2A} agonists. The modulation of 5-HT_{1A} activity could be accomplished by “designing in” 5-HT_{1A} activity of a given drug molecule (agonist to tune down and antagonist to tune up psychedelic-like effects) or by co-administration of a clinically available 5-HT_{1A} ligand of choice (e.g., buspirone or pindolol). Clinical data support the modulatory effect of 5-HT_{1A} activity since co-administration of buspirone with psilocybin reduces subjective psychedelic effects in human volunteers, while pindolol prior to intravenous DMT enhances subjective psychedelic effects.^{29, 30} Therefore optimizing the 5-HT_{1A/2A} preference or taking advantage of the existing polypharmacology of these substances can offer avenues for potentially tailoring the intensity of subjective effects for patients engaged in psychedelic-assisted therapies.

CONCLUSION

In the present study, we confirm that 5-MeO-DMT analogs varying in *N*-alkyl, *N*-allyl, and 2-position ring substitution are non-selective 5-HT agonists with high potencies and efficacies at 5-HT_{1A} and 5-HT_{2A} *in vitro*. The preference of the compounds for 5-HT_{1A} vs. 5-HT_{2A} functional activities varied across the series, with only the 2-methyl, 5-MeO-iPTs (5-MeO-NiPT, -MiPT, -DiPT), and 5-MeO-MALT displaying 5-HT_{2A}-preferring or balanced potency ratios. Interestingly, many of the compounds exhibit binding and functional effects across a multitude of non-5-HT receptor targets (e.g., alpha adrenergic, dopamine, melatonin, SERT) that may impact their biological effects.

In mice, analogs with an *N,N*-dimethyl moiety or larger alkyl/allyl substitutions produced dose-related psychedelic-like effects to increase HTR, while all compounds induced 5-HT syndrome-like hypothermia and hypolocomotion at high doses (> 3 mg/kg). In the context of recent investigations of 5-MeO-DMT analogs in mice, our results point out that mouse strain, length of behavioral observation period, route of drug administration, and other factors may be important for comparing HTR studies across laboratories. The psychedelic-like effects of 5-MeO-DMT analogs were blocked by pretreatment with a selective 5-HT_{2A} antagonist, while the 5-HT syndrome-like effects were partially to fully blocked by pretreatment with a 5-HT_{1A} antagonist. Perhaps most importantly, blockade of 5-HT_{1A} prior to high-dose administration of HTR-active compounds enhanced maximal HTRs, or even revealed latent HTRs, supporting a notable suppressive influence of 5-HT_{1A} on expression of 5-HT_{2A}-mediated effects. The fact that 5-HT_{1A} blockade only enhanced HTRs after high doses of 5-MeO-DMT suggests other receptor activities might influence psychedelic-like effects (e.g., alpha_{2A} adrenergic activity). Collectively, our data reveal important SAR differences for 5-MeO-DMT analogs, such as 5-HT_{1A}/5-HT_{2A} preference, which may be useful for understanding the unique psychopharmacology of the compounds in both rodents and humans.

METHODS

Drugs

Drugs were dissolved in 0.9% saline and administered s.c. as the weight of the salt form in an injection volume of 0.01 mL/g body weight. Hemifumarate salts of 5-MeO-T, 5-MeO-NMT, 5-MeO-DMT, 5-MeO-DPT, 5-MeO-NET, 5-MeO-MiPT, and 5-MeO-NiPT,

and fumarate salts of 5-MeO-DET and 5-MeO-DiPT were synthesized by B.E.B. and A.L. as described previously.^{9, 40} 5-MeO-T HCl, 5-MeO-DALT hydrofumarate, 5-MeO-2-Me-DMT hydrofumarate and 5-MeO-2-Me-TMT iodide were synthesized and characterized by D.R.M., J.A.G., & M.N. as described previously.^{11, 68, 128} 5-MeO-MET hydrofumarate was purchased from ChemLogix and was purified by recrystallization from ethanol/isopropanol. 5-MeO-MALT hydrofumarate was synthesized via the neutralization reaction of freebase 5-MeO-MALT and fumaric acid in methanol, and purified by recrystallization in water/acetone.

Target Screening - Binding and Functional

Target screening was performed by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP) as described in their assay protocol book available online.⁶⁶ Data were plotted as previously described.^{28, 87, 88} The following targets were assessed in 10 micromolar screens for competition binding: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT₃, 5-HT_{5A}, 5-HT₆, 5-HT_{7A}, Alpha_{1A}, Alpha_{1B}, Alpha_{1D}, Alpha_{2A}, Alpha_{2B}, Alpha_{2C}, Beta₁, Beta₂, Beta₃, BZP Rat Brain Site, D₁, D₂, D₃, D₄, D₅, DAT, DOR, GABA_A, H₁, H₂, H₃, H₄, KOR, M₁, M₂, M₃, M₄, M₅, MOR, NET, PBR, SERT, Sigma 1, Sigma 2, AMPA, Kainate (Rat Brain), NMDA, NR2B.

Bioluminescence resonance energy transfer (BRET) Functional Assays

For human 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and mouse 5-HT_{2A} Gq dissociation assays as measured by BRET, HEK 293T cells (ATCC) were transfected in a 1:1:1:1 ratio of receptor: Gαq-Rluc8:β3:GFP2-γ9, as previously reported.¹⁰⁸ For 5-HT_{2A} β-arrestin2 recruitment as measured by BRET, HEK 293T cells were transfected in a 1:15 ratio of human 5-HT_{2A}-Rluc8: GFP2-human βarr2. For human and mouse 5-HT_{1A}, GoB dissociation assays as measured by BRET, HEK 293T cells were transfected in a 1:1:1:1 ratio of receptor: GαoB-Rluc8:β1:GFP2-γ2. Cells at approximately 60–80% confluency were transfected in 10% dialyzed FBS (dFBS; Omega Scientific), and all transfection mixes were prepared in Opti-MEM (Invitrogen) using a 3:1 ratio of TransIT-2020 (Mirus Bio) μL:μg total DNA. Next day, cells were detached, centrifuged, resuspended and plated in 1% dFBS at an approximate density of 30–50,000 cells per well into poly-L-lysine-coated 96-well white assay plates (Greiner Bio-One). After approximately 24 hours, media was decanted and replaced with 60 μL per well of drug buffer (1× HBSS, 20 mM HEPES, pH 7.4), and incubated for at least 15 minutes at 37°C in a humidified incubator before receiving drug stimulation. Compounds were diluted at 3× in drug buffer containing 0.3% BSA fatty acid free and 0.03% ascorbic acid. Diluted compounds were dispensed as 30 μL into each well using multi-channels and plates were incubated at 37°C in a humidified incubator until reading. Next, plates were briefly taken out and coelenterazine 400a (5 μM final concentration; Nanolight Technology) was added 15 minutes before reading. After 60 minutes total time of compound incubation in the assays plates, plates were read in a PheraStarFSX or ClarioStar Plus (BMG Labtech; Cary, NC) at 1 second per well for at least 15 minutes for 3–5 cycles. BRET ratios of 510/400 luminescence were calculated per well and were plotted as a function of compound concentration. Data were normalized to % positive control (5-HT) stimulation and analyzed using nonlinear regression “log(agonist) vs. response” to yield E_{max} and EC₅₀ parameter

estimates. All assays were performed in duplicate with at least three independent cell culture preparations.

Mouse Brain Competition Binding

Mouse brain competition binding studies were conducted as previously described.⁸⁷ Briefly, the experiments utilized C57BL/6 mouse brain (BioIVT, Westbury, NY, USA) to assess ability of the 5-MeO-DMT analogs to compete for [³H]8-OH-DPAT binding (0.5 nM, 5-HT_{1A} receptors) or [³H]M100907 (1 nM, 5-HT_{2A} receptors). Both radioligands were purchased from PerkinElmer (Boston, MA, USA). Nonspecific binding was determined using 10 μ M serotonin for 5-HT_{1A} and 10 micromolar ketanserin for 5-HT_{2A} binding. Dissociation constant values used for one site - fit K_i determinations in GraphPad Prism were from previously published studies using mouse brain (K_d = 1.03 and 0.35 nM respectively). Data represent 3 runs performed in triplicate for each value.

Mouse Studies

Mouse studies were conducted using male C57BL/6J mice (The Jackson Laboratory #000664) housed at the NIDA IRP facilities in Baltimore, MD. Animals were purchased at 6 weeks of age, housed in a 12:12 light-dark cycle (lights on at 0700 local time), and allowed ad libitum access to food and water outside of acute drug effect testing. All experiments were approved by the NIDA IRP Animal Care and Use Committee.

Studies testing acute drug effects of 5-MeO-DMT analogs in mice were conducted as previously described without modification.^{28, 87, 88, 118} Briefly a temperature transponder (14 × 2 mm, model IPTT-300, Bio Medic Data Systems, Inc., Seaford, DE, USA) was implanted in each mouse under brief isoflurane immobilization to facilitate remote body temperature recordings before and after each experiment using a handheld reader. After a one-week recovery, mice were tested once every 7–14 days (to avoid tolerance)^{122, 123, 129} for up to 6 – 8 treatments to complete dose-response and antagonist reversal experiments.

To start dose-response experiments, a short 5 min period of acclimation to the testing arenas was utilized, followed by baseline temperature recording. Test drugs were then administered, and mice were returned to the test chambers for a 30 min session to monitor locomotor activity using modified open field chambers with photobeam arrays (Coulbourn Instruments, Holliston, MA, USA) that facilitate video recordings (GoPro Hero 7 camera - 960p resolution at 120 frames per sec) of each mouse. After each experiment, videos are used to quantify HTR counts for each mouse via a commercially available software platform (Clever Sys Inc. Reston, VA, USA).¹³⁰ Antagonist studies mirrored dose-response testing, but with a 30 min pretreatment with either M100907 (0.01 mg/kg) or WAY100635 (3 mg/kg), or their respective vehicles, prior to 5-MeO-DMT analog administration.

For mouse experiments, mean total number of HTRs over the 30 min session, change in body temperature from pre to post session (Δ °C), and the total distance traveled (cm) were calculated for each drug dose. Values for dose-response studies were compared via one-way ANOVA with Dunnett's post hoc test ($p < 0.05$), comparing all doses to respective saline vehicle controls (0 mg/kg). For antagonist studies, groups were compared via one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Dose-response relationships for HTR and

in some cases, locomotor activities, were fit using bell-shaped curve fits, while temperature change data were fit using four-parameter non-linear regression curve fits. The rising phase of the HTR curve, the descending phase of the locomotor activity dose-response curves, and the whole curve for temperature change data were used to determine potency values from four parameter non-linear regression fits respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

HTR	Head twitch Response
5-MeO-DMT	5-methoxy- <i>N,N</i> -dimethyltryptamine
5-MeO-T	5-methoxy- <i>N,N</i> -tryptamine
5-MeO-NMT	5-methoxy- <i>N</i> -methyltryptamine
5-MeO-NET	5-methoxy- <i>N</i> -ethyltryptamine
5-MeO-MET	5-methoxy- <i>N</i> -methyl- <i>N</i> -ethyltryptamine
5-MeO-DET	5-methoxy- <i>N,N</i> -diethyltryptamine
5-MeO-NiPT	5-methoxy- <i>N</i> -isopropyltryptamine
5-MeO-MiPT	5-methoxy- <i>N</i> -methyl- <i>N</i> -isopropyltryptamine
5-MeO-DiPT	5-methoxy- <i>N,N</i> -diisopropyltryptamine
5-MeO-MALT	5-methoxy- <i>N</i> -methyl- <i>N</i> -allyltryptamine
5-MeO-DALT	5-methoxy- <i>N,N</i> -diallyltryptamine
5-MeO-2-Me-DMT	5-methoxy-2-methyl- <i>N,N</i> -dimethyltryptamine
5-MeO-2-Me-TMT	5-methoxy-2-methyl- <i>N,N,N</i> -trimethyltryptammonium
5-MeO-PyrT	5-methoxy- <i>N,N</i> -tetramethylenetryptamine
4F-5-MeO-PyrT	4-Fluoro-5-methoxy- <i>N,N</i> -tetramethylenetryptamine

5-HT	Serotonin
DOI	2,5-dimethoxy-4-iodoamphetamine
DOM	2,5-dimethoxy-4-methylamphetamine
LSD	Lysergic acid diethylamide
5-HT_{1A}	Serotonin 1A receptor
h5-HT_{1A}	Human 5-HT _{1A} receptor
m5-HT_{1A}	Mouse 5-HT _{1A} receptor
5-HT_{1B}	Serotonin 1B receptor
5-HT_{1D}	Serotonin 1D receptor
5-HT_{1E}	Serotonin 1E receptor
5-HT_{2A}	Serotonin 2A receptor
h5-HT_{2A}	Human 5-HT _{2A} receptor
m5-HT_{2A}	Mouse 5-HT _{2A} receptor
5-HT_{2B}	Serotonin 2B receptor
5-HT_{2C}	Serotonin 2C receptor
5-HT₃	Serotonin 3 receptor
5-HT_{5A}	Serotonin 5A receptor
5-HT₆	Serotonin 6 receptor
5-HT_{7A}	Serotonin 7A receptor
5-HT_{7B}	Serotonin 7B receptor
5-HT_{7D}	Serotonin 7D receptor
SERT	Serotonin transporter
alpha_{2A}	Alpha 2A receptors
alpha_{2B}	Alpha 2B receptors
alpha_{2C}	Alpha 2C receptors
D₂	Dopamine 2 receptors
D₃	Dopamine 3 receptors
D₄	Dopamine 4 receptors
MT₁	Melatonin receptor 1

MT₂	Melatonin receptor 2
MOR	Mu opioid receptor
KOR	Kappa opioid receptor
DOR	Delta opioid receptor

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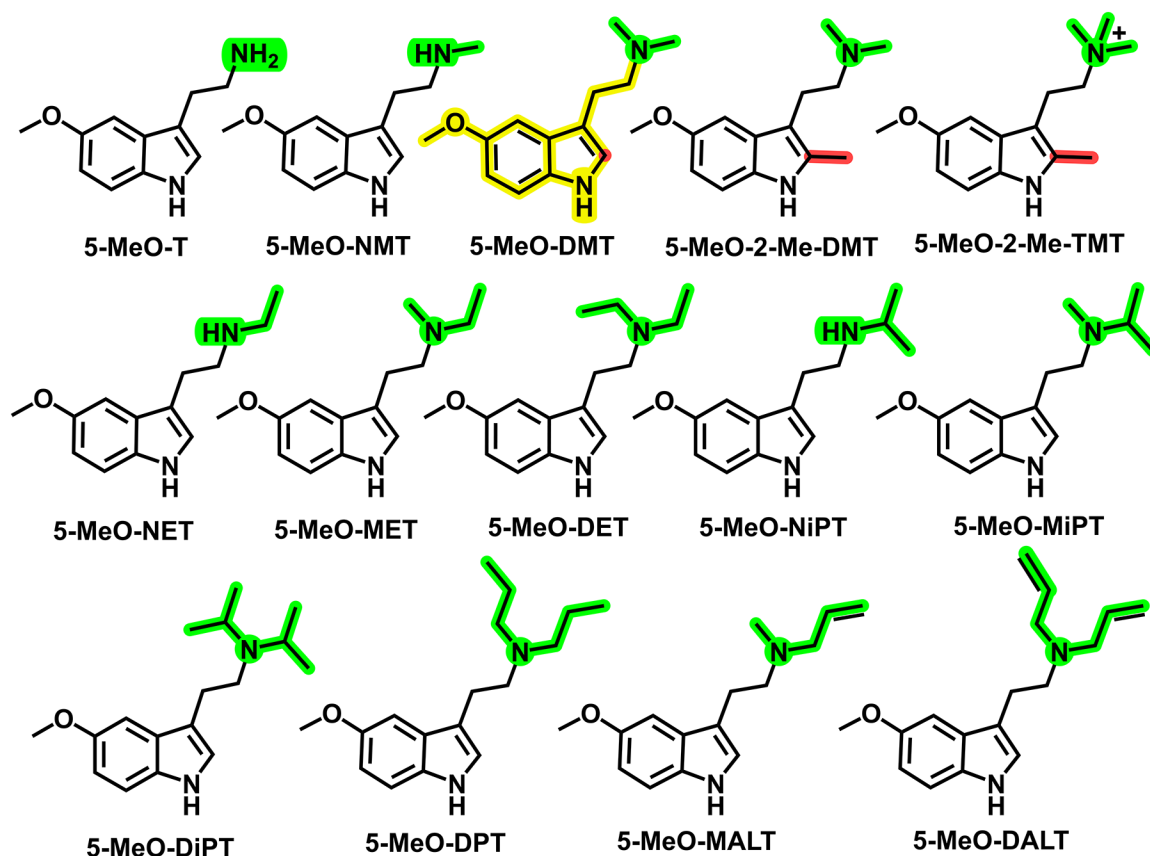
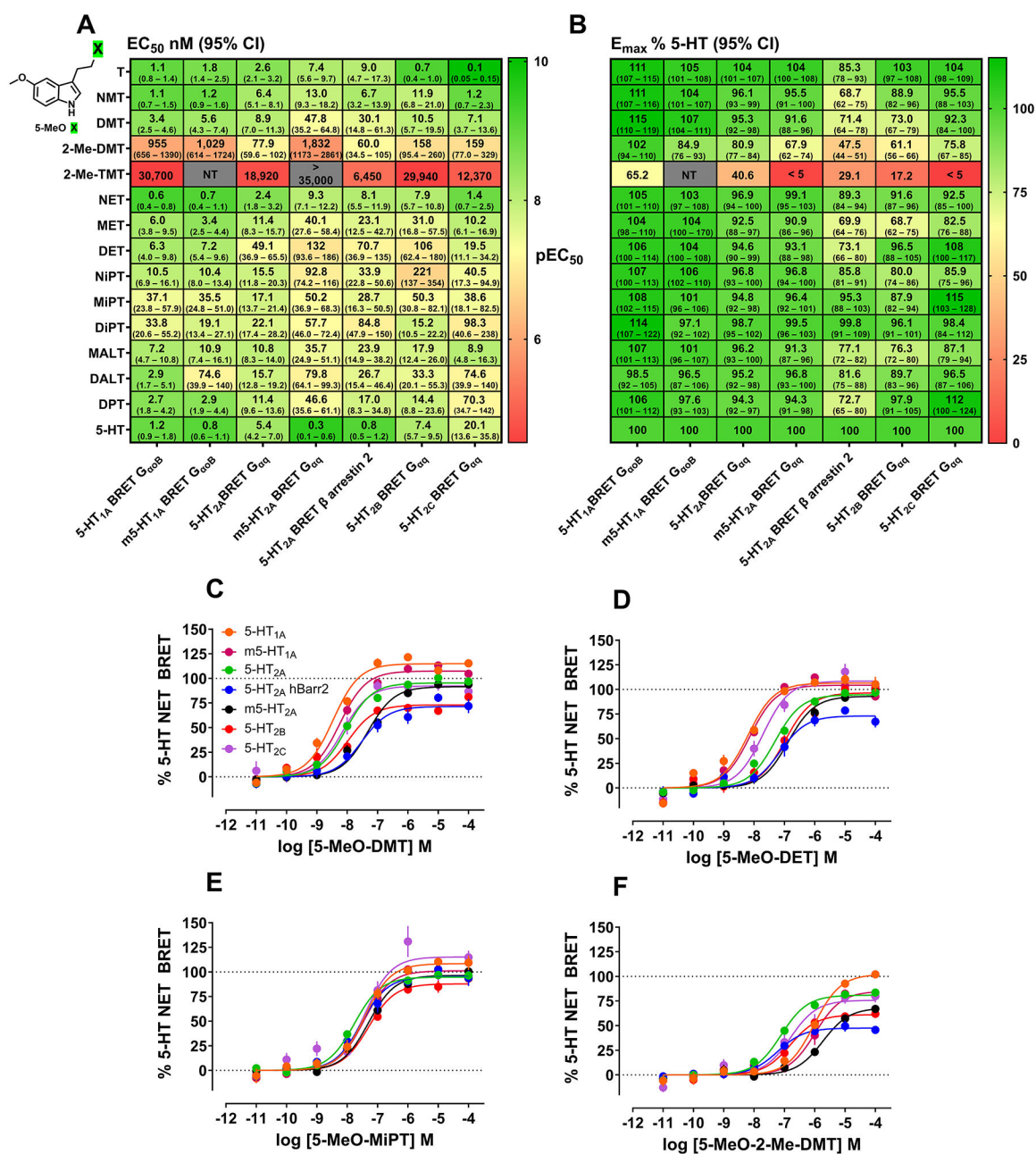


Figure 1.

Structures of 5-MeO-DMT and related analogs included in the study. The parent compound 5-MeO-DMT is highlighted yellow with the *N*-alkyl/allyl substitutions shown in green and the 2-position ring substitutions in red. The freebase structures are shown for all compounds except the quaternary ammonium with salt forms used listed in Methods.

**Figure 2.**

Potencies (EC₅₀) and maximum efficacies (E_{max}) of 5-MeO-DMT analogs in BRET functional assays measuring G protein dissociation at 5-HT_{1A} (human and mouse), 5-HT_{2A} (human and mouse), 5-HT_{2B}, and 5-HT_{2C} relative to the reference agonist 5-HT. (**A - B**) Data are expressed as means, with 95% confidence intervals noted in parentheses. Data represent 3 – 4 experiments with triplicate determinations. Concentration-effect curves are shown in panels **C – F** with additional plots shown in Figure S3. Values in each block represent EC₅₀ (**A**) or E_{max} values (**B**), while color mapping represents pEC₅₀ (**A**) or E_{max} (% reference, **B**) according to the scale shown.

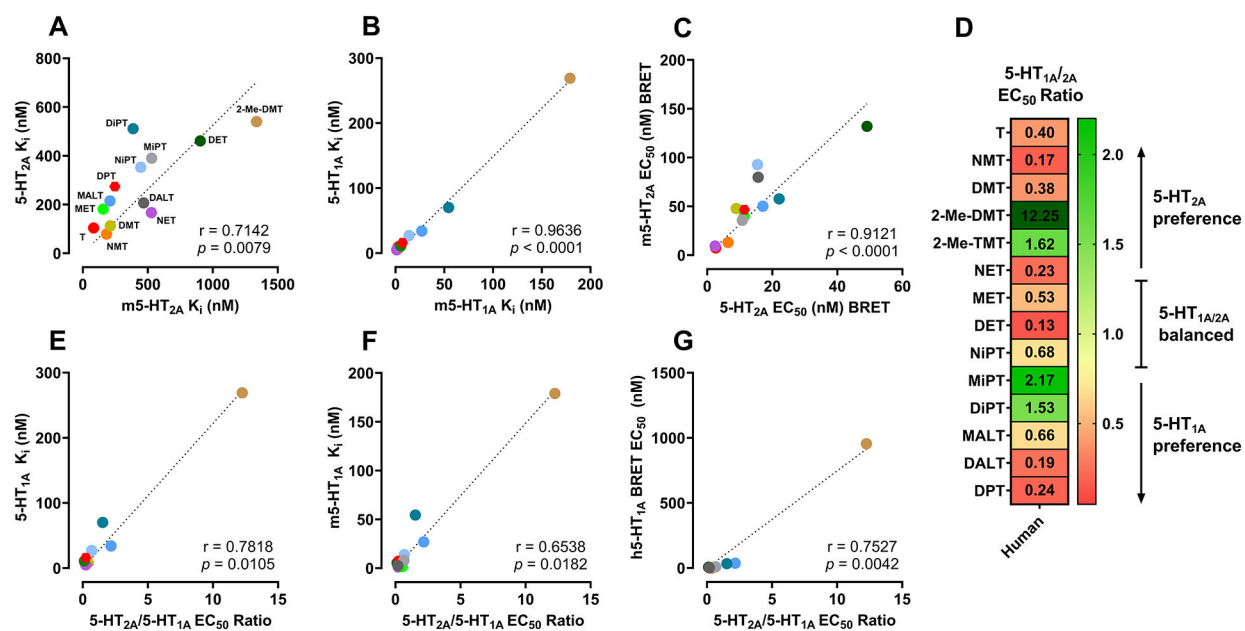


Figure 3. Relationships between *in vitro* affinities, functional activities, and the 5-HT_{1A}/5-HT_{2A} potency (EC₅₀) ratios across all tested 5-MeO-DMT analogs.

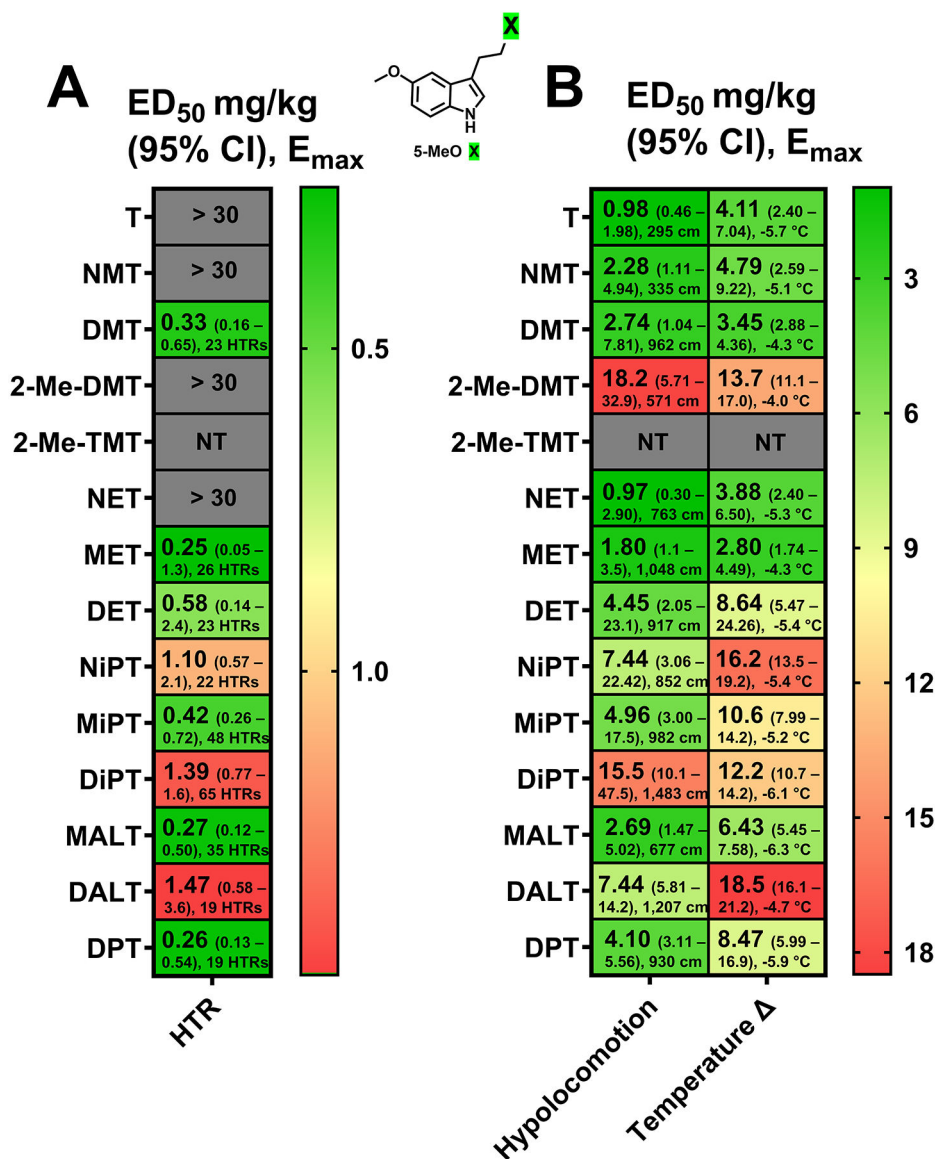


Figure 4. Potencies (ED₅₀) and maximal effects (E_{max}) of 5-MeO-DMT analogs for inducing the HTR (A) as well as hypolocomotion and hypothermia (B). Data are expressed with 95% confidence intervals listed below in parentheses and maximum effect values for each endpoint.

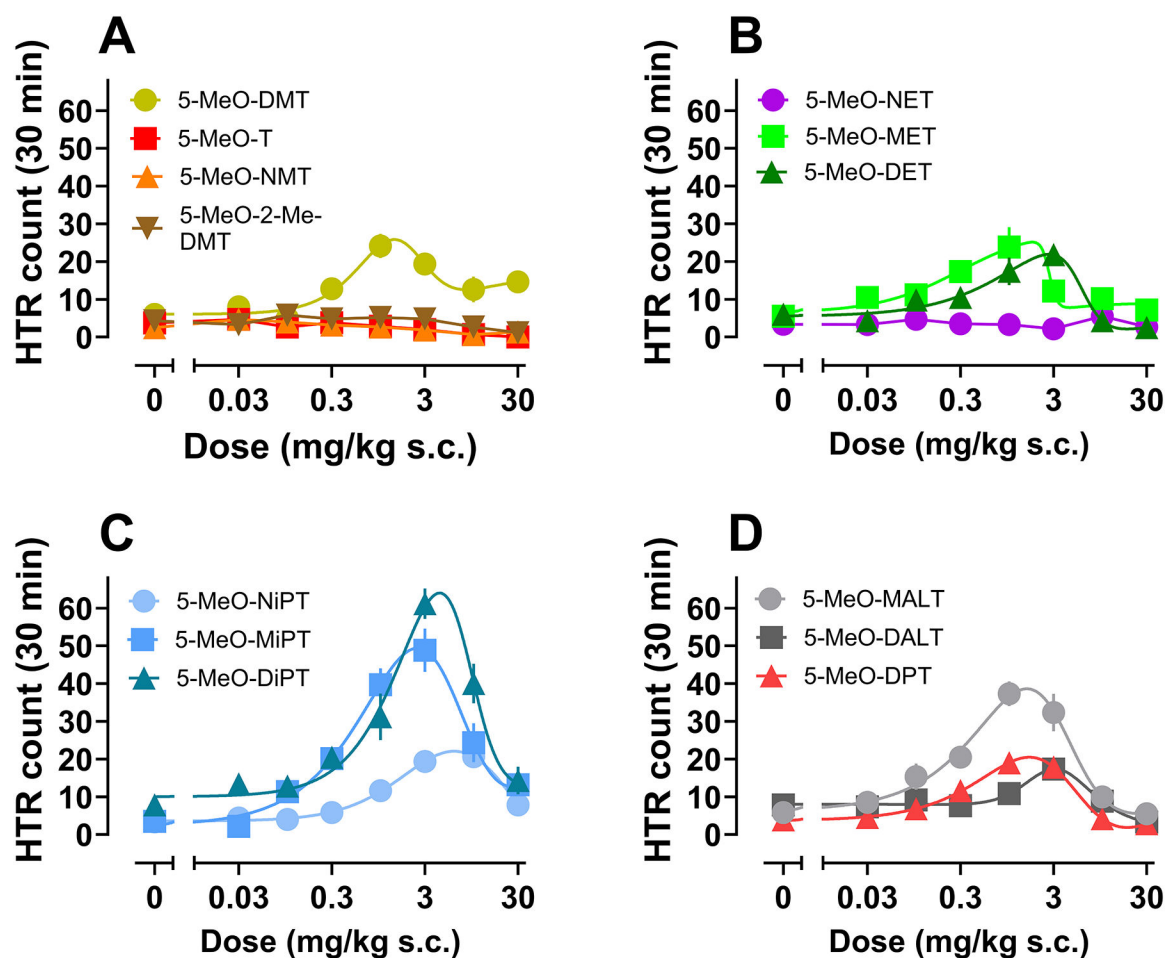


Figure 5.
Dose-response curves for HTRs induced by 5-MeO-DMT analogs. Data are mean $\pm$ SEM.
Further information on statistical comparisons for each drug vs. saline vehicle controls ("0")
and descriptive statistics can be found in Table S3.

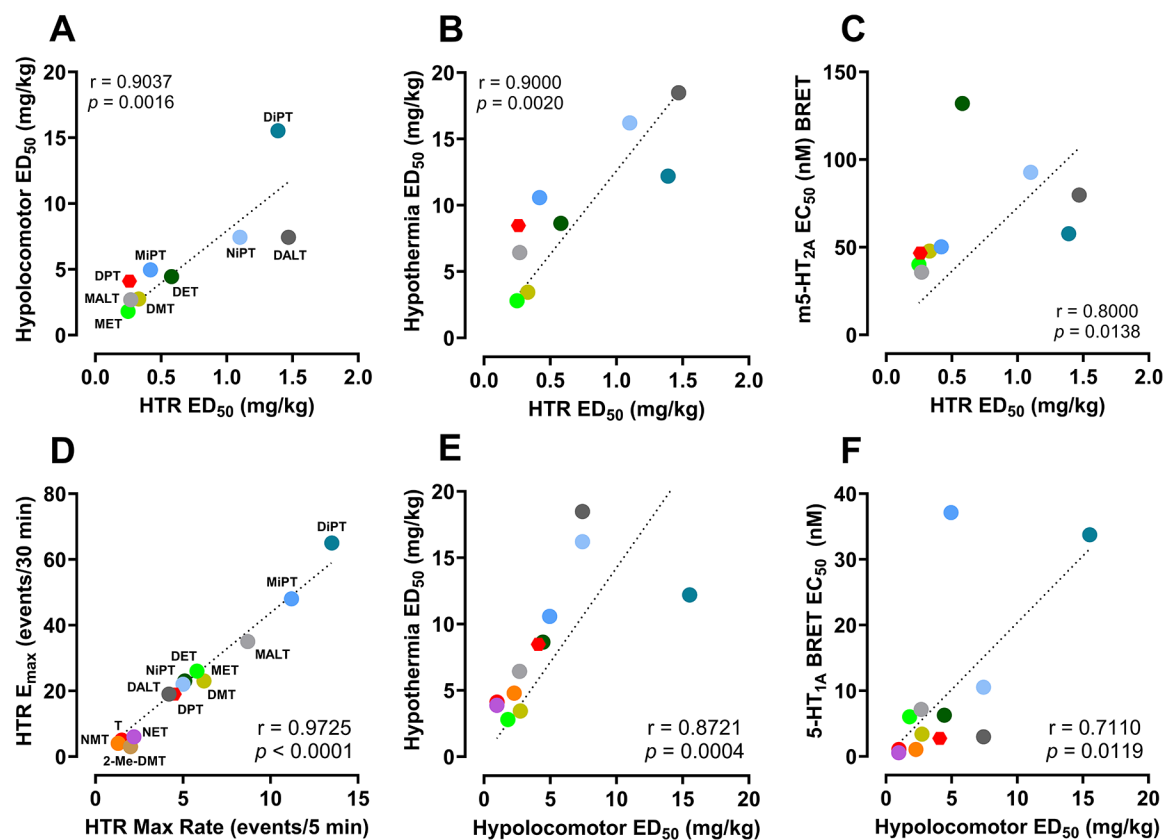


Figure 6.
Relationships between in vitro and in vivo pharmacological parameters.

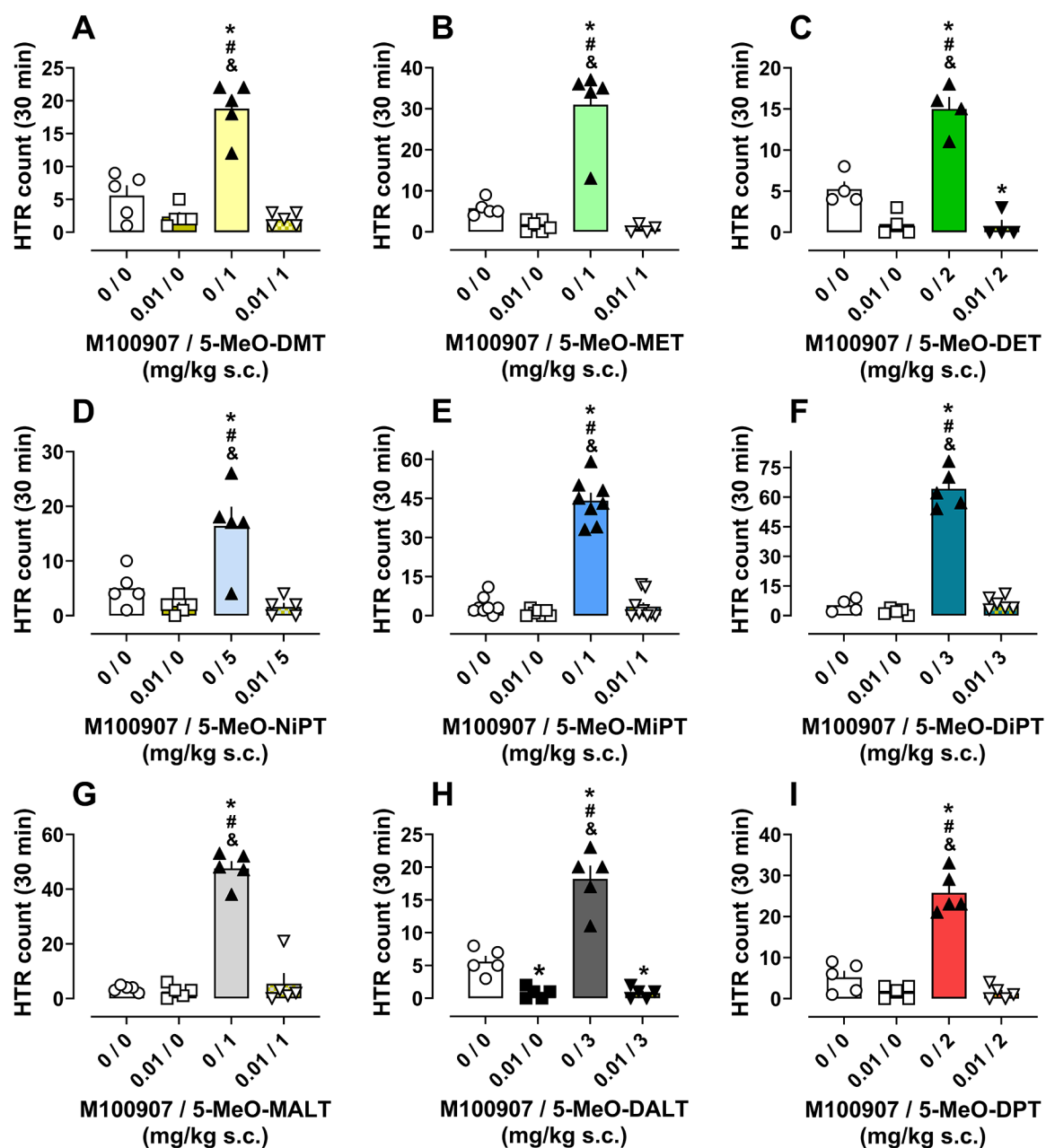
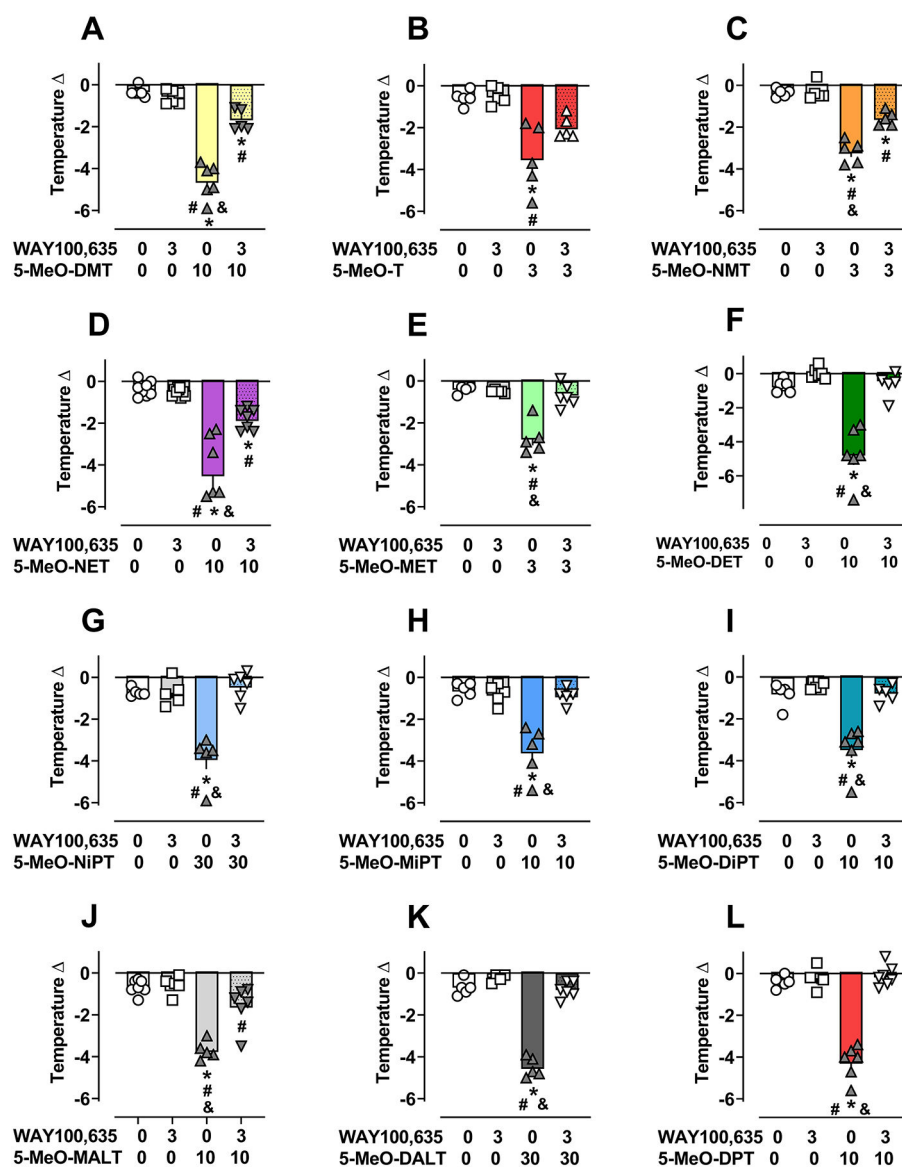


Figure 7.

Effects of 5-HT_{2A} antagonist pretreatment on HTR induced by 5-MeO-DMT analogs. Data are mean $\pm$ SEM. Comparisons were made via one-way ANOVA with Tukey's post hoc test ($p < 0.05$) as follows: * vs. 0 / 0, # vs. 0.01 / 0, & vs. 0.01 / 5-MeO compound. Other details regarding statistical comparisons including descriptive statistics can be found in Materials and Methods, Table S4, and Table S5.

**Figure 8.**

Effects of 5-HT_{1A} antagonist pretreatment on the hypothermia induced by 5-MeO-DMT analogs. Data are mean $\pm$ SEM. Comparisons were made via one-way ANOVA with Tukey's post hoc test ($p < 0.05$) as follows: * vs. 0 / 0, # vs. 3 / 0, & vs. 3 / 5-MeO compound. More details regarding descriptive statistics and statistical comparisons can be found in Materials and Methods, Table S6, and Table S7.

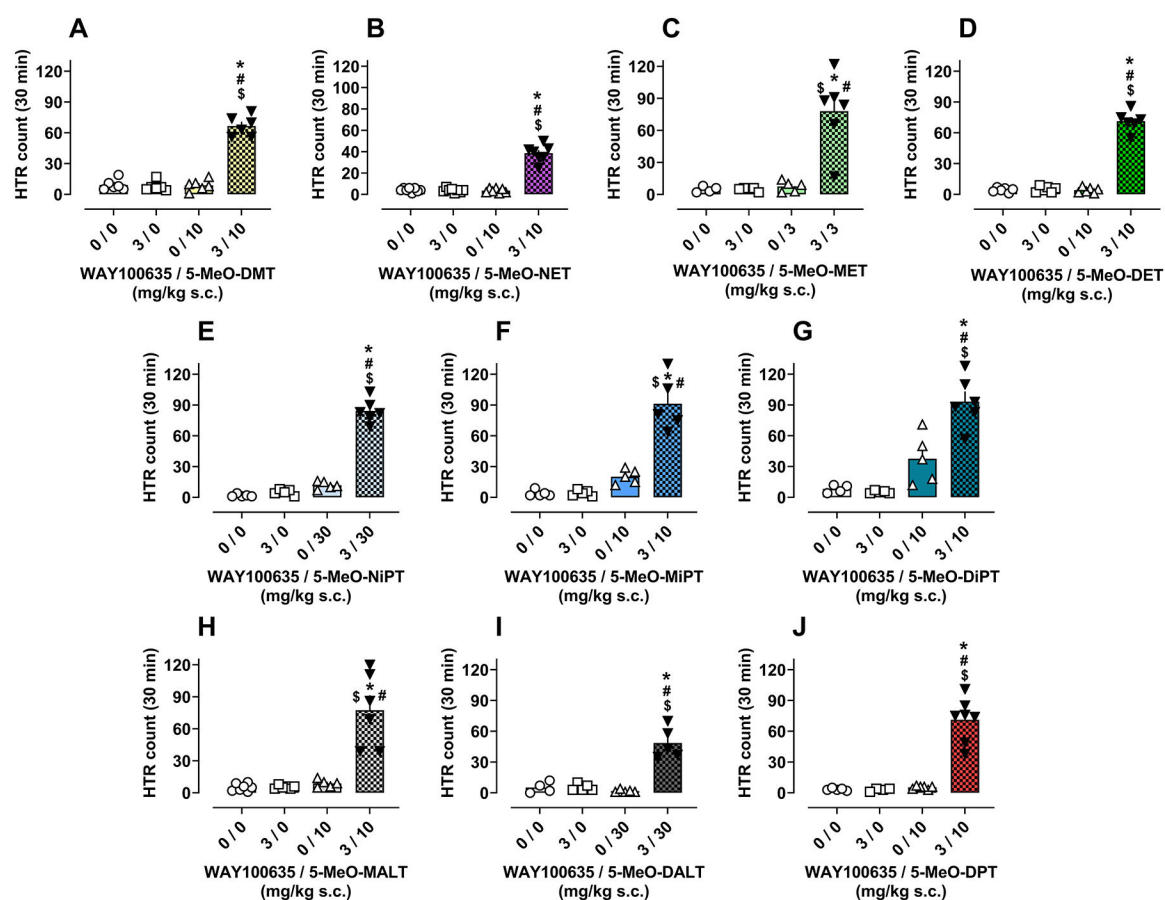


Figure 9.

Effects of 5-HT_{1A} antagonist pretreatment on HTR induced by 5-MeO-DMT analogs. Data are mean $\pm$ SEM. Comparisons were made via one-way ANOVA with Tukey's post hoc test ($p < 0.05$) as follows: * vs. 0 / 0, # vs. 3 / 0, \$ vs. 0 / 5-MeO compound. Further details regarding statistical comparisons and descriptive statistics can be found in Materials and Methods, Table S6 – S7.

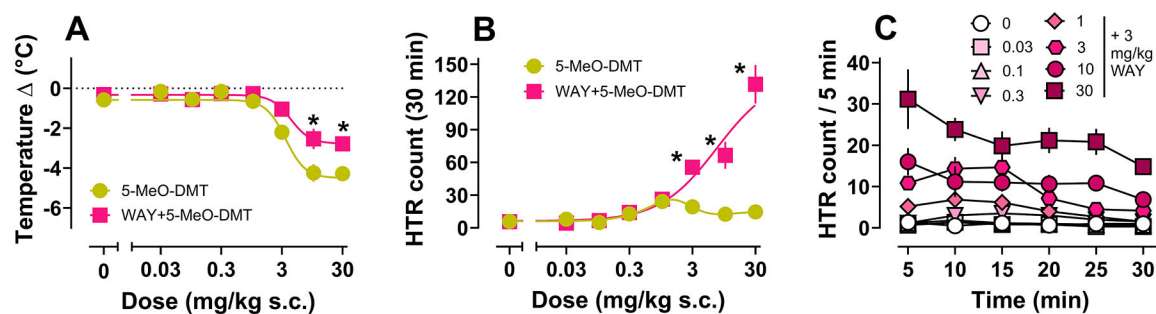


Figure 10.

Effects of 5-HT_{1A} antagonist pretreatment with WAY100635 (WAY) on the temperature change and HTR dose-response curves of 5-MeO-DMT. Effects of 5-MeO-DMT alone are redrawn from Figure 5A and Figure S18 for visual comparison. Data are mean $\pm$ SEM with comparisons for WAY+5-MeO-DMT made via one-way ANOVA with Dunnett's post hoc test (* $p < 0.05$). More details regarding statistical comparisons and descriptive statistics can be found in Materials and Methods and Table S8.