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Article

# Receptor Binding Profiles for Tryptamine Psychedelics and Effects of 4-Propionoxy-*N,N*-dimethyltryptamine in Mice

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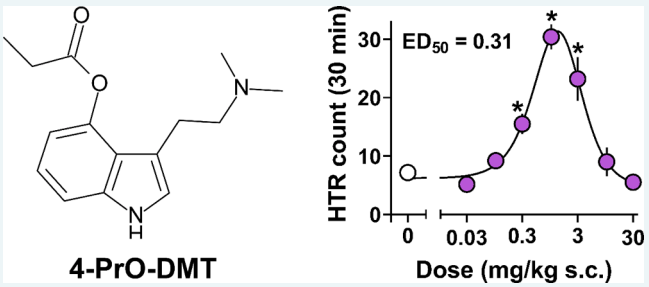
**ABSTRACT:** Analogues of 4-phosphoryloxy-*N,N*-dimethyltryptamine (psilocybin) are being sold on recreational drug markets and developed as potential medications for psychedelic-assisted therapies. Many of these tryptamine-based psilocybin analogues produce psychedelic-like effects in rodents and humans primarily by agonist activity at serotonin 2A receptors (5-$HT_{2A}$). However, the comprehensive pharmacological target profiles for these compounds compared to psilocybin and its active metabolite 4-hydroxy-*N,N*-dimethyltryptamine (psilocin) are unknown. The present study determined the receptor binding profiles of various tryptamine-based psychedelics structurally related to psilocybin across a broad range of potential targets. Specifically, we examined tryptamine psychedelics with different 4-position (hydroxy, acetoxy, propionoxy) and *N,N*-dialkyl (dimethyl, methyl-ethyl, diethyl, methyl-propyl, ethyl-propyl, diisopropyl, methyl-allyl, diallyl) substitutions. Further, the psilocybin analogue 4-propionoxy-*N,N*-dimethyltryptamine (4-PrO-DMT) was administered to mice in experiments measuring head twitch response (HTR), locomotor activity, and body temperature. Overall, the present pharmacological profile screening data show that the tryptamine psychedelics target multiple serotonin receptors, including serotonin 1A receptors (5-$HT_{1A}$). 4-Acetoxy and 4-propionoxy analogues of 4-hydroxy compounds displayed somewhat weaker binding affinities but similar target profiles across 5-HT receptors and other identified targets. Additionally, differential binding screen profiles were observed with *N,N*-dialkyl position variations across several non-5-HT receptor targets (i.e., alpha receptors, dopamine receptors, histamine receptors, and serotonin transporters), which could impact *in vivo* pharmacological effects of the compounds. In mouse experiments, 4-PrO-DMT displayed dose-related psilocybin-like effects to produce 5-$HT_{2A}$-mediated HTR (0.3−3 mg/kg s.c.) as well as 5-$HT_{1A}$-mediated hypothermia and hypolocomotion (3−30 mg/kg s.c.). Lastly, our data support a growing body of evidence that the 5-$HT_{2A}$-mediated HTR induced by tryptamine psychedelics is attenuated by 5-$HT_{1A}$ receptor agonist activity at high doses in mice.




**KEYWORDS:** *tryptamines, psychedelics, psilocybin analogues, receptor binding, head twitch response*

Classic psychedelics, such as 4-phosphoryloxy-*N,N*-dimethyltryptamine (4-PO-DMT or psilocybin), have traditionally been used by various indigenous groups as religious sacraments to facilitate mystical or spiritual experiences.[1,2] Psychedelics are also used recreationally in various non-medical contexts, which can pose public health risks.[3−5] More recently, psilocybin given in conjunction with psychotherapy is being investigated for the treatment of depression, obsessive compulsive disorder, chronic pain, substance use disorders, and psychological distress in terminally ill cancer patients.[2,6−9]

Aside from classic tryptamine-based psychedelics like psilocybin, many new psychoactive substances (NPS) from this drug class have emerged on recreational drug markets, and some of these compounds are used in scientific research.[2,10−14] Many of these psilocybin analogues have substitutions at the 4-position (e.g., acetoxy, hydroxy) and various symmetrical or asymmetrical *N,N*-dialkyl groups (e.g., diethyl or methyl-ethyl).[14,15] The psychedelic-like effects in rodent models and *in vitro* functional activities at serotonin 2 (5-$HT_2$) receptors have been reported for a large number of tryptamines.[15−17] However, the comprehensive pharmacological target profiles for many of the newer synthetic tryptamines are unknown, especially how they compare to the natural product psilocybin and its active metabolite 4-hydroxy-*N,N*-dimethyltryptamine (4-HO-DMT or psilocin).

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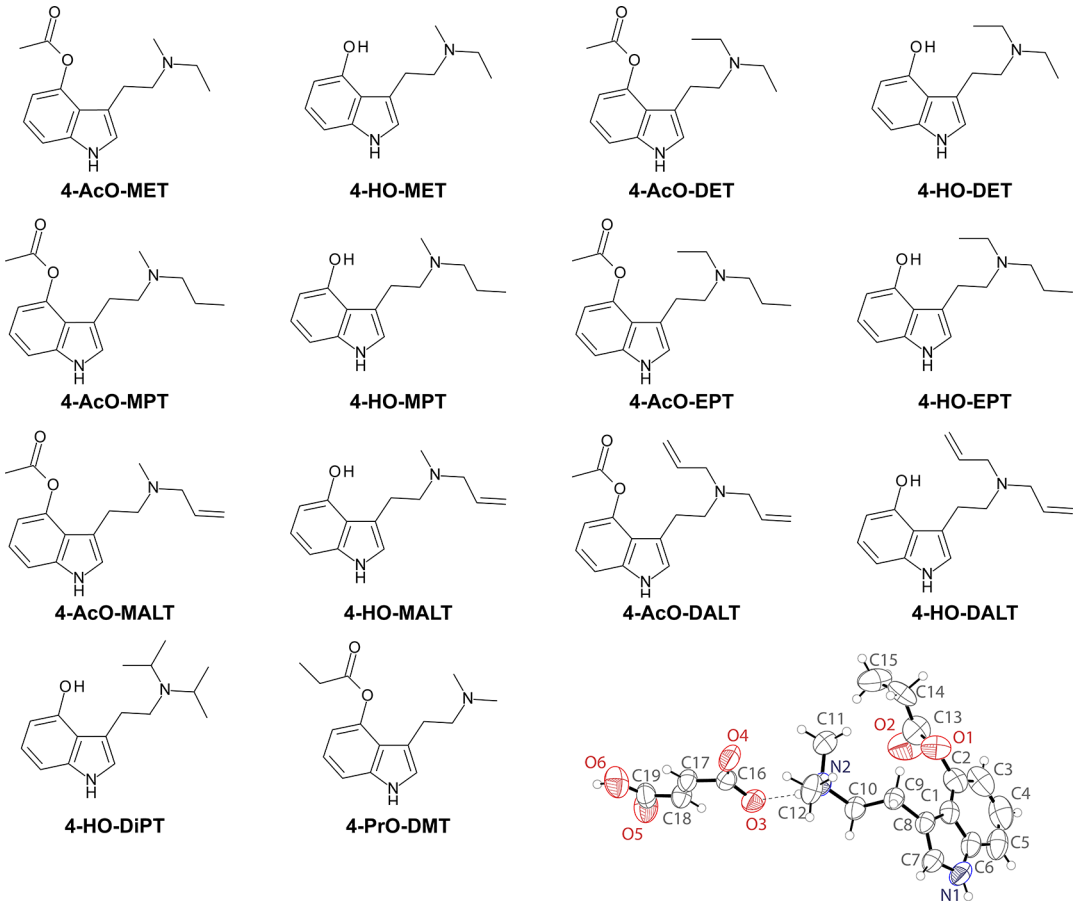


**Figure 1.** Chemical structures of tryptamine psychedelic NPS compounds included in the study along with the crystal structure of 4-PrO-DMT hydrofumarate. The freebase forms of the compounds are shown, though the samples studied were in a protonated state as either fumarate or hydrofumarate salts as outlined in the Methods.

4-Propionoxy-*N*,*N*-dimethyltryptamine (4-PrO-DMT) is an example of an obscure NPS sold as a psychedelic on recreational drug markets with little information available about its pharmacological activity.[18] Similar to 4-acetoxy-*N*,*N*-dimethyltryptamine (4-AcO-DMT or psilacetin) and psilocybin, it has been postulated that 4-PrO-DMT is a prodrug for psilocin. Anecdotal reports from online sources indicate that consumption of purported 4-PrO-DMT samples induces subjective experiences that are similar to those produced by psilocin, psilocybin, and psilacetin.[19,20]

In this paper, we report the comprehensive target binding profiles and 5-$HT_2$ receptor functional activities for several tryptamine psychedelic NPS with variations at the 4-position (hydroxy, acetoxy, propionoxy) and the *N*,*N*-dialkyl group (dimethyl, methyl-ethyl, diethyl, methyl-propyl, ethyl-propyl, diisopropyl, methyl-allyl, diallyl) (Figure 1). Further, 4-PrO-DMT was evaluated in mouse studies measuring acute effects of the drug on head twitch response (HTR), locomotor activity, and body temperature. Together the results from these experiments demonstrate structure−activity relationships (SARs) for *in vitro* pharmacological profiles and inhibition constants ($K_i$) at identified 5-HT receptors as well as non-5-HT targets of tryptamine psychedelics. Furthermore, the mouse studies demonstrate that 4-PrO-DMT is a psilocybin-like compound comparable to other tryptamine psychedelics.

## RESULTS AND DISCUSSION

**Target Profiles of Tryptamine Psychedelics.** Compounds were sent for comprehensive target profiling across 50 receptors and other targets of interest.[21] The first stage is a "primary competition binding screen" where 10 $\mu$M concentrations of each compound are assessed in radioligand binding assays to determine potential targets. "Hits" for various targets were defined by >50% average inhibition of radioligand binding from a single experiment with quadruplicate determinations. The identified targets from the 10 $\mu$M screen for each compound are shown in Table 1. Across all compounds, the predominant targets identified were 5-HT receptors. Non-5-HT targets included kappa opioid receptors (KOR), histamine receptors ($H_1$ and $H_2$), alpha receptors ($alpha_{2A}$, $alpha_{2B}$, $alpha_{2C}$), dopamine receptors ($D_2$, $D_3$, $D_4$, $D_5$), the dopamine transporter (DAT), the serotonin transporter (SERT), muscarinic receptors ($M_2$, $M_3$, $M_4$), sigma receptors (sigma 1 and sigma 2), and NMDA glutamate receptors (NR2B subunit). The primary screening data also revealed potential differences between compounds regarding the number of targets identified. For instance, 4-hydroxy-*N*,*N*-diisopropyltryptamine (4-HO-DiPT) inhibited binding to fewer 5-HT receptors compared to the other compounds assessed.

Next, full concentration−effect curves for inhibition of radioligand binding for each compound were generated to determine the affinities ($K_i$) at each target identified in primary


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**Table 1. List of Target and Receptor Hits for Tryptamine Psychedelic NPS in Primary Competition Binding Screens[a]**

| Compound | Targets identified in competition binding screen at 10 $\mu$M |
|---|---|
| 4-PrO-DMT | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $H_1$, KOR, NR2B, Sigma 2 |
| 4-HO-MET | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $H_1$, SERT, NR2B, $Alpha_{2A}$ |
| 4-AcO-MET | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_6$, $5HT_{7a}$, $H_1$, $D_4$, $Alpha_{2B}$, Sigma 1 |
| 4-HO-DET | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $H_1$, $H_2$, $M_3$, SERT, NR2B, Sigma 2 |
| 4-AcO-DET | $5HT_{1B,D,e}$, $5HT_{2B,C}$, $5HT_6$, $5HT_{7a}$, $Alpha_{2A,B}$, NR2B, $D_2$ |
| 4-HO-MPT | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $H_1$, $D_3$, $Alpha_{2A,B}$, NR2B, SERT, Sigma 1 and 2 |
| 4-AcO-MPT | $5HT_{1B,D}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $H_1$, $Alpha_{2A,B,C}$, Sigma 1 |
| 4-HO-EPT | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $H_1$, $D_2$, $Alpha_{2A}$, NR2B, SERT, Sigma 1 and 2 |
| 4-AcO-EPT | $5HT_{1B,D,e}$, $5HT_{2A,B,C}$, $5HT_6$, $5HT_{7a}$, $H_1$, $D_3$, $Alpha_{2A,B,C}$, NR2B, Sigma 2 |
| 4-HO-DiPT | $5HT_{1D}$, $5HT_{2A,B}$, $Alpha_{2A,B}$, DAT, $M_4$, SERT, Sigma 1 and 2 |
| 4-HO-MALT | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $Alpha_{2A}$, $D_5$, $H_{1,2}$, SERT, Sigma 1 and 2 |
| 4-AcO-MALT | $5HT_{1A,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $Alpha_{2A,B}$, $H_1$, Sigma 1 |
| 4-HO-DALT | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_{5A}$, $5HT_6$, $5HT_{7a}$, $Alpha_{2A,B,C}$, $D_{2,3,4}$, $H_1$, $M_4$, SERT, Sigma 1 and 2 |
| 4-AcO-DALT | $5HT_{1A,B,D,e}$, $5HT_{2A,B,C}$, $5HT_6$, $5HT_{7a}$, $Alpha_{2A,B,C}$, $D_{2,3}$, $H_1$, $M_2$, Sigma 2 |

[a]Data represent receptors and targets where compounds exhibited >50% average inhibition at the 10 $\mu$M test concentration.

screens.[21] Inhibition constants for 5-HT receptors assessed are shown in Table 2, while inhibition constants for the most common non-5-HT receptor targets are listed in Table 3 and Table 4. Many of the compounds displayed affinity for all 5-HT receptors assessed, similar to the findings for psilocin and psilacetin.[22−24] In general, the receptor binding results show that 4-hydroxy compounds have higher affinity across all 5-HT receptor targets when compared to 4-acetoxy analogues with the same *N*-alkyl groups. We previously demonstrated that 4-hydroxy and 4-acetoxy analogues of psilocybin display higher affinity for more 5-HT receptor subtypes when compared to their 4-phosphoryloxy counterparts.[23] Others have found similar potency trends for agonist activity at 5-$HT_2$ receptors and HTR responses in mice for 4-hydroxy vs 4-acetoxy compounds with the same *N,N*-dialkyl substitutions.[15] The 4-propionoxy analogue, 4-PrO-DMT, also displayed inhibition constants across 5-HT receptors more comparable to psilocin and psilacetin as opposed to psilocybin. These findings suggests that 4-PrO-DMT could be an active prodrug for psilocin, as has been suggested for psilacetin, depending on the speed of enzymatic hydrolysis *in vivo*.[15]

Variations of the *N,N*-dialkyl constituents of the tested tryptamines had modest effects on the inhibition constants and receptor binding profiles across 5-HT receptors. Most compounds displayed comparable inhibition constants across 5-HT receptor subtypes in the ∼100−1,000 nM range, that were within 10-fold of one another. One exception was the finding that 4-PrO-DMT, 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-HO-MET), and 4-hydroxy-*N*-methyl-*N*-propyltryptamine (4-HO-MPT) had higher inhibition constants at serotonin 6 receptors (5-$HT_6$), which were more than 10-fold greater than the inhibition constants of 4-acetoxy-*N,N*-diethyltryptamine (4-AcO-DET) and 4-acetoxy-*N*-ethyl-*N*-propyltryptamine (4-AcO-EPT) at this site.

Additionally, inhibition constants at serotonin 7a (5-$HT_{7a}$) for 4-PrO-DMT, 4-HO-MET, and 4-HO-MPT were more than 10-fold greater relative to the affinity of 4-AcO-DET. The present binding results are consistent with known differences in agonist activities (i.e., potency and efficacy) at 5-$HT_2$ receptors and behavioral effects for many of the tested tryptamine analogues in mouse HTR studies.[15] The present receptor binding data for 4-HO-DiPT also agree with the

**Table 2. Inhibition Constants of Tryptamine Psychedelic NPS in Competition Binding Assays for Human 5-HT Receptors[a]**

| | $K_i$ (nM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| drug | 5-$HT_{1A}$ | 5-$HT_{1B}$ | 5-$HT_{1D}$ | 5-$ht_{1e}$ | 5-$HT_{2A}$ | 5-$HT_{2B}$ | 5-$HT_{2C}$ | 5-$HT_{5A}$ | 5-$HT_6$ | 5-$HT_{7a}$ |
| 4-PrO-DMT | 396 | 2,410 | 274 | 229 | 336 | 17 | 228 | 325 | 54 | 73 |
| 4-HO-MET | 135 | 331 | 197 | 161 | 177 | 12 | 164 | 304 | 70 | 60 |
| 4-AcO-MET | 950 | 960 | 667 | 500 | 514 | 17 | 370 | - | 345 | 310 |
| 4-HO-DET | 414 | 2,242 | 585 | 568 | 400 | 73 | 436 | 1,429 | 230 | 826 |
| 4-AcO-DET | - | 3,817 | 445 | 1,162 | - | 53 | 875 | - | 860 | 1,680 |
| 4-HO-MPT | 106 | 224 | 170 | 246 | 114 | 8 | 150 | 664 | 48 | 99 |
| 4-AcO-MPT | - | 9,108 | 1,900 | - | 830 | 22 | 694 | 1,805 | 383 | 220 |
| 4-HO-EPT | 163 | 1,097 | 644 | 591 | 546 | 62 | 1,272 | 1,576 | 284 | 438 |
| 4-AcO-EPT | - | 9,259 | 1,674 | 2,225 | 2,459 | 43 | 967 | - | 1,787 | 321 |
| 4-HO-DiPT | - | - | 1,860 | - | 922 | 85 | - | - | - | - |
| 4-HO-MALT | 402 | 1,500 | 159 | 165 | 357 | 20 | 392 | 492 | 195 | 179 |
| 4-AcO-MALT | 1,141 | - | 699 | 775 | 1,006 | 9 | 1,556 | 1,246 | 241 | 582 |
| 4-HO-DALT | 131 | 978 | 432 | 367 | 452 | 63 | 1,550 | 2,966 | 578 | 454 |
| 4-AcO-DALT | 582 | 2,689 | 2,099 | 2,116 | 958 | 137 | 824 | - | 479 | 406 |

[a]Radioligands used, reference control compound used, and control $K_i$ values for each 5-HT receptor were as follow: 5-$HT_{1A}$ = [$^3$H]WAY100635 vs 8-HO-DPAT ($K_i$ = 0.6−0.7 nM), 5-$HT_{1B}$ = [$^3$H]GR125743 vs ergotamine tartrate ($K_i$ = 4−12 nM), 5-$HT_{1D}$ = [$^3$H]GR125743 vs ergotamine tartrate ($K_i$ = 3−6 nM), 5-$ht_{1e}$ = [$^3$H]5-HT vs 5-HT ($K_i$ = 5−15 nM), 5-$HT_{2A}$ = [$^3$H]ketanserin vs clozapine ($K_i$ = 5−8 nM), 5-$HT_{2B}$ = [$^3$H]LSD vs SB206553 ($K_i$ = 7−12 nM), 5-$HT_{2C}$ = [$^3$H]mesulergine vs ritanserin ($K_i$ = 1−3 nM), 5-$HT_{5A}$ = [$^3$H]LSD vs ergotamine tartrate ($K_i$ = 12−37 nM), 5-$HT_6$ = [$^3$H]LSD vs clozapine ($K_i$ = 7−23 nM), 5-$HT_{7a}$ = [$^3$H]LSD vs clozapine ($K_i$ = 12−36 nM). Control $K_i$ values represent the range of inhibition constants across 3−4 experiments with triplicate determinations. Dash indicates <50% inhibition in the primary radioligand binding screen.



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**Table 3. Inhibition Constants of Tryptamine Psychedelic NPS in Competition Binding Assays for Non-5-HT Receptor Targets[a]**

| | $K_i$ (nM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Drug | $H_1$ | KOR | NR2B | Sigma 1 | Sigma 2 | $Alpha_{2A}$ | $Alpha_{2B}$ | $Alpha_{2C}$ | SERT |
| 4-PrO-DMT | 1,481 | 4,745 | 6,250 | - | 1,349 | - | - | - | - |
| 4-HO-MET | 483 | - | >10,000 | - | - | 1,666 | - | - | 1,830 |
| 4-AcO-MET | 1,720 | - | - | 7,077 | - | - | 1,681 | - | - |
| 4-HO-DET | 1,079 | - | 8,720 | - | 3,026 | - | - | - | 1,800 |
| 4-AcO-DET | - | - | 2,936 | - | - | 1,965 | 1,095 | - | - |
| 4-HO-MPT | 92 | - | 3,658 | 891 | 1,166 | 3,625 | 1,844 | - | 910 |
| 4-AcO-MPT | 493 | - | - | 1,512 | - | 1,300 | 453 | 1,447 | - |
| 4-HO-EPT | 406 | - | 5,947 | 1,400 | 1,773 | 2,073 | - | - | 1,257 |
| 4-AcO-EPT | 1,024 | - | 8,111 | - | 3,497 | 1,135 | 274 | 1,329 | - |
| 4-HO-DiPT | - | - | - | 1,063 | 2,215 | - | - | - | 816 |
| 4-HO-MALT | 815 | - | NT | 1,413 | 2,525 | 535 | - | - | 355 |
| 4-AcO-MALT | 2,603 | - | NT | 3,471 | - | 387 | 1,169 | - | - |
| 4-HO-DALT | 355 | - | - | 1,846 | 1,770 | 2,670 | 2,084 | 2,280 | 657 |
| 4-AcO-DALT | 874 | - | - | - | 4,190 | 482 | 214 | 803 | - |

[a]Radioligands, reference control compound, and control $K_i$ values for each radiolabeled binding site were as follow: $H_1$ = [$^3$H]pyrilamine vs chlorpheniramine maleate ($K_i$ = 2−6 nM), KOR = [$^3$H]U69593 (2007-07-27) vs Salvinorin A ($K_i$ = 2−6 nM), NR2B = [$^3$H]ifenprodil vs ifenprodil ($K_i$ = 2−7 nM), sigma 1 = [$^3$H]pentazocine vs haloperidol ($K_i$ = 12−32 nM), sigma 2 = [$^3$H]DTG vs haloperidol ($K_i$ = 36−61 nM), $alpha_{2A}$ = [$^3$H]rauwolscin vs oxymetazoline HCl ($K_i$ = 5−14 nM), $alpha_{2B}$ = [$^3$H]rauwolscin vs yohimbine ($K_i$ = 6−10 nM), $alpha_{2C}$ = [$^3$H]rauwolscin vs oxymetazoline HCl ($K_i$ = 23−74 nM), SERT = [$^3$H]citalopram vs amitriptyline ($K_i$ = 12−43 nM). Control $K_i$ values represent the range of inhibition constants across 2−3 experiments run in triplicate. NT = not tested. Dash indicates <50% inhibition in the primary radioligand binding screen.

**Table 4. Inhibition Constants of Tryptamine Psychedelic NPS in Competition Binding Assays for Non-5-HT Receptor Targets (Continued)[a]**

| | $K_i$ (nM) | | | | | | |
|---|---|---|---|---|---|---|---|
| Drug | DAT | $M_4$ | $H_2$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ |
| 4-PrO-DMT | - | - | - | - | - | - | - |
| 4-HO-MET | - | - | - | - | - | - | - |
| 4-AcO-MET | - | - | - | - | - | 1,371 | - |
| 4-HO-DET | - | - | 9,984 | - | - | - | - |
| 4-AcO-DET | - | - | - | 1,534 | - | - | - |
| 4-HO-MPT | - | - | - | - | 921 | - | - |
| 4-AcO-MPT | - | - | - | - | - | - | - |
| 4-HO-EPT | - | - | - | 3,010 | 985 | - | - |
| 4-AcO-EPT | - | - | - | - | - | - | - |
| 4-HO-DiPT | >10,000 | 1,725 | - | - | - | - | - |
| 4-HO-MALT | - | - | 1,448 | - | - | - | >10,000 |
| 4-AcO-MALT | - | - | - | - | - | - | - |
| 4-HO-DALT | - | 5,280 | - | >10,000 | 578 | 2,256 | - |
| 4-AcO-DALT | - | - | - | 4,522 | 1,907 | - | - |

[a]Radioligands, reference control compound, and control $K_i$ values for each radiolabeled binding site were: DAT = [$^3$H]WIN35428 vs GBR12909 ($K_i$ = 14 nM), $M_4$ = [$^3$H]QNB vs atropine ($K_i$ = 0.2 nM), $H_2$ = [$^{125}$I]aminopotentidine vs asenapine maleate ($K_i$ = 3 nM), $D_2$ = [$^3$H]*N*-methylspiperone vs haloperidol ($K_i$ = 15 nM), $D_3$ = [$^3$H] *N*-methylspiperone vs nemonapride ($K_i$ = 1 nM), $D_4$ = [$^3$H] *N*-methylspiperone vs nemonapride ($K_i$ = 2 nM), $D_5$ = [$^3$H]SCH23390 vs SKF83566 ($K_i$ = 5 nM). Dash indicates <50% inhibition in the primary radioligand binding screen.

findings of others who showed weak serotonin 2C receptor (5-$HT_{2C}$) agonist potency as well as binding affinity.[15,24,25]

Regarding inhibition constants for non-5-HT receptors and potential sites of action, most of the inhibition constants were >1,000 nM with a few exceptions. 4-HO-MET, 4-HO-MPT, 4-acetoxy-*N*-methyl-*N*-propyltryptamine (4-AcO-MPT), 4-HO-EPT, 4-hydroxy-*N*-methyl-*N*-allyltryptamine (4-HO-MALT), 4-hydroxy-*N*,*N*-diallyltryptamine (4-HO-DALT), and 4-acetoxy-*N*,*N*-diallyltryptamine (4-AcO-DALT) all had notable affinity at $H_1$ which was comparable to inhibition constants at some 5-HT receptors including 5-$HT_{2A}$. This finding is consistent with prior data for psilocin showing competition for receptor binding at $H_1$ of ∼300−700 nM.[22,23] 4-HO-MALT and 4-acetoxy-*N*-methyl-*N*-allyltryptamine (4-AcO-MALT) also had mid nM inhibition constants at $alpha_{2A}$, but not at other alpha receptors. 4-AcO-MPT and 4-AcO-EPT displayed low to mid nM affinities at $alpha_{2B}$ receptors in contrast to the corresponding 4-hydroxy analogues. 4-AcO-DALT competed for receptor binding at all 3 alpha receptors with inhibition constants from 200−800 nM, in contrast to its 4-hydroxy analogue which displayed weak μM affinities for these sites. At SERT, 4-HO-MPT, 4-HO-DiPT, 4-HO-MALT,


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and 4-HO-DALT had notable mid to high nM affinities. 4-PrO-DMT was the only compound to compete for binding at KOR, a site of action associated with the unique psychoactive effects of Salvinorin A, setting it apart from related 4-substituted analogues with the *N,N*-dimethyl moiety.[23,26] 4-HO-MPT, 4-HO-EPT, and 4-HO-DALT all had mid to high nM affinities for $D_3$. Lastly, several of the compounds displayed inhibition constants for the NR2B subunit of NMDA receptors, both sigma receptors, $H_2$, $M_4$, $D_2$, and $D_4$, but inhibition constants were all >1,000 nM. The present data support the notion that psychedelic tryptamines are mostly serotonergic compounds, but each derivative has its own set of non-5-HT targets that might influence its subjective and physiological effects. The role of various non-5-HT receptors in modulating *in vivo* pharmacological effects of psychedelic tryptamine analogues is largely unexplored and warrants further investigation.

The present studies are the first to report the comprehensive receptor binding and target profiles for a set of tryptamine psychedelic NPS being used as alternatives to psilocybin in recreational drug markets and being explored as potential new medications, including 4-PrO-DMT.[10−13] Partial receptor profiles for 4-HO-DALT, 4-AcO-DALT, 4-HO-DiPT, and 4-HO-MET have been reported previously.[24,25,27] Prior results generally agree with the receptor binding affinity data reported here, but there are a few discrepancies worth noting. 4-HO-DALT was previously shown to have affinity at SERT of ∼5 $\mu$M in contrast to the affinity of ∼700 nM shown here.[27] Additionally, another previous study found that 4-HO-MET had an affinity of ∼200 nM at SERT vs an affinity of ∼1.8 $\mu$M that we observed.[25] Despite some differences, most of the receptor binding affinity data described in the present report are consistent with available SAR trends for the tested psychedelic tryptamines.

**Agonist Potencies and Efficacies of Tryptamine Psychedelics at 5-HT$_2$ Receptors.** To confirm functional activities at 5-HT$_2$ receptors, 4-PrO-DMT and several other 4-hydroxytryptamines (4-HO-MET, 4-HO-DET, 4-HO-EPT, 4-HO-MPT, 4-HO-MALT, 4-HO-DALT, 4-HO-DiPT) were also tested for agonist activity *in vitro* relative to the activity of 5-HT using a $G_q$-calcium mobilization assay.[21] Concentration−response curves are shown in Figure S1, while potency and efficacy values are shown in Table S1. All tryptamine psychedelics displayed agonist activity in this assay at 5-HT$_{2A}$ ($EC_{50}$ = 3−93 nM), 5-HT$_{2B}$ ($EC_{50}$ = 3−72 nM), and 5-HT$_{2C}$ receptors ($EC_{50}$ = 50−6,400 nM). All compounds also displayed partial to full agonist efficacies at 5-HT$_{2A}$ ($E_{max}$ = 93−104%), 5-HT$_{2B}$ ($E_{max}$ = 71−110%), and 5-HT$_{2C}$ ($E_{max}$ = 57−91%) receptors. These data are generally consistent with other reports of agonist activities of these tryptamines at 5-HT$_2$ receptors in the $G_q$-calcium mobilization assay.[15] Agonist activity of 4-PrO-DMT has not previously been reported but is consistent with agonist actions of related compounds in this assay.[15,23] As predicted by the present primary binding screen and previously reported by others,[15,24] 4-HO-DiPT had weak ($EC_{50}$ ≈ 6,400 nM) potency and/or partial agonist efficacy (∼72%) at 5-HT$_{2C}$ relative to 5-HT and the other psychedelic tryptamines tested.

One limitation of our receptor screening data is the lack of functional assessments to determine efficacies for the compounds (i.e., agonist, antagonist, or inverse agonist) at the identified non-5-HT$_2$receptors and targets. In addition to the present data showing agonist activities of several of the psychedelic tryptamines at 5-HT$_2$ receptors (Figure S1, Table S1), functional agonist potencies for $G_{\alpha q}$-calcium signaling at 5-HT$_2$ receptor subtypes as well as the potencies to induce HTR in mice, have been reported by another group for most of the tested compounds.[15] Other studies have examined the binding affinities as well as the potencies and efficacies of a large set of tryptamine psychedelics at 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_{1A}$ receptors.[24,28] However, the functional activities for other signaling pathways linked to 5-HT$_{2A}$ and at other 5-HT receptors and targets are still largely unknown. This study fills an important gap in knowledge by providing a comprehensive evaluation of the potential non-5-HT$_{2A}$ targets for psychedelic tryptamines, and complements the existing data regarding functional activities for compounds at 5-HT$_2$ receptors.[15] Future studies should determine the intrinsic efficacies of these tryptamine psychedelics at identified 5-HT and non-5-HT sites of action.

Importantly, ligand induced receptor signaling does not always correlate with receptor binding at 5-HT$_2$ receptors.[29,30] At the present time, however, it is unclear which 5-HT$_{2A}$-linked functional assay(s) are capable of predicting psychedelic-like or potential therapeutic effects of psychedelic compounds. One study reported a positive correlation between potencies for psychedelic induced $G_{\alpha q}$-calcium mobilization at rat 5-HT$_{2A}$ *in vitro* and potencies for producing HTR in mice,[31] while another study found that potencies for $\beta$-arrestin 2 recruitment at 5-HT$_{2A}$ *in vitro* predicts bioactive doses of phenethylamine psychedelics in humans.[32] However, potencies of psychedelics for *in vitro* phosphoinositide hydrolysis linked to 5-HT$_{2A}$ receptor function do not correlate with their potencies for drug discrimination in rats.[33] Rodent studies assessing the role of various signaling pathways in mediating behavioral effects of psychedelics have revealed modest effects of some 5-HT$_{2A}$ receptor-mediated signaling cascades, but the summed findings suggest that multiple signaling pathways could be involved.[34−39] In contrast to the enigmatic role of specific signaling pathways, receptor binding data from multiple laboratories show that affinity for [$^3$H]ketanserin-labeled 5-HT$_{2A}$ receptors predicts psychedelic-like effects of drugs in rodents and subjective psychedelic effects in humans.[25,40−44] One study found no correlation between HTR potencies of *N,N*-diallyltryptamines and 5-HT$_{2A}$ binding affinity alone, but found a significant correlation in a multiple regression analysis when also including 5-HT$_{1A}$ affinity.[27] Therefore, understanding the complex pharmacology of tryptamine psychedelics will require resolving these discrepancies and clarifying the relationships between *in vitro* affinity, *in vitro* functional potency/efficacy, and *in vivo* effects.

**Effects of 4-PrO-DMT in Mice.** The recreational use of 4-PrO-DMT has been reported in humans and there is interest in developing related psilocybin analogues as novel medications. However, no previous studies of the pharmacological effects of 4-PrO-DMT in animal models have been conducted. Based on the reported subjective experience in humans, as well as the receptor binding profile and 5-HT$_{2A}$ agonist activity of 4-PrO-DMT shown here, we surmised that the drug would produce psychedelic-like effects akin to psilocybin *in vivo*. To test this hypothesis, we conducted mouse studies to measure acute dose-related (0.03−30 mg/kg s.c.) effects of 4-PrO-DMT on HTR, temperature change, and locomotor activity over a 30 min testing period.

Mice treated with 4-PrO-DMT exhibited increases in HTR frequency at doses from 0.3−3 mg/kg s.c. that were statistically


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higher than HTR in vehicle controls (Figure 2A; Table S2). 4-PrO-DMT induced a typical inverted U shape dose−response

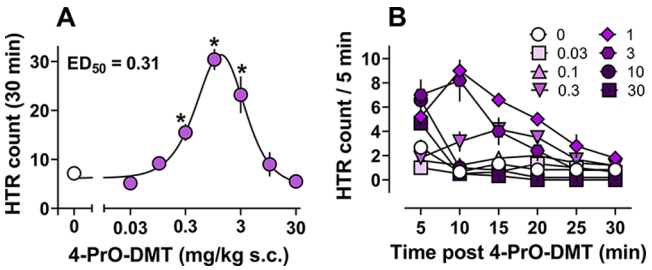


**Figure 2.** Dose−response of 4-PrO-DMT (0.03−30 mg/kg s.c.) to produce HTR. (**A**) Dose−response curve and potency for 4-PrO-DMT ) to produce HTR. * = statistically significant values ($p < 0.05$) compared to vehicle controls (0 mg/kg). (**B**) Time-course of dose-related HTR produced by 4-PrO-DMT over the 30 min session. Data represent mean ± SEM HTR count for $n$ = 5−6/dose.

curve for HTR, with an ascending limb potency to produce HTR of 0.31 mg/kg s.c., similar to previous studies examining the effects of psilocin, psilocybin, and psilacetin in mice.[15,23,45] The time-course of HTR effects for 4-PrO-DMT was similar to the time-course effects of other related psilocybin analogues with the *N*,*N*-dimethyl moiety. Time-course effects across the session are shown in Figure 2B and reveal that the HTR counts peaked from 5−10 min post injection and waned to control levels by the end of the session. At the highest doses tested (10 and 30 mg/kg s.c.), total HTR count was reduced to vehicle control levels, and these mice only displayed HTR activity for the first 5 min of the session.

The descending limb of the inverted U shaped HTR curve coincided with significant decreases in locomotor activity and body temperature (3−30 mg/kg s.c.) relative to vehicle controls. The dose−response curves for temperature and locomotor effects of 4-PrO-DMT are depicted in Figure 3 with

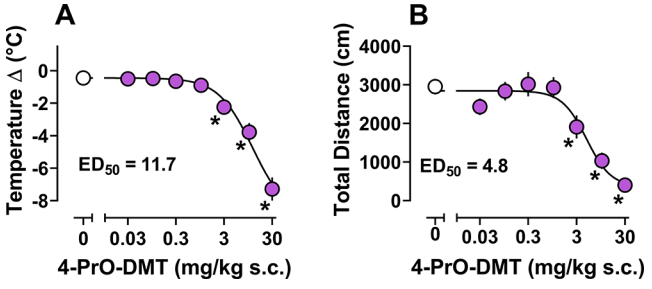


**Figure 3.** Dose−response of 4-PrO-DMT (0.03−30 mg/kg s.c.) for effects on body temperature and locomotor activity. (**A**) Dose−response curve and potency for 4-PrO-DMT to change body temperature across the 30 min pre to post session. (**B**) Dose−response curve and potency for 4-PrO-DMT to decrease distance traveled. * = statistically significant values ($p < 0.05$) compared to vehicle controls (0 mg/kg). Data represent mean ± SEM for $n$ = 5−6/dose.

additional information in Table S2. Potencies for temperature change and total distance traveled across the session were 11.7 and 4.8 mg/kg s.c. respectively. Notably, these effects were exhibited at doses 3−10-fold higher than those that producing reliable HTR. Thus, these 5-HT syndrome-like effects are probably most relevant to overdose situations. These results are also consistent with other studies of psilocybin and related analogues showing hypolocomotion and hypothermia at high doses.[23,46] Importantly, combinations of drug-induced symptoms like hypothermia and hypolocomotion in rodents have been shown to predict compounds that will produce 5-HT syndrome in humans.[47]

**Receptor Contributions to Effects of 4-PrO-DMT in Mice.** We also sought to determine the receptors mediating HTR, locomotor suppression, and hypothermic effects of 4-PrO-DMT in mice. To do this, we utilized antagonist pretreatment experiments to assess receptor contributions to effects of 4-PrO-DMT. Based on previous studies with psilocybin and related analogues, we surmised that HTR induced by 4-PrO-DMT would be blocked by pretreatment with the 5-$HT_{2A}$ antagonist, MDL100907 (M100907), while pretreatment with the 5-$HT_{1A}$ antagonist, WAY100635, would block locomotor and temperature related effects of the drug.

The effects of 0.01 mg/kg s.c. M100907 pretreatment on HTR induced by 0.6 mg/kg s.c. 4-PrO-DMT given 30 min later are shown in Figure 4A and further summarized in Tables

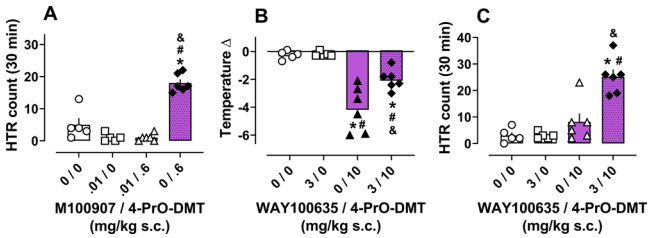


**Figure 4.** Effects of 5-$HT_{2A}$ and 5-$HT_{1A}$ antagonist pretreatment on HTR and hypothermia induced by 4-PrO-DMT. (A) M100907 pretreatment blocked HTR induced by 4-PrO-DMT. (B - C) WAY100635 pretreatment partially blocked hypothermia produced by 4-PrO-DMT (B) and revealed HTR (C). *, #, or & = statistically significant values ($p < 0.05$) compared to 0/0, antagonist/0, or 0/4-PrO-DMT conditions, respectively. Data represent mean ± SEM for $n$ = 5−6/dose.

S3 and S4. Results show that the vehicle/4-PrO-DMT condition produced significantly higher HTR counts compared to all other groups, including saline vehicle controls. Blockade of 5-$HT_{2A}$ receptors in the M100907/4-PrO-DMT group prevented the increase in HTR produced by 4-PrO-DMT, demonstrating involvement of this receptor in the HTR. These results are consistent with many studies implicating the 5-$HT_{2A}$ receptor as a critical target in psychedelic-like effects in rodents and psychedelic subjective effects in humans.[48−52] M100907 pretreatment did not produce any changes in temperature or locomotor activity over the session (Figure S2A,B).

The effects of 3 mg/kg s.c. WAY100635 pretreatment on temperature change, HTR count, and locomotor activity are shown in Figure 4B,C and further summarized in Figure S2C,D, Table S5, and Table S6. Temperature decreases induced by the vehicle/4-PrO-DMT condition were significantly lower than vehicle/vehicle and WAY100635/vehicle conditions. The hypothermic effect was partially, but not fully blocked in the WAY100635/4-PrO-DMT vs vehicle/4-PrO-DMT condition, suggesting a partial role for 5-$HT_{1A}$ receptors in effects of 10 mg/kg s.c. 4-PrO-DMT. This result supports findings from multiple laboratories showing a role of 5-$HT_{1A}$ in hypothermic responses to psilocybin and related analogues at 1−3 mg/kg.[23,46] WAY100635 may have only partially reduced hypothermic responses in the present studies due to the high dose of 4-PrO-DMT used (10 mg/kg s.c.). At higher doses, the


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hypothermic effects of 4-PrO-DMT and other psilocybin analogues may involve non-5-HT$_{1A}$ receptor interactions, such as those observed in the radioligand binding assays shown here and elsewhere.[22,23,52] Additionally, vehicle/4-PrO-DMT treatment reduced distance traveled (Figure 4D), but this effect was not statistically significant. The lack of a significant effect could in part be due to the extra habituation to the chambers during the 30 min after WAY100635, but before agonist administration. Habituation to the testing chamber has been previously shown to impact locomotor effects of serotonergic drugs and psychedelics.[53,54] Pretreatment with 3 mg/kg s.c. WAY100635 prior to agonist administration appeared to rescue the reduced distance traveled seen in the vehicle/4-PrO-DMT group (Figure S2C), but this effect was not statistically significant, also likely due to habituation differences between dose−response and antagonist study designs.

WAY100635 pretreatment also modulated the HTR activity induced by high dose 4-PrO-DMT (10 mg/kg s.c.) (Figure 4C). Specifically, the vehicle/4-PrO-DMT condition did not produce a HTR that was significant compared to vehicle controls, but the WAY100635/4-PrO-DMT condition displayed robust HTR (∼25 HTR counts/30 min). This intriguing finding suggests that agonist activity of 4-PrO-DMT at 5-HT$_{1A}$ at high doses (3−30 mg/kg s.c.) might be causally related to the descending limb of the HTR dose−response curve. Further, this finding supports an established role of the 5-HT$_{1A}$ receptor in modulating 5-HT$_{2A}$ receptor activity of psychedelics.[27,55−57] In particular, previous studies found that 5-HT$_{1A}$ agonists reduce, while antagonists enhance, 5-HT$_{2A}$ mediated effects of psychedelics. It has been shown that HTR produced by psilocybin and related analogues is unchanged or slightly increased after the same WAY100635 pretreatment used here, but these studies used lower doses of WAY100635 and lower doses of test agonists which may account for the discrepancy. One prior study found that the obscure lysergamide, lysergic acid morpholide (LSM-775), only produced the HTR in mice when animals were pretreated with WAY100635.[55] Another previous study showed that HTR potencies of *N*,*N*-diallyl tryptamines in mice are negatively related to 5-HT$_{1A}$ and positively related to 5-HT$_{2A}$ affinities in a multiple regression analysis.[27] These previous findings in combination with the present results further support the regulatory relationship between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in psychedelic drug action of tryptamines.

One other potential limitation regarding the WAY100635 antagonist experiments, is the finding that this drug and its major metabolite are only mildly selective at 5-HT$_{1A}$ vs D$_4$ receptors, where they act as agonists rather than as antagonists.[58] 4-PrO-DMT did not display any affinity for D$_4$ receptors in primary target screening, but it cannot be fully ruled out that agonist activity of WAY100635 at D$_4$ did not contribute to the observed blockade of hypothermic effects of 4-PrO-DMT at high doses. On the other hand, evidence in the literature suggests that D$_4$ agonist activity does not play a major role in body temperature regulation in other rodents,[59] suggesting that D$_4$ agonist effects did not play a role in the results from the present WAY100635 experiments.

## CONCLUSION

The present data reveal that 4-acetoxy and 4-propionoxy compounds display similar, albeit weaker, binding affinities across 5-HT receptors compared to their 4-hydroxy analogues having the same *N*,*N*-dialkyl groups. In addition to 5-HT receptor affinities, structure-related differences in inhibition constants for non-5-HT receptors and targets (histamine, dopamine, adrenergic, and the serotonin transporter) were observed. 4-PrO-DMT and several 4-hydroxy tryptamine psychedelics also displayed agonist activities *in vitro* at 5-HT$_2$ receptors. In mice, 4-PrO-DMT produced a dose-dependent increase in HTR, which was similar to previous reports for psilocybin, and consistent with its reported subjective effects as an NPS in human users. Additionally, at higher doses, 4-PrO-DMT produced hypolocomotion and hypothermia corresponding with the descending limb of the HTR dose−response curve. The HTR produced by 4-PrO-DMT was blocked by antagonist pretreatment with the 5-HT$_{2A}$ antagonist M100907, indicating involvement of this receptor. Hypothermic and hypolocomotive effects observed upon administering high doses of 4-PrO-DMT were partially to fully blocked in mice pretreated with 5-HT$_{1A}$ antagonist WAY100635, suggesting a role for this receptor in these effects. WAY100635 pretreatment significantly increased the total number of HTR seen after administering a high dose of 4-PrO-DMT, suggesting that 5-HT$_{1A}$ effects can counteract 5-HT$_{2A}$ mediated HTR under certain conditions. Overall, these results highlight pharmacological target profile differences between tryptamine psychedelics and provide data that will be useful in the continued monitoring of emerging NPS and informed development of psychedelic-assisted therapies.

## METHODS

**Drugs.** Commercial samples of 4-AcO-MET hydrofumarate, 4-HO-MET hydrofumarate, 4-AcO-EPT hydrofumarate, 4-HO-EPT hydrofumarate and 4-AcO-MALT hydrofumarate (The Indole Shop, Canada) as well as 4-PrO-DMT hydrofumarate, 4-AcO-MPT hydrofumarate, 4-HO-MPT hydrofumarate, 4-HO-MALT hydrofumarate, 4-AcO-DALT hydrofumarate and 4-HO-DiPT hydrofumarate (ChemLogix, Canada) were purified via recrystallization.[60] 4-HO-DALT hydrofumarate was synthesized via hydrolysis of 4-AcO-DALT hydrofumarate. Synthetic details are provided in the Supporting Information. (+)M100907 was generously provided by Kenner Rice, Ph.D. and Agnieszka Sulima, Ph.D. (NIDA). WAY100635 maleate was purchased from Cayman Chemical (Item No. 14599). For *in vitro* studies, compounds were initially dissolved in 100% DMSO at 10 mM and subsequently diluted in assay buffer for experiments. For *in vivo* studies, drug doses represent the weight of the salt. Drugs were administered to mice at 0.01 mL/g body weight via s.c. injection on the back. Saline was used as the vehicle control for all drugs except M100907, which utilized a 1% DMSO 99% saline vehicle.

**Comprehensive receptor binding screen.** A comprehensive binding screen was provided by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP). The initial primary screening phase tested 10 $\mu$M concentrations of test ligand against radioligands for each of the targets listed below:

5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-ht$_{1e}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_{5A}$, 5-HT$_6$, 5-HT$_{7a}$, Alpha$_{1A}$, Alpha$_{1B}$, Alpha$_{1D}$, Alpha$_{2A}$, Alpha$_{2B}$, Alpha$_{2C}$, Beta$_1$, Beta$_2$, Beta$_3$, BZP Rat Brain Site, D$_1$, D$_2$, D$_3$, D$_4$, D$_5$, DAT, DOR, GABA$_A$, H$_1$, H$_2$, H$_3$, H$_4$, KOR, M$_1$, M$_2$, M$_3$, M$_4$, M$_5$, MOR, NET, PBR, SERT, Sigma 1, Sigma 2, AMPA, Kainate (Rat Brain), NMDA, NR2B

In all instances where radioligand binding was reduced by 50% or greater in primary screening, secondary screening was initiated to construct full concentration-effect curves for


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inhibition of radioligand binding and determine inhibition constants at each target identified.

In addition to the comprehensive binding screen, NIMH PDSP also constructed concentration−response curves for agonist activity of several of the test compounds at 5-$HT_2$ receptors using calcium mobilization assays with results relative to the standard ligand 5-HT.

All details of the competition binding assays and 5-$HT_2$ receptor functional assays run by PDSP can be found online in the assay protocol book.[21]

**Mouse Studies.** *Details and Design.* A single cohort of 12 male C57BL/6J mice (The Jackson Laboratory # 000664) was purchased for *in vivo* studies and group housed 3 per cage for acclimation to the facility. Mice were single housed after temperature transponder implantation. Mice had ad libitum access to food and water and were housed in a 12/12 light-dark cycle with lights on at 0700 local time. All experiments were approved by the NIDA IRP Animal Care and Use Committee and conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in Baltimore, MD, USA.

Mice were tested every 1−2 weeks with a minimum of 7 days between treatments to mitigate any influence of tolerance on behavioral study measures.[61−63] Dose−response studies for 4-PrO-DMT (0.03−30 mg/kg s.c.) were conducted first, followed by antagonist reversal studies with 0.01 mg/kg s.c. M100907 and 3 mg/kg s.c. WAY100635 pretreatments. Experiments were conducted between 0900−1700 local time during the light phase. Prior to each experiment, all mice were acclimated to the testing room in their home cage for at least 1 h prior to testing.

*Temperature Transponder Implantation.* All mice were implanted subcutaneously with temperature transponders (14 × 2 mm, model IPTT-300, Bio Medic Data Systems, Inc., Seaford, DE, USA) at least 1 week prior to any experimental sessions as previously described .[64,65] The implanted temperature transponders allowed body temperature (° C) of each mouse to be measured non-invasively using a hand-held receiver designed to read signal from the transponders.

*Behavioral Testing.* Behavioral testing to assess the dose-related effects of 4-PrO-DMT on HTR, temperature change, and locomotor activity was conducted as previously described.[23] Briefly, mouse body weight and baseline temperature were recorded, followed by a brief 5 min acclimation to the testing chamber. After brief acclimation, mouse body temperature was again recorded for baseline measurement, mice were injected with various doses of 4-PrO-DMT, and returned to the testing chambers for 30 min. During the session, locomotor activity was measured with photobeam tracking of distance traveled in cm in the horizontal plane. HTR events were recorded simultaneously using an overhead GoPro Hero 7 Black camera (960p resolution at 120 frames per sec) and analyzed after each experiment using a computer software-based scoring platform (Clever Sys Inc. TopScan) validated in our laboratory.[66] Open field chambers used for the studies (Coulbourn Instruments, Holliston, MA, USA) were modified with cylindrical inserts and custom flooring useful in aiding software-based detection of HTR events. After each session, mice were returned to their home cage after recording post experiment temperature, and temperature data represent the change from baseline to the end of the session.

*Antagonist Studies.* Antagonist studies involved s.c. administration of either receptor antagonists or solvent vehicle after a brief 5 min acclimation period to the test chamber. Before returning mice to the testing chamber, baseline temperature was recorded to allow later assessment of any change in temperature induced by receptor antagonists. Locomotor activity during the 30 min after antagonist pretreatment was recorded to detect any potential adverse effects of antagonist treatments. Thirty min after antagonist pretreatment or vehicle, mice received s.c. 4-PrO-DMT or its vehicle, were subjected to temperature testing, then returned to the testing chamber for another 30 min of recording (video and photobeam). The remainder of the antagonist experiments were analogous to the dose−response study conditions.

*Data Analyses.* GraphPad Prism 9 (La Jolla, CA, USA) was used to conduct all statistical analyses and graphically represent data. Non-linear regression analyses were used to determine the receptor inhibition constants/affinities ($K_i$) and thepotencies for calcium mobilization ($EC_{50}$) as well as effects in mice ($ED_{50}$). HTR potency data was determined using the rising phase of the inverted U dose−response curve. In mouse studies, one-way ANOVA with either Tukey's or Dunnett's post hoc tests were used to compare groups to controls. The mean HTR count over the 30 min testing period, temperature change from baseline, and distance traveled in the horizontal plane (cm) were used for the statistical comparisons conducted. For all analyses, the alpha level was set at 0.05. Group sizes, exact *p*-values for post hoc comparisons, and descriptive statistics for each data set can be found in either the figure legends or the Supporting Information.

*Crystallographic Characterization of 4-PrO-DMT Hydrofumarate.* Single crystals of 4-PrO-DMT hydrofumarate were obtained from the slow evaporation of an aqueous solution, and data was collected on a Bruker D8 Venture diffractometer. Crystallographic details are provided in the Supporting Information.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.2c00222.

**Figure S1**, concentration−response curves for 5-$HT_2$ functional activity; **Table S1**, potency and efficacy values for 5-$HT_2$ functional activity; **Table S2**, statistical info for 4-PrO-DMT dose−response mouse experiments; **Table S3**, descriptive statistics for 4-PrO-DMT and M100907 antagonist experiments; **Table S4**, ANOVA post test results for 4-PrO-DMT and M100907 antagonist experiments; **Table S5**, descriptive statistics for 4-PrO-DMT and WAY100635 antagonist experiments; **Table S6**, ANOVA post test results for 4-PrO-DMT and WAY100635 antagonist experiments; **Figure S2**, control measures for antagonist studies in mice; **Figure S3**, crystal structure of 4-PrO-DMT hydrofumarate; **Tables S7−S11**, analytical characterization of 4-PrO-DMT hydrofumarate (PDF)

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Study design: G.C.G., M.H.B., D.R.M. Chemical synthesis and analysis: D.R.M., J.A.G., D.N.K.P., M.N. Mouse experiments: G.C.G. Manuscript was drafted by G.C.G., critically reviewed by M.H.B., A.R.C., D.R.M., and final version approved by all authors.


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### Notes

The authors declare the following competing financial interest(s): A.R.C. has an ownership stake in CaaMTech, Inc., owning patent applications concerning new tryptamine compounds, their compositions, formulations, method of use, and their syntheses. No other authors report any competing financial interests related to the data in this publication.

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## ■ ABBREVIATIONS

5-HT, Serotonin; 5-$HT_{1A}$, Serotonin 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; 5-$HT_{2B}$, Serotonin 2B receptor; 5-$HT_{2C}$, Serotonin 2C receptor; 5-$HT_{3}$, Serotonin 3 receptor; 5-$HT_{5A}$, Serotonin 5A receptor; 5-$HT_{6}$, Serotonin 6 receptor; 5-$HT_{7a}$, Serotonin 7a receptor; $Alpha_{2A}$, Alpha 2A receptor; $Alpha_{2B}$, Alpha 2A receptor; $Alpha_{2C}$, Alpha 2C receptor; DAT, Dopamine transporter; SERT, Serotonin transporter; $H_1$, Histamine H1 receptor; $H_2$, Histamine H1 receptor; $M_4$, Muscarinic M4 receptor; $D_2$, Dopamine D2 receptor; $D_3$, Dopamine D3 receptor; $D_4$, Dopamine D4 receptor; $D_5$, Dopamine D5 receptor; KOR, Kappa opioid receptor; psilocybin, 4-phosphoryloxy-*N*,*N*-dimethyltryptamine; psilocin, 4-hydroxy-*N*,*N*-dimethyltryptamine; psilacetin or 4-AcO-DMT, 4-acetoxy-*N*,*N*-dimethyltryptamine; 4-PrO-DMT, 4-propionoxy-*N*,*N*-dimethyltryptamine; 4-AcO-MET, 4-acetoxy-*N*-methyl-*N*-ethyltryptamine; 4-HO-MET, 4-hydroxy-*N*-methyl-*N*-ethyltryptamine; 4-AcO-DET, 4-acetoxy-*N*,*N*-diethyltryptamine; 4-HO-DET, 4-hydroxy-*N*,*N*-diethyltryptamine; 4-AcO-MPT, 4-acetoxy-*N*-methyl-*N*-propyltryptamine; 4-HO-MPT, 4-hydroxy-*N*-methyl-*N*-propyltryptamine; 4-AcO-EPT, 4-acetoxy-*N*-ethyl-*N*-propyltryptamine; 4-HO-EPT, 4-hydroxy-*N*-ethyl-*N*-propyltryptamine; 4-AcO-MALT, 4-acetoxy-*N*-methyl-*N*-allyltryptamine; 4-HO-MALT, 4-hydroxy-*N*-methyl-*N*-allyltryptamine; 4-AcO-DALT, 4-acetoxy-*N*,*N*-diallyltryptamine; 4-HO-DALT, 4-hydroxy-*N*,*N*-diallyltryptamine; 4-HO-DiPT, 4-hydroxy-*N*,*N*-diisopropyltryptamine; M100907, (+)MDL 100907; WAY100635, WAY-100653; NIMH PDSP, National Institute of Mental Health Psychoactive Drug Screening Program

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