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Investigation of the structure-activity relationships of psilocybin analogues

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ABSTRACT

The 5-HT_{2A} receptor is thought to be the primary target for psilocybin (4-phosphoryloxy-*N,N*-dimethyltryptamine) and other serotonergic hallucinogens (psychedelic drugs). Although a large amount of experimental work has been conducted to characterize the pharmacology of psilocybin and its dephosphorylated metabolite psilocin (4-hydroxy-*N,N*-dimethyltryptamine), there has been little systematic investigation of the structure-activity relationships (SAR) of 4-substituted tryptamine derivatives. In addition, structural analogs of psilocybin containing a 4-acetoxy group, such as 4-acetoxy-*N,N*-dimethyltryptamine (4-AcO-DMT), have appeared as new designer drugs, but almost nothing is known about their pharmacological effects. To address the gap of information, SAR studies were conducted with 16 tryptamines containing a variety of symmetrical and asymmetrical *N,N*-dialkyl substituents and either a 4-hydroxy or 4-acetoxy group. Calcium mobilization assays were conducted to assess functional activity at human and mouse 5-HT_2 subtypes. Head-twitch response (HTR) studies were conducted in C57BL/6J mice to assess 5-HT_{2A} activation *in vivo*. All of the compounds acted as full or partial agonists at 5-HT_2 subtypes, displaying similar potencies at 5-HT_{2A} and 5-HT_{2B} receptors, but some tryptamines with bulkier *N*-alkyl groups had lower potency at 5-HT_{2C} receptors and higher 5-HT_{2B} receptor efficacy. In addition, *O*-acetylation reduced the *in vitro* 5-HT_{2A} potency of 4-hydroxy-*N,N*-dialkyltryptamines by about 10-20-fold but did not alter agonist efficacy. All of the compounds induce head twitches in mice, consistent with an LSD-like behavioral profile. In contrast to the functional data, acetylation of the 4-hydroxy group had little effect on HTR potency, suggesting that *O*-acetylated tryptamines may be deacetylated *in vivo*, acting as pro-drugs. In summary, the tryptamine derivatives have psilocybin-like pharmacological properties, supporting their classification as psychedelic drugs.

Psilocybin (4-phosphoryloxy-*N,N*-dimethyltryptamine), a prototypical serotonergic hallucinogen that produces effects similar to lysergic acid diethylamide (LSD) and mescaline, is the major active constituent of *Psilocybe mexicana* and other species of hallucinogenic mushrooms ("magic mushrooms"). Psilocybin is rapidly dephosphorylated to psilocin (4-hydroxy-*N,N*-dimethyltryptamine, 4-HO-DMT) by alkaline phosphatase *in vitro*[15,37,38] and *in vivo*[32,39]. Although psilocybin and psilocin have equivalent molar potencies *in* vivo[77], psilocybin has considerably lower potency at the receptor level[69], indicating that it may serve as a prodrug for psilocin. Over the past decade there has been a renewed interest in the pharmacology and effects of psilocybin due to accumulating evidence that it possesses therapeutic efficacy against disorders such as anxiety, depression, obsessive-compulsive disorder, and substance abuse[6]. In addition, psilocybin continues to be a popular recreational drug[49,53,59].

Following the isolation of psilocybin and psilocin by Hofmann and colleagues in 1957[34], various 4-substituted tryptamines were reported in the literature. For example, Hofmann synthesized 4-hydroxy-*N,N*-diethyltryptamine (CZ-74, 4-HO-DET) and 4-phosphoryloxy-*N,N*-diethyltryptamine (CEY-19)[75]. Similar to psilocybin and psilocin, CEY-19 and CZ-74 have equivalent molar potencies in humans[3,50]. Repke synthesized several other psilocin homologues, including 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-HO-MET), 4-hydroxy-*N*-methyl-*N*-isopropyltryptamine (4-HO-MIPT), 4-hydroxy-*N,N*-dipropyltryptamine (4-HO-DPT), and 4-hydroxy-*N,N*-diisopropyltryptamine (4-HO-DIPT)[63,64]. Tryptamines containing 4-acetoxy groups have also been synthesized. 4-Acetoxy-*N,N*-dimethyltryptamine (*O*-acetylpsilocin, 4-AcO-DMT) was patented by Hofmann in 1963[33]. Shulgin and Shulgin[71] experimented with 4-AcO-DMT and its *N,N*-diethyl (4-AcO-DET) and *N*-methyl-*N*-isopropyl (4-AcO-MIPT) homologues and found them to be potent psychedelic drugs. Subsequently, Nichols published an improved synthesis for 4-AcO-DMT and proposed that it could serve as an alternative prodrug for psilocin in scientific studies[56].

Although psilocin and psilocybin have been available on the illicit market since the 1960s, the recreational use of other 4-substituted tryptamines is a more recent development, fueled by marketing and distribution via the Internet. 4-Acetoxy-*N,N*-diisopropyltryptamine (4-AcO-DIPT) was first detected in Europe in 2005[16], followed by 4-AcO-DMT[17], 4-AcO-MET[17], and 4-AcO-DPT[18]. 4-HO-MET and 4-HO-DPT have also been detected[17,18]. In 2011, Kjellgran and Soussan published a detailed description of the phenomenological effects of 4-HO-MET in Swedish users[43]. Overall, reports indicate that most 4-substituted tryptamines produce psilocybin-like psychedelic effects[26,58,74].

Despite the increasing popularity and availability of 4-acetoxy-*N,N*-dialkyltryptamines, there is a lack of information about their pharmacological and behavioral properties. Furthermore, although numerous *N,N*-dialkyltryptamines have been explored[5,24,51,54], there has been little systematic investigation of the effect of *N*-alkyl substitution on their activity. The goal of the present investigation was to address the gap of knowledge regarding the structure-activity relationships (SAR) of tryptamine hallucinogens containing an oxygenated substituent at the 4-position of the indole ring. We focused on activity at the 5-HT_{2A} receptor, which is thought to be the primary target for psilocybin and other psychedelic drugs in humans and rodents[27,55]. Calcium mobilization assays were conducted to assess functional activation at human and mouse 5-HT_{2A}, and human 5-HT_{2B} and 5-HT_{2C} receptors. Behavioral data from the mouse head-twitch response (HTR) assay were used as a measure of 5-HT_{2A} receptor activation *in vivo*[9,29]. The HTR is widely used as a behavioral proxy in rodents for human hallucinogenic effects because it is one of only a few behaviors that can reliably distinguish hallucinogenic and non-hallucinogenic 5-HT_{2A} receptor agonists[25]. The studies were conducted with 16 tryptamine derivatives containing a variety of symmetrical and asymmetrical *N,N*-dialkyl substituents and either a 4-hydroxy or a 4-acetoxy group. Activity was assessed *in vitro* and *in vivo* to generate converging evidence and to evaluate the likelihood that the 4-acetoxy-*N,N*-dialkyltryptamines are serving as prodrugs for their 4-hydroxy counterparts.

METHODS

Animals. Male C57BL/6J mice (6–8 weeks old) obtained from Jackson Laboratories (Bar Harbor, ME, USA) were housed in a vivarium at the University of California San Diego, an AAALAC-approved animal facility that meets all Federal and State requirements for care and treatment of laboratory animals. Mice were housed up to four per cage in a climate-controlled room on a reverse-light cycle (lights on at 1900 h, off at 0700 h) and were provided with ad libitum access to food and water, except during behavioral testing. Testing was conducted between 1000 and 1800 h. All animal experiments were carried out in accordance with NIH guidelines and were approved by the UCSD Institutional Animal Care and Use Committee.

Drugs. 4-Acetoxy-*N,N*-dimethyltryptamine (4-AcO-DMT) fumarate, 4-hydroxy-*N,N*-diethyltryptamine (4-HO-DET) hydrochloride, 4-acetoxy-*N,N*-diethyltryptamine (4-AcO-DET) fumarate, 4-hydroxy-*N*-methyl-*N*-ethyltryptamine (4-HO-MET) hemifumarate, 4-acetoxy-*N*-methyl-*N*-ethyltryptamine (4-AcO-MET) fumarate, 4-hydroxy-*N*-methyl-*N*-propyltryptamine (4-HO-MPT) fumarate, 4-acetoxy-*N*-methyl-*N*-propyltryptamine (4-AcO-MPT) fumarate, 4-hydroxy-*N*-ethyl-*N*-propyltryptamine (4-HO-EPT) 3:2 fumarate, 4-acetoxy-*N*-ethyl-*N*-propyltryptamine (4-AcO-EPT) fumarate, 4-hydroxy-*N,N*-dipropyltryptamine (4-HO-DPT) hemifumarate, 4-acetoxy-*N,N*-dipropyltryptamine (4-AcO-DPT) fumarate, 4-hydroxy-*N*-methyl-*N*-isopropyltryptamine (4-HO-MIPT) hemifumarate, 4-acetoxy-*N*-methyl-*N*-isopropyltryptamine (4-AcO-MIPT) fumarate, 4-hydroxy-*N,N*-diisopropyltryptamine (4-HO-DIPT) hydrochloride, and 4-hydroxy-*N*-methyl-*N*-allyltryptamine (4-HO-MALT) 3:2 fumarate were available from previous studies performed in our laboratories. 4-Acetoxy-*N,N*-diisopropyltryptamine (4-AcO-DIPT) acetate was obtained from Cayman Chemical (Ann Arbor, MI, USA). Psilocin was obtained from the National Institute on Drug Abuse (Rockville, MD, USA). The identity and analytical purity of the test substances were

confirmed using mass spectrometry and nuclear magnetic resonance spectroscopy. All test substances had a minimum purity of >95%. For behavioral studies, psilocin was dissolved in water containing 5 mM tartaric acid (pH ~5.0), and all other compounds were dissolved in isotonic saline. Test substances were administered intraperitoneally (IP) at a volume of 5 mL/kg. For *in vitro* studies, all compounds were dissolved in DMSO at 10 mM concentration before serial dilution.

Head-Twitch Response Studies. The head-twitch response (HTR) was assessed using a head-mounted neodymium magnet and a magnetometer coil[29,30]. Briefly, mice were anesthetized, a small incision was made in the scalp, and a neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a one-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. After magnet implantation, mice were tested in multiple HTR experiments, for up to 4–5 months. Test compounds were injected immediately prior to testing and then HTR activity was recorded for 30 min in a 12.5-cm glass cylinder surrounded by a magnetometer coil. Coil voltage was low-pass filtered (2–10 kHz cutoff frequency), amplified, and digitized (20 kHz sampling rate) using a Powerlab/8SP data acquisition system with LabChart v 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). Head twitches were identified based on the following criteria: 1) sinusoidal wavelets; 2) evidence of at least three sequential head movements (usually exhibited as bipolar peaks) with frequency ≥ 40 Hz; 3) amplitude exceeding the level of background noise; 4) duration < 0.15 s; and 5) stable coil voltage immediately preceding and following each response.

The entire 30-min recordings were examined for head twitches, but in some instances a shorter block of time was used for analysis to accommodate compounds with a relatively brief duration of action. HTR counts were analyzed using one-way analyses of variance (ANOVA).

Post hoc pairwise comparisons between selected groups were performed using Tukey's studentized range method. Significance was demonstrated when an α-level of 0.05 was surpassed. Median effective doses (ED_{50} values) and 95% confidence intervals (95% CI) for HTR dose-response experiments were calculated by nonlinear regression (Prism 7.00, GraphPad Software, San Diego, CA, USA).

5-HT_2 Receptor Functional Assays. 5-HT_2 functional experiments (measuring Gq-mediated calcium flux) were performed with Flp-In T-REx 293 cells (Invitrogen, Carlsbad, CA, USA) expressing either human 5-HT_{2A} (h5-HT_{2A}), mouse 5-HT_{2A} (m5-HT_{2A}), human 5-HT_{2B} (h5-HT_{2B}) or human 5-HT_{2C} I-N-I (h5-HT_{2C}) receptor cDNA under the tetracycline repressor protein. Cells were plated into black 384-well clear-bottom tissue culture plates in 40 µL of DMEM containing 1% dialyzed foetal bovine serum (FBS) at a density of approximately 10,000 cells per well, and receptor expression was induced with 2 µg/mL tetracycline. After approximately 20-24 hours, medium was decanted and replaced with 20 µL per well of drug buffer (HBSS, 20 mM HEPES, pH 7.4) containing Fluo-4 Direct dye (Invitrogen) and incubated for between 1 and 2 h at 37°C. Test substances were diluted in drug buffer (HBSS, 20 mM HEPES, 0.1% bovine serum albumin, 0.01% ascorbic acid, pH 7.4). Before the experiment, plates were allowed to equilibrate to room temperature and calcium flux was measured using a FLIPR^{TETRA} cellular screening system (Molecular Devices, Sunnyvale, CA, USA). Plates were read for fluorescence initially for 10 seconds (1 read per second) to establish a baseline, and then stimulated with drug dilutions or buffer and read for an additional 120 seconds. Peak fluorescence in each well was normalized to maximum-fold increase over baseline. Data were normalized to the maximum peak fold-over-basal fluorescence produced by 5-HT (100%) and baseline fluorescence (0%). Data were analyzed using the sigmoidal dose-response function of Prism 5.0 or 8.0 (GraphPad Software, San Diego, CA, USA). Relative activity (RA) was expressed as the logarithm of the ratio of E_{MAX} over EC_{50} parameter estimates.

RESULTS

4-Hydroxy-*N,N*-dialkyltryptamines induce the head-twitch response. Previous studies, conducted using traditional experimental methods, have shown that psilocin is active in the HTR paradigm[11,25,31]. Using a magnetometer assessment method[29], we confirmed that psilocin induces the HTR in C57BL/6J mice with ED_{50} = 0.17 mg/kg, which is equivalent to 0.81 μmol/kg. As shown in Table 1, all of the 4-hydroxy-*N,N*-dialkyltryptamines induced the HTR (full experimental details are provided in Table S1). Similarly to other tryptamine hallucinogens[19,20,28,44], the HTR followed an inverted-U-shaped dose-response function. The dose-response curves for psilocin and 4-HO-MET are shown in Figure 1A as representative examples. Overall, some variation in potency was noted, ranging from 4-HO-MET (ED_{50} = 0.65 μmol/kg) to 4-HO-DIPT (ED_{50} = 3.46 μmol/kg). Potency in the HTR assay appears to be related to the steric properties of the alkyl groups on the amine nitrogen. For example, the rank order of potency for 4-hydroxytryptamines with symmetrical alkyl chains was as follows: psilocin (ED_{50} = 0.81 μmol/kg) > 4-HO-DET (ED_{50} = 1.56 μmol/kg) > 4-HO-DPT (ED_{50} = 2.47 μmol/kg) > 4-HO-DIPT (ED_{50} = 3.46 μmol/kg). A similar relationship exists for the psilocin analogs with asymmetrical alkyl substituents: 4-HO-MET (ED_{50} = 0.65 μmol/kg) > 4-HO-MPT (ED_{50} = 1.92 μmol/kg) > 4-HO-MIPT (ED_{50} = 2.97 μmol/kg). We examined the relationship between HTR potency and the steric properties of the amine substituents using Charton's upsilon parameter υ (which is based on van der Waals radii) as a steric descriptor[10]. For the eight *N,N*-dialkyl-4-hydroxytryptamines, -log ED_{50} values in the HTR assay were negatively correlated (R = –0.8283, p = 0.011) with the sum of the values of υ for the two amine substituents.

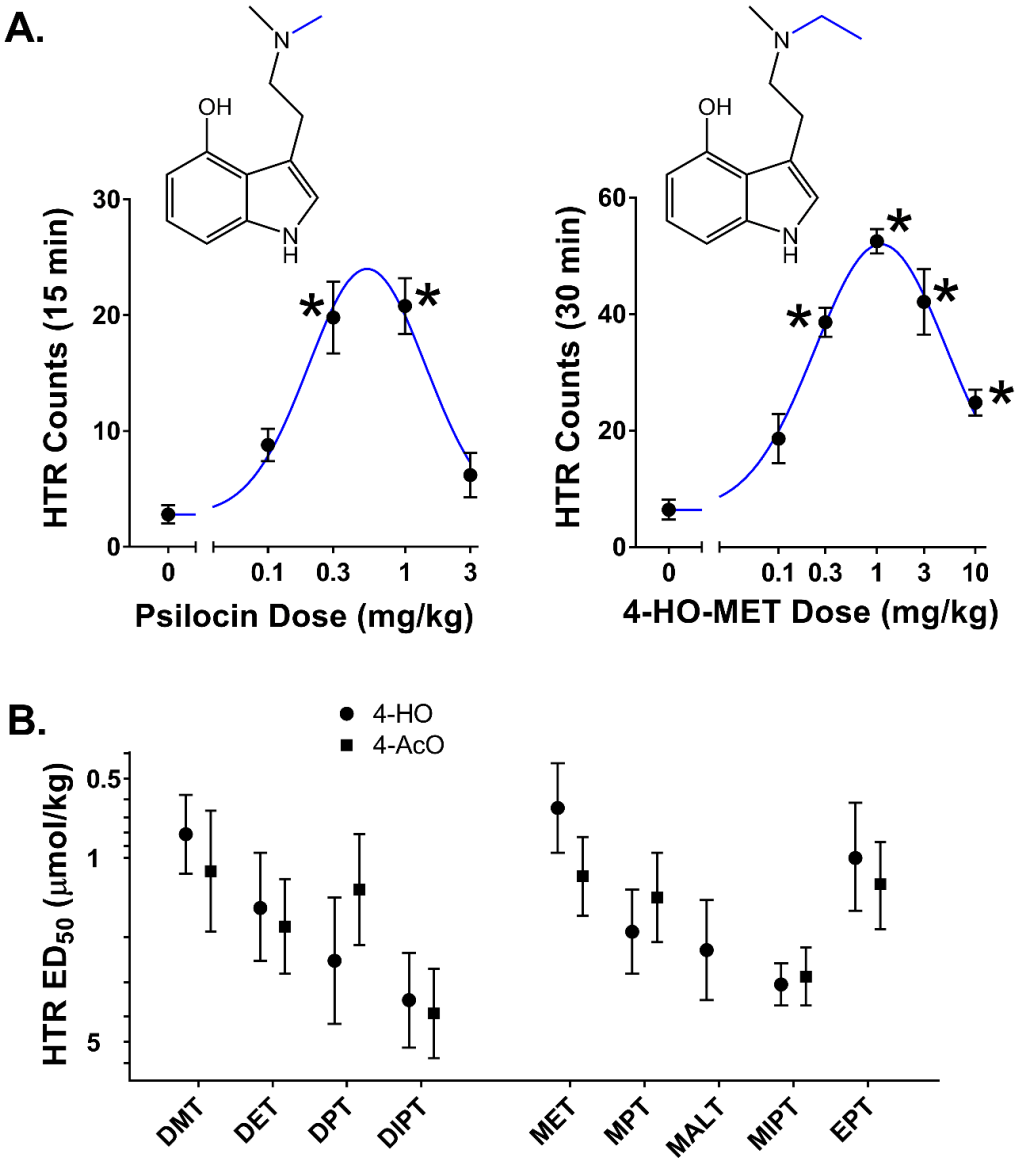

Figure 1. 4-Substituted tryptamines induce the head-twitch response (HTR) in C57BL/6J mice. (A) Effect of psilocin (*left*) and 4-HO-MET (*right*) on the HTR. Data are presented as group means ± SEM for the entire test session. *$p < 0.05$, significant difference from the vehicle control group (Tukey's test). **(B)** Comparison of the potencies (ED_{50} values, in µmol/kg) of 4-hydroxytryptamines (●) and 4-acetoxytryptamines (■) in HTR experiments.

Effect of *O*-acetylation on activity in the HTR paradigm. Similarly to the 4-hydroxy-*N,N*-dialkyltryptamines, the 4-acetoxy-*N,N*-dialkyltryptamines were also active in the HTR paradigm (see Table 2). *O*-Acetylation did not reliably alter the potency of psilocin or its

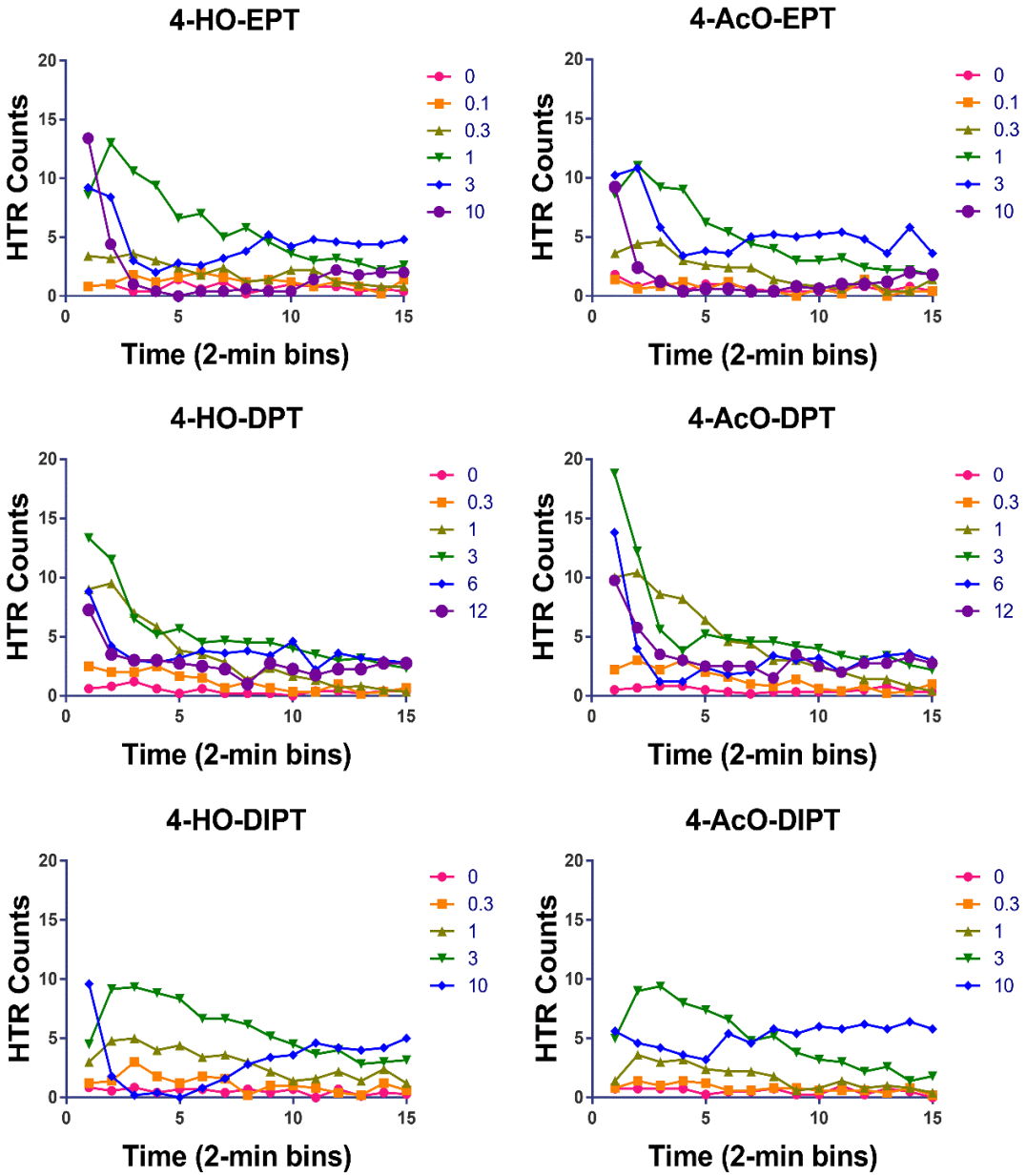

Figure 2. Time-course of the head-twitch response (HTR) induced by 4-substituted tryptamines. Data are presented as group means during consecutive 2-min time bins. Drug doses are shown in mg/kg.

homologues; potency was increased in some cases and reduced in others (Fig. 1B). In general, however, there was little difference in potency between the 4-hydroxy-*N,N*-dialkyltryptamines and their acetate esters. For the eight 4-acetoxy-*N,N*-dialkyltryptamines, -log ED_{50} values in the

HTR assay were also negatively correlated (R = –0.7208, p = 0.043) with the sum of the values of υ for the two amine substituents, although the relationship was not as robust as was found for the 4-hydroxy-*N,N*-dialkyltryptamines.

Comparison of the time course and magnitude of the *in vivo* responses. Figure 2 depicts the time course of effects on the HTR for a subset of the compounds. After binning all of the experiments in 2-min time blocks, a few of the acetate esters appeared to produce a greater maximal response than the corresponding 4-hydroxytryptamines. Nevertheless, as shown in Fig. 2, *O*-acetylation did not alter the time-course of the response, with the maximal response typically occurring during the first 10 min after drug administration. There was some variation in the duration of action of the tryptamines; for example, the response to 4-HO-DPT and 4-AcO-DPT began to decline within the first 10-min, whereas 4-HO-DIPT and 4-AcO-DIPT produced longer-lasting effects.

4-Substituted-*N,N*-dialkyltryptamines act as 5-HT$_2$ receptor agonists. *In vitro* functional activity at 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors was assessed using calcium flux assays (see Fig. 3 and Table 3). All of the 4-substituted tryptamines stimulated calcium mobilization via activation of human and mouse 5-HT$_{2A}$ receptors. In fact, most of the 4-hydroxy tryptamines had similar potency and efficacy at mouse and human 5-HT$_{2A}$ receptors. Almost all of the compounds behaved as highly efficacious 5-HT$_{2A}$ agonists (E_{max} range of 90–100% relative to 5-HT), with the exception of the *O*-acetylated tryptamines 4-AcO-DMT (E_{max} = 79.2%) and 4-AcO-DIPT (E_{max} = 74.6%). Notably, the 4-hydroxy tryptamines had high potency at 5-HT$_{2A}$ (EC_{50} values ranging from about 1–10 nM), while the potency of the *O*-acetylated tryptamines was about an order of magnitude weaker (ranging from 10-40-fold) compared to their 4-hydroxy counterparts.

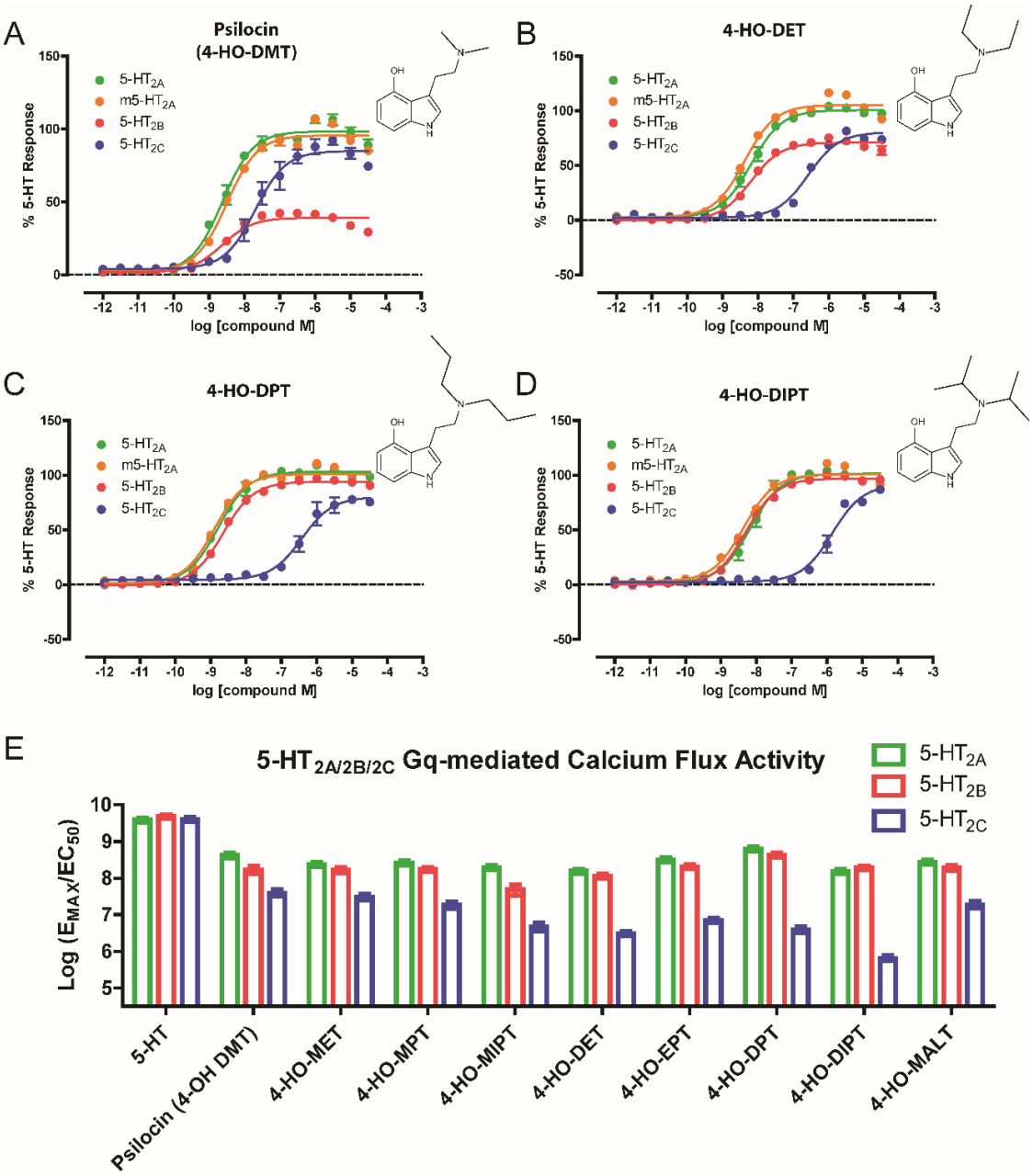

Figure 3. *In vitro* activity of 4-substituted tryptamines at 5-HT$_2$ receptors. All compounds were assayed in parallel measuring Gq-mediated calcium flux using the same drug dilutions for psilocin **(A)**, 4-HO-DET **(B)**, 4-HO-DPT **(C)**, and 4-HO-DIPT **(D)** at h5-HT$_{2A}$ (green), m5-HT$_{2A}$ (orange), h5-HT$_{2B}$ (red), and h5-HT$_{2C}$ (blue) receptors expressing Flp-In T-REx 293 stable cell lines. Data represent concentration-response curves plotting mean and SEM of data points performed in triplicate from at least three independent experiments. **(E)** The relative activities of the compounds (log(E_{MAX}/EC_{50}) are plotted for h5-HT$_{2A}$ (green), h5-HT$_{2B}$ (red), and h5-HT$_{2C}$ (blue) and represent data from at least three independent experiments.

Results at the 5-HT_{2B} receptor were similar to those observed at 5-HT_{2A}, although efficacy at 5-HT_{2B} was more variable, ranging from 22.1% to 97.4%. There was also a relationship between 5-HT_{2B} efficacy and the *N,N*-dialkyl substitution pattern: tryptamines containing *N*-ethyl-*N*-propyl*, N,N*-dipropyl, or *N,N*-diisopropyl groups had high efficacy (E_{max} of about 90–100%), whereas psilocin and other compounds containing shorter alkyl chains had lower efficacy at the 5-HT_{2B} receptor.

The tryptamines had relatively lower potency at 5-HT_{2C} receptors compared to 5-HT_{2A} receptors. *O*-Acetylation tended to reduce potency and efficacy at 5-HT_{2C}, whereas *N*-alkyl chain length had striking effects on 5-HT_{2C} activity. For example, *N,N*-diisopropyl substitution was optimal for efficacy at 5-HT_{2C} (e.g., 4-HO-DIPT E_{max} = 92.1%) but also caused a marked reduction of potency at that site (e.g., 4-HO-DIPT EC_{50} = 1,408 nM). These results indicate that the 5-HT_{2C} receptor does not tolerate longer and bulkier *N*-substitutions to the same degree as the 5-HT_{2A} receptor (Fig. 3). Notably, because *N,N*-dipropyl and *N,N*-diisopropyl substitution is detrimental for activity at 5-HT_{2C} but has little effect on activity at 5-HT_{2A}, 4-HO-DPT and 4-HO-DIPT show considerable selectivity for 5-HT_{2A} over 5-HT_{2C} (4-HO-DPT is 129-fold selective for 5-HT_{2A} and 4-HO-DIPT is 206-fold selective for 5-HT_{2A}).

DISCUSSION AND CONCLUSIONS

The present investigation examined the pharmacology and behavioral effects of *N,N*-dialkyltryptamines containing either a hydroxy or acetoxy group at the 4-position. One of the main findings of these studies is that psilocin and its homologues activate calcium mobilization via 5-HT_{2A} with high efficacy and nanomolar potency, whereas the acetate esters have about 10-fold lower potency. All of the compounds induced head twitches in mice, a behavior known to be mediated by the 5-HT_{2A} receptor. In contrast to the *in vitro* functional assays, however, *O*-acetylation of the 4-hydroxy group had little effect on potency in the HTR assay. In summary, 4-

acetoxy-*N,N*-dialkyltryptamines have LSD-like pharmacological activity, supporting their classification as psychedelic drugs. Similarly to the present results, *N,N*-dialkyltryptamines containing 4- acetoxy and 4-hydroxy groups reportedly have identical potencies in humans[71]. Hence, there appears to be a discrepancy between the activity of 4-acetoxy-*N,N*-dialkyltryptamines at the receptor level and after *in vivo* administration to mice and humans.

It has been known for several decades that psilocybin acts as a pro-drug for psilocin. According to Sard et al.[69], psilocin (EC_{50} = 24 nM) has more than 100-fold higher potency than psilocybin (EC_{50} = 3,475 nM) at h5-HT_{2A} receptors. By contrast, psilocin and psilocybin have equivalent molar potencies in humans[77]. Because psilocybin is rapidly metabolized to psilocin in human and animal tissues[15,32,37-39], the most parsimonious explanation for the discrepancy between the activity of psilocybin *in vivo* and *in vitro* is that psilocin is the active species in the CNS. Indeed, blocking the enzyme alkaline phosphatase using a competitive substrate (β-glycerophosphate) attenuates the behavioral response to psilocybin[39]. Similarly to psilocybin, there has been speculation that 4-AcO-DMT and its homologues may also act as prodrugs[56,71]. In the case of the 4-acetoxy-*N,N*-dialkyltryptamines, however, definite conclusions regarding their mechanism of action have not been possible because little was known about their pharmacological properties. Based on the present results, the 4-acetoxy-*N,N*-dialkyltryptamines have higher behavioral potency than would be anticipated based on their activity at the receptor level, which is consistant with the expectation that these compounds serve as pro-drugs for their 4-hydroxy counterparts. Nevertheless, controlled biotransformation studies are necessary to definitively show that the 4-acetoxy-*N,N*-dialkyltryptamines are acting as pro-drugs.

4-Hydroxy- and 4-acetoxy-*N,N*-dialkyltryptamines reportedly produce very similar effects in humans[71]. These two sets of compounds also produced very similar effects on the HTR. Nevertheless, a few of the 4-acetoxy-*N,N*-dialkyltryptamines (e.g., 4-AcO-DMT and 4-AcO-DET) produced larger peak responses in the HTR assay compared to their *O*-desacetyl counterparts. One possible explaination for these differences is that the acetoxy group may be facilitating

brain uptake. Transport of drugs across the blood-brain barrier is largely dependent on their lipophilicity[57]. In tryptamines, esterification of a free phenolic group can markedly enhance lipid solubility[22]. Alternatively, the 4-acetoxy group may enhance absorption from the injection site. The same phenomenon is believed to explain why the concentration of 6-monoacetylmorphine (6-MAM) in the brain is higher after heroin (3,6-diacetylmorphine) administration than after administration of an equimolar dose of 6-MAM[1]. Similarly to heroin, hydrolysis of 4-AcO-DMT and other homologues may occur rapidly in peripheral tissues and blood prior to brain uptake.

As far as we are aware, these are the first studies conducted with the *N*-allyl-*N*-methyl substituted tryptamine 4-HO-MALT. Structurally, this compound is closely related to 4-HO-MPT, with the *N*-propyl group in the latter compound replaced with an *N*-allyl group. Interestingly, 4-HO-MALT had about the same potency and efficacy as 4-HO-MPT at 5-HT_2 subtypes. These two compounds also had fairly similar potencies in the HTR assay (4-HO-MPT: ED_{50} = 1.92 μmol/kg; 4-HO-MALT: 2.24 μmol/kg). Thus, in *N,N*-disubstituted tryptamines, the presence of a single allyl substituent on the terminal amine does not have a detrimental effect on activity at the 5-HT_{2A} receptor. 4-HO-MALT likely acts as a serotonergic hallucinogen, with a potency similar to that of 4-HO-MPT. Consistent with these predictions, both 4-HO-MALT and its acetate ester are currently available online as New Psychoactive Substances.

The interaction of tryptamine hallucinogens with the 5-HT_{2B} receptor is noteworthy. Psilocin and its homologues have similar nanomolar potency at 5-HT_{2B} and act as less efficacious partial agonists compared to their activities at 5-HT_{2A}. Similar findings have been reported previously with psilocin[69], although conflicting data have also appeared[65]. These interactions are potentially significant because 5-HT_{2B} activation is responsible for valvular heart disease in patients treated chronically with ergot alkaloids such as methysergide, pergolide, cabergoline, ergonovine, and ergotamine[21,40,66,68]. Several other 5-HT_{2B} agonists have been linked to cardiac-valve disorders, including fenfluramine, dexfenfluramine, and 3,4-methylenedioxymethamphetamine (MDMA)[14,42]. The primary pulmonary hypertension observed

in patients treated chronically with fenfluramine and aminorex may also be mediated by 5-HT_{2B}[48,67]. Notably, some of the medications linked to these effects have about the same 5-HT_{2B} efficacy as psilocin. Ergonovine, for example, activates calcium flux via h5-HT_{2B} with an E_{max} of 39.7%[40]. Methylergonovine, the primary metabolite of methysergide[7,8,73], also acts as a partial agonist at h5-HT_{2B} (E_{max} = 49.5%)[40]. Recreational use of hallucinogens probably poses little risk of valvular heart disease because hallucinogen intake for recreational purposes is usually limited and occurs sporadically. However, repeated ingestion of low doses of hallucinogens (a practice known as *microdosing*) is becoming more common[1,41,60]. Given the high potency of psilocin and its homologues at 5-HT_{2B}, it should not be assumed that repeated, daily use of low doses poses no risk of valvular heart disease, especially considering our results with 4-HO-DPT and 4-HO-DIPT which show greater 5-HT_{2B} agonist efficacy compared to psilocin.

One of the goals of these studies was to examine how the *N,N*-dialkyl substitution pattern influences the interaction of tryptamine hallucinogens with 5-HT_2 subtypes. Previous SAR studies with tryptamine hallucinogens have focused on the influence of ring-substituents[4,13,24,44,51]. By contrast, there has been relatively little systematic investigation of the effect of the substituents on the side-chain nitrogen. To determine how the *N,N*-dialkyl substitution pattern influences potency at 5-HT_2 sites, we examined the effect of progressively lengthening one or both of the *N*-methyl groups in psilocin. Our studies showed that the size of the *N*-alkyl group has little effect on agonist potency at 5-HT_{2A} or 5-HT_{2B}, whereas potency at 5-HT_{2C} declined when there was a relatively bulky substituent on the terminal amine. Similar to our results, *N,N*-dimethyltryptamine (EC_{50} = 38.3 nM), *N,N*-diethyltryptamine (EC_{50} = 67.8 nM), *N,N*-dipropyltryptamine (EC_{50} = 26.1 nM), *N,N*-diisopropyltryptamine (EC_{50} = 33.5 nM), and *N*-methyl-*N*-isopropyltryptamine (EC_{50} = 44.9 nM) all have about the same potency in h5-HT_{2A} calcium flux assays[5]. Likewise, McKenna et al.[54] found that the *N*-alkyl group in had little effect on the 5-HT_{2A} affinity of *N,N*-dialkyltryptamines unless the groups were larger than isopropyl. Although the *N,N*-dialkyl substitution pattern does not appear to be an important determinant of

5-HT_{2A} agonist potency, it does seem to have an effect on potency in the HTR assay. Increasing the size or bulk of one or both of the alkyl chains tends to reduce HTR potency. It is not clear why the steric properties of the alkyl chains would affect activity *in vivo* but not *in vitro*, but DMT seems to be actively transported into brain tissue[78], so steric factors could potentially influence central transport mechanisms.

In summary, 4-substituted *N,N*-dialkyltryptamines activate 5-HT_{2A} receptors *in vitro* and *in vivo*. These findings support the classification of these compounds as psychedelic drugs. Indeed, the psychedelic effects produced by psilocybin and other hallucinogens are largely attributable to 5-HT_{2A} activation because ketanserin (a 5-HT_{2A} antagonist) blocks the response[35,45,46,61,76]. Additionally, the intensity of the subjective response to psilocybin is correlated with the level of central 5-HT_{2A} occupation, measured using the PET tracer [^{11}C]Cimbi-36 ([^{11}C]25B-NBOMe)[52]. We also found that 4-acetoxytryptamines are likely serving as prodrugs for the corresponding 4-hydroxytryptamines. In addition to activating 5-HT_{2A}, psilocin and its homologues also act as 5-HT_{2B} agonists, which is a potentially worrisome property. The findings in this report will facilitate predictions regarding the psychoactive potential of new tryptamine derivatives based on the substitution pattern on the terminal amine and the indole ring.

SUPPORTING INFORMATION

The results of the behavioral experiments, including HTR counts, statistical analyses, and ED_{50} values and 95% CI, are summarized in Table S1.

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Table 1. Potency of 4-hydroxy-*N,N*-dialkyltryptamines in head-twitch response (HTR) experiments conducted in C57BL/6J mice.

Drug	ED_{50} (95% CI)	
	mg/kg	μmol/kg
Psilocin (4-HO-DMT)	0.17 (0.12 – 0.23)	0.81 (0.57 – 1.15)
4-HO-MET	0.18 (0.12 – 0.27)	0.65 (0.44 – 0.97)
4-HO-MPT	0.67 (0.46 – 0.97)	1.92 (1.33 – 2.78)
4-HO-MIPT	0.86 (0.72 – 1.21)	2.97 (2.49 – 3.54)
4-HO-DET	0.42 (0.26 – 0.67)	1.56 (0.98 – 2.51)
4-HO-EPT	0.42 (0.26 – 0.68)	1.01 (0.63 – 1.62)
4-HO-DPT	0.79 (0.46 – 1.35)	2.47 (1.43 – 4.25)
4-HO-DIPT	1.03 (0.67 – 1.57)	3.46 (2.27 – 5.28)
4-HO-MALT	0.91 (0.59 – 1.40)	2.24 (1.45 – 3.46)

Table 2. Potency of 4-acetoxy-*N,N*-dialkyltryptamines in head-twitch response (HTR) experiments conducted in C57BL/6J mice.

Drug	ED_{50} (95% CI)	
	mg/kg	μmol/kg
4-AcO-DMT	0.41 (0.24 – 0.69)	1.12 (0.66 – 1.90)
4-AcO-MET	0.44 (0.31 – 0.63)	1.17 (0.82 – 1.67)
4-AcO-MPT	0.55 (0.37 – 0.81)	1.41 (0.96 – 2.08)
4-AcO-MIPT	1.11 (0.87 – 1.41)	2.84 (2.22 - 3.62)
4-AcO-DET	0.71 (0.47 – 1.07)	1.81 (1.20 – 2.74)
4-AcO-EPT	0.51 (0.35 – 0.74)	1.26 (0.86 – 1.84)
4-AcO-DPT	0.55 (0.35 – 0.89)	1.32 (0.82 – 2.12)
4-AcO-DIPT	1.41 (0.95 – 2.08)	3.88 (2.63 – 5.72)

Table 3. Gq-mediated calcium flux of 4-substituted tryptamines at 5-HT_2 receptor subtypes.
All data were acquired with at least three independent experiments performed in triplicate and in parallel. *N.T.*, not tested

Compound	**5-HT_{2A}**			**m5-HT_{2A}**			**5-HT_{2B}**			**5-HT_{2C}**		
	EC_{50} (nM)	pEC_{50} (± SEM)	E_{max} % 5-HT (± SEM)	EC_{50} (nM)	pEC_{50} (± SEM)	E_{max} % 5-HT (± SEM)	EC_{50} (nM)	pEC_{50} (± SEM)	E_{max} % 5-HT (± SEM)	EC_{50} (nM)	pEC_{50} (± SEM)	E_{max} % 5-HT (± SEM)
5-HT	0.26	9.58±0.04	100	0.16	9.81±0.06	100	0.21	9.67±0.03	100	0.25	9.60±0.04	100
Psilocin	2.40	8.62±0.05	98.4±1.3	3.21	8.49±0.04	95.7±1.0	2.37	8.63±0.08	39.2±0.8	21.8	7.66±0.07	85.1±1.9
4-HO-MET	4.04	8.39±0.04	96.8±1.1	2.49	8.60±0.04	98.2±1.2	2.64	8.58±0.05	43.8±0.6	29.7	7.53±0.06	90.8±1.7
4-HO-MPT	3.82	8.42±0.04	98.1±1.1	2.85	8.55±0.05	99.9±1.3	3.40	8.47±0.04	58.4±0.7	45.8	7.34±0.07	82.9±2.1
4-HO-MIPT	5.20	8.28±0.04	99.6±1.2	6.51	8.21±0.03	103±1.0	10.3	7.99±0.12	49.1±1.8	166	6.78±0.10	76.2±2.8
4-HO-DET	6.47	8.19±0.04	100±1.2	4.19	8.38±0.04	105±1.1	6.27	8.20±0.04	71.1±0.8	264	6.58±0.04	80.4±1.3
4-HO-EPT	3.15	8.50±0.04	99.5±1.2	1.88	8.73±0.04	101±1.1	4.34	8.36±0.03	89.2±0.7	129	6.89±0.06	89.0±2.1
4-HO-DPT	1.64	8.79±0.04	103±1.0	1.28	8.89±0.04	101±0.9	2.23	8.65±0.03	94.1±0.7	212	6.67±0.06	83.3±2.0
4-HO-DIPT	6.82	8.17±0.04	102±1.3	4.37	8.36±0.04	101±1.1	5.12	8.29±0.02	97.0±0.6	1408	5.85±0.05	91.4±2.6
4-HO-MALT	3.67	8.43±0.04	101±1.2	3.81	8.42±0.04	102±1.2	3.16	8.50±0.05	60.4±0.8	44.2	7.36±0.07	82.7±2.0
4-AcO-DMT	103	6.99±0.02	79.2±0.7	*N.T.*			100	7.00±0.13	22.1±1.0	268	6.57±0.08	22.8±0.6
4-AcO-MET	92.3	7.04±0.03	94.3±1.1	*N.T.*			45.7	7.34±0.06	48.9±1.0	575	6.24±0.06	41.2±0.9
4-AcO-MPT	42.4	7.37±0.03	96.0±1.1	*N.T.*			28.8	7.54±0.06	62.7±1.2	401	6.40±0.05	32.7±0.6
4-AcO-MIPT	43.9	7.36±0.04	93.2±1.4	*N.T.*			44.7	7.35±0.08	49.1±1.3	542	6.27±0.16	33.7±2.1
4-AcO-DET	184	6.74±0.02	90.7±0.8	*N.T.*			79.4	7.10±0.07	64.2±1.6	851	6.07±0.04	53.5±1.0
4-AcO-EPT	66.4	7.18±0.03	98.1±0.9	*N.T.*			24.0	7.62±0.04	95.8±1.2	315	6.50±0.04	87.6±1.4
4-AcO-DPT	23.7	7.63±0.04	100±1.0	*N.T.*			5.50	8.26±0.06	97.4±1.8	991	6.00±0.05	68.2±1.5
4-AcO-DIPT	70.7	7.15±0.16	74.6±4.3	*N.T.*			70.8	7.15±0.04	93.0±1.2	1303	5.89±0.06	80.8±2.4

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