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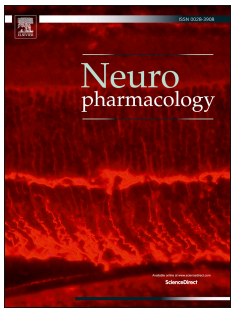

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Receptor binding profiles and behavioral pharmacology of ring-substituted *N*,*N*-diallyltryptamine analogs

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ABSTRACT

Substantial effort has been devoted toward understanding the psychopharmacological effects of tryptamine hallucinogens, which are thought to be mediated by activation of 5-HT_{2A} and 5-HT_{1A} receptors. Recently, several psychoactive tryptamines based on the *N*,*N*-diallyltryptamine (DALT) scaffold have been encountered as recreational drugs. Despite the apparent widespread use of DALT derivatives in humans, little is known about their pharmacological properties. We compared the binding affinities of DALT and its 2-phenyl-, 4-acetoxy-, 4-hydroxy-, 5-methoxy-, 5-methoxy-2-methyl-, 5-fluoro-, 5-fluoro-2-methyl-, 5-bromo-, and 7-ethyl-derivatives at 45 receptor and transporter binding sites. Additionally, studies in C57BL/6J mice examined whether these substances induce the head twitch response (HTR), a 5-HT2A receptor-mediated response that is widely used as a behavioral proxy for hallucinogen effects in humans. Most of the test drugs bound to serotonin receptors, σ sites, α2-adrenoceptors, dopaminergic D3 receptors, histaminergic H1 receptors, and the serotonin transporter. DALT and several of the ring-substituted derivatives were active in the HTR assay with the following rank order of potency: 4-acetoxy-DALT > 5-fluoro-DALT > 5-methoxy-DALT > 4-hydroxy-DALT > DALT > 5-bromo-DALT. 2-Phenyl-DALT, 5-methoxy-2-methyl-DALT, 5-fluoro-2-methyl-DALT, and 7-ethyl-DALT did not induce the HTR. HTR potency was not correlated with either 5-HT1A or 5-HT2A receptor binding affinity, but a multiple regression analysis indicted that 5-HT2A and 5-HT1A receptors make positive and negative contributions, respectively, to HTR potency (R^2 = 0.8729). In addition to supporting the established role of 5-HT2A receptors in the HTR, these findings are consistent with evidence that 5-HT1A activation by tryptamine hallucinogens buffers their effects on HTR.

Keywords: hallucinogen; psychedelic; mice; head twitch; 5-methoxy-N,N-diallyltryptamine; 5-MeO-DALT; 4-acetoxy-N,N-diallyltryptamine; 4-AcO-DALT; 4-hydroxy-N,N-diallyltryptamine.

1. INTRODUCTION

Over the past decade there has been a renewed focus on the pharmacology and effects of serotonergic hallucinogens. This focus has been driven, in part, by accumulating evidence that serotonergic hallucinogens may have therapeutic efficacy against anxiety, depression, substance abuse, and obsessive-compulsive disorder (Bogenschutz and Ross 2017). Additionally, although hallucinogen use has remained relatively stable over the past few decades, there has been a marked increase in the availability and diversity of hallucinogens in recent years that has resulted in numerous reports of untoward effects. Some of these hallucinogens are derived from *N*,*N*-diallyltryptamine (DALT). 5-Methoxy-*N*,*N*-diallyltryptamine (5-MeO-DALT), for example, was first synthesized by Alexander T. Shulgin (A.T. Shulgin, personal communication), and was first marketed via the Internet in 2004 (Corkery et al. 2012). According to Shulgin, oral doses of 12-20 mg produce psychoactive effects with a rapid onset and a relatively brief duration of 2-4 h (Shulgin and Shulgin 2004). Subsequently, 5-MeO-DALT and other DALT derivatives have become popular recreational hallucinogen; 5-MeO-DALT has been identified in many seized samples (Nagai et al. 2007; Rasanen et al. 2014; Strano Rossi et al. 2014; Odoardi et al. 2016; Brunt et al. 2017) and DALT and 4-acetoxy-*N*,*N*-diallyltryptamine (4-AcO-DALT) have also been detected (EMCDDA 2008,2013,2015).

Despite the widespread distribution and nonmedical use of diallyltryptamines (DALTs), very little is known about their pharmacology. It was previously reported that six DALT compounds bind non-selectively to 27 different receptors including 5-HT receptors (Cozzi and Daley 2016), and 5-MeO-DALT has been shown to act as a 5-HT_{2A} agonist (Arunotayanun et al. 2013). However, few animal behavioral assessments have been performed with these compounds, and the resulting information could provide insight into the relationship between

receptor binding and the behavioral effects of these drugs. Hence, the binding of DALT and nine ring-substituted DALTs (see Fig. 1) were assessed at 45 receptor and transporter binding sites.

Serotonergic hallucinogens produce the head twitch response (HTR), a brief paroxysmal head rotation in rats and mice, via activation of the 5-HT_{2A} receptor (Schreiber et al. 1995; Canal and Morgan 2012; Halberstadt and Geyer 2013), the same receptor responsible for the psychedelic effects of hallucinogens in humans (Quednow et al. 2012; Kometer et al. 2013; Valle et al. 2016; Kraehenmann et al. 2017; Preller et al. 2017b,a). 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 (Gonzalez-Maeso et al. 2007). We employed HTR studies with the ten DALT compounds in C57BL/6J mice to test whether these tryptamines produce LSD-like behavioral effects *in vivo*.

In addition to producing effects via the 5-HT_{2A} receptor, tryptamine hallucinogens also bind to 5-HT_{1A} receptors with moderate to high affinity and efficacy (McKenna et al. 1990; Blough et al. 2014; Rickli et al. 2016). The HTR induced by hallucinogens is attenuated by administration of 5-HT_{1A} receptor agonists such as 8-OH-DPAT, ipsapirone, and buspirone (Darmani et al. 1990; Schreiber et al. 1995; Kleven et al. 1997), which is consistent with evidence for countervailing interactions between 5-HT_{1A} and 5-HT_{2A} receptors (Araneda and Andrade 1991; Ashby et al. 1994; Krebs-Thomson and Geyer 1998; Amargos-Bosch et al. 2004; Li et al. 2011). In light of this apparent cross-talk, one unanswered question is whether the ability of tryptamine hallucinogens to induce the HTR via 5-HT_{2A} activation is modulated by their concurrent effects on 5-HT_{1A} receptors. Pretreatment with the mixed 5-HT_{1A}/β-adrenergic antagonist pindolol markedly augments the subjective response induced by the hallucinogen

N,*N*-dimethyltryptamine (DMT) in human volunteers, suggesting that 5-HT_{1A} activation by DMT may blunt its 5-HT_{2A}-mediated effects (Strassman 1996). Based on those findings, we hypothesized that 5-HT_{1A} activation by tryptamine hallucinogens may buffer their ability to induce the HTR in mice.

One way to gauge the involvement of 5-HT_{1A} receptors in the behavioral response to hallucinogens is to assess the effect of combined administration with a 5-HT_{1A} antagonist. The possibility exists, however, that 5-HT_{1A} antagonists might alter the potency of 5-HT_{2A} receptor-mediated responses due to interactions that are known to occur between the receptors (Krebs-Thomson and Geyer 1998; Salmi and Ahlenius 1998; Li et al. 2011). Indeed, 5-HT_{1A} antagonists can augment the HTR induced by hallucinogen administration (Willins and Meltzer 1997), and under certain conditions can even induce head twitches through a mechanism involving indirect activation of 5-HT_{2A} receptors (Darmani and Reeves 1996; Darmani 1998; Fox et al. 2010). As an alternative to conducting antagonist blockade studies, receptor binding studies were conducted with DALT derivatives and regression analyses were performed to determine whether potency in the HTR assay is correlated with 5-HT_{2A} and/or 5-HT_{1A} receptor affinities.

2. MATERIALS AND METHODS

2.1. Subjects

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 conducted in accordance with NIH guidelines and were approved by the UCSD animal care committee.

2.2. Drugs

The following drugs were tested: *N*,*N*-diallyltryptamine hydrochloride (DALT), 5-methoxy-*N*,*N*-diallyltryptamine hydrochloride (5-MeO-DALT), 5-fluoro-*N*,*N*-diallyltryptamine hydrochloride (5-F-DALT), 5-bromo-*N*,*N*-diallyltryptamine hydrochloride (5-Br-DALT), 4-hydroxy-*N*,*N*-diallyltryptamine fumarate (4-HO-DALT), 4-acetoxy-*N*,*N*-diallyltryptamine fumarate (4-AcO-DALT), 2-phenyl-*N*,*N*-diallyltryptamine hydrochloride (2-Ph-DALT), 5-methoxy-2-methyl-*N*,*N*-diallyltryptamine hydrochloride (5-MeO-2-Me-DALT), 5-fluoro-2-methyl-*N*,*N*-diallyltryptamine hydrochloride (5-F-2-Me-DALT), and 7-ethyl-*N*,*N*-diallyltryptamine hydrochloride (7-Et-DALT). 4-AcO-DALT fumarate and 4-HO-DALT hemifumarate were obtained from Scientific Supplies (London, UK); the other tryptamines were synthesized, fully characterized, and available from previous studies (Meyer et al. 2014; Michely et al. 2015; Dinger et al. 2016; Brandt et al. 2017a; Caspar et al. 2017; Michely et al. 2017).

2.3. Binding studies

A screening at 45 receptor and transporter binding sites was performed by the NIMH Psychoactive Drug Screening Program (NIMH PDSP). Most of these screenings were performed with cloned human receptors; exceptions are listed in Table 1. Test compounds were dissolved in DMSO and were tested at 10 µM in competition assays against radioactive probe compounds. Sites exhibiting > 50% inhibition at 10 µM were tested in secondary assays at the identified receptor or transporter using 12 concentrations of the DALT compound, measured in triplicate, to generate competition binding isotherms. K_i values were obtained from nonlinear regression of these binding isotherms from best-fit IC_{50} values using the Cheng-Prusoff equation (Cheng and Prusoff 1973). K_i values were converted to pK_i values for data analysis. The radioligands used were as follows: [^{3}H]8-OH-DPAT (5-HT_{1A}), [^{3}H]GR125743 (5-$HT_{1B/1D}$), [^{3}H]5-HT (5-HT_{1E}), [^{3}H]ketanserin (5-HT_{2A}), [^{3}H]LSD (5-$HT_{2B/5A/6/7}$), [^{3}H]mesulergine (5-HT_{2C}), [^{3}H]citalopram (serotonin transporter), [^{3}H]prazocin ($\alpha_{1A/1B/1D}$), [^{3}H]rauwolscine ($\alpha_{2A/2B/2C}$), [^{125}I]pindolol (β_1), [^{3}H]CGP12177 (β_2, β_3), [^{3}H]nisoxetine (norepinephrine transporter), [^{3}H]SCH23390 (D_1, D_5), [^{3}H]*N*-methylspiperone ($D_{2/3/4}$), [^{3}H]WIN35428 (dopamine transporter), [^{3}H]DAMGO (μ-opioid), [^{3}H]DADLE (δ-opioid), [^{3}H]U69593 (κ-opioid), [^{3}H]muscimol ($GABA_A$), [^{3}H]funitrazepam (central benzodiazepine), [^{3}H]PK11195 (peripheral benzodiazepine), [^{3}H]pyrilamine (H_1), [^{3}H]tiotidine (H_2), [^{3}H]α-methylhistamine (H_3), [^{3}H]histamine (H_4), [^{3}H]QNB ($M_{1–5}$), [^{3}H](+)-pentazocine (σ_1), and [^{3}H]DTG (σ_2). The experimental protocols are available from the NIMH PDSP website (Roth 2013).

2.4. Head-twitch response

The head twitch response (HTR) was assessed using a head-mounted magnet and a magnetometer detection coil (Halberstadt and Geyer 2013,2014; Nichols et al. 2015). Briefly, mice were anesthetized and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a two-week recovery period, HTR experiments were carried out in a well-lit room with at least 7-days between sessions to avoid carryover effects. Test compounds were dissolved in water containing 5% Tween 80 and administered IP at a volume of 5 or 10 mL/kg body weight immediately prior to testing. Mice (n=5–6/group) were injected with drug or vehicle and then HTR activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 minutes. Coil voltage was low-pass filtered (2–10 kHz cutoff frequency), amplified, and digitized (20 kHz sampling rate) using a Powerlab/8SP with LabChart v 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). Head twitches were identified manually based on the following criteria: 1) sinusoidal wavelets; 2) evidence of at least two 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 succeeding each response.

2.5. Data analysis

Head twitch 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. The entire 30-min recordings were examined for head twitches, but in some cases a shorter block of time was used for analysis to accommodate compounds with a brief duration-

of-action (potency calculations can be confounded by extended periods of inactivity). ED_{50} values and 95% confidence limits were calculated using nonlinear regression. Relationships between HTR potency and binding affinities were assessed using linear regression and ordinary least-squares regression. For all analyses, significance was demonstrated by surpassing an α-level of 0.05.

3. RESULTS

3.1. Receptor binding

DALT and 9 ring-substituted derivatives were submitted to the NIMH PDSP for examination of their binding profiles at 45 neurotransmitter receptors and transporters. K_i values were determined for compounds that produced > 50% displacement of a radioactive probe compound at a concentration of 10,000 nM. The results are summarized in Table 1. The data for DALT and several of its 5-substituted derivatives (5-MeO-DALT, 5-F-DALT, and 5-Br-DALT) were reported in a previous publication (Cozzi and Daley 2016). All of the compounds were devoid of 50% displacement at M_1-M_5 muscarinic, β_1-β_3 adrenergic, H_4 histaminergic, central benzodiazepine sites (labeled with [^{3}H]flunitrazepam), and $GABA_A$ receptors.

As reported previously (Cozzi and Daley 2016), DALT binds relatively non-selectively to 5-HT_1 and 5-HT_2 subtypes, σ_1 and σ_2 sites, α_2-adrenoceptors, dopaminergic D_3 receptors, histaminergic H_1 receptors, and the 5-HT transporter (SERT). DALT had the highest measured affinities for 5-HT_{2B} (K_i = 61 nM), 5-HT_{1A} (K_i = 100 nM), σ_1 (K_i = 101 nM), α_{2A} (K_i = 124 nM), H_1 (K_i = 127 nM) and SERT (K_i = 150 nM). Incorporation of an oxygenated substituent at the 4-

position altered the binding pattern of DALT. Compared to DALT, the 4-hydroxy and 4-acetoxy derivatives showed several-fold lower affinities for 5-HT_{1A}, 5-HT_{2C}, α_{2A}-adrenergic receptors, σ_1 and σ_2 sites, and SERT, whereas 5-HT_7 receptor affinity was increased by at least an order of magnitude. 4-Hydroxy-DALT also had low affinity for 5-HT_{2B} receptors (K_i = 2593 nM) and moderately high affinity for 5-HT_6 receptors (K_i = 213 nM).

The 2-phenyl-substituted DALT derivative (2-Ph-DALT) showed a notable binding profile. The 5-HT_{2A} binding affinity of 2-Ph-DALT (K_i = 13 nM) was 54-fold higher than the affinity of DALT (K_i = 701 nM) and at least 10-fold higher than the affinity of any other DALT derivative. According to a previous report (Stevenson et al. 2000), 2-aryl-tryptamines such as 2-phenyl-*N*,*N*-dimethyltryptamine and 2-phenyl-*N*,*N*-diethyltryptamine act as 5-HT_{2A} receptor antagonists and have high affinity (K_i values of 4.4 nM and 2.8 nM, respectively, vs. [^{3}H]ketanserin). 2-Ph-DALT was the only compound tested herein that bound to D_1, D_4, D_5, H_2, δ-opioid, and peripheral benzodiazepine receptors with a K_i value < 10 µM. Compared to the other compounds, 2-Ph-DALT also had relatively high affinity for α_{1A} and α_{1D} adrenoceptors and D_2 receptors. By contrast, 2-phenyl substitution abolished binding to σ_1 sites and SERT.

The 2-methyl derivatives of 5-MeO-DALT and 5-F-DALT were also examined. Incorporation of a 2-methyl group tended to reduce the affinity of those DALT derivatives for 5-HT receptors and SERT. The affinities of 5-MeO-DALT and 5-F-DALT for 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, and 5-HT_{2C} receptors were consistently reduced by 2-methylation (see Table 1). Likewise, the binding of 5-MeO-DALT to SERT (K_i = 499 nM) was abolished by 2-methylation (5-MeO-2-Me-DALT: < 50% displacement at 10,000 nM), whereas the affinity of 5-F-DALT (K_i = 36 nM) was reduced almost 30-fold (5-F-2-Me-DALT; K_i = 983 nM).

Although 7-ethyl-substitution tended to reduce the binding affinity of DALT for most sites (including 5-HT_{1A} and 5-HT_{2A} receptors), the affinity of 7-Et-DALT for σ_1 sites (K_i = 22 nM) was nearly 5-fold higher than the parent compound.

3.2. Head twitch response

DALT induced the HTR in mice with an ED_{50} of 3.42 mg/kg. Compared to other *N*,*N*-disubstituted tryptamines such as *N*,*N*-dipropyltryptamine and *N*,*N*-diisopropyltryptamine (Smith et al. 2014), DALT had relatively low potency. Similar to other tryptamine derivatives (Fantegrossi et al. 2008a), the response to DALT followed an inverted-U-shaped dose-response function (see Table 2).

Ring-substitution on the DALT molecule resulted in active compounds, some of which were more potent than DALT (see Table 2). The 4-hydroxy and 5-methoxy derivatives induced the HTR with almost twice the potency of DALT. 4-Acetoxy- or 5-fluoro-substitution produced even greater increases in potency. By contrast, 5-bromo substitution did not significantly alter HTR potency relative to DALT. Substitution at the 2-position with either a methyl or a phenyl group (e.g., 2-Ph-DALT, 2-Me-5-MeO-DALT, 2-Me-5-F-DALT) abolished activity in the HTR assay. Similarly, 7-Et-DALT did not induce the HTR. In addition to having higher potency than DALT, the 4-hydroxy and 4-acetoxy derivatives produced a HTR with an extremely rapid onset (data not shown).

For DALT and its active derivatives, there was no correlation between HTR potency (ED_{50} values) and 5-HT_{1A} receptor affinity (R^2 = 0.2804; $F(1,4)$ = 1.56, *NS*) or 5-HT_{2A} receptor affinity (R^2 = 0.1646; $F(1,4)$ = 0.79, *NS*). A multiple regression analysis was performed to test

whether HTR potency is predicted by both 5-HT_{1A} and 5-HT_{2A} affinity. The ordinary least-squares (OLS) regression revealed that 5-HT_{1A} and 5-HT_{2A} binding affinities significantly predicted HTR potency (R^2 = 0.8729; $F(2,3) = 10.31$, $p < 0.05$; Figure 2). Both 5-HT_{2A} affinity ($\beta = 0.741$, $t(3) = 3.74$, $p < 0.04$) and 5-HT_{1A} affinity ($\beta = –0.279$, $t(3) = –4.09$, $p < 0.03$) contributed significantly to the prediction, indicating that 5-HT_{2A} and 5-HT_{1A} receptors make positive and negative contributions, respectively, to HTR potency. In addition to 5-HT_{1A} and 5-HT_{2A} receptors, several other monoaminergic sites can influence HTR expression, including 5-HT_{2C} receptors (Fantegrossi et al. 2010), SERT (Basselin et al. 2009), and α_2-adrenoceptors (Schreiber et al. 1995). To test whether these other receptors play a role in the HTR induced by DALT derivatives, additional regression analyses were performed for sites with K_i < 10,000 nM. There was no correlation between HTR potency and affinity at 5-HT_{2C} (R^2 = 0.0292; $F(1,4)$ = 0.12, *NS*), SERT (R^2 = 0.0661; $F(1,4) = 0.28$, *NS*), or α_{2A} sites (R^2 = 0.2197; $F(1,4) = 1.12$, *NS*). Furthermore, affinity for these sites did not significantly predict HTR potency when analyzed in combination with 5-HT_{2A} receptor affinity using multiple regression (data not shown).

4. DISCUSSION

The potency and 5-HT receptor affinities of tryptamine hallucinogens are influenced by the substituent groups present on the indole nucleus and amine nitrogen. Most compounds in this structural class contain *N*,*N*-dialkyl substituents, but tryptamines containing *N*,*N*-diallyl groups have also been synthesized (Brandt et al. 2017a). Although the structure-activity relationships and pharmacology of dialkyltryptamines such as DMT and psilocybin have been widely investigated, relatively little is known about the comparative properties of diallyltryptamines.

The present studies were conducted to investigate the pharmacology and behavioral effects of DALT and a variety of ring-substituted derivatives, some of which are used recreationally as new psychoactive substances or “research chemicals" and reportedly have hallucinogenic effects.

Consistent with the effects of other tryptamine hallucinogens (Fantegrossi et al. 2006; Fantegrossi et al. 2008b; Halberstadt et al. 2011; Carbonaro et al. 2015; Nichols et al. 2015), DALT and several of its derivatives substituted at the 4 or 5 position induced head twitches in mice. Although our studies measured 5-HT_{2A} binding affinity and did not include a functional assessment of receptor activation, DALT, 4-HO-DALT, 4-AcO-DALT, 5-Br-DALT, 5-F-DALT and 5-MeO-DALT are likely to be 5-HT_{2A} agonists based on their effects in the HTR assay. Importantly, 5-MeO-DALT was previously reported to act as an agonist at recombinant human 5-HT_{2A} receptors (Arunotayanun et al. 2013). Similarly, it was recently reported (Gatch et al. 2017) that 5-MeO-DALT produces full substitution in rats trained to discriminate the hallucinogenic 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-methylamphetamine (DOM). Since the head twitch assay is routinely used to test whether 5-HT_{2A} agonists produce LSD-like behavioral effects (Gonzalez-Maeso et al. 2007), the ability of diallyltryptamines to induce the HTR and produce DOM-like stimulus effects is thus consistent with their classification as serotonergic hallucinogens. However, few details have been published regarding the effects of these compounds in humans.

Notably, the potency of the diallyltryptamines in the HTR assay is not correlated with 5-HT_{2A} receptor binding affinity alone but is dependent on activity at *both* 5-HT_{1A} and 5-HT_{2A} receptors. According to the multiple regression analysis, there is a positive relationship between HTR potency and 5-HT_{2A} affinity and a negative relationship between HTR potency and 5-HT_{1A} affinity; in other words, HTR potency increases as 5-HT_{2A} affinity increases and decreases as 5-

HT_{1A} affinity increases. As noted earlier, the hallucinogen HTR occurs as a result of 5-HT_{2A} activation and can be suppressed by concurrent administration of a 5-HT_{1A} agonist (Darmani et al. 1990; Schreiber et al. 1995; Kleven et al. 1997). Based on the roles that 5-HT_{1A} and 5-HT_{2A} receptors are known to play in the hallucinogen HTR, the regression analysis can be interpreted as showing that 5-HT_{2A} activation by DALT and its derivatives mediates the HTR, whereas their interaction with the 5-HT_{1A} receptor has a countervailing influence that inhibits expression of head twitch behavior. Hence, the potency of diallyltryptamines in the HTR assay may ultimately be determined by their combined activities at 5-HT_{1A} and 5-HT_{2A} receptors. These findings support the hypothesis that 5-HT_{1A} activation by tryptamine hallucinogens buffers their effects on the HTR.

Based on the ability of 5-HT_{1A} agonists to inhibit the HTR, there has been speculation that 5-HT_{1A} stimulation by nonselective tryptamine and lysergamide hallucinogens may reduce or inhibit the frequency of their induced head twitch behavior (Darmani et al. 1990). Our recent work has demonstrated that the LSD analog and non-selective 5-HT_{1A}/5-HT_{2A} agonist lysergic acid morpholide (LSM-775) does not induce the HTR in mice unless the animals are pretreated with the 5-HT_{1A} antagonist WAY-100635 (Brandt et al. 2017b), indicating that 5-HT_{1A} activation by LSM-775 masks its ability to induce the HTR. As far as we are aware, however, the present study is the first to show that the *potency* of the HTR induced by tryptamine hallucinogens may be influenced by their 5-HT_{1A} interactions. Nevertheless, these findings remain tentative given to the small number of compounds tested; follow-up studies with a larger group of tryptamines are necessary to achieve more definitive results.

~~The absence of a direct correlation between 5-HT_{2A} binding affinity and HTR ED_{50} values is surprising because the potency of hallucinogens in the drug discrimination paradigm is~~

known to correlate with 5-HT_{2A} binding affinity (Glennon et al. 1984; Titeler et al. 1988). For example, there is a significant correlation (r = 0.938) between the 5-HT_{2A} affinities of 22 hallucinogens and their ED_{50} values in rats trained to discriminate 1.0 mg/kg DOM (Glennon et al. 1984). Although these discrepant findings could potentially reflect mechanistic differences between HTR and drug discrimination, a simpler explanation is that the lack of congruence is a consequence of testing hallucinogens with different degrees of selectivity for 5-HT_{1A} and 5-HT_{2A} receptors. Most of the hallucinogens included in the Glennon et al. (1984) study are phenylalkylamine derivatives — compounds with high 5-HT_{2A}/5-HT_{1A} selectivity — so their correlation analysis was designed to minimize confounding interactions with 5-HT_{1A} receptors. By contrast, the diallyltryptamines are relatively nonselective and hence testing these compounds would increase the likelihood of detecting functional interactions between 5-HT_{1A} and 5-HT_{2A} receptors. One caveat is that it is not clear whether the 5-HT_{1A} receptor can regulate hallucinogen discriminative stimulus effects in the same manner as the HTR. 8-OH-DPAT reportedly inhibits the stimulus effects of DOM in monkeys, but the same interaction has not been observed in rats (Li et al. 2010). According to Kleven et al. (1997), pretreatment with certain doses of the 5-HT_{1A} agonists 8-OH-DPAT and buspirone produced a slight reduction of drug-lever selection in rats trained to discriminate 0.63 mg/kg DOI, although the reduction did not exceed 33%. Nevertheless, we have found robust correlations between drug discrimination- and HTR-derived ED_{50} values for both phenylalkylamine and tryptamine hallucinogens (Halberstadt et al., unpublished findings), demonstrating that potencies in these two behavioral assays are likely governed by similar mechanistic factors.

One potential confound for the regression analysis is that the binding studies were performed with cloned human 5-HT receptors whereas the behavioral experiments were

performed in mice. Sequence differences between rodent and human 5-HT receptors can result in ligand binding affinity differences (Kao et al. 1992; Oksenberg et al. 1992; Parker et al. 1993; Smolyar and Osman 1993). There are reportedly species differences in the affinities of 4-hydroxytryptamines for the 5-HT_{2A} receptor, which are potentially relevant to our studies with 4-HO-DALT and 4-AcO-DALT. Specifically, according to Gallaher et al. (1993), who studied human and rat 5-HT_{2A} receptors labeled with [^{3}H]ketanserin, 4-hydroxy-DMT (psilocin) has 15-fold higher affinity for the human receptor (K_i = 340 nM) than for the rat receptor (K_i = 5,100 nM), whereas its 5-hydroxy isomer bufotenine has nearly equal affinities for the human and rat receptors (K_i values of 300 nM and 520 nM, respectively). The human 5-HT_{2A} receptor contains a serine at position 242 in helix V whereas alanine is present in the receptor in rodents, leading Gallaher et al. (1993) to speculate that psilocin may have higher affinity for the human receptor because Ser-242$^{(5.42)}$ can form a hydrogen-bond with the 4-hydroxyl group in psilocin. Other studies, however, failed to confirm their findings. Another group reported that both psilocin and bufotenine displace [^{125}I]*R*-(–)-DOI binding to 5-HT_{2A} receptors in rat cortex with high affinity and have nearly equivalent IC_{50} values (McKenna et al. 1990). Furthermore, Ser-242$^{(5.42)}$ in the human 5-HT_{2A} receptor is believed to form a hydrogen-bond with the indole *N*1 nitrogen of tryptamines and ergolines based on mutagenesis experiments and molecular modeling (Nelson et al. 1993; Johnson et al. 1994; Almaula et al. 1996; Wacker et al. 2017), abrogating the structural basis for the species differences posited by Gallaher. Therefore, although there is no clear evidence indicating that differences between human and mouse 5-HT receptors are likely to confound our regression analysis, especially with regard to 4-substituted DALT derivatives, the potential existence of cross-species differences in 5-HT receptor pharmacology must be acknowledged as a source of potential error for the regression.

DALT and derivatives substituted at the 5-position have been shown to bind to multiple 5-HT receptors, as well as α_2 adrenergic subtypes, σ_1 and σ_2 sites, histamine H_1 receptors, and SERT (Cozzi and Daley 2016). As shown in the present investigation, substitution at other positions in the indole ring can markedly alter the binding profile of DALT. The 4-substituted derivatives displayed reduced affinity at 5-HT_{1A} receptors compared to DALT and the 5-substituted derivatives. This is consistent with reports demonstrating that 4-hydroxy-DMT (psilocin) binds to 5-HT_{1A} sites with 20-fold lower affinity compared to its 5-hydroxy isomer (bufotenine) or the 5-hydroxy *O*-methyl derivative (5-methoxy-DMT), whereas there is little difference between their 5-HT_{2A} receptor affinities (McKenna et al. 1990; Blair et al. 2000).

Addition of a methyl group to the 2-position of 5-MeO-DALT reduced its affinity for most 5-HT binding sites, including 5-HT_{1A} and 5-HT_{2A} receptors, and abolished its ability to induce the HTR in mice at doses up to 14 mg/kg. These findings parallel those of Glennon et al. (2000), who found that 2-methylation or 2-ethylation of 5-methoxy-DMT reduced its affinity for 5-HT_{2A} receptors. Similarly, although 2-methyl-5-methoxy-DMT is a hallucinogen in humans, it reportedly has significantly lower potency than 5-methoxy-DMT (Shulgin and Shulgin 1997). The 5-HT_{2A} receptor apparently has difficulty accommodating tryptamines with a 2-alkyl substituent.

2-Ph-DALT did not induce the HTR despite having the highest 5-HT_{2A} affinity of any compound screened (K_i = 13 nM). According to Stevenson et al. (2000), various 2-phenyl-*N*,*N*-dialkyltryptamines including the *N*,*N*-dimethyl, *N*,*N*-diethyl, and *N*-methyl-*N*-ethyl homologues bind to the 5-HT_{2A} receptor with high (nM) affinities. However, all of these compounds blocked the stimulatory effect of 5-HT on phosphoinositide hydrolysis in CHO cells expressing the human 5-HT_{2A} receptor. In light of the fact that other 2-phenyl-*N*,*N*-disubstituted tryptamines act

as antagonists, the failure of 2-Ph-DALT to induce the HTR suggests that it may also act as a 5-HT_{2A} antagonist.

The 7-ethyl-substituted derivative of DALT also had low affinity for 5-HT_{1A} and 5-HT_{2A} receptors and did not induce the HTR in mice when tested at 15 mg/kg. These findings are consistent with the behavioral effects of other 7-ethyl-substituted tryptamines. 7-Ethyl-DMT produces only partial substitution in rats trained to discriminate 5-MeO-DMT from vehicle (Glennon et al. 1980a). Rats trained to discriminate the interoceptive cue produced by 5-MeO-DMT generalize to other serotonergic hallucinogens (Glennon et al. 1980b; Young et al. 1982); hence, the absence of full substitution with 7-ethyl-DMT indicates that it does not produce hallucinogen-like stimulus effects in rodents.

~~In addition to their relatively high potency in the HTR assay, the responses produced by 4-HO-DALT and 4-AcO-DALT had a rapid onset relative to other diallyltryptamines, with effects occurring almost immediately following administration. It is thus likely that these compounds enter the brain rapidly. Given the relatively rapid onset of the effects of 5-MeO-DALT in humans (e.g. peak effects occurring 30 min after p.o. dosing), one may expect an even faster onset for the 4-substituted DALT derivatives.~~

~~The almost doubled molar potency of 4-AcO-DALT relative to 4-HO-DALT in mice is another notable finding. 4-Hydroxytryptamine esters are rapidly hydrolyzed *in vivo* and are thought to serve as prodrugs. Psilocybin and psilocin are equipotent in humans on a molar basis (Wolbach et al. 1962) and psilocybin is known to be rapidly dephosphorylated *in vivo* and *in vitro* (Horita and Weber 1961; Eivindvik et al. 1989; Hasler et al. 1997; Hasler et al. 2002; Brown et al. 2017). 4-Acetoxy-DMT, the acetate ester of psilocin, is also believed to serve as a prodrug (Shulgin and Shulgin 1997; Nichols and Fescas 1999). By contrast, based on the present~~

~~results, 4-AcO-DALT may not solely function as a prodrug given that it has moderate affinity for the 5-HT$_{2A}$ receptor and is more potent than 4-HO-DALT in the HTR paradigm. Hence, certain 4-hydroxytryptamine esters may be active drugs in the absence of hydrolysis.~~

~~Given the link between 5-HT$_{2A}$ receptor activation and hallucinogenic effects (Halberstadt 2015; Nichols 2016), it is surprising that virtually all of the DALT compounds had higher affinities for 5-HT$_{1A}$ sites than for 5-HT$_{2A}$ sites. The binding data reported herein, however, likely underestimate the potency of the interactions between DALT compounds and the 5-HT$_{2A}$ receptor. 5-HT$_{2A}$ receptors exist in high-affinity and low-affinity agonist binding conformations depending on whether they are coupled to G proteins. Antagonist radioligands (such as [^{3}H]ketanserin) label both states non-selectively, whereas agonist radioligands (such as [^{3}H]DOB and [^{125}I]DOI) are selective for the subset of receptors in the G protein-coupled state (Lyon et al. 1987; Glennon et al. 1988). In competitive binding studies, the affinity of 5-HT$_{2A}$ agonists can vary depending on whether an agonist or an antagonist radioligand is used, and agonists typically display 10–100-fold higher affinity when agonist radioligands are used compared to antagonist radioligands (Titeler et al. 1988; Glennon et al. 1992; Glennon et al. 1994). Hence, the binding data listed in Table 1 likely overestimate the selectivity of DALT compounds for 5-HT$_{1A}$ versus 5-HT$_{2A}$ sites because the former receptor was labeled with an agonist radioligand ([^{3}H]8-OH-DPAT) whereas the latter receptor was labeled with an antagonist radioligand ([^{3}H]ketanserin).~~

The present findings also suggest that while 4- and 5-substituted DALT compounds may produce hallucinogenic effects in humans, 2- and 7-substituted DALT compounds may lack hallucinogenic effects, although further studies are necessary to test this hypothesis. While DALT, 5-MeO-DALT, and 4-AcO-DALT have already been detected by the European Early-

Warning System and reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA 2013, 2015), no such reports have arisen for 2- or 7-substituted DALT compounds.

To our knowledge, this analysis is the first to quantify the relative contributions of 5-HT_{2A} and 5-HT_{1A} receptors to the induction of HTR by a class of tryptamine hallucinogens. These findings may allow us to better predict the psychoactive potential of DALT derivatives based on their behavioral pharmacology, and suggest that similar analyses could be attempted for other classes of tryptamine hallucinogens. However, although 5-MeO-DALT produces hallucinogen-like behavioral responses in rodent behavioral paradigms including mouse HTR (the present studies) and rat drug discrimination (Gatch et al. 2017), it is not yet clear whether DALT derivatives can fully mimic the psychedelic effects produced by classical hallucinogens, allowing the possibility of subtle pharmacological differences relative to other tryptamine hallucinogens. Hence, it is not known whether the observed relationship between HTR potency and 5-HT_{2A} and 5-HT_{1A} binding affinities is consistent across the entire class of tryptamine hallucinogens. Nevertheless, if similar relationships do exist for other tryptamines, performing similar analyses on those classes should improve our understanding of their complex pharmacology and facilitate predictions regarding their psychoactive potencies.

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FIGURE CAPTIONS

Figure 1. Chemical structures of *N*,*N*-diallyltryptamine (DALT) and several ring-substituted derivatives.

Figure 2. Correlation between potency in the head twitch response (HTR) assay (pED_{50} values) and serotonin receptor binding affinities (pK_i values) for *N*,*N*-diallyltryptamine (DALT) and five ring-substituted derivatives. (A) Correlation between HTR potency and 5-HT_{1A} receptor affinity. (B) Correlation between HTR potency and 5-HT_{2A} receptor affinity. (C) Correlation between HTR potency and 5-HT_{1A} and 5-HT_{2A} receptor affinity.

Table 1. Summary of binding data for *N*,*N*-diallyltryptamine (DALT) and ring-substituted derivatives at 33 receptors and transporters.

Site	Species[a]	Binding Affinity (K_i, nM)									
		DALT	**5-MeO**	**5-F**	**5-Br**	**4-HO**	**4-AcO**	**2-Ph**	**5-MeO-2-Me**	**5-F-2-Me**	**7-Et**
5-HT_{1A}	Human	100	19	80	11	319	383	402	267	318	1,013
5-HT_{1B}	Human	> 10,000	735	1,787	950	2,494	> 10,000	273	2,267	2,011	> 10,000
5-HT_{1D}	Human	689	107	816	130	693	801	204	900	1,592	2,691
5-HT_{1E}	Human	378	500	474	512	238	467	> 10,000	1,594	1,273	> 10,000
5-HT_{2A}	Human	701	218	247	477	652	565	13	1,153	655	1,515
5-HT_{2B}	Human	61	59	16	53	2,593	63	192	241	17	65
5-HT_{2C}	Human	385	456	102	358	2,113	1,515	278	> 10,000	541	443
5-HT_{5A}	Human	> 10,000	3,312	4,299	2,389	> 10,000	5,844	1,670	1,822	1,916	> 10,000
5-HT_{6}	Human	1,718	153	74	133	213	1,791	68	206	168	> 10,000
5-HT_{7}	Human	> 10,000	90	402	49	600	724	> 10,000	> 10,000	493	> 10,000
SERT	Human	150	499	36	127	5,210	1,089	> 10,000	> 10,000	983	795
α_{1A}	Human	1,663	> 10,000	1,251	637	> 10,000	> 10,000	75	1,198	1,570	> 10,000
α_{1B}	Human	1,369	> 10,000	> 10,000	2,050	> 10,000	> 10,000	904	> 10,000	> 10,000	> 10,000
α_{1D}	Human	> 10,000	> 10,000	> 10,000	1,124	> 10,000	> 10,000	243	2,405	> 10,000	> 10,000
α_{2A}	Human	124	215	119	83	1,206	342	85	189	53	141
α_{2B}	Human	305	726	218	227	> 10,000	170	78	335	108	489
α_{2C}	Human	901	1,467	848	356	> 10,000	748	159	888	184	682
NET	Human	1,121	> 10,000	1,818	964	> 10,000	> 10,000	420	> 10,000	> 10,000	1,879
D_1	Human	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	2,793	> 10,000	> 10,000	> 10,000
D_2	Human	> 10,000	> 10,000	2,463	4,349	> 10,000	> 10,000	388	> 10,000	4,416	> 10,000
D_3	Human	672	> 10,000	120	240	1,570	> 10,000	342	2,399	414	1,082
D_4	Human	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	1,000	> 10,000	> 10,000	> 10,000
D_5	Human	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	2,003	> 10,000	> 10,000	> 10,000
DAT	Human	1,406	3,378	2,150	2,455	> 10,000	> 10,000	746	2,413	2,208	1,725
MOR	Human	> 10,000	> 10,000	> 10,000	1,726	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	2,674
DOR	Human	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	6,789	> 10,000	> 10,000	> 10,000
KOR	Human	2,477	1,132	2,184	898	> 10,000	5,235	589	391	580	580
PBR	Rat kidney[b]	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	1,929	> 10,000	> 10,000	> 10,000
H_1	Human	127	505	83	106	> 10,000	353	79	847	435	913
H_2	Human	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000	367	> 10,000	> 10,000	> 10,000
H_3	Guinea pig	> 10,000	1,712	2,093	1,495	> 10,000	> 10,000	> 10,000	1,134	1,397	> 10,000
σ_1	Rat brain[b]	101	301	86	101	2,765	299	> 10,000	427	531	22
σ_2	Rat PC12[b]	356	253	303	224	> 10,000	> 10,000	717	1,235	396	136

[a]The experiments were performed using cloned receptors from the species indicated. [b]The experiment was performed using tissues or cells natively expressing the receptor.
Abbreviations: **2-Ph**, 2-phenyl-*N*,*N*-diallyltryptamine; **4-AcO**, 4-acetoxy-*N*,*N*-diallyltryptamine; **4-HO**, 4-hydroxy-*N*,*N*-diallyltryptamine; **5-Br**, 5-bromo-*N*,*N*-diallyltryptamine; **5-F**, 5-fluoro-*N*,*N*-diallyltryptamine; **5-F-2-Me**, 5-methoxy-2-fluoro-*N*,*N*-diallyltryptamine; **5-MeO**, 5-methoxy-*N*,*N*-diallyltryptamine; **5-MeO-2-Me**, 5-methoxy-2-methyl-*N*,*N*-diallyltryptamine; **7-Et**, 7-ethyl-*N*,*N*-diallyltryptamine**; DALT**, *N*,*N*-diallyltryptamine; **DAT**, dopamine transporter; **DOR**, δ-opioid receptor; **KOR**, κ-opioid receptor; **MOR**, μ-opioid receptor; **NET**, norepinephrine transporter; **PBR**, peripheral benzodiazepine receptor; **SERT**, serotonin transporter.

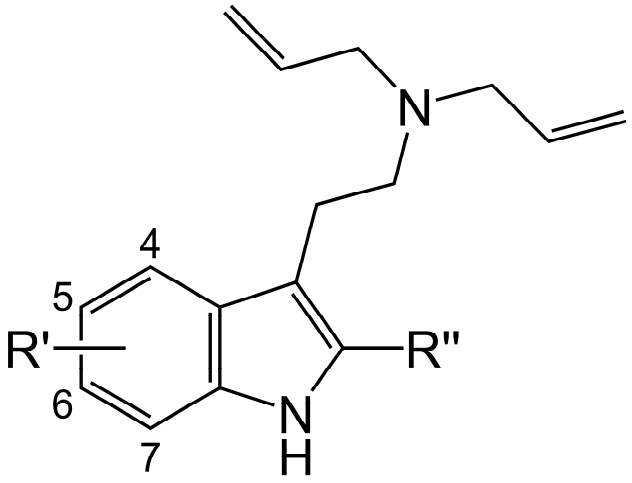
Table 2. Summary of head twitch response (HTR) data for *N*,*N*-diallyltryptamine (DALT) and ring-substituted derivatives.

Drug	One-Way ANOVA	Duration (min)	*N*	Dose (mg/kg)	HTR Counts (mean ± SEM)	ED_{50} (95% CI) (mg/kg)	ED_{50} (95% CI) (µmol/kg)
DALT	$F(5,24) = 5.71$, $p < 0.002$	30	5	0	3.6 ± 0.9	3.42 (2.44-4.79)	12.3 (8.8-17.3)
			5	0.875	8.2 ± 2.8		
			5	1.75	6.8 ± 2.6		
			5	3.5	14.2 ± 4.3		
			5	7	21.8 ± 4.4 **		
			5	14	20.6 ± 2.7 **		
5-MeO-DALT	$F(5,24) = 6.63$, $p=0.0005$	20	5	0	3.0 ± 1.5	2.25 (1.82-2.78)	7.3 (5.9-9.1)
			5	1.75	6.6 ± 1.0		
			5	3.5	19.8 ± 1.5 **		
			5	7	8.8 ± 2.6		
			5	14	8.0 ± 4.9		
5-F-DALT	$F(5,24) = 5.12$, $p < 0.003$	30	5	0	4.4 ± 0.6	1.58 (1.09-2.28)	5.4 (3.7-7.7)
			5	0.875	9.8 ± 2.6		
			5	1.75	21.0 ± 5.7		
			5	3.5	36.0 ± 6.8 **		
			5	7	26.8 ± 7.1 *		
			5	14	21.0 ± 4.0		
5-Br-DALT	$F(5,24) = 5.21$, $p < 0.003$	30	5	0	3.4 ± 0.5	4.80 (2.70-8.54)	13.5 (7.6-24.0)
			5	3.5	5.0 ± 0.3		
			5	7	10.8 ± 2.7 *		
			5	14	8.6 ± 2.7		
			5	28	1.4 ± 0.4		
			5	56	1.4 ± 1.4		
4-HO-DALT	$F(5,24) = 12.07$, $p < 0.0001$	5	5	0	1.2 ± 0.4	2.60 (2.01-3.35)	8.3 (6.4-10.6)
			5	0.875	4.0 ± 3.3		
			5	1.75	9.2 ± 3.7		
			5	3.5	28.6 ± 4.1 **		
			5	7	31.6 ± 4.9 **		
			5	14	24.6 ± 4.7 **		
4-AcO-DALT	$F(5,24) = 6.87$, $p=0.0004$	30	5	0	4.8 ± 1.0	1.99 (1.35-2.95)	4.8 (3.3-7.1)
			5	0.875	10.4 ± 1.4		
			5	1.75	42.0 ± 9.5 *		
			5	3.5	39.0 ± 14.1 *		
			5	7	65.0 ± 8.4 **		
			5	14	47.8 ± 10.0 **		

2-Ph-DALT	$F(5,24) = 2.20$, *NS*	30	5 5 5 5 5 5	0 0.875 1.75 3.5 7 14	3.8 ± 0.8 2.8 ± 0.5 3.6 ± 1.3 1.4 ± 0.5 2.0 ± 0.5 1.2 ± 0.5	*ND*	*ND*
2-Me-5-MeO-DALT	$F(5,24) = 1.02$, *NS*	30	5 5 5 5 5 5	0 0.875 1.75 3.5 7 14	3.8 ± 1.3 4.4 ± 0.2 7.4 ± 2.2 4.2 ± 1.0 4.2 ± 0.9 5.0 ± 1.4	*ND*[1]	*ND*
2-Me-5-F-DALT	$F(5,24) = 0.19$, *NS*	30	5 5 5 5 5 5	0 0.875 1.75 3.5 7 14	5.4 ± 1.7 6.2 ± 1.0 6.8 ± 0.9 5.8 ± 2.5 6.4 ± 1.6 7.2 ± 0.8	*ND*	*ND*
7-Et-DALT	$F(1,10) = 0.11$, *NS*	30	6 6	0 15	10.7 ± 1.7 9.8 ± 1.8	*ND*	*ND*

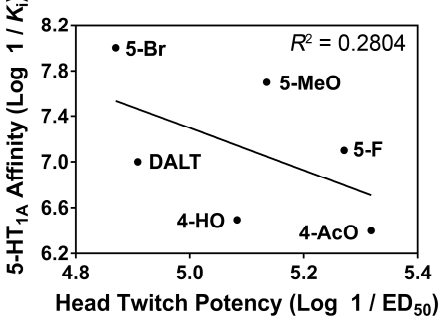
[1]*ND* = not determined (the compound was not active within the dose range tested).

*$p < 0.05$, **$p < 0.01$, significant difference from the vehicle control group (Tukey's test).



R'	R''	Abbreviation
H	H	DALT
H	C_6H_5	2-Ph-DALT
4-OAc	H	4-AcO-DALT
4-OH	H	4-HO-DALT
5-OCH_3	H	5-MeO-DALT
5-OCH_3	CH_3	5-MeO-2-Me-DALT
5-F	H	5-F-DALT
5-F	CH_3	5-F-2-Me-DALT
5-Br	H	5-Br-DALT
7-C_2H_5	H	7-Et-DALT

A.



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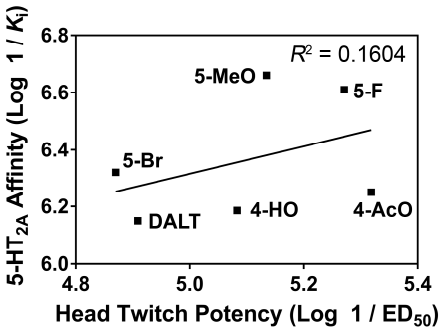

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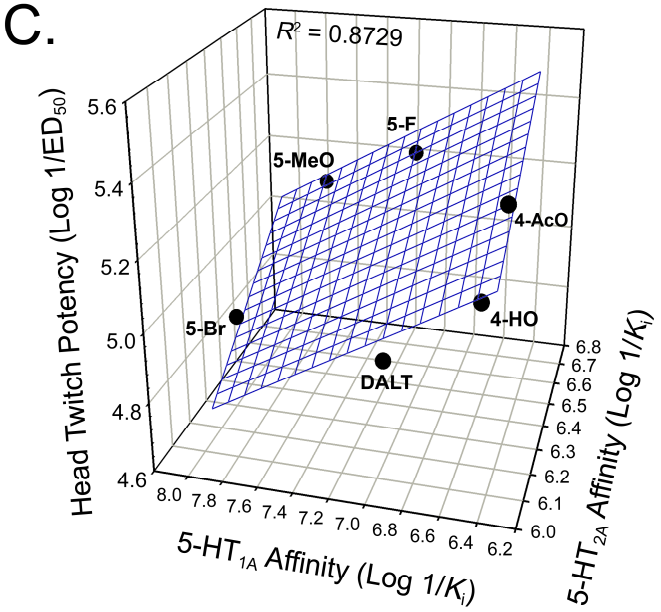

$pED_{50} = 2.367 + (0.741)(5\text{-}HT_{2A}\ pK_i) - (0.279)(5\text{-}HT_{1A}\ pK_i)$

HIGHLIGHTS

A new class of recreational drugs are derived from *N*,*N*-Diallyltryptamine (DALT)

DALT derivatives are relatively nonselective for serotonin receptors

DALT derivatives induce the head twitch response (a 5-HT_{2A}-mediated behavior) in mice

Both 5-HT_{2A} and 5-HT_{1A} receptors contribute to head twitch potency