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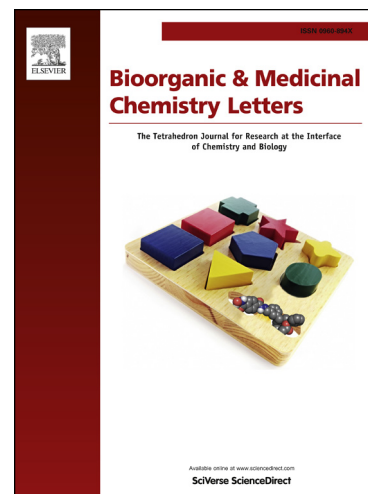
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RECEPTOR BINDING PROFILES AND QUANTITATIVE STRUCTURE-AFFINITY RELATIONSHIPS OF
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ABSTRACT

N,N-Diallyltryptamine (DALT) and 5-methoxy-*N,N*-diallyltryptamine (5-MeO-DALT) are two tryptamines synthesized and tested by Alexander Shulgin. In self-experiments, 5-MeO-DALT was reported to be psychoactive in the 12-20 mg range, while the unsubstituted compound DALT had few discernible effects in the 42-80 mg range. Recently, 5-MeO-DALT has been used in nonmedical settings for its psychoactive effects, but these effects have been poorly characterized and little is known of its pharmacological properties. We extended the work of Shulgin by synthesizing additional 5-substituted-DALTs. We then compared them to DALT and 5-MeO-DALT for their binding affinities at 45 cloned receptors and transporter proteins. Based on *in vitro* binding affinity, we identified 27 potential receptor targets for the 5-substituted-DALT compounds. Five of the DALT compounds had affinity in the 10-80 nM range for serotonin 5-HT_{1A} and 5-HT_{2B} receptors, while the affinity of DALT itself at 5-HT_{1A} receptors was slightly lower at 100 nM. Among the 5-HT₂ subtypes, the weakest affinity was at 5-HT_{2A} receptors, spanning 250-730 nM. Five of the DALT compounds had affinity in the 50-400 nanomolar range for serotonin 5-HT_{1D}, 5-HT₆, and 5-HT₇ receptors; again, it was the unsubstituted DALT that had the weakest affinity at all three subtypes. The test drugs had even weaker affinity for 5-HT_{1B}, 5-HT_{1E}, and 5-HT_{5A} subtypes and little or no affinity for the 5-HT₃ subtype. These compounds also had generally nanomolar affinities for adrenergic α_{2A} , α_{2B} , and α_{2C} receptors, sigma receptors σ_1 and σ_2 , histamine H₁ receptors, and norepinephrine and serotonin uptake transporters. They also bound to other targets in the nanomolar-to-low micromolar range. Based on these binding results, it is likely that multiple serotonin receptors, as well as several nonserotonergic sites are important for the psychoactive effects of DALT drugs. To learn whether any quantitative structure-affinity relationships existed, we evaluated correlations among physicochemical properties of the congeneric 5-substituted-DALT compounds. The descriptors included electronic (σ_p), hydrophobic (π), and steric (CMR) parameters. The binding affinity at 5-HT_{1A}, 5-HT_{1D}, 5-HT₇, and κ opioid receptors was positively correlated with the steric volume parameter CMR. At α_{2A} , α_{2B} , and α_{2C} receptors, and at the histamine H₁ receptor, binding affinity was correlated with the Hammett substituent parameter σ_p ; higher affinity was associated with larger σ_p values. At the σ_2 receptor, higher affinity was correlated with increasing π . These correlations should aid in the development of more potent and selective drugs within this family of compounds.

The pharmacology of naturally occurring and synthetic psychoactive *N,N*-dialkyltryptamines has been a matter of investigation since the 1950s.¹⁻³ The discovery of serotonin in the brain⁴ and the subsequent discovery that hallucinogenic snuffs prepared by people in South America, Haiti, and Puerto Rico contained a close congener of serotonin, namely *N,N*-dimethyltryptamine (DMT),⁵ led Szára in Hungary to perform self-experiments with DMT. After his discovery that parenteral DMT produced psychedelic effects similar to those produced by mescaline and LSD,⁶ the synthesis and pharmacological characterization of numerous *N,N*-disubstituted tryptamines, including *N,N*-diallyltryptamine (DALT), followed. However, with the exception of some early reports on DALT,⁷⁻⁹ information about the chemistry and pharmacology of DALT and ring-substituted DALT compounds remains sparse.

As part of his systematic structure-activity studies of hallucinogenic tryptamines, Alexander Shulgin synthesized and self-tested two *N,N*-diallyltryptamines, namely DALT itself and the ring-substituted 5-MeO-DALT (Fig. 1). While Szára reported that DALT was "psychotropic" at oral or intramuscular doses of 60 mg,⁹ Shulgin concluded that DALT had few discernible effects when taken orally in the 42-80 mg range and that 5-MeO-DALT had short-lived psychoactive effects in a range of 12-20 mg orally (A.T. Shulgin, personal communication). The psychoactive effects of 5-MeO-DALT reported by Shulgin were not well-characterized but they seemed to consist of an intoxication that was devoid of the usual visual imagery and cognitive effects associated with psychedelic agents. Nevertheless, 5-MeO-DALT has recently been identified as a compound used outside of medical settings for its psychoactive effects.^{10,11} The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) received the first notification of 5-MeO-DALT detection in 2007,¹² followed by 4-acetoxy-*N,N*-diallyltryptamine (4-AcO-DALT) in 2012,¹³ and DALT itself in 2014.¹⁴ The availability of 5-MeO-DALT in adult shops in Tokyo was reported in 2007¹⁵ and analytical data was published in 2008.¹⁶ Since then, the detection and analysis of 5-MeO-DALT, either on its own or present in other products, has been reported relatively frequently.¹⁷⁻²¹

The pharmacological properties of DALT compounds are largely unexplored. A recent metabolic study of DALT and 5-MeO-DALT in rats identified numerous metabolites and proposed the involvement of several metabolic pathways in the biotransformation of DALT compounds.²² Several pharmacodynamic studies of 5-MeO-DALT have been reported to date. In one study, 5-MeO-DALT did not exhibit appreciable monoamine uptake inhibition or releasing activity in synaptosomes,¹⁵ while in another study, 5-MeO-DALT was observed to stimulate [³⁵S]GTPγS binding in rat brain membranes, possibly including agonist activity at the 5-HT_{1A} receptor.²³ A third study evaluating the binding affinity of 5-MeO-DALT at various cloned receptors and transport proteins, as well as its ability to mobilize calcium via cloned serotonin 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, has been reported.¹⁸ In this report, 5-MeO-DALT had the highest affinity

at 5-HT_{2B}, 5-HT₆, and 5-HT₇ receptor subtypes and displayed the weakest affinity at 5-HT_{2A} and 5-HT_{2C} receptors. In a calcium mobilization assay, 5-MeO-DALT was a full 5-HT₂ agonist at low nanomolar concentrations but was less potent than serotonin.¹⁸

The relative lack of pharmacological data for DALT compounds, especially given their growing occurrence and recreational use, motivated the present study. To identify other biological targets for these tryptamines, we synthesized DALT compounds with various indole ring substitutions and presented these substances to the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH PDSP) for binding analysis at 45 cloned human receptors and transport proteins. Here, we report the binding profiles and quantitative structure-affinity analysis (QSAR) of a congeneric set of six DALT compounds, namely DALT, 5-F-DALT, 5-Cl-DALT, 5-Br-DALT, 5-Me-DALT, and 5-MeO-DALT (Fig. 1).

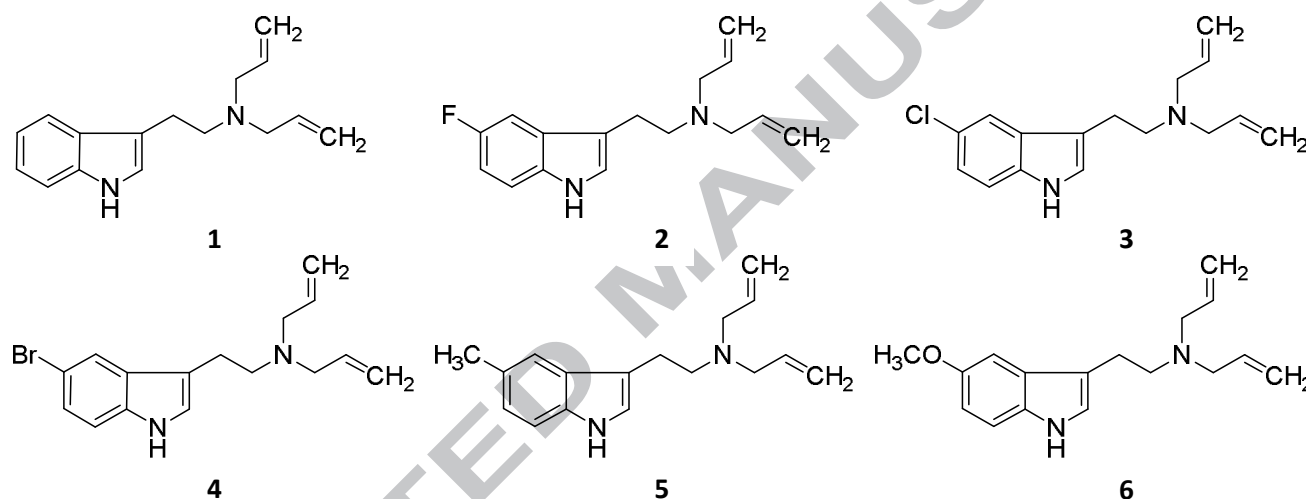
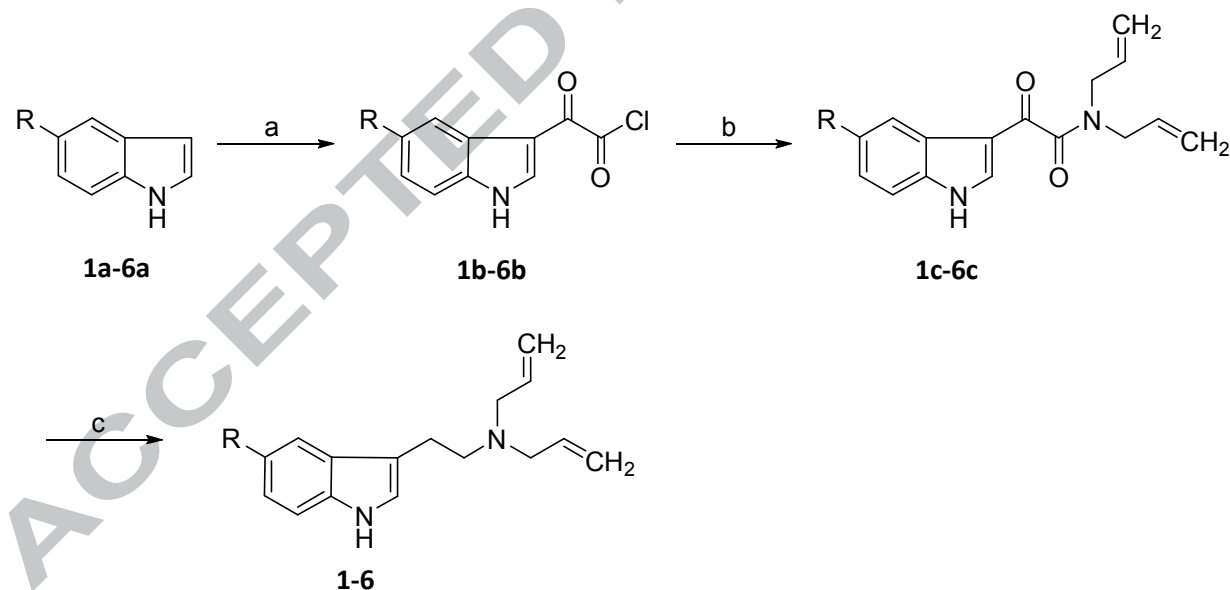


Fig. 1. Chemical structures of 5-substituted-*N,N*-diallyltryptamines.

After binding targets were identified, we examined the data set for correlations among physicochemical descriptors and binding affinity. The physicochemical properties analyzed were electronic (σ_p), hydrophobic (π), and steric (calculated molar refractivity; CMR) parameters. The electronic value σ_p , also called the Hammett substituent constant, is a measure of the change in free-energy that accompanies formation of complexes between ligands and their target proteins.²⁴⁻²⁶ Positive σ values are associated with electron-withdrawing substituents, negative σ values indicate electron-donating substituents, and the absolute value of σ indicates the strength of the electronic effect. In our analysis, we treat the sigma value of the 5-substituent as *para* (σ_p) to the indole nitrogen (N1). There are several reports that in indole-containing compounds, N1 is involved in hydrogen bond formation with binding targets, and that the electronic influences of 5-substituents are best explained if they are transmitted to N1 via both

inductive and resonance effects, i.e. the 5-substituent is *para* to N1.²⁷⁻²⁹ The hydrophobic parameter π quantifies the degree to which the addition of a given substituent changes the partitioning of a compound between water and *n*-octanol and thus reflects the relative tendency of a compound to partition into nonpolar environments compared to the unsubstituted parent compound.^{25,26,30} Finally, the calculated molar refractivity (CMR) estimates the spatial volume occupied by an atom or substituent group, with higher CMR values representing greater steric bulk. Correlations of binding with substituent CMR reveals potential steric interactions between a ligand and its binding site.^{26,31}

The DALT compounds were synthesized by adapting our published methods (Scheme 1).³² Briefly, using the method of Speeter and Anthony,³³ the 5-substituted-glyoxylamides (**1b-6b**) were obtained by acylation of the 5-substituted-indoles (**1a-6a**) with oxalyl chloride, followed by reaction with *N,N*-diallylamine to give the 5-substituted-*N,N*-diallylglyoxylamides (**1c-6c**). The *N,N*-diallylglyoxylamides were rapidly reduced to the *N,N*-diallyltryptamines (**1-6**) using lithium aluminum hydride in sealed glass tubes under microwave-accelerated conditions as described.³² Compounds were isolated as hydrochloride salts and were analyzed by gas chromatography-ion trap-mass spectrometry, liquid chromatography-high-resolution electrospray quadrupole time-of-flight mass spectrometry (LC-HR-MS/MS) with diode array detection, ¹H nuclear magnetic resonance spectroscopy, and infrared spectroscopy. Analytical results agreed with the predicted structures and will be published elsewhere.



Scheme 1. Synthesis of 5-substituted-*N,N*-diallyltryptamines. Reagents and conditions: (a) oxalyl chloride, diethyl ether, 0 °C, 4 h, 90-92%; (b) *N,N*-diallylamine, THF, 0 °C, 4 h, 55-64%; (c) LAH, THF, 150 °C, microwave power 250 W, maximum pressure 280 psi, ramp time 5 min, hold time 5 min, 68-70%. R = H, F, Cl, Br, CH₃, CH₃O.

Binding assays were performed by the National Institute of Mental Health's Psychoactive Drug Screening Program (NIMH PDSP, Contract # HHSN-271-2013-00017-C). Details of individual binding assay conditions and protocols are available at the NIMH PDSP website: <http://pdsp.med.unc.edu/pdspw/binding.php>. Briefly, DALT compounds were screened at 10 μM for their abilities to compete with various target-selective radioactive probe compounds at 45 cloned receptors and transporter proteins. In this primary assay target screening, an IC_{50} value of 10 μM was established as the threshold for further analysis. If a DALT compound displaced more than 50% of the radioligand probe at 10 μM , secondary assays were performed at the identified receptor or transporter using 12 concentrations of the DALT compound to generate competition binding isotherm curves. K_i values were then obtained from nonlinear regression of these binding isotherms. K_i values are calculated by the NIMH PDSP from best-fit IC_{50} values using the Cheng-Prusoff equation.³⁴ K_i values were converted to $\text{p}K_i$ values for data analysis.

Correlations were evaluated between *in vitro* $\text{p}K_i$ values and physicochemical parameters to assess quantitative structure-affinity relationships (QSAR). Specifically, electronic (σ_p), hydrophobic (π), and molar volume (CMR) values of the substituents at the 5-position of the tryptamine ring were examined by linear regression analysis for correlations to binding affinity at target proteins using commercial computer software (GraphPad Prism, San Diego, CA, USA). The regression analysis tests the null hypothesis that the slope of the least-squares regression line is different from zero, with $P < 0.05$ considered significant. Hammett σ_p and π values were obtained from Hansch *et al.*²⁶, and CMR values were calculated using commercial computer software (ChemBioDraw Ultra 13, PerkinElmer Informatics, Waltham Massachusetts 02451 USA).

Although *N,N*-diallyltryptamine compounds were briefly explored over 50 years ago, it is only recently that one of them, 5-MeO-DALT, has been reportedly used for its psychoactive effects.^{11,12} Very little is known of its pharmacology.^{15,18,23} To more completely characterize the binding targets for this substance and related DALT compounds, we synthesized and characterized 5-substituted-DALTs and engaged the NIMH PDSP to identify protein binding sites. We then employed QSAR analysis to elucidate structural determinants for affinity at the identified binding sites. There were four main findings. First, all six DALT compounds exhibited less than 10 μM binding affinity at most sites tested, with some of the highest affinities observed at 5-HT_{1A}, 5-HT_{2B}, 5-HT₇, H₁, and σ_1 receptors, and the serotonin uptake transporter (SERT) (Table 1). Second, QSAR analysis identified a significant correlation between the steric parameter (CMR) of the 5-substituents and binding affinity at 5-HT_{1A}, 5-HT_{1D}, 5-HT₇, and κ opioid receptors (Table 1, Fig. 2). Third, pK_i values at α_{2A} , α_{2B} , α_{2C} , and H₁ receptors were positively correlated with the Hammett constant, σ_p (Table 1, Fig. 3). Finally, binding at σ_2 receptors was correlated with the hydrophobic constant, π (Table 1, Fig. 4). These results show that steric bulk, electron-withdrawing power, and hydrophobicity of the 5-substituent of the DALT scaffold are key determinants for *in vitro* selectivity among these binding sites.

Table 1. *In vitro* binding constants ($pK_i \pm \text{SEM}$) for DALT compounds with K_i values of 10 μM or lower at various receptors and transporters.^a

	5-substituted DALT compounds					
	1	2	3	4	5	6
Physicochemical descriptor^b						
π	0	0.14	0.71	0.86	0.56	-0.02
σ_p	0	0.06	0.23	0.23	-0.17	-0.27
CMR	7.8344	7.8499	8.3258	8.6114	8.2982	8.4513
Binding site	$pK_i \pm \text{SEM}$ at binding site^a					
5-HT _{1A} ^c	7.00 \pm 0.06	7.10 \pm 0.07	7.23 \pm 0.05	8.00 \pm 0.10	7.50 \pm 0.10	7.70 \pm 0.10
5-HT _{1B}	> 5.00	5.75 \pm 0.04	6.05 \pm 0.04	6.02 \pm 0.04	5.87 \pm 0.04	6.13 \pm 0.04
5-HT _{1D} ^c	6.16 \pm 0.09	6.12 \pm 0.09	6.67 \pm 0.09	6.90 \pm 0.10	6.40 \pm 0.10	7.00 \pm 0.10
5-HT _{1E}	6.42 \pm 0.04	6.32 \pm 0.04	6.45 \pm 0.05	6.29 \pm 0.04	6.31 \pm 0.03	6.30 \pm 0.05
5-HT _{2A}	6.15 \pm 0.05	6.61 \pm 0.05	6.39 \pm 0.05	6.32 \pm 0.05	6.14 \pm 0.05	6.66 \pm 0.08
5-HT _{2B}	7.21 \pm 0.05	7.78 \pm 0.06	7.52 \pm 0.04	7.28 \pm 0.05	7.22 \pm 0.05	7.23 \pm 0.05
5-HT _{2C}	6.41 \pm 0.05	6.99 \pm 0.05	6.69 \pm 0.05	6.45 \pm 0.05	6.50 \pm 0.08	6.34 \pm 0.08
5-HT _{5A}	> 5.00	5.37 \pm 0.07	5.68 \pm 0.06	5.62 \pm 0.06	5.49 \pm 0.04	5.48 \pm 0.04
5-HT ₆	5.76 \pm 0.04	7.13 \pm 0.06	7.16 \pm 0.07	6.88 \pm 0.08	6.13 \pm 0.03	6.81 \pm 0.03
5-HT ₇ ^c	> 5.00 ^f	6.40 \pm 0.06	7.13 \pm 0.06	7.31 \pm 0.06	6.81 \pm 0.07	7.05 \pm 0.07
α_{1A}	5.78 \pm 0.05	5.90 \pm 0.06	6.28 \pm 0.05	6.20 \pm 0.06	5.90 \pm 0.06	> 5.00
α_{1B}	5.90 \pm 0.20	> 5.00	6.10 \pm 0.20	5.70 \pm 0.20	5.75 \pm 0.09	> 5.00
α_{1D}	> 5.00	> 5.00	5.88 \pm 0.09	5.95 \pm 0.09	> 5.00	> 5.00
α_{2A} ^d	6.91 \pm 0.06	6.93 \pm 0.05	7.09 \pm 0.05	7.08 \pm 0.06	6.71 \pm 0.06	6.67 \pm 0.07
α_{2B} ^d	6.52 \pm 0.06	6.66 \pm 0.04	6.77 \pm 0.04	6.64 \pm 0.04	6.36 \pm 0.04	6.14 \pm 0.04
α_{2C} ^d	6.05 \pm 0.06	6.10 \pm 0.10	6.20 \pm 0.10	6.40 \pm 0.10	5.96 \pm 0.07	5.83 \pm 0.06
D ₂	> 5.00	5.61 \pm 0.04	5.73 \pm 0.04	5.36 \pm 0.05	5.29 \pm 0.09	> 5.00
D ₃	6.17 \pm 0.06	6.92 \pm 0.06	6.85 \pm 0.04	6.62 \pm 0.04	6.16 \pm 0.07	> 5.00
H ₁ ^d	6.90 \pm 0.10	7.10 \pm 0.20	7.00 \pm 0.10	7.00 \pm 0.20	6.17 \pm 0.05	6.30 \pm 0.06
H ₃	> 5.00	5.68 \pm 0.04	5.80 \pm 0.04	5.83 \pm 0.04	> 5.00	5.77 \pm 0.04
κOR ^c	5.61 \pm 0.08	5.66 \pm 0.07	6.09 \pm 0.07	6.05 \pm 0.07	5.91 \pm 0.07	5.95 \pm 0.07
μOR	> 5.00	> 5.00	5.55 \pm 0.04	5.76 \pm 0.03	> 5.00	> 5.00
σ_1	7.00 \pm 0.06	7.07 \pm 0.06	7.01 \pm 0.06	6.99 \pm 0.06	7.13 \pm 0.08	6.52 \pm 0.06
σ_2 ^e	6.45 \pm 0.09	6.52 \pm 0.05	6.98 \pm 0.08	7.01 \pm 0.06	6.66 \pm 0.06	6.60 \pm 0.05
DAT	5.90 \pm 0.10	5.70 \pm 0.10	6.10 \pm 0.20	5.60 \pm 0.10	5.50 \pm 0.10	5.50 \pm 0.20
NET	6.00 \pm 0.20	5.70 \pm 0.20	6.20 \pm 0.20	6.00 \pm 0.20	> 5.00	> 5.00
SERT ^d	6.82 \pm 0.05	7.44 \pm 0.06	7.39 \pm 0.06	6.90 \pm 0.05	6.44 \pm 0.06	6.30 \pm 0.05

^a pK_i values determined by NIMH PDSP. ^bValues of π and σ_p from Hansch *et al.*, 1973; values of CMR from ChemBioDraw Ultra 13. ^c pK_i correlates with CMR. ^d pK_i correlates with Hammett substituent constant σ_p . ^e pK_i correlates with π . ^fValue excluded from QSAR analysis; see text.

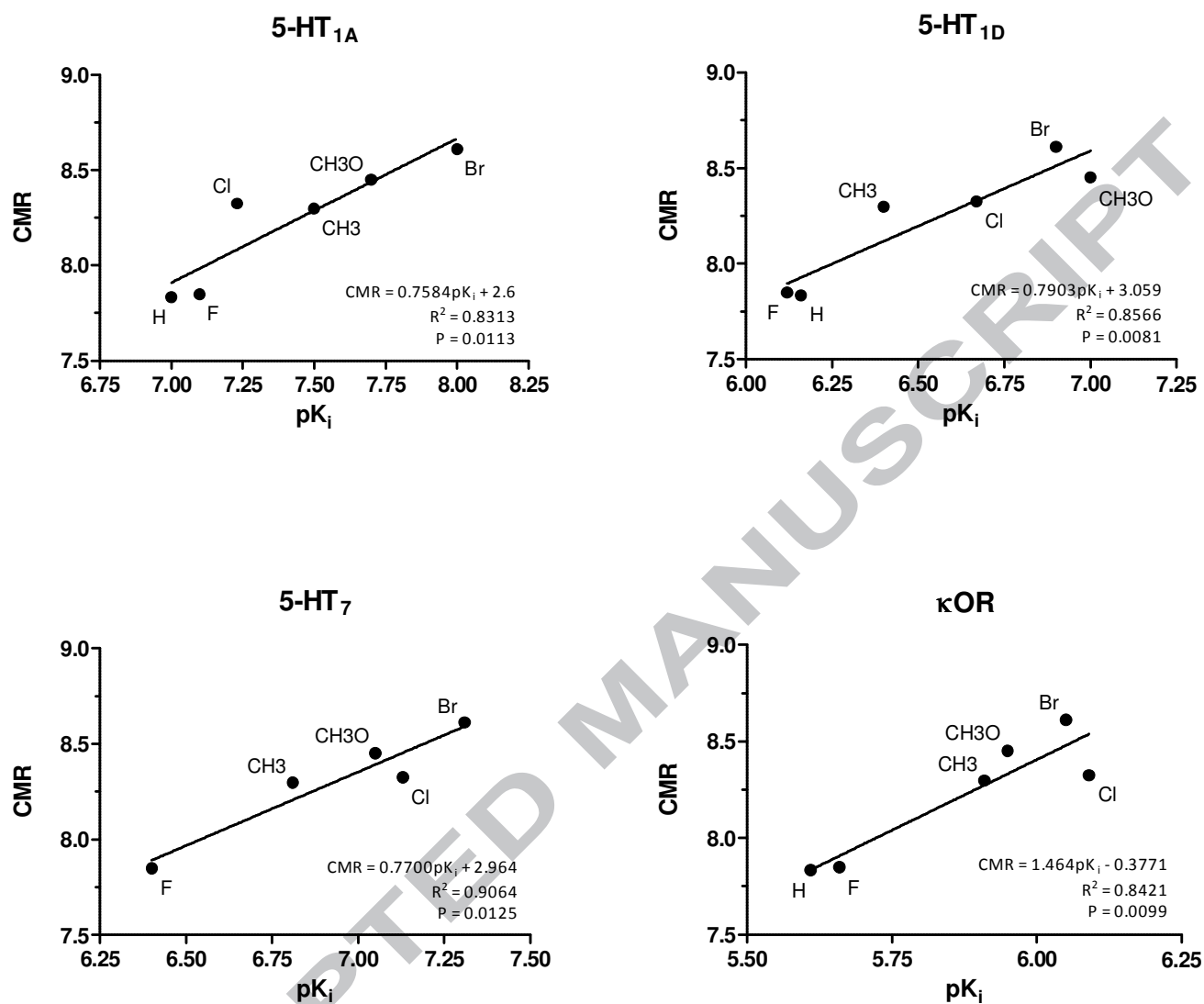


Fig. 2. Correlational analysis between CMR and pK_i values from Table 1. The figure shows the correlation between spatial volume (CMR, ordinate) of the 5-substituent and binding affinity (pK_i, abscissa) at 5-HT_{1A}, 5-HT_{1D}, 5-HT₇, and κ opioid receptors.

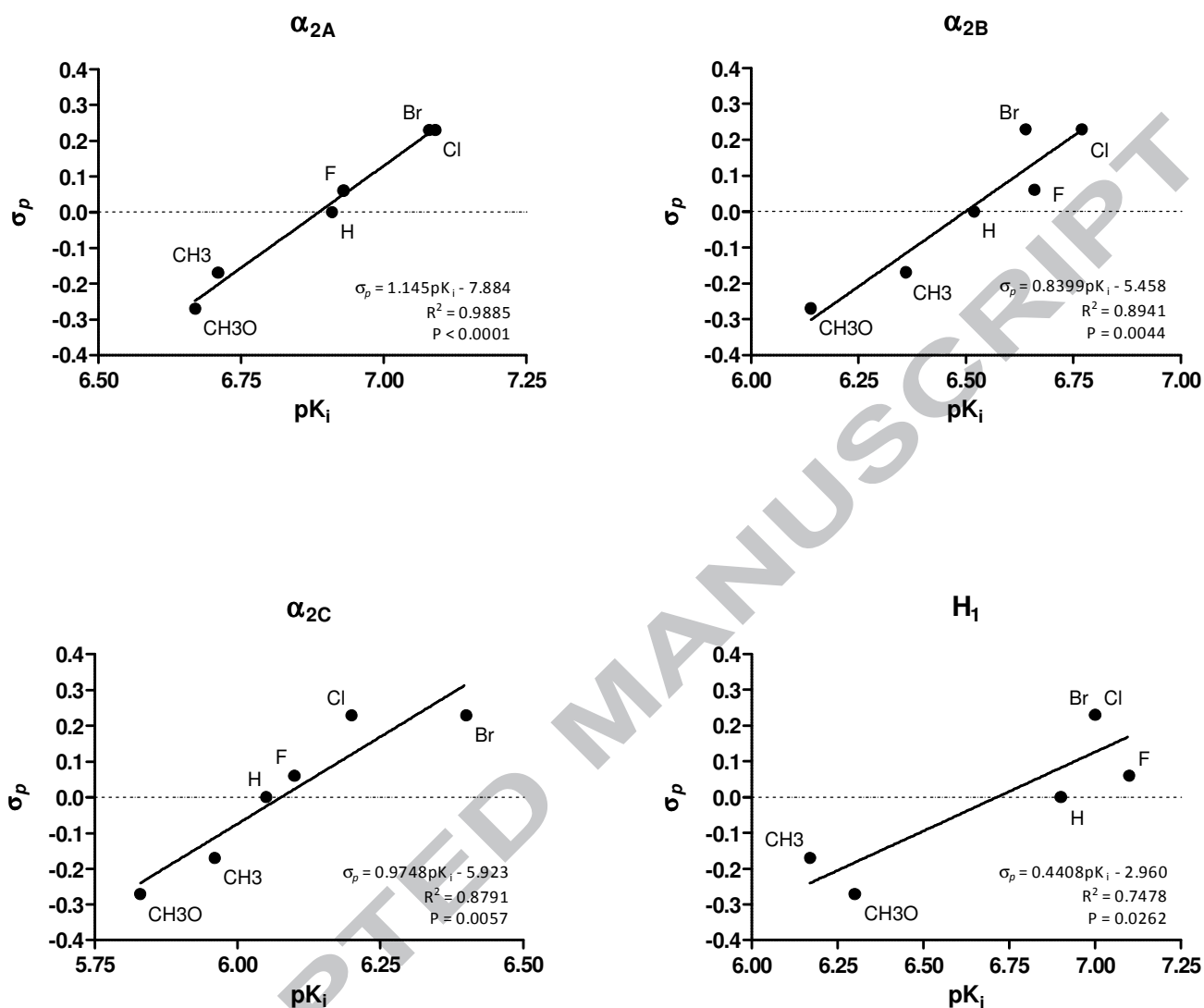


Fig. 3. Correlational analysis between the Hammett parameter σ_p and pK_i values from Table 1. The figure shows the correlation between electron-withdrawing strength (σ_p , ordinate) of the 5-substituent and binding affinity (pK_i , abscissa) at α_{2A} , α_{2B} , and α_{2C} adrenergic receptors, and at histamine H_1 receptors.

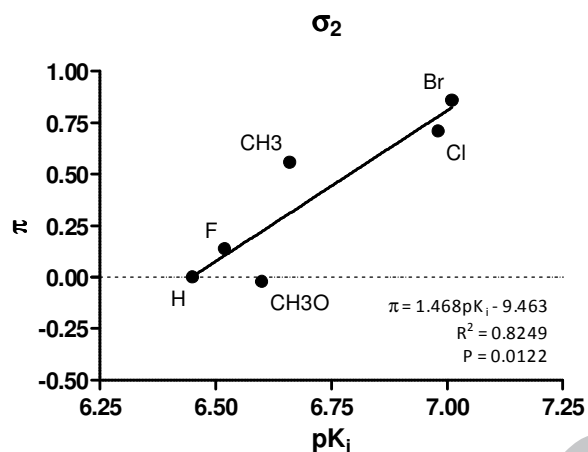


Fig. 4. Correlational analysis between π and pK_i values from Table 1. The figure shows the correlation between hydrophobicity (π , ordinate) of the 5-substituent and binding affinity (pK_i , abscissa) at σ_2 receptors.

The DALT compounds were initially screened at 45 different receptors and transporters. From this set, 27 sites met the 10 μM threshold criterion for secondary analysis and K_i determination. Notably, none of the following sites met the 10 μM threshold: beta adrenergic (β_1 - β_3); dopaminergic D_1 , D_4 , D_5 ; GABAergic (flunitrazepam GABA_A , muscimol GABA_A , and peripheral benzodiazepine sites); muscarinic (M_1 - M_5); 5-HT₃; δ -opioid; histaminergic H_2 and H_4 receptors. All of the test compounds exhibited K_i values of less than 10 μM at the following targets: serotonergic receptors 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT₆; adrenergic receptors α_{2A} , α_{2B} , and α_{2C} ; histamine receptor H_1 ; κ opioid receptor (κOR); sigma receptors σ_1 and σ_2 ; DAT and SERT monoamine uptake transporters. The highest measured affinities, which were in the 10-100 nM range, were at the 5-HT_{1A} and 5-HT_{2B} subtypes. In addition, several compounds had sub-100 nM affinity at 5-HT₆, 5-HT₇, α_{2A} , and σ_1 receptors and the SERT. The 5-substituted-DALT compounds are thus relatively nonselective over a broad range of biogenic amine receptors and transporters, as well as opioid and sigma receptors.

Evidence from both human and animal studies indicates that, at a minimum, binding and partial agonist activity at serotonergic 5-HT_{1A} and 5-HT_{2A} receptors are required for the manifestation of psychoactive effects from psychedelic tryptamines such as DMT, 5-MeO-DMT, and psilocin.³⁵⁻³⁸ These two receptors, as well as several other 5-HT receptors, σ_1 receptors, and the SERT were previously identified as high affinity targets for 5-MeO-DALT.^{18,23} The present study is in good agreement with these earlier findings and identifies additional binding sites that are likely to play a role in the psychoactive effects of DALT compounds. In particular, our data indicate that 5-MeO-DALT binds with the highest affinity at 5-HT_{1A} sites (19 nM) and has 150 nM or better affinity at 5-HT_{1D}, 5-HT_{2B}, 5-HT₆, and 5-HT₇ receptors; its affinity for 5-HT_{2A} receptors is somewhat weaker at 218 nM (Table 1). Nonserotonergic targets identified in the present study are also likely to contribute to the psychoactive effects of DALT drugs. Notably, binding potency at some of these other sites is as high or higher than potency at serotonergic sites. Thus, α_{2A} , H_1 , σ_1 , and σ_2 receptors are all comparatively high-affinity targets for the DALT compounds (Table 1). Although the present work did not examine the functional consequences of 5-substituted-DALT binding, the receptor and transporter targets identified herein are all expressed in brain tissue and drugs that bind to these receptors are well-known to affect neuronal function, producing effects on mood, alertness, perception, memory, and cognition.³⁹⁻⁴³ Our results therefore suggest that multiple serotonin receptors, as well as several nonserotonergic sites, are involved in the psychoactive effects of DALT drugs. While it is not possible to predict from our data whether the DALT binding profiles correlate with specific cognitive changes, it seems likely that perhaps nine 5-HT receptors, as well as α_{2A} , H_1 , σ_1 , and σ_2 receptors and the SERT all contribute to the psychoactive effects of these drugs at physiologically meaningful concentrations. Additional studies, including behavioral and

pharmacokinetic studies, will be needed to clarify what roles these binding sites play in the psychopharmacology of DALT compounds.

To identify factors that influence binding affinity, a quantitative structure-affinity analysis was conducted for the six DALT compounds with respect to three physicochemical parameters. These physicochemical parameters are steric bulk (CMR), electronic (σ_p), and hydrophobic (π) constants. Of the parameters analyzed, CMR and the Hammett constant σ_p correlated with binding affinity at multiple receptors, and one correlation was detected between π and binding affinity at the σ_2 receptor. Note that NIMH PDSP reported that DALT (**1**) had no appreciable affinity for 5-HT₇ receptors, although all of the other compounds had pK_i values in the 6.4-7.3 (50-400 nM) range. Compound **1** was therefore excluded from the QSAR analysis at this particular receptor. Significant correlations were found between CMR of the 5-substituent and pK_i values at 5-HT_{1A} ($R^2 = 0.8313$, $P = 0.0113$), 5-HT_{1D} ($R^2 = 0.8566$, $P = 0.0081$), 5-HT₇ ($R^2 = 0.9064$, $P = 0.0125$), and κ OR ($R^2 = 0.8421$, $P = 0.0099$) receptors (Fig. 2, Table 1). At these receptors, increased steric bulk is associated with higher affinity. The spatial volume of the 5-substituent thus plays a key role in binding at these sites, suggesting a topological correspondence between the DALT 5-position and the binding pocket within these four receptors. As long as the spatial limits of the binding pocket are not exceeded, we would anticipate that DALT compounds with even larger CMR values (e.g. 5-I-DALT, 5-EtO-DALT, 5-Pr-DALT) would have very high, possibly subnanomolar, affinity at 5-HT_{1A} receptors and increased affinity at the other three receptors as well. The electron-withdrawing capacity of the 5-substituent, described by the Hammett constant σ_p , was positively correlated with pK_i values at α_{2A} ($R^2 = 0.9885$, $P < 0.0001$), α_{2B} ($R^2 = 0.8941$, $P = 0.0044$), and α_{2C} ($R^2 = 0.8791$, $P = 0.0057$) adrenergic receptors, and the histamine H₁ ($R^2 = 0.7478$, $P = 0.0262$) receptor (Fig. 3, Table 2). At SERT, there was a trend to increased affinity with σ_p , but this correlation did not quite reach significance ($R^2 = 0.6427$, $P = 0.0551$). We would expect that DALT compounds having 5-substituents with larger σ_p values, (e.g. 5-CN-DALT, 5-CF₃-DALT, 5-NO₂-DALT), would exhibit even higher affinity at these sites. There are several plausible mechanisms whereby larger σ_p values are associated with higher binding affinity. First, one or more positively charged amino acid residues within the target binding pockets in the vicinity of the DALT 5-substituent could account for the correlation between σ_p and binding affinity at the receptors. Based upon multiple sequence alignments among these four receptors, six positively-charged conserved arginine and lysine residues were identified as potential candidates at positions 3.50, 4.41, 5.60, 6.29, 6.31, and 6.32.⁴⁴⁻⁴⁶ Second, favorable π - π stacking interactions between the indole ring of the DALT compounds and conserved binding site aromatic amino acid residues could also contribute to the observed higher affinity with electron-withdrawing 5-substituents, either through an electrostatic mechanism^{47,48} or through a direct interaction of the 5-substituent with hydrogen atoms of the amino acid aromatic nucleus.⁴⁹ To this point, two conserved aromatic residues are located at positions 5.47 and 5.48 and six additional conserved aromatic

residues are located nearby within a stretch of eight amino acids in the 6.48-6.55 ligand-binding regions of these receptors.⁴⁴⁻⁴⁶ Finally, hydrogen bond strength between the tryptamine N1 and a fully conserved threonine residue (3.37)^{29,44-46} within the orthosteric binding site of these four receptors could be increased by higher electron-withdrawing power of the 5-substituent, transmitted to N1 via inductive and resonance effects.

In summary, we have identified a number of receptor and monoamine transporter binding sites for a series of 5-substituted-DALT compounds, and we found associations between binding affinity and physicochemical properties at some of these sites. The correlations between CMR, σ_p , and π and receptor binding affinity should be of value in the design of more potent and selective drugs within the DALT family of compounds.

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