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EXAMINING AYAHUASCA CONSTITUENTS AT 5-HT2A RECEPTORS IN SEARCH OF ANTIDEPRESSANT ACTION

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EXAMINING AYAHUASCA CONSTITUENTS AT 5-HT_{2A} RECEPTORS IN SEARCH OF ANTIDEPRESSANT ACTION

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Medicinal Chemistry at Virginia Commonwealth University

by

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Dedication

This thesis is dedicated to my beloved parents Jim and Monica, as well as my best friend and dog, Oscar.

Thank you for all of your support and love.

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List of Abbreviations

AA	Arachidonic Acid
ACRI	Addiction Research Center Inventory
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid
APZ	Abnormal Mental States
ASI-R	Anxiety-Sensitivity Index-Revised
BBB	Blood-Brain Barrier
BCA	Bicinchoninic Acid
BDNF	Brain-derived Neurotrophic Factor
BHS	Beck Hopelessness Scale
BPCS	Brief Psychiatric Rating Scale
CHN	Elemental Analysis
CIS-R	Clinical Interview Schedule – Revised Edition
DA	Dopamine
DAG	Diacylglycerol
DHBC	Dihydro β-carboline
DMSO	Dimethyl Sulfoxide
DMT	*N*,*N*-Dimethyltryptamine
DOI	1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane
DOM	1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane
EBP	Extended Binding Pocket
EC_{50}	Effective Concentration (half-maximal effect)
Et_2O	Diethyl Ether
FDA	Food and Drug Administration
FST	Forced Swim Test
GABA	Gamma-Aminobutyric Acid
GOLD	Genetic Optimization of Ligand Docking
GPCRs	G-Protein Coupled Receptors
GRK	G Protein-Coupled Receptor Kinases
HDRS	Hamilton Depression Rating Scale
HEK-293	Human Embryonic Kidney 293 Cells

HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HINT	Hydropathic INTeraction
HRS	Hallucinogen Rating Scale
IC_{50}	Inhibition Concentration (half-maximal effect)
IP	Inositol Triphosphate
i-PrOH	Isopropyl Alcohol
K_i	Disassociation Constant
LGIC	Ligand-Gated Ion Channel
LSD	Lysergic Acid Diethylamide
MADRS	Montgomery-Åsberg Depression Rating Scale
MAO	Monoamine Oxidase
MAO-A	Monoamine Oxidase A
MAO-B	Monoamine Oxidase B
MAOI	Monoamine Oxidase Inhibitor
MDD	Major Depressive Disorder
MeOH	Methanol
mp	Melting Point
mTor	Mechanistic Target of Rapamycin
NE	Norepinephrine
NMDA	N-Methyl-D-aspartate
NMT	*N*-Methyltryptamine
OCD	Obsessive-Compulsive Disorder
PDB	Protein Data Bank
PKC	Protein Kinase C
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLP	Piecewise Linear Potential
RIMAs	Reversible Inhibitors of Monoamine Oxidase
RMSD	Root-Mean-Square Deviation
SAR	Structure-Activity Relationship
SEM	Standard Error of the Mean

SNRI	Serotonin Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitors
TCA	Tricyclic Antidepressant
THBC	Tetrahydro β-carboline
THH	Tetrahydroharmine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TTCT	Torrance Tests of Creative Thinking
YMRS	Young Mania Rating Scale
2-CN	2,5-Dimethoxy-4-Cyanophenethylamine
5-HT	Serotonin
5-OMe DMT	5-Methoxy-*N*,*N*-Dimethyltryptamine

Abstract

EXAMINING AYAHUASCA CONSTITUENTS AT 5-HT_{2A} RECEPTORS IN SEARCH OF ANTIDEPRESSANT ACTION

By Jeremy Derham Rolquin

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Major Director: Małgorzata Dukat, Associate Professor
Department of Medicinal Chemistry

Recently, it has been reported that ligands of the 5-HT_{2A} receptor can have drastic and fast-acting efficacy towards a number of different mental health disorders, such as depression and anxiety. A mixture of Amazonian plants called *ayahuasca* contains multiple compounds which have been shown to interact with the serotonergic system, and the 5-HT_{2A} receptor in particular.

The structurally similar compounds (DMT, harmine, harmaline, and tetrahydroharmine) found in ayahuasca were examined for differences in their physiochemical properties that might contribute to their binding affinity for the 5-HT_{2A} receptor. 3D Molecular modeling and docking studies of these compounds were conducted to predict their relative binding modes in relation to one another, and to validate previously proposed 2D binding modes.

Published studies of the binding affinities of both dimethyltryptamine (i.e., DMT) derivatives as well as the harmala alkaloids (harmine, harmaline, tetrahydroharmine) provided data points from which correlations were used to support the relative binding modes. Additional bromo-substituted DMT analogs were synthesized and assayed at cloned human 5-HT_{2A} receptors for binding affinity. The binding affinity data provided additional data points for our QSAR studies necessary to provide statistical significance to these correlations. Our results indicated that 5-Br

DMT (K_i = 45 nM) binds with the highest affinity for this receptor, followed by 4-Br DMT (K_i = 62 nM), 7-Br DMT (K_i = 353 nM), and 6-Br DMT (K_i = 814 nM). The major constituents of ayahuasca were also tested in a calcium mobilization assay at the 5-HT_{2A} receptor to determine general agonist or antagonist activity. DMT was found to be a partial agonist, while harmine and harmaline produced no calcium mobilization in this assay. Tetrahydroharmine was shown for the first time to be an antagonist in any known 5-HT_{2A} receptor downstream signaling assays. Full characterization of each of these compounds at all major downstream signaling assays will need to be conducted to determine whether they might elicit an antidepressant effect via any 5-HT_{2A} mediated pathway.

I. Introduction

Ayahuasca is an ancient Amazonian traditional brew which has recently been examined for its possible efficacy in the treatment of psychological disorders such as depression. The main chemical constituents of this brew (*N*,*N*-dimethyltryptamine, harmine, harmaline, and tetrahydroharmine) have been shown to be individually efficacious in animal models of depression. The presence and demonstrated antidepressant effect of the potent tryptamine hallucinogen *N*,*N*-dimethyltryptamine (DMT), which acts as an agonist at the human 5-HT_{2A} serotonin receptor, suggests that the combined antidepressant effect of this brew is a result of action at the 5-HT_{2A} receptor.

The antidepressant effects brought about by ayahuasca as well as other 5-HT_{2A} ligands are both efficacious and fast acting when compared to the antidepressants currently on the market, most of which exert some effect on the serotonergic system. This has led to a drastic increase in the amount of interest in these types of compounds, most of which had been discarded due to hallucinogenic side effects. There is great potential for selective 5-HT_{2A} ligands that produce antidepressant effects, without the hallucinogenic side effects. Developing a model of the interactions between the 5-HT_{2A} receptor and ligands that interact with it, as well as downstream signaling pathways, will go a long way towards developing more specific and efficacious compounds for the treatment of depression.

Two previous relative binding modes between DMT and harmine, harmaline, and tetrahydroharmine were proposed by Glennon and co-workers, and it is of interest to us to further investigate these two relative binding modes using molecular modeling and docking of these compounds to 3D models of the 5-HT_{2A} receptor. In addition to molecular modeling, binding affinities of analogs of DMT and harmine, harmaline, and tetrahydroharmine will be correlated to

one another, to determine if there is a linear relationship between similar substitutions on each set of compounds which would support or refute either binding mode. While it is possible to make these correlations using binding data of previously published compounds, the number of analogs for comparison falls short of the number required for statistical significance.

In order to make these comparisons statistically significant, analogs of DMT will be synthesized for comparison to previously synthesized harmaline and tetrahydroharmine analogs. The strength of the correlation seen in these studies will further support or refute either of the binding poses that had been proposed in previous studies. The relative orientation of these compounds in the binding pocket of the 5-HT_{2A} receptor should provide insight into which residues of the protein are interacting with which functional groups of ligands, which should help explain their physiological effects. Determining the binding pose and residue interactions of these compounds will go a long way in determining whether these compounds could be possible biased agonists for specific downstream signaling pathways.

It is also of interest to determine the functional activity of the compounds found in ayahuasca at the 5-HT_{2A} receptor. Very little data currently exist for ayahuasca compounds at this receptor, but so far DMT has been shown to be an agonist, while harmine and harmaline were both shown to be inactive. To establish whether these compounds are exhibiting any of their therapeutic potential through the 5-HT_{2A} receptor, determining their functional activity is essential. At the conclusion of this study, the relationship between the constituents of ayahuasca and the 5-HT_{2A} serotonin receptor should be much clearer than it is currently.

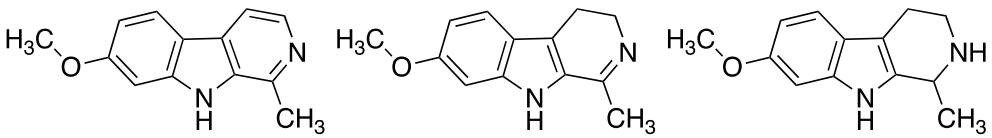
II. Background

A. History of Ayahuasca

1. Traditional Use

Ayahuasca, known also as *caapi*, or *yagé*, is a traditional Amazonian brew with a long and rich history of human use.[1,2] The term ayahuasca comes from the Quechan word *ayawaska*, meaning "Spirit Vine", which is indicative of the reverence that the various Amazonian religious groups had for this psychoactive medicine. Ayahuasca was first introduced to the western world by British botanist Richard Spruce, who found widespread use of a beverage called *caapi* among various tribes of the Amazon River basin. This brew has been used by native people of the South American continent for spiritual, social, healing, and recreational purposes, and in a medical context, it was used often as a way to determine the natural or supernatural origins of an ailment.[3–5] The exact origin of ayahuasca is unknown, but artifacts including both pottery and artwork, and practical items such as vessels used to contain alkaline hallucinogenic snuffs and stems used to inhale said snuffs are abundant in the regions of South America associated with ayahuasca usage.[6] As ayahuasca is not exclusively restricted to one particular area of the Amazon adjacent regions of South America, many different recipes and ingredients have been combined under the same name. The discovery of this combination of plant materials seems unlikely to have been intentional, as neither of the ingredients of the brew are acutely psychoactive on their own in the quantities commonly found in preparations of ayahuasca. The most commonly used combination of plant ingredients includes the wooden liana *Banisteriopsis caapi*, as well as a member of the coffee plant family *Psychotria viridis*, but plants such as *Mimosa tenuiflora*, *Diplopterys cabrerana,* and *Nicotinia rustica,* and many others have also been used. In total, over 80

admixtures of different Amazonian plant species have been recorded.[5,7–9] The preparation of ayahuasca consists of the pulverization of the wooden *caapi* vine, which contains a number of β-carboline compounds such as harmine (**1**), harmaline (**2**), and tetrahydroharmine (THH, **3**) (Figure 1), and whichever admixture plant is being used, followed by boiling the vegetable matter in boiling water and subsequent reduction of this liquid to yield a bitter tasting tea. In spite of any differences in composition and classification, ayahuasca is generally considered to be made up of a plant which contains monoamine oxidase inhibitor (MAOI) compounds known as the harmala alkaloids, and a plant which contains the hallucinogen *N*,*N*-dimethyltryptamine (DMT, **4,** Figure 2).[10,11]

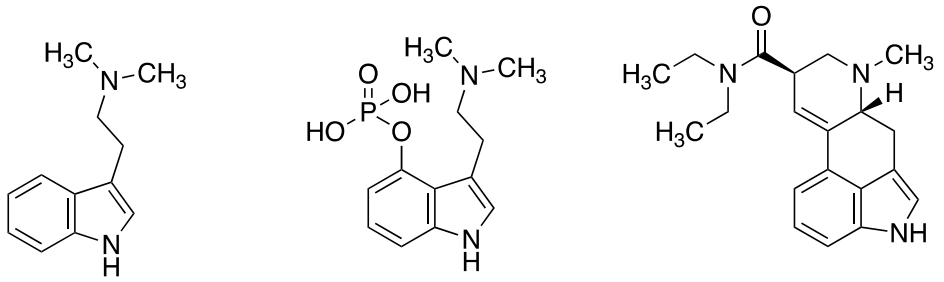


Harmine (**1**) Harmaline (**2**) Tetrahydroharmine (**3**)

Figure 1. Harmine (**1**), harmaline (**2**), and tetrahydroharmine (THH, **3**), the three β-carbolines most commonly found in preparations of *Banisteriopsis caapi* vine.

The use of ayahuasca by native groups both traditionally and medicinally has brought attention to its potential as a treatment for a variety of both physical and psychological ailments. Until recently, the study and use of almost all psychoactive plant preparations was outlawed by the signing of the United Nations Convention on Psychotropic Substances (1971) without special permissions. However, since then a handful of clinical trials have taken place to determine the therapeutic potentials of psychoactive natural products or derivatives thereof, including but not limited to DMT (**4**, ayahuasca, *Psychotria viridis*, *Mimosa hostilis*), psilocybin (**5**, *Psilocybe* genus of mushrooms), and lysergic acid diethylamide (**6**, LSD, an analogue of lysergic acid found in

Claviceps purpurea, Figure 2) for conditions as diverse as alcoholism, smoking cessation, end-of-life anxiety, major depressive disorder (MDD), and others. [12–14]



N,*N*-Dimethyltryptamine (**4**) Psilocybin (**5**) Lysergic Acid Diethylamide (**6**)

Figure 2. The hallucinogenic compounds DMT (**4**), psilocybin (**5**), and LSD (**6**), which have been examined in clinical trials for numerous psychiatric disorders.

2. *In Vivo* Research

The number of clinical trials and the interest in these psychoactive compounds and others like them, specifically for the treatment of psychiatric disorders, has increased steadily over time since they were banned. The interest in ayahuasca has increased significantly enough in Western culture for its purported spiritual and medicinal benefits, that drug tourism from both the United States and Europe to South America has become a common phenomenon.[15] A review of published human studies with ayahuasca in 2016 by dos Santos and co-workers found twenty-eight published human studies examining either the subjective effects/psychiatric symptoms, five which evaluated neurophysiological functioning, and five which used neuroimaging.[16] Many of these *in vivo* studies of ayahuasca have examined the effects of ayahuasca treatment on self-reported changes in perception, such as the Hallucinogenic Rating Scale (HRS), developed by Rick Strassman during some of the first clinical trials examining the subjective effects of DMT (**4**) *in vivo*. The HRS

measures six separate factors which have been found to be commonly altered by the "hallucinogenic" experience, and attempts to quantify the experience via questionnaire. This questionnaire has become the gold standard in assessing hallucinogenic effects since its inception.[17]

The HRS factors are as follows:

1. Affect/Emotion: questions concerning "anxiety, fear, euphoria, urge to laugh, urge to cry"
2. Somasthesia/Interoception: questions addressing "feeling flushed, effect on bodily weight, shaky feelings, effect on body temperature"
3. Intensity: questions concerning "how high were you, how intense was the experience, how strong was the rush"
4. Perception: questions addressing primary visual and auditory effects such as "visual imagery, visual effects, presence of a geometric grid over objects, something sounds different"
5. Cognition: questions concerning effects on thought processes or content, such as "thoughts speeded up, effect on quality of thinking, insights into occupational or personal life"
6. Volition: effects pertaining to "loss of control, feeling sane or insane, ability to move around if asked to"

This scale is just one of the multiple measures which have been employed to quantify the subjective effects of ayahuasca as well as other hallucinogens. All ayahuasca *in vivo* studies reviewed by dos Santos et al.[16] found dose dependent increases of every subscale excluding

volition. Several of these also examined secondary rating scales such as the Addiction Research Center Inventory (ACRI), the Brief Psychiatric Rating Scale (BPCS), the Young Mania Rating Scale (YMRS), and Abnormal Mental States (APZ), which aim to either quantify the subjective effects of a drug, or compare the altered state of consciousness brought on by hallucinogenic drugs to those induced by mental illness. Ayahuasca consumption produced increased scores in each of these scales in a dose-dependent manner. Decreases in scores were obtained for the Anxiety-Sensitivity Index-Revised (ASI-R), and the Beck Hopelessness Scale (BHS), which indicated decreases in feelings of both anxiety and hopelessness. Generally, the short-term psychoactive effects produced by ayahuasca were able to be monitored and scored using previously established tests of psychoactive drug activity, although some of these studies lacked quantification of the active ayahuasca constituents and/or a placebo group.

The number of clinical trials and reviews of users of ayahuasca draws a clear correlation between the use of ayahuasca and positive mental health outcomes. Studies on short term acute psychiatric effects of ayahuasca which examined results from the Clinical Interview Schedule – Revised Edition (CIS-R) and Torrance Tests of Creative Thinking (TTCT), indicated a decrease in mental illness side effects and an increase in creativity.[18,19] Treatment of patients with a single dose of ayahuasca was able to return levels of cortisol, the bodies' main stress hormone and a biomarker reliably linked with MDD, to levels which were equal to those of a healthy control group.[20] A survey of adolescent users of ayahuasca also found markedly low incidence of common psychiatric disorders compared to the general adolescent population.[21]

The most promising trial to date linking ayahuasca with antidepressant effects was a randomized, placebo controlled study conducted by Palhano-Fontes et al.,[22] that found significant decreases in depressive symptoms as measured by the Montgomery-Åsberg Depression Rating

Scale (MADRS) one day after treatment, with results remaining significant seven days after treatment. The short time frame over which significant decreases in depressive symptoms were seen is something that is unique when compared to traditional antidepressant therapies. This is by no means the first trial of ayahuasca and its antidepressant effects in humans, but previous trials were conducted on small populations, had low returning response rates, did not have a placebo control group, or the active constituents of the brew were not quantified and controlled between patients.[23–31] Examination of the both the short- and long-term psychiatric benefits of ayahuasca show great potential for the treatment of mental illnesses as well as improvements in overall cognition, and that it is for the most part extremely well tolerated in patient populations.

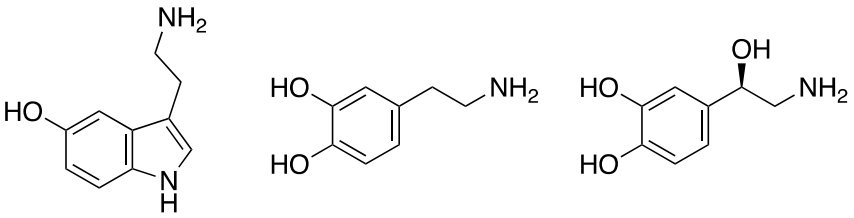
B. Depression

1. Biological Basis for Depression

Major Depressive Disorder is a disorder characterized by anhedonia, decreased mood, and decreased energy, as well as an increased rate of morbidity and mortality, for a minimum of two weeks.[32] MDD affects more than 264 million people worldwide, of which most are left untreated.[33] Depression is linked to a large increase in the likelihood of a suicidal episode, and suicide is the second leading cause of death between the ages of 15-29 in both America and worldwide.[33] Suicide is the 10th overall cause of death in the United States among all age groups.[33] This disorder is as debilitating as it is costly, with the average cost of treatment for a depressive disorder in the United States reaching $1200-$2200 annually per person.[34] MDD patients are also particularly likely to suffer from anxiety disorders and to a lesser extent, substance abuse disorders, particularly alcohol misuse.[35] Nearly 20% of individuals experience depression at some point, and for a considerable portion of the population, depression is a chronic illness which can persist for over ten years.[36]

These statistics are likely to fall short of the actual prevalence of depressive disorders and related comorbidities, as mental health issues are often underreported, and under-diagnosed.

One of the most popular theories for the biological basis for depressive disorders is the monoamine hypothesis proposed in the mid 20th century.[37,38] This hypothesis stated that those suffering from depressive symptoms were precipitated by a functional deficiency of the major aminergic neurotransmitters: serotonin (5-HT, **7**), dopamine (DA, **8**), and norepinephrine (NE, **9**) (Figure 3).

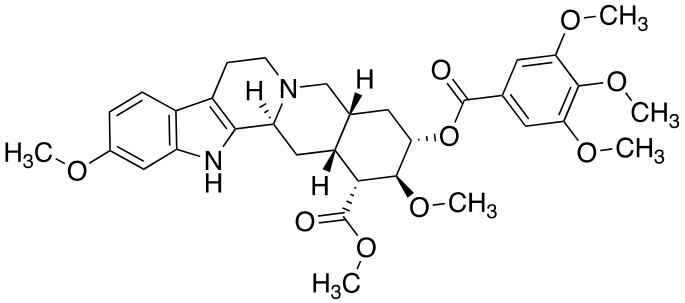


Serotonin (5-HT, **7**) Dopamine (DA, **8**) Norepinephrine (NE, **9**)

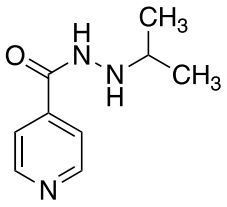
Figure 3. The aminergic neurotransmitters serotonin (5-HT, **7**), dopamine (DA, **8**), and norepinephrine (NE, **9**).

This hypothesis was first supported by two observations:

1. Reserpine (**10,** Figure 4), an alkaloid of the Indian snakeroot plant *Rauwolfia serpentina* used commonly to treat hypertension, was found to induce symptoms resembling depression. It was then discovered that reserpine also depletes presynaptic storage vesicles of 5-HT, NE, and DA.[39]
2. Iproniazid (**11,** Figure 4), a tuberculosis drug which also inhibits the enzyme monoamine oxidase (MAO), was found to cause symptoms of euphoria and hyperactivity.[40]



Reserpine (**10**)



Iproniazid (**11**)

Figure 4. Reserpine (**10**), an alkaloid from the Indian snakeroot plant *Rauwolfia serpentina*, and the first antidepressant drug, iproniazid (**11**).

The monoamine hypothesis is not a complete explanation of the biological basis of depression, and the treatment of depression is not as simple as increasing the functional level of monoamine neurotransmitters. For example, it was found that decreased levels of monoaminergic neurotransmitters did not increase the severity of depressive symptoms in untreated patients with MDD.[41,42] In spite of the issues with the monoamine hypothesis, most approved antidepressant drugs have modulatory effects on the concentrations of one or more of the aminergic transmitters. There are many classes of antidepressant drugs which have been brought to market, but the main focus of this thesis will be on the antidepressant classes of compounds which have an effect on the serotonergic system.[43] These compounds are the monoamine oxidase inhibitors, tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), and the serotonin norepinephrine reuptake inhibitors (SNRI).

2. Current Antidepressants

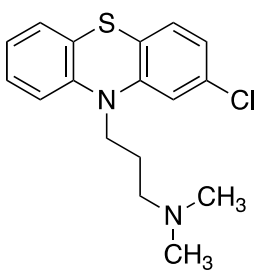
The first class of compounds discovered for the treatment of depression were monoamine oxidase inhibitors, which were discovered serendipitously during trials for antituberculosis compounds by Fox and Gibas in 1953.[44] An alkylation of isoniazid, from isonicotinyl hydrazide

to isopropyl isonicotinyl hydrazide (Iproniazid, **11**) produced enhanced mood and euphoria among the patients administered the drug, which would go on to be marketed for its anti-tuberculosis effects, but was used off-label as a treatment for MDD. This compound was discovered to be a potent inhibitor of monoamine oxidase soon thereafter.[45] Although the serotonergic system is not directly targeted by monoamine oxidase inhibitors, it is indirectly impacted, as the levels of all monoamine neurotransmitters are increased after treatment, including serotonin (**7**). The first clinical trials of iproniazid (**11**) in patients classified with severe depression found a 70% response rate to the drug in treatment of their depression (n=17).[46] There are two separate isoforms of monoamine oxidase, monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B), that selectively deaminate specific neurotransmitters. MAO-A preferentially deaminates serotonin (**7**), melatonin, norepinephrine (**9**), and epinephrine, while MAO-B preferentially deaminates phenethylamine and benzylamine. The monoamine oxidase isoforms share responsibilities in deamination of dopamine (**8**), tryptamine (**60**), and tyramine.[47,48] These isoforms differ not only in their preferred substrate, but also in their distribution. Isoform A is more populous in the liver, placenta, intestine, adrenal gland, kidney, heart, lungs, spleen, and thyroid, while isoform B is more populous in the pancreas, brain, and equally represented in skeletal muscle.[49]

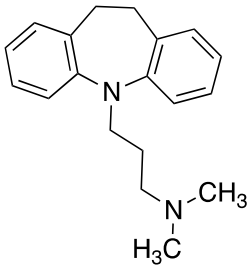
Long after the discovery of iproniazid (**11**), it was determined that MAO-A was responsible for the antidepressant effects of monoamine oxidase inhibitors.[50] The first MAOIs such as iproniazid (**11**), isocarboxazid, tranylcypromine, and phenelzine are irreversible inhibitors of monoamine oxidase, which have problematic safety profiles due to their permanent inhibition of the enzyme. Cases of jaundice, nephrotoxicity, hepatic cell damage, and hypertensive crisis leading to intracranial hemorrhages were all noted.[51–53] Initially believed to be caused by alcohol, these side effects were eventually attributed to decreased activity of MAO on certain types of aged

or fermented foods containing the amino acid tyramine, which is essential in the regulation of blood pressure. The search for MAOIs with more favorable safety profiles with antidepressant effects led to reversible MAO-A inhibitors (RIMAs) such as selegiline and moclobemide, which were able to be displaced by tyramine and were therefore much safer. Due to continued issues with efficacy and side effects, almost all forms of MAOIs were withdrawn from the market, although some are still used as a second-choice drug for the treatment of MDD or in cases of intolerance or lack of reaction to typically prescribed drugs.[54]

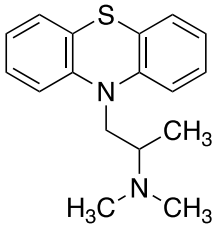
The safety profile of irreversible monoamine oxidase inhibitors and the efficacy issues demonstrated by RIMAs provided a prime opportunity for development of a more tolerable and efficacious class of compounds. The breakthrough discovery of chlorpromazine (**12**, Figure 5) by French chemist Paul Charpentier in 1950 for use as a non-narcotic sedative opened the door for the elaboration on the antihistamine structure in search of additional neuroleptic compounds.[55] One of these compounds was imipramine (**13**, Figure 5) , another derivative of the antihistamine compound promethazine (**14**, Figure 5) that was shown to be ineffective at treating psychotic episodes in patients with schizophrenia, but was effective in reducing symptoms of severe depression in some patients.[56] In the 500 psychiatric patients treated by Kuhn in the first clinical trial of imipramine (**13**), no serious side effects were observed.[57]



Chlorpromazine (**12**)



Imipramine (**13**)



Promethazine (**14**)

Figure 5. The antipsychotic drug chlorpromazine (**12**), the first tricyclic antidepressant drug imipramine (**13**), and the tricyclic antihistamine drug promethazine (**14**).

TCAs are the only antidepressants classified by their structure instead of their mechanism of action, as they do not all alleviate depressive symptoms through the same pathways. Eight tricyclic antidepressants have been approved for treatment of depression in the United States, each with their own distinct binding profile and side effects. Most of the interest in these compounds can be attributed to their ability to block re-uptake of both serotonin and norepinephrine from the synapse into the pre-synaptic neuron.[43] Tricyclic antidepressants also have non-negligible antagonist activity at multiple classes of serotonin receptors, adrenergic receptors, and NMDA receptors, and agonist activity at sigma receptors. Of particular note are the potent antihistamine and anticholinergic effects of these compounds, which can sometimes precipitate unwanted side effects of drowsiness, weight gain, memory loss, and dizziness.[58]

Unfortunately, TCAs posed a significant risk to the prescribed population as the therapeutic window is quite narrow in comparison to other drugs on the market. Most of the TCAs are non-toxic in doses under 5.0 mg/kg, although there are a few which are non-toxic only below 2.5 mg/kg.[59] Overdose via TCA typically presents itself through anticholinergic side effects such as delirium, tachycardia, pupil dilation, hypertension, cardiac arrest, seizure, coma, and hyperventilation. The toxicity of this class of drugs is even more pronounced among children of

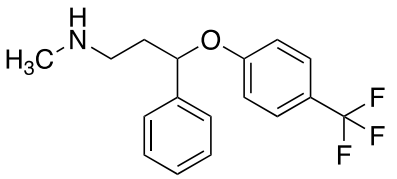
patients who gain access to their medications, as just a few pills can prove deadly.[60] It is also less than ideal to provide a medication that is so easily overdosed to a patient population in which suicidal ideation is already higher than average, as it presents an opportunity for self-harm to the patient in a state of mental crisis that they may not have had access to otherwise.

While TCAs were generally effective in the treatment of MDD, their nonspecific binding profiles and myriad of off-target effects, as well as the emerging role of serotonin (**7**) in the pathophysiology of depression, led to a search for more specific compounds. Shaw, Camps, and Eccleston[61] found a decreased concentration of serotonin (**7**) in the brains of post-mortem patients who had been diagnosed with depression and had committed suicide, compared to those who had died of wounds which were not self-inflicted. The introduction of fluoxetine (**15**) by Eli Lilly in 1974 as a drug which had significant specificity for the serotonin transporter over the norepinephrine transporter, and its subsequent FDA approval in 1987, presented an alternative to the TCA and MAOI drugs which had previously been approved.[62]

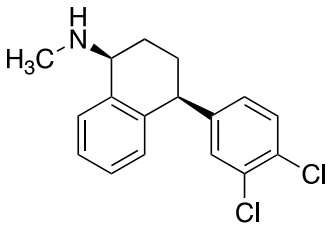
The discovery of fluoxetine (**15**) paved the way for the development of further SSRIs which continue to be the first choice for the treatment by physicians in the United States to this day, such as sertraline (**16**), paroxetine (**17**), citalopram (**18**), and escitalopram (**19**) **(**Figure 6).[63] The binding affinities for these drugs are preferential to the TCAs due to their high specificity to the serotonin transporter, with minimal binding at any other population of receptor.[64] Notably, these compounds have anywhere between 20-1500-fold selectivity for the serotonin transporter compared to the norepinephrine transporter, and lack the binding affinities for muscarinic and adrenergic receptors which were involved in the acute toxicity and overdose potential of the TCAs.

The reduction in acute toxicity is evident in the decrease in deaths by antidepressant overdose, even though the number of total overdoses has increased over time as SSRIs have

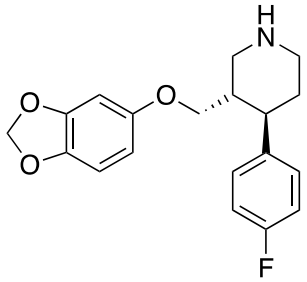
become the most prescribed class of drugs in treating MDD. In a direct comparison, TCAs had a higher rate of hospitalization after overdose (79% vs 65%) and higher rate of fatalities from overdose (0.73% vs 0.14%) when compared to SSRIs.[65] That is not to say that SSRIs are completely safe, there are a number of side effects associated with this class of compounds including drowsiness, sexual dysfunction, weight gain, gastrointestinal disturbance, anxiety, agitation, and sleep disruption. Discontinuation of these drugs without proper tapering of dosage can also lead to numerous withdrawal effects.[66]



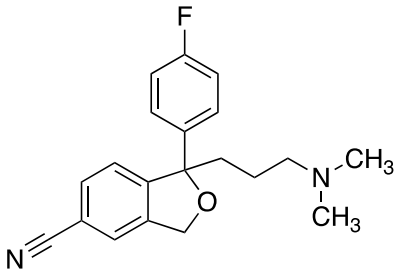
Fluoxetine (**15**)



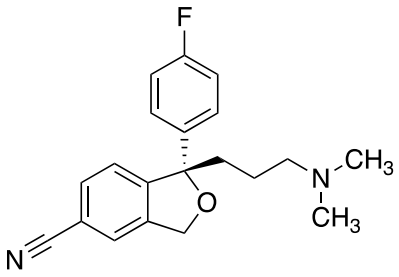
Sertraline (**16**)



Paroxetine (**17**)



Citalopram (**18**)

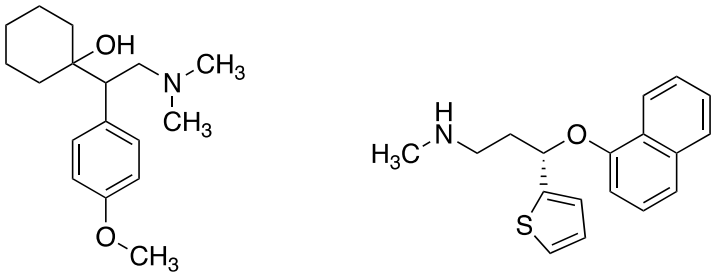


Escitalopram (**19**)

Figure 6. FDA approved SSRI antidepressants fluoxetine (Prozac ®, **15**), sertraline (Zoloft ®, **16**), paroxetine (Paxil ®, **17**), citalopram (Celexa ®, **18**), and escitalopram (Lexapro ®, **19)**.

Following the approval of SSRI compounds for the treatment of MDD, SNRI drugs such as venlafaxine (Effexor ®, **20**) and duloxetine (Cymbalta ®, **21**) were developed (Figure 7), which

combined some of the key pharmacological actions of both the SSRIs and TCAs. SNRI drugs inhibit both serotonin and norepinephrine transporters like previously mentioned SSRIs and TCAs, but like the SSRI class of drugs, lack affinity for any of the muscarinic, adrenergic, and histamine receptors that have been identified as being problematic.[67] It was hypothesized that inhibiting the reuptake of both neurotransmitters would lead to higher efficacy in the treatment of depression by these compounds, and response rates for SNRI drugs in MDD was found to be slightly higher when compared to response rates for SSRIs.[68] The side effects associated with SNRIs are considered to be even less serious than those induced by SSRIs, such as anxiety, insomnia, sexual disfunction, and headaches; but other side effects such as nausea, dry mouth, and elevated blood pressure are more common.[69]



Venlafaxine (**20**) Duloxetine (**21**)

Figure 7. FDA approved SNRI antidepressants venlafaxine (Effexor ®, **20**) and duloxetine (Cymbalta ®, **21**).

3. Issues with Current Antidepressants

Although all of the previously mentioned classes of antidepressants have been used extensively or are still in use, there are some very serious adverse effects brought about by these drugs. In one study, it was found that up to 43% of patients with MDD discontinued treatment due to side effects.[70] In addition to the previously stated specific side effects of each class of drug mentioned, antidepressant compounds are associated with increases in rates of gastrointestinal

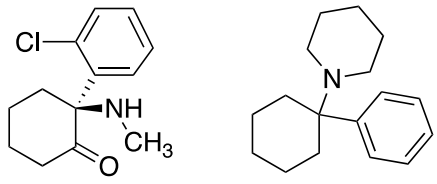
bleeding, cardiovascular effects, dry mouth, gastrointestinal disturbances, hepatotoxicity, seizure, suicide, overdose, sexual dysfunction, weight gain, sleep disruptions, hyponatremia, and sweating.[71] Overall, these factors contribute to a rate of mortality that is 33% higher than that of the general population.[72]

A further issue with current antidepressant therapies is the long "lag time" between the first dose of an antidepressant drug and therapeutic activity. Despite the wide variety of physiological targets affected by different members of this class of drugs, all have been found to take anywhere from two weeks to two months to improve symptoms. It has been hypothesized that the cause of this period between the beginning of an antidepressant medication regimen, and the alleviation of depressive symptoms is due to the release of the $G\alpha_s$ from lipid raft localization in the cell membrane, as the time period where changes in this signaling pathway are seen match up with the time needed for these cellular changes to occur.[73,74] This hypothesis was further supported by the ability of antidepressant compounds, but not structurally similar compounds without known antidepressant activity, to bind to these lipid rafts independently of the monoamine transporters that their effects are usually associated with.[75]

The mechanism behind this common yet complex antidepressant issue is still not fully understood. This is a pressing issue with current antidepressant therapies, as the nature of disease can require urgent alleviation of symptoms to reduce the risk of suicide or other self-inflicted harm. The numerous issues aforementioned along with an increasing concern that the effects precipitated by traditional antidepressants are not much more efficacious than placebo has increased interest in new types of therapies that are both fast-acting and novel in their mechanism of action.[76]

4. Breakthrough in MDD Treatment – Ketamine (22)

The most recent breakthrough in antidepressant therapeutics was the recent approval in March of 2019 of *Sprovato* ®, also known as esketamine or S(+)-ketamine (**22**, Figure 8), for treatment-resistant depression. Ketamine is an NMDA (*N*-Methyl-D-Aspartate) receptor antagonist which was originally developed by Dr. Calvin Stevens for Parke-Davis in search of an anesthetic alternative to phencyclidine (PCP, **23**, Figure 8) with a preferential safety profile. Ketamine, as well as phencyclidine (**23**), have long histories of human use both medicinally as anesthetics, and recreationally as dissociative hallucinogens. Ketamine has many desirable characteristics over traditional antidepressant drugs, in that it is very fast acting, and associated side effects are generally less serious and more transient than those caused by previously mentioned classes of antidepressants.[77] The development of S(+)-ketamine (**22**) as an antidepressant was significant in that it demonstrated the potential of new targets for antidepressant activity, as well as the potential for compounds which were previously considered to be recreationally appealing with a history of abuse to be transitioned to medications approved for the treatment of psychiatric disorders.



S(+)-Ketamine (**22**) Phencyclidine (**23**)

Figure 8. FDA approved antidepressant and NMDA antagonist S(+)-ketamine (**22**), and discontinued dissociative anesthetic phencyclidine (**23**).

Although ketamine is primarily known as an NMDA receptor antagonist, there are a number of different proposed mechanisms responsible for its antidepressant effects which could introduce new targets for which ligands can be synthesized in an attempt to treat depressive symptoms. For instance, the acute and long-lasting antidepressant effects of ketamine have been shown to be mediated through a 5-HT_{1B} dependent mechanism, and subanesthetic doses of ketamine have been shown to decrease serotonin transporter activity in non-human primates.[78–80] Additional targets of ketamine that have been implicated in antidepressant effects include AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) ionotropic receptors, BDNF (brain-derived neurotrophic factor) production cascades, and mTor (mechanistic target of rapamycin) kinase inhibition, although that is beyond the scope of this thesis.[81]

The most promising aspect of ketamine treatment is the lack of lag-time associated with the time that the drug is given to the first sign of therapeutic effects. A review of ten different studies using ketamine on patients with MDD and treatment-resistant MDD found significant improvements in depressive symptoms quantified by the Hamilton Depression Rating Scale (HDRS), with improvements in one trial being seen as early as 90 minutes post-treatment, and in another study lasting as long as 28 days after treatment.[82–84] The parameters of these studies varied greatly, but the general conclusion was consistent: ketamine is an efficacious and fast acting antidepressant with potential for significant effects long after initial dosing. The benefit of lessened lag time and decreased risk of long-term side effects has pushed ketamine to the forefront of antidepressant research, to the point where in 2015 there were more than twenty clinical trials evaluating ketamine for the treatment of MDD.[82]

Ketamine treatment differs in many ways from traditional antidepressant therapy besides the lack of lag-time. Instead of a daily consumption of a tablet, ketamine is most commonly given

in infusions, typically on a weekly or bi-weekly schedule after initial "loading doses" given over a shorter period of time. The approval of *Sprovato* ®, which is formulated as a nasal spray for even greater patient convenience, follows a similar dosing regimen. The frequency of treatments is variable and subject to change to suit the needs of the patients and the provider, but this treatment regimen nonetheless represents a deviation from typical antidepressant care.

C. 5-HT$_{2A}$ Serotonin Receptor

1. Receptor History

Serotonin (**7**) was first discovered by Vittorio Erspamer in 1935, as a biological amine extracted from enterochromaffin cells which he called "enteramine", which had vasoconstrictive and muscle-contracting properties.[85] Over a decade later, the discovery of a vasoconstrictive compound extracted from bovine serum, henceforth named "serotonin" (serum + tonic) was found that was hypothesized to contain an indolic structure similar to that of tryptamine or tryptophan by Rappaport, Green, and Page.[86] It was eventually established that the structure of this vasoconstrictive biogenic amine was 5-hydroxytryptamine (5-HT, serotonin, **7**) and that the chemical being researched by both Erspamer and Rappaport was one and the same.[87] Shortly thereafter, the serotonin (**7**) content of mammalian brain tissue (dog, rabbit) was confirmed by Twarog and Page,[88] suggesting that this compound was not solely acting as a vasoconstrictive compound, and that it may have neurotransmission properties as well. It was Woolley and Shaw[89,90] who first made the connection between the occurrence of this compound in the brain and the action of similarly structured therapeutic compounds as agents of interference with the biological actions of neurotransmitters and hormones.

The ability of LSD (**6**), a compound with a similar indolic structure, to block the effects of serotonin (**7**) in smooth muscle contraction assays, and the ability of LSD to cause disturbances in mental state similar to those caused by schizophrenia led to Woolley hypothesizing that the cause of LSD-precipitated mental disturbances were due to its action as an "anti-metabolite" that interfered with the natural function of serotonin in the brain.[91] Compounds that also shared structural similarities to serotonin, namely yohimbine and the harmala alkaloid harmine (**1**), were also shown to antagonize the activity of serotonin in arterial preparations.[89] Differences in the abilities to antagonize the actions of serotonin by ergot compounds among differing tissue preparations led to the hypothesis that there is more than one site of serotonergic binding. Gaddum and Picarelli[92] found that preparations of guinea pig ileum appeared to contain two separate types of serotonin receptors, which they named D and M, after the antagonists used to block serotonin (**7**) activity at each site (dibenzyline and morphine), with the D receptors thought to be related to smooth muscle, and the M receptors thought to be related to the nervous system. This finding was further supported by the discovery of two distinct binding sites for both [^{3}H]5-hydroxytryptamine and [^{3}H]LSD with unique binding affinities by Fillion et al.[93] The discovery that [^{3}H]5-hydroxytryptamine selectively interacted with what was named the 5-HT$_1$ receptor, and [^{3}H]spiperone to what was named the 5-HT$_2$ receptor by Peroutka and Snyder[94] supported this hypothesis as well.

Through extensive radioligand binding studies, the two 5-HT receptor subtypes were eventually separated into 15 different receptors, divided unequally amongst 7 different subfamilies of receptor from 5-HT$_1$ to 5-HT$_7$. Almost all of the serotonin family of receptors are G-Protein Coupled Receptors (GPCRs), with the lone exception being the 5-HT$_3$ receptor, which is a ligand-gated ion channel (LGIC), which is the same receptor that was denoted as the M receptor by

Gaddum and Picarelli. Humphrey et al.[95] proposed that the characteristics for separation of these subgroups of receptors should be based on three different criteria: operational data on both agonists and antagonists of each receptor type and their binding affinities; structural data of the amino acid sequence of the specific receptor subtype and its homology with other receptors in the family; and transductional data on the intracellular signaling pathways utilized by the receptor subtype.

2. Structure and Distribution

5-HT_2 receptors are a class of three receptors which display between 46-50% sequence identity with one another: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. These receptors are made up of seven transmembrane helices, with three extracellular loops, three intracellular loops, an extracellular N-terminus and intracellular C-terminus. These receptor proteins preferentially couple with $G_{q/11}$ to upregulate hydrolysis of inositol phosphate (IP), accumulate diacylglycerol (DAG) and consequentially release cytosolic stores of calcium, increase influx of extracellular calcium, and activate protein kinase C (PKC) through phospholipase C (PLC).[96,97] For the sake of brevity, the 5-HT_{2A} receptor will be the only receptor examined for this thesis, though there are many reviews covering the 5-HT_2 receptor family in great detail. [96,98–100]

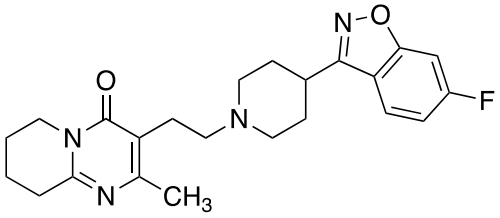
The 5-HT_{2A} receptor is most highly localized in the brain of humans and other mammals, with the highest concentrations of 5-HT_{2A} mitochondrial RNA occurring in layer 1, 4, and 5a of the cerebral cortex, the entorhinal, and the piriform cortex, particularly on pyramidal neurons. Appreciable levels of 5-HT_{2A} are also found in the diencephalon, mesencephalon, metencephalon, and myelencephalon. In peripheral tissues 5-HT_{2A} was nearly indetectable, but its presence has been demonstrated in both gut and platelet cells.[98,101] These regions of the brain are most generally associated with perception, cognitive function, and social interaction, and therefore the localization

of this receptor in these areas suggests that regulation of 5-HT_{2A} receptor function in these areas may be key in treating conditions which affect the aforementioned biological functions.

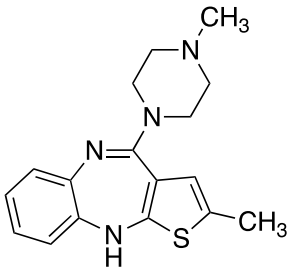
3. Potential as an Antidepressant Target

Glennon et al.[102,103] first discovered the link between hallucinogenic potency of certain compounds and their binding affinities at 5-HT_{2A} receptors in 1984, whereas previously the nature of hallucinogenic action was much more obfuscated due to the promiscuous binding of most hallucinogenic compounds. This discovery was later validated in humans, who were given the 5-HT_{2A} antagonists ketanserin and risperidone (**24**) prior to treatment with psilocybin (**5**), and found that pretreatment with these 5-HT_{2A} antagonists abolished the psychosis-like effects of psilocybin.[104] Since this discovery, 5-HT_{2A} receptor agonists have become known for their link to hallucinogenic compounds, and it has been largely avoided as a target for therapeutic drugs, outside of certain 5-HT_{2A} antagonist antipsychotic compounds. However, the similarities in the changes in perception brought about by classical hallucinogens and schizophrenia has kept the 5-HT_{2A} receptor as a topic of interest in the treatment of various mental disorders.[105–107] 5-HT_{2A} receptors are particularly intertwined in the treatment of mental disorders such as depression, schizophrenia, obsessive-compulsive disorder (OCD), obesity, Parkinson's, and anorexia nervosa.[98] It has been shown with 5-HT_{2A} selective radioligands that the densities of receptor in the human brain are altered significantly in patients who are diagnosed with schizophrenia, anorexia, bulimia, and untreated obsessive compulsive disorder.[101] In patients diagnosed with MDD, the density of the 5-HT_{2A} receptor in the brain and in platelets was also shown to be decreased.[108]

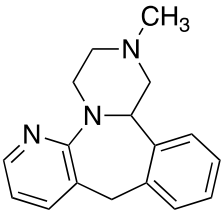
In the case of depression, many connections can be drawn between the activity or expression of the receptor and positive or negative outcomes in treatment of MDD. For instance, higher amounts of 5-HT_{2A} receptors were found in the postmortem brains of teen suicide victims as compared to controls.[109] Chronic treatment with antidepressant drugs is also associated with a decrease in expression of 5-HT_{2A} receptors after prolonged treatment.[110] It has also been reported that 5-HT_{2A} antagonists (risperidone **24**, olanzapine **25**, mirtazapine **26**, mianserin **27**; Figure 9) improve the response to traditional antidepressant treatment.[111]



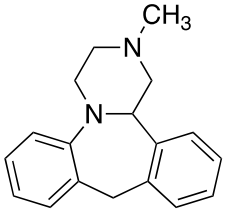
Risperidone (**24**)



Olanzapine (**25**)



Mirtazapine (**26**)



Mianserin (**27**)

Figure 9. 5-HT_{2A} antagonists risperidone (antipsychotic, **24**), olanzapine (antipsychotic, **25**), mirtazapine (antidepressant, **26**), and mianserin (antidepressant, **27**).

The association between disfunction of 5-HT_{2A} receptors and depression has been supported in preclinical trials with animal models of depression. Treatment with selective 5-HT_{2A} agonists such as 2,4-dimethoxy-4-iodoamphetamine (DOI) significantly increased the amount of time spent by mice immobilized in the forced swim test (FST), a model for evaluating the efficacy

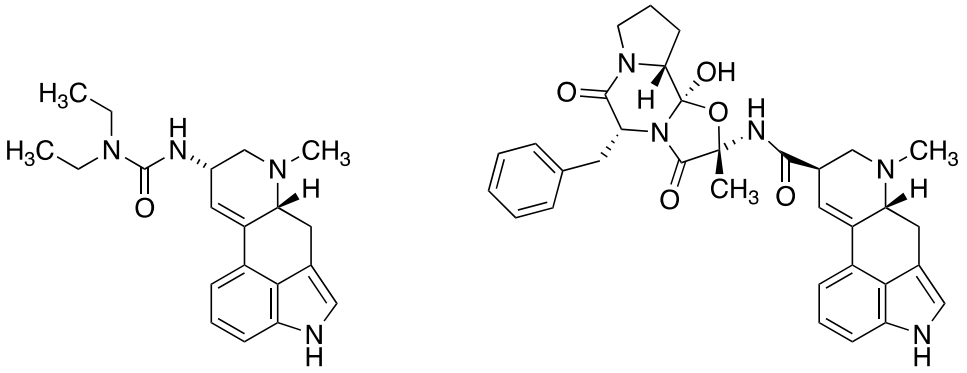
of antidepressant compounds where less time spent immobilized is found to correspond with a decrease in depressive-like behavior in rodents.[112] Pre-treatment with the selective 5-HT_{2A} antagonist volinanserin (MDL100907) abolished the increases in immobility time caused by DOI completely.[113] Other preclinical trials examining selective 5-HT_{2A} antagonists (i.e. 5-((4-benzo [α] isothiazol-3-yl) piperazin-1- yl)methyl)-6-chloroindolin-2-one and volinanserin) in the FST found antidepressant-like effects, suggesting that inactivation of this receptor type may be key in reducing depressive symptoms.[114,115]

More recently, the ability of 5-HT_{2A} agonists to cause antidepressant-like effects has also been demonstrated in animal models. DMT (**4**), psilocybin (**5**), LSD (**6**), and DOI have all been shown to produce antidepressant-like effects in rodents by themselves. A dose of ayahuasca, containing the β-carbolines harmine (**1**), harmaline (**2**), and tetrahydroharmine (**3**), as well as DMT (**4**), was also shown to have antidepressant effects in a primate model of human depression.[116] The aforementioned clinical trials in humans of the hallucinogenic compounds DMT (**4**), psilocybin (**5**), and LSD (**6**) have all shown great promise in the treatment of MDD, but none have made it beyond clinical trials as of the writing of this thesis.

4. Signal Transduction / Biased Agonism

Since the activity of the 5-HT_{2A} receptor has been shown to modulate the appearance of depressive symptoms and the efficacy of antidepressant treatment, large amounts of research have been and are currently being done to elucidate the mechanism of action by which 5-HT_{2A} agonists and antagonists' effect MDD. Of particular interest are the multitude of signaling pathways that are activated by the receptor. Interestingly, while all serotonergic hallucinogenic compounds are agonists at the 5-HT_{2A} receptor subtype, not all 5-HT_{2A} agonists are hallucinogenic. There are few

examples of compounds that have been shown to be agonists, but have not been shown to produce the same altered states of perception except in very rare cases. Examples of these kinds of compounds are the ergot alkaloids lisuride (**28**, Figure 10) and ergotamine (**29**, Figure 10), which are used as treatments for Parkinson's disease and migraines respectively. One cannot say that these compounds are without hallucinogenic activity of some kind, as there are case reports of both causing visual or auditory hallucinations in very particular circumstances, but they are generally without psychoactive activity.[117,118] The existence of non-hallucinogenic 5-HT_{2A} agonist compounds is strong evidence that agonism at this receptor is not the sole cause of hallucinogenic effects, and suggests that there are more specific causes at play. Furthermore, if the possibility of agonism of 5-HT_{2A} without hallucinogenic effects exists, and some hallucinogenic 5-HT_{2A} agonists have been shown to have antidepressant effects in humans, it may be possible to design an agonist of the 5-HT_{2A} receptor that is non-hallucinogenic, that also maintains antidepressant activity.



Lisuride (**28**) Ergotamine (**29**)

Figure 10. Non-hallucinogenic 5-HT_{2A} agonists lisuride (**28**) and ergotamine (**29**).

The key to separating antidepressant effects from hallucinogenic effects brought upon by these compounds, if at all possible, lies in understanding the second-messenger systems that are

selectively activated or not activated by ligands of 5-HT_{2A} receptors. Ligand-directed signaling has become increasingly popular in the world of medicinal chemistry, as it allows the possibility of designing medications which are even more selective via stimulation of downstream signaling pathways, rather than just being agonists or antagonists at a receptor population, possibly providing more specific therapeutic effects and/or decreased side effects. Ligands for a receptor can affect multiple pathways at once, with varying efficacies, or may be very selective for only one pathway. This concept has been proven to apply to various different types of receptors, including serotonin, opioid, dopamine, and adrenergic receptors, and it has been described by various names; such as biased agonism, ligand directed signaling, agonist-directed trafficking, and functional selectivity.[119,120] For the sake of clarity and consistency, this phenomenon will henceforth be referred to as biased agonism.

5-HT_{2A} receptors have two main downstream signaling pathways: the previously mentioned phospholipase C-mediated inositol phosphate and diacylglycerol accumulation, as well as phospholipase A_2 (PLA_2)-mediated arachidonic acid (AA) release.[97,121] The first mention of biased agonism in reference to the 5-HT_{2A} receptor was by Berg and co-workers[122] in 1998, who discovered that the 5-HT_{2A} receptor agonists were able to stimulate these pathways independently and with different maximal efficacies (Table 1). These findings were further supported by Kurrasch-Orbaugh and co-workers[123], who found similar differences in the efficacies for each signaling pathway among hallucinogenic and non-hallucinogenic 5-HT_{2A} receptor agonists, and that these two pathways were activated independently of one another. This represented a large shift from previously accepted theory of rank order receptor activation and intrinsic efficacy, because ligands of a certain receptor could no longer adequately be described by their activation of a single receptor pathway. For example, ranked efficacy of ligands at the IP pathway of 5-HT_{2A} receptors

would not reflect the ranked efficacy of the same ligands at the AA pathway. The two-state receptor model, which proposed that the efficacy of ligands of a receptor are dependent on the ability of the ligands to switch the conformation of a receptor from active to inactive or vice versa, would then also no longer be an accurate description of ligand-receptor interactions.[124,125] The implications of this discovery for not only serotonin receptors, but any receptor family with multiple downstream signaling cascades, cannot be overstated.

Table 1. Relative 5-HT_{2A} agonist maximal efficacies for both IP accumulation and AA release in comparison to the endogenous ligand serotonin (5-HT, **7**) as determined by Berg et al.[122]

Agonist	E_{max} agonist/E_{max} 5-HT IP	E_{max} agonist/E_{max} 5-HT AA
5-HT	1.00	1.00
Bufotenine	0.78	1.08
DOI	0.61	0.97
LSD	0.17	0.39
Quipazine	0.89	0.59
Trifluoromethylphenylpiperazine	1.02	0.59

The difference in the ability of ligands to activate downstream signaling pathways based on small changes in their chemical structure led researchers to hypothesize about the existence of "molecular switches" in the binding pocket of the 5-HT_{2A} receptor which would activate one of these pathways when interacting with the bound ligand. Molecular modeling and binding studies confirmed that certain residues in transmembrane helix 5 had a significant effect not only on the binding of 5-HT_{2A} ligands, but also their ability to induce IP hydrolysis.[126] Substituents on the

indolealkylamine structure were found to alter the binding affinity as well as the level of IP hydrolysis when certain residues of the binding pocket were mutated.[127]

Using the knowledge that the endogenous ligand serotonin (**7**) activates these pathways with roughly equal efficacy, and that 2,5-dimethoxy-4-cyanophenethylamine (2-CN) stimulates the AA pathway while having little efficacy for the IP pathway, Marti-Solano et al.[128] were able to design analogs of serotonin (**7**), making slight modifications of the indolealkylamine structure-based modeled interactions of the previously mentioned compounds to the receptor to selectively enhance the efficacy for one pathway over the other. By synthesizing ligands which were unable to make electrostatic interactions with a key asparagine residue in the binding pocket, they were able to create ligands which were highly selective for the IP downstream signaling cascade which lacked efficacy for the AA pathway entirely. Discovery of key residues in the binding pocket of the 5-HT_{2A} receptor that selectively activate downstream signaling cascades would be essential in designing new agonists for the receptor if these pathways can be linked to specific therapeutic effects.

One of the most critical groups of signaling pathways are those that regulate receptor expression and downregulation. Down-regulation of GPCR receptor populations is thought to be a cellular shut-off switch to halt overstimulation, which can lead to toxic cellular environments or uncontrolled cell growth, and all GPCR receptor populations have mechanisms by which they may be internalized and degraded, including the 5-HT_{2A} receptor.[129]

There are two separate types of desensitization: homologous or agonist-specific, which is desensitization to a specific singular agonist, and heterologous, which is desensitization to structurally distinct agonists following stimulation of that receptor.[110] Homologous desensitization is thought to be dependent on beta-arrestin proteins and G protein-coupled receptor kinases (GRK),

while heterologous desensitization is thought to be coupled to the second messenger kinases protein kinase A (PKA) and protein kinase C.[110,130] It is common for receptor groups to be prone to receptor down-regulation after interaction with agonist ligands in order to prevent overstimulation, but in contrast to most other types of receptors, the down-regulation of 5-HT_{2A} receptors has been shown to be caused by both agonists and antagonists.

Buckholtz and co-workers[131] found that the administration of hallucinogenic 5-HT_{2A} agonists such as LSD (**6**) and DOI were able to significantly reduce the total receptor population in rat models within 24 hours after administration. They also found that chronic administration of LSD and psilocybin (**5**) were able to decrease 5-HT_{2A} receptor populations without reducing binding levels of other serotonin receptor and non-serotonin receptor (adrenergic, dopaminergic) populations, while the non-hallucinogenic 5-HT_{2A} neutral antagonist and LSD analogue bromo-LSD did not affect 5-HT_{2A} receptor binding levels at all.[132] Many antidepressant and antipsychotic compounds across different classes with varying affinities for the 5-HT_{2A} receptor have also been shown to cause reductions in receptor binding populations.[133–135] It would be misleading to suggest that these compounds cause their therapeutic benefits entirely through this mechanism, as some SSRI antidepressants have been shown to produce no decrease in 5-HT_{2A} receptor population, and even increase them in some instances. There are also many other compounds which have no known antidepressant action which have been shown to cause 5-HT_{2A} receptor population decreases as well.[136,137]

The mechanism of action by which these compounds down-regulate 5-HT_{2A} is not fully understood, but beta-arrestins, lysosomal degradation, and gene transcription factors are all hypothesized to play a role. Desensitization caused by beta-arrestins and GRKs is an acute response which occurs over minutes, while lysosomal degradation and receptor ubiquitination

occurs over hours or days.[129] The ability of cells expressing 5-HT_{2A} receptors to re-express or re-synthesize the original amount of receptor after exposure to agonist or antagonist ligands occurs on a longer timescale than the down-regulation of the receptor population, meaning that any therapeutic benefit brought about by this phenomenon would theoretically be longer lasting than the acute effects of the agonist or antagonist being used.

Another vital part of the activation of GPCRs is gene expression, which is in many cases the end result of a downstream signaling cascade. A study by González-Maeso et al.[138] found that each agonist of 5-HT_{2A} induced a unique profile of gene transcription, and further analysis determined that particular gene transcription was associated with different activity patterns. The induction of the *c-fos* gene was associated with agonist activity at the 5-HT_{2A} receptor regardless of hallucinogenic activity, while *egr-1* and *egr-2* induction was found to be reliably produced only by compounds with hallucinogenic activity. Inhibition of PLC attenuated the response of both hallucinogenic and non-hallucinogenic agonist gene transcription, but pre-treatment of cortical cell cultures with pertussis-toxin only decreased the gene transcription response seen from LSD (**6**), but not lisuride. Although this was only performed on one pair of structurally similar hallucinogenic/non-hallucinogenic 5-HT_{2A} agonists, the results of this experiment suggest that the hallucinogenic agonist gene response could possibly be mediated by pertussis toxin sensitive $G_{i/o}$, as well as $G_{q//11}$.

One particular 5-HT_{2A} receptor downstream signaling-linked protein which has been associated with antidepressant effects as well as positive changes in both memory and cognition is brain-derived neurotrophic factor (BDNF).[139,140] This protein has been shown to promote synaptic plasticity, which is known to reduce the effects aging, Alzheimer's, chronic stress, and psychiatric disorders have on cognition and memory.[141] It has even been suggested that BDNF is

capable of acting as a biomarker for MDD: chronic stress, which has a high degree of correlation with MDD, decreases the amount of BDNF in the brain, while patients with MDD on antidepressant medications have seen increases in the expression of BDNF normalize after treatment.[142] BDNF has also been shown to reduce the level of 5-HT_{2A} in hippocampal cell cultures, which mimics the decrease seen in 5-HT_{2A} receptor levels after treatment with antidepressants.[143] Synaptic plasticity has recently become a topic of interest in the treatment of MDD, as there is overwhelming evidence for the implication of neuronal atrophy in the prefrontal cortex in MDD and related psychiatric disorders.[144] Antidepressant such as SSRIs, esketamine (**22**), and exploratory hallucinogenic therapies have been shown to significantly increase the levels of BDNF, and to consequently counteract neuronal decay and increase the concentration of synapses in the brain.[145–147] Further support for what has been termed the "neural plasticity theory of depression" comes from the revelation that the time period by which chronic antidepressant treatment increases BDNF has been linked to the "lag time" experienced at the start of traditional antidepressant treatment before benefits are seen.[43,148] While these correlations may seem promising, the link between antidepressant compounds of various classes and BDNF expression is still not completely understood, and compounds which have been screened specifically for neurogenesis properties have yet to pass clinical trials as antidepressants.[149]

D. Chemical Constituents of Ayahuasca

1. Tryptamines: NMT and DMT

Tryptamines are a class of compounds which are found endogenously in humans and in other mammalian species, which are mainly produced via dietary sources of the amino acid L-tryptophan. Once tryptophan has been transported across the blood-brain barrier (BBB),

tryptophan hydroxylase and tryptophan decarboxylase enzymes are used to produce the endogenous neurotransmitter serotonin (**7**). There are a number of compounds related to serotonin that are synthesized depending on location and availability of enzymes which act upon the tryptamine structure to add or remove constituents. In addition to the previously mentioned enzymes which are used in the synthesis of serotonin from tryptophan, indolethylamine-*N*-methyltransferase is used to methylate the basic amine of the tryptamine structure. Studies using tryptamine as substrate for indolethylamine-*N*-methyltransferase found that it was consequently converted to *N*-methyltryptamine (NMT) and then DMT (**4**).[150] DMT was first discovered in human blood along with the structurally related compounds serotonin (**7**), tryptamine (**60**), *N*,*N*-dimethyl-5-hydroxytryptamine, and 5-methoxytryptamine in 1965.[151] It was originally postulated to be a "schizotoxin", but the only real connection that can be drawn between DMT-induced hallucinations and schizophrenia are the remarkable perceptual changes that can occur.[152]

The first thorough analytical examination of ayahuasca and specifically various members of the genus *Psychotria* as ingredients of ayahuasca was conducted by Rivier and Lindgren in 1972. In various specimens of *Psychotria*, the tryptamines NMT and DMT (**4**) were found. In the two specimens that were found to contain DMT (**4**), it was found to make up the vast majority of the alkaloidal content of the plant (99%), while NMT was represented only in trace quantities.[11] Interestingly, only certain members of the *Psychotria* genus contained DMT (**4)** or any indolic alkaloid, while others were completely devoid of alkaloids (*P. emetica, P. bacteriophylla, P. undulata*). Out of a total of nine ayahuasca mixtures which were examined in their study, six contained quantifiable amounts of DMT (**4**), whereas NMT was not reported for any of the beverages. The amount of DMT (**4**) in the mixture was found to be as low as 5.4 mg / 100 mL of beverage, and as high as 16 mg / 100 mL. Since then, many studies have been conducted on various

specimens of ayahuasca from different regions of the Amazon River basin, and all have found similar concentrations of DMT (**4**) as the only active tryptamine present in any significant quantity (Table 2).[153–155]

Table 2. Concentration of DMT (**4**) in ayahuasca preparations examined by Rivier and Lindgren.[11]

Preparation	Total alkaloid makeup	mg DMT / 100 mL
"White Ayahuasca"	*B. caapi, P. viridis*	13
"Crude Black Ayahuasca"	*B. caapi, P. viridis, L. venustum*	0
"Boiled Black Ayahuasca"	*B. caapi, P. viridis, L. venustum*	12
"Crude Black Ayahuasca"	*B. caapi, P. viridis, L. venustum*	0
"Boiled Black Ayahuasca"	*B. caapi, P. sp.*	5.4
"Black Ayahuasca"	*B. caapi, P. viridis, L. venustum*	9.8
"Red Ayahuasca"	*B. caapi, P. viridis, L. venustum*	16
"Ayahuasca"	*Unknown*	14
"Ayahuasca"	*B. caapi, P. viridis*	0

The first clinical trials in humans conducted with DMT (**4**) were in 1956 by Szára, who found that it produced profound hallucinations comparable to LSD (**6**) and mescaline, with a much shorter duration of action.[156] It was not until the 1990s that Strassman elaborated on the clinical trial conducted by Szára, giving DMT (**4**) to more than 60 participants over 5 years. These trials studied the psychological and physiological responses of humans to DMT (**4**) administration, developed a dose-response curve for the effects of DMT (**4**), and produced the HRS for the

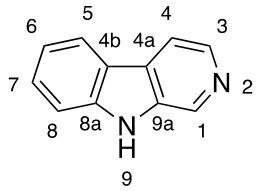
description of hallucinogenic experiences.[17,157,158] In addition, Sźara showed that DMT (**4**) can be administered safely to experienced users of hallucinogenic compounds, and is generally well tolerated with a lack of non-transient side effects.

Recreational use of DMT (**4**) and other dialkylated tryptamines is relatively common, while recreational use of NMT and other monoalkylated tryptamines is almost unheard of. DMT (**4**) when taken by itself is commonly smoked or injected, with active smoked doses ranging from 15-60 mg and produce intense psychoactive effects lasting roughly 15 minutes.[159] There are anecdotal reports of NMT activity from Dr. Alexander Shulgin, who suggested that the vaporization of 100 mg gave brief visuals lasting for less than one minute, but this compound has not been well studied in a controlled setting.[160] The dialkylation of the basic nitrogen of the tryptamine structure is likely important in preventing first-pass metabolism when ingested orally, as NMT and DMT (**4**) are both orally inactive without the co-ingestion of a MAO inhibitor, while increasing the aliphatic bulk of the substituents of the basic nitrogen (for example: *N,N*-diethyltryptamine) is shown to make these compounds active when taken via this route. The very minor presence of NMT in analytically examined preparations of ayahuasca suggest that it plays a very small role in the effects of the brew, if it contributes at all, and for that reason DMT (**4**) will be the only tryptamine structure which is examined in this thesis.

2. β-Carbolines: The Harmala Alkaloids

β-Carbolines are a class of compounds that are distributed widely in nature containing a tricyclic indolic ring structure, which are the cyclization product of tryptamines. Compounds containing this ring structure have been of interest to medicinal chemists for decades, for various uses such as gamma-aminobutyric acid (GABA) receptor antagonists, intercalation of DNA,

inhibitors of MAO, antiparasitic activity, antitumor, antiseizure, antiviral, and antimicrobial properties.[161] The β-carboline scaffold is the major substructure of over 60 "simple" alkaloids found widely in nature, in both fungi, angiosperms, arachnids, marine invertebrates, and mammals.[162–164] For clarity, the numbering system of the simplest general β-carboline scaffold compound (norharmane, **30**) is shown below (Figure 11).



Norharmane (**30**)

Figure 11. Numbering system for the β-carboline structure shown on norharmane (**30**).

β-Carbolines are also present in the brains of humans.[165,166] The cyclization of tryptamines has been shown to be possible through a number of different mechanisms with distinct precursors. The biosynthesis of these compounds is thought to proceed predominantly through a Pictet-Spengler-type cyclization, using the readily available biogenic tryptamines discussed previously, as well as endogenous aldehydes. β-Carbolines which are unsubstituted at the 1-position are formed through the reaction of tryptamines with 5-methyl-tetrahydrofolate and the corresponding available tryptamine (**60**). Synthesis of β-carbolines substituted at the 1-position is proposed to occur through three distinct biosynthetic pathways, intramolecular Bischler-Napieralski-type cyclization, cyclization of tryptamines via condensation with biogenic aldehydes such as acetaldehyde in a Pictet-Spengler cyclization, and condensations with alpha-keto acids such as pyruvic acid which result in methyl and carboxyl substitution at the 1-position (Figure 12).[167] These 1-carboxyl β-carbolines were readily decarboxylated into 1-methyl β-carbolines in mouse

brain tissue homogenate when compared to lung tissue homogenate, suggesting that there may be a biological need for the synthesis of these compounds in brain tissue.[168]

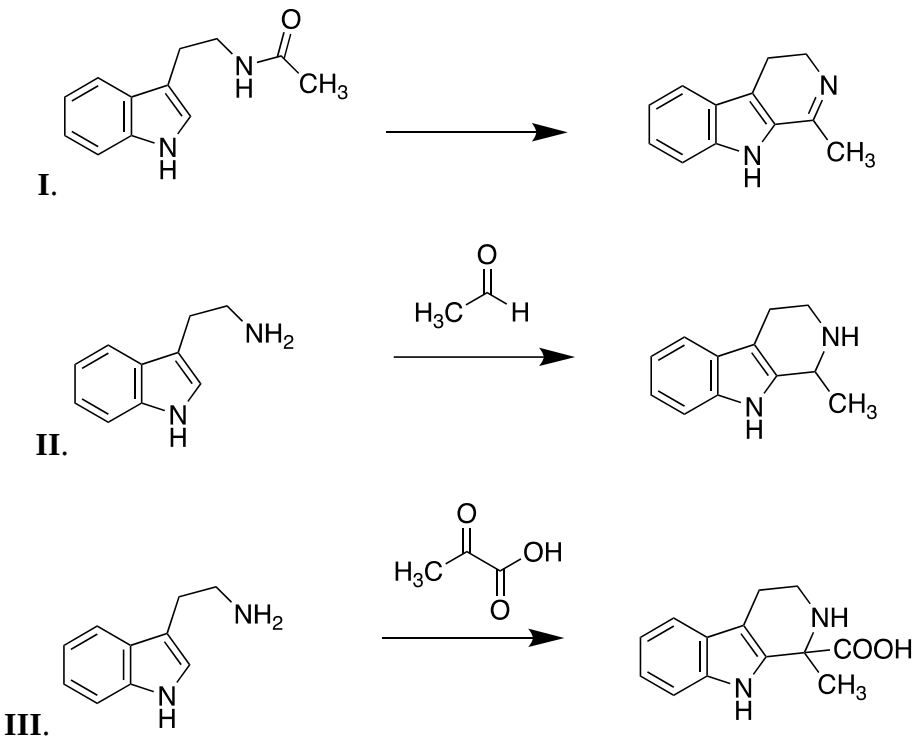


Figure 12. Biosynthetic pathways for the conversion of tryptamine compounds to β-carbolines using Bischler-Napieralski-like cyclization (**I**), Pictet-Spengler-like cyclization (**II**), and condensations with alpha-keto acids (**III**).[167]

The presence of β-carboline alkaloids in plants was first discovered in the 1840's by German chemists Goebel and Fritzche, who extracted and isolated harmaline (**2**) and harmine (**1**, Figure 1), respectively, from the seed husks of *Peganum harmala*, hence the name "harmala" alkaloids and "harm-" prefix of the common names of these alkaloids.[169] It was later revealed that harmine (**1**) found in *Peganum harmala* and the major alkaloid of the hallucinogenic ayahuasca brew from *Banisteriopsis caapi* were identical. The previously mentioned study by Rivier and

Lindgren found harmine (**1**), harmaline (**2**), and tetrahydroharmine (**3**) in almost all preparations that were examined (Table 3).[11] It was determined that the R-isomer was the naturally occurring form of tetrahydroharmine (**3**) found in all species of *B. caapi*.[170]

Table 3. Concentration of harmala alkaloids in ayahuasca preparations examined by Rivier and Lindgren.[11]

Preparation	Total alkaloid makeup	mg / 100 mL
"White Ayahuasca"	Harmine – 26%	Harmine – 17
	THH – 11%	THH – 7.2
"Boiled Black Ayahuasca"	Harmine – 47%	Harmine – 18
	Harmaline – 4%	Harmaline – 1.5
	THH – 6%	THH – 2.3
"Boiled Black Ayahuasca"	Harmine – 43%	Harmine – 6.6
	Harmaline – Trace	Harmaline – 0
	THH – 10%	THH – 1.5
"Black Ayahuasca"	Harmine – 37%	Harmine – 18
	Harmaline – 2%	Harmaline – 1.1
	THH – 20%	THH – 9.8
"Red Ayahuasca"	Harmine – 37%	Harmine – 19
	Harmaline – 3%	Harmaline – 1.6
	THH – 14%	THH – 7.2
"Ayahuasca"	Harmine – 22%	Harmine – 7.1
	Harmaline – 1%	Harmaline – 0.3
	THH – 9%	THH – 2.9
"Ayahuasca"	Harmine – 21%	
	Harmaline – 4%	Not measured
	THH – 40%	

The general abundance of harmala alkaloids in these preparations seems to follow a consistent pattern of quantity of each alkaloid, no matter where the plant material is taken from or

how the beverage is prepared. In general, harmine (**1**) is the most abundant alkaloid, followed by tetrahydroharmine (**3**), and then harmaline (**2**). This trend has been validated by later studies using more accurate analytical techniques using a larger number of distinct ayahuasca samples.[155,171]

The presence of these alkaloids in a beverage that is known to be hallucinogenic, along with a compound which is not known to be hallucinogenic by itself when taken orally, i.e., DMT (**4**), begs the question of whether the harmala alkaloids are hallucinogenic on their own. Claudio Naranjo, noting that Pennes and Hoch had found harmine (**1**) to be hallucinogenic in its own right, discovered that upon administration of harmaline (**2**) to 30 volunteers, hallucinations which were distinct from those brought about by mescaline were observed.[172] In comparison to mescaline, harmaline (**2**) intoxication was described as being more physical than visual, although closed-eyed visuals were abundant, and there was a lack of synesthesia or time dilations which are known to occur with classical psychedelics. The threshold psychoactive dose for harmaline (**2**) was found to be one-half of that reported for harmine (**1**) by Pennes and Hoch, which was in agreement with the observation by James Gunn that non-psychoactive effects of harmaline were precipitated at half the dose needed for harmine.[173] A 300 mg dose of tetrahydroharmine (**3**) was also administered to a single volunteer, and it was reported by the volunteer that the subjective effects were similar to those brought about by a 100 mg dose of harmaline (**2**).[172] Harmaline (**2**) and its' structural isomer 6-methoxyharmalan were also shown to substitute for the hallucinogen 2,5-dimethoxy-4-methylamphetamine (DOM, **37**) in trained animals.[174] Interestingly, harmaline (**2**) did not substitute for LSD, and only partially substituted for DOM (**37**) in another study.[175] Beyond these studies, human reports of isolated harmala alkaloid psychoactive activity have not been conducted in any detail, although plenty of personal anecdotes exist online.

3. Binding and Functional Data at 5-HT$_{2A}$ and Related Receptors

With the discovery that the harmala compounds, when taken individually, are hallucinogenic on their own, the relationship to classical hallucinogens needed to be developed further. DMT (**4**) is also known to be a hallucinogenic compound, but it is inactive orally. As mentioned previously, Glennon et al.[102] discovered that 5-HT$_{2A}$ agonism was essential for hallucinogenic activity, and it was therefore of interest to determined what affinity these compounds had for the human 5-HT$_{2A}$ receptor. Glennon at al.[176] also determined the binding affinity for these compounds at [^{3}H] agonist labeled cloned rat 5-HT$_{2A}$ receptors (Table 4).

Table 4. Binding affinities of the major chemical constituents of ayahuasca at [^{3}H] agonist labeled rat 5-HT$_{2A}$ receptors.[176]

Compound	Affinity (nM)
Harmine	397 ± 13
Harmaline	5,010 ± 85
R(+)-THH	>10,000
S(-)-THH	5,000 ± 950
DMT	323

The determined binding affinity for these compounds at the 5-HT$_{2A}$ receptor is difficult to explain in reference to their reported hallucinogenic activity. Harmine (**1**) has relatively high affinity for the 5-HT$_{2A}$ receptor, roughly equal to that of mescaline, but lower than other classical hallucinogens, which typically possess single or double-digit nanomolar affinity for the receptor.[176–179] The reported potency of two of these compounds is also counterintuitive to their

binding affinities, with harmaline (**2**) reportedly being twice as potent as harmine (**1**), but having over an order of magnitude lower affinity for the 5-HT_{2A} receptor.

The harmala alkaloids were also examined at the 5-HT_{1A} and 5-HT_{2C} serotonin receptor populations, for which indolealkylamine hallucinogens have modest affinity. Harmine (**1**) and harmaline (**2**) showed poor affinity for the 5-HT_{2C} receptor (5,300 nM and 9,400 nM respectively), whereas *R*(+)-THH (**3**) showed no affinity for 5-HT_{2C} receptors. All three harmala alkaloids showed no affinity for the 5-HT_{1A} receptor (>10,000 nM), while DMT (**4**) showed modest affinity for both the 5-HT_{2C} and 5-HT_{1A} receptors (1450 nM and 200 nM respectively).[176]

The lack of agreement between the 5-HT_{2A} binding affinities of the harmala alkaloids and their reported hallucinogenic potency suggests that binding at the 5-HT_{2A} receptor may not be the cause of their activity. Combined with the observation that hallucinations brought about by harmala alkaloid ingestion varied noticeably from those produced by the classical hallucinogen mescaline, it is possible, if not likely, that there are other biomolecular targets which are attributing to this effect. As of yet, there have been no other receptor populations with proven links to the hallucinogenic action of the harmala alkaloids.

In terms of functional data at the 5-HT_{2A} receptor, the only component of ayahuasca that has been studied in great detail is DMT (**4**), while harmine (**1**) and harmaline (**2**) have only been looked minimally in a single downstream signaling assay. As mentioned previously, there are a number of downstream signaling pathways linked to the 5-HT_{2A} receptor, of which most attention is focused on inositol triphosphate accumulation and arachidonic acid release.[97,121] DMT (**4**) has been shown to be an agonist in both of these downstream signaling pathways,[175,180] but harmine (**1**) and harmaline (**2**) are the only harmala alkaloids that have been examined for functional activity at the 5-HT_{2A} receptor. Both of these compounds were shown to not have any activity in a

phospotidyl inositol hydrolysis assay, which is linked to inositol triphosphate accumulation, but as mentioned previously, it is possible that these compounds could have agonist effects through another downstream signaling pathway, while having no effect on inositol triphosphate accumulation.[175,176] In order to truly determine the functional activity of these compounds at the 5-HT_{2A} receptor and determine if they could possibly be biased agonists, or antagonists, biological assays assessing the activity at multiple known downstream signaling pathways would need to be completed.

4. MAO Inhibition and Serotonin Transporter Activity

The most likely cause of the hallucinogenic effects of the ayahuasca brew is the activity of the harmala alkaloids at MAO-A, preventing first pass metabolism of DMT (**4**), and allowing it to become orally active. Rivier and Lindgren estimated that in a typical 200 mL ayahuasca dose, there are 65 mg of alkaloids present, of which 30 mg are harmine (**1**), 10 mg are THH (**3**), and 25 mg are DMT (**4**).[11] These doses of harmala alkaloids are much lower than the hallucinogenic doses discussed previously by a large margin, and DMT is not known to be orally active by itself at such a low dose.

The harmala alkaloids and their action at MAO was first investigated by Buckholtz and Boggan,[181] who found that the activity of these compounds at the MAO enzyme was related to the level of unsaturation of the pyridine moiety of the harmala alkaloids in mouse brain preparations (Table 5). Comparison of the activities of numerous β-carbolines allowed for the generation of a precursory SAR relationship between the structure of these compounds, which found that in addition to the effect of the saturation of the pyridine ring, substitution of the 7-position with a

methoxy group which harmine (**1**) and other harmala alkaloids contain, was also preferential for inhibitory activity at this enzyme.[181]

Table 5. Inhibition of MAO by harmala alkaloids in rat brain preparations as determined by Buckholtz and Boggan.[181]

Compound	IC_{50} (nM)
Harmine	80
Harmaline	60
THH	14,000

McKenna et al.[153] found that that a combination of harmala alkaloids equimolar to the ratio seen in ayahuasca preparations produced an IC_{50} value approximately halfway between the most abundant (harmine, **1** and THH, **3**) compounds, which demonstrated that these compounds do not act synergistically in their inhibition of MAO, and instead compete with one another for binding at this enzyme. An "ayahuasca analogue" consisting of doses of harmine (**1**) and THH (**3**) representative of the levels of these compounds in ayahuasca mixtures was found to have an almost identical IC_{50} value to the equimolar sample of harmala alkaloids, suggesting that the activity of harmine (**1**) and THH (**3**) is probably wholly responsible for the MAO effects of ayahuasca preparations.

Many follow-up studies examining the binding of harmine and harmaline at MAO-A have been conducted, consistently showing significant potency towards inhibition of the enzyme. Harmine (**1**) was determined to have IC_{50} values ranging from 1.0 nM to 25 nM, while the IC_{50} values for harmaline (**2**) ranged between 7.0 and 48 nM.[182–185] Neither of these compounds were shown to have any significant effect at MAO-B in any of the studies mentioned. It is surprising

that THH (**3**) has not been included in these studies as a member of the harmala alkaloid family, but it was probably not examined due to its previously established low potency at MAO compared to harmine (**1**) and harmaline (**2**).

A naturally occurring β-carboline compound, 6-methoxy-tetrahydro-β-carboline, a metabolism product of melatonin (*N*-acetyl-5-methoxytryptamine), was shown to increase brain concentrations of serotonin (**7**) without increasing the amount of 5-hydroxyindoleacetic acid, which is the main metabolite of serotonin produced by MAO-A. It was proposed that since this structurally related β-carboline was capable of inhibiting the reuptake of serotonin (**7**), that the harmala alkaloids would be likely to possess some degree of inhibition of serotonin reuptake as well. Buckholtz and Boggan[186] found that the trend in serotonin reuptake inhibition potency was not the same as that shown for MAO inhibition, and that the degree of reuptake inhibition increased with the degree of saturation of the pyridine moiety. Although the β-carbolines show some level of activity as serotonin reuptake inhibitors, they are substantially less active in this regard than the standard SSRI drugs that are currently on the market for the treatment of MDD, as shown in the comparison between the harmala alkaloids and fluoxetine (**15**, Table 6).

Table 6. Inhibition of serotonin (**7**) reuptake by harmala alkaloids and the SSRI fluoxetine (**15**) in synaptosomal suspension from mouse brain preparations as determined by Buckholtz and Boggan.[186]

Compound	IC_{50} (nM)
Harmine	11,000
Harmaline	15,000
THH	3,400
Fluoxetine	25

5. Animal Studies

There have been extensive *in vivo* animal studies of harmala alkaloids and their analogs for a variety of different ailments, but few have been done analyzing possible antidepressant effects. A review of the literature found that harmine (**1**) was the only harmala alkaloid found in ayahuasca that had been examined in more than one study for its antidepressant effects, with harmaline (**2**) being the only other alkaloid examined in any capacity.

Harmine (**1**), when given in acute 5, 10, and 15 mg/kg doses in mice, decreased immobility times in the forced swim test in a dose depended manner. This decreased immobility time was found to be antagonized by the indirect GABA antagonist flumazenil, but not the 5-HT_{2A} antagonist risperidone (**24**), suggesting that this effect was mediated through GABA receptor stimulation.[187] Another study using the same acute doses of harmine (**1**) found decreased immobility time in the FST, as well as increased swimming and climbing times. The 10 and 15 mg/kg doses of harmine (**1**) were found to significantly increase the amount of BDNF when compared to the tricyclic antidepressant imipramine (**13**), which also produced decreased immobility time.[188]

The findings of this study were further supported by identical results in terms of both decreased immobility in the FST and increased levels of BDNF when harmine (**1**) was administered chronically over 14 days, and the reversal of symptoms of anhedonia produced by a chronic mild stress program.[189,190] Causation between the levels of BDNF produced by harmine (**1**) administration and antidepressant effects seen in the FST were not proven, but as discussed earlier, increases in BDNF are often associated with positive mental health outcomes.

A further study examining the effects of chronic harmine (**1**) administration over 10 days (10 and 20 mg/kg) found that improvements in chronic unpredictable stress-induced depressive

symptoms were similar to those produced by administration of the antidepressant drug fluoxetine. Decreases in hippocampal BDNF levels and impairment of astrocyte function produced by chronic unpredictable stress were also returned to normal levels by harmine (**1**) administration. Harmaline (**2**) was also examined in the FST, and was found to produce antidepressant-like effects, as well as anxiolytic-like effects in an elevated plus maze test.[191]

Recently, it has been shown that DMT (**4**) is also capable of producing antidepressant-like effects in animal models. When rats were treated with sub-hallucinogenic (1 mg/kg) doses of DMT (**4**), the extinction of a learned-fear response was more likely to occur, and the time spent immobilized in the FST was also decreased.[192] This sub-hallucinogenic dose also did not cause increases in anxiogenesis-related behavior patterns, unlike larger doses of DMT (10 mg/kg, **4**). The effects of DMT (**4**) in the FST were also noted to be nearly indistinguishable from those of ketamine (**22**).[193] Since DMT (**4**) and ayahuasca both reduce locomotive activity in open-field tests, the increase in swimming behavior can be interpreted as true antidepressant activity, rather than stimulation. Antidepressant effects of numerous other serotonergic psychedelic compounds have been analyzed in animal models of depression, but there is a relative lack of studies examining DMT (**4**) in animal models of depression by itself, instead of as an active ingredient of ayahuasca.[194]

Naturally, since both subsets of compounds present in ayahuasca have been examined for antidepressant action, the concoction itself has also been tested in animals for the same effects. The first study examining the ayahuasca found that it produced antidepressant-like effects when administered to rats, with an inverse relationship between the dosage given and increased swimming behavior, while also reducing locomotive activity. Dosages of 2.5 mg/kg reduced immobility time by 32%, while a 10 mg/kg doses reduced immobility time by only 4.3%.[195]

Ayahuasca was given to juvenile primates who had been socially isolated to precipitate symptoms matching many of those seen in humans with depression, such as self-grooming, somnolence, decreased appetite, and anhedonia. Acute administration of ayahuasca produced significant and rapid improvement in the expression of these symptoms.[196]

These findings demonstrate that ayahuasca as well as some of the studied active ingredients in ayahuasca brews: DMT (**4**), harmine (**1**), and harmaline (**2**) elicit antidepressant-like action in animal models. Although data are lacking for the third most abundant active constituent, THH (**3**), its demonstrated action as a weak MAO-A inhibitor and serotonin reuptake inhibitor and structural similarity to the other three compounds mentioned suggest that animal studies with this compound may be warranted.

6. Human Studies

As of the writing of this thesis, there are no published studies with any of the harmala alkaloids or DMT (**4**) examining their antidepressant properties in humans. As previously mentioned, DMT (**4**) was famously administered in clinical trials by Sźara and Strassman[156,157] to examine its physiological effects, but its potential benefits in relation to depressive symptoms were never studied. However, there is currently at least one clinical trial underway to determine the potential efficacy of DMT (**4**) combined with psychotherapy in the treatment of major depression. The lack of human data surrounding these compounds, particularly in relation to their antidepressant effects, is surprising considering the multitude of positive animal studies using a wide variety of methodologies, models, and species. Even further support for examination of these compounds comes from the numerous clinical trials of ayahuasca in humans for depression or depression-linked conditions which were discussed previously. It would be of interest to compare

the imminent results of the first human trial of DMT (**4**) for antidepressant action to the study by Palhano-Fontes et al.[22] on the antidepressant effects of ayahuasca to see if isolating the active hallucinogenic component produces similar effects without the harmala alkaloids. It is possible that DMT (**4**) will be shown to have antidepressant activity, but with different efficacy, or a longer period before the therapeutic effects occur.

Presently, the mechanism of action for the antidepressant effects of ayahuasca, as well as the assumption that the antidepressant effects therein are produced through a singular biological process, are speculative at best. Until further human data has been collected, it will be impossible to separate the possible therapeutic effects of this concoction from the hallucinogenic activity that it possesses. Ideally, the antidepressant action of ayahuasca lies in just one of the β-carboline or tryptamine scaffolds, that can be modified and expanded upon to elucidate a pharmacophore for antidepressant action. It is also entirely possible that an entourage effect exists between all active components of ayahuasca that makes the divorce between hallucinogenic action and therapeutic action impossible.

III. Specific Aims and Rationale

Although the therapeutic utility of ayahuasca for the treatment of neuropsychiatric disorders is still under investigation, and whereas its mechanism of action in this regard is still under debate, there is no question that ayahuasca constituents can bind at serotonin receptors, and in particular, 5-HT_{2A} receptors. Hence, the present investigation focuses on the nature of the interaction of ayahuasca constituents (i.e., harmine (**1**), harmaline (**2**), THH (**3**), and DMT (**4**)) and some of their analogs at 5-HT_{2A} receptors.

A. Specific Aim 1: To determine if the binding affinity at 5-HT_{2A} receptors of the structurally similar constituents of ayahuasca are related to either the strength of their basic amine, or their planarity.

Since DMT (**4**) and the harmala alkaloids all bind at the 5-HT_{2A} receptor, and have a high degree of structural similarity, the small differences in their structure must account for the differences in their binding affinity.

The first area of interest was the interaction between the basic amine of the compounds and the highly conserved so-called "anchoring residue", ASP155, which is responsible for the interaction between aminergic 5-HT_{2A} ligands and the receptor.[197] Under physiological conditions, this aspartic acid residue exists in a negatively charged state (pK_a ~3.9), while the basic amine moiety of 5-HT_{2A} ligands exists in a positively charged (i.e., protonated) state. *If the strength of the interaction between the ligands of 5-HT_{2A} and the receptor could be correlated to the percentage of ligand that is existing as a cation under physiological conditions, the pK_a of the basic nitrogen of each active component should then correlate to the binding affinity of each compound at the receptor*. Although the pK_a of the basic amine of THH (**3**) has never been

experimentally determined, the presence of a conjugated system in both harmine (**1**) and harmaline (**2**) dictates that their ability to be protonated would be less favorable than an unconjugated alkylamine such as the one found in THH. It was therefore assumed that the pK_a of THH (**3**) was somewhere above the p*K*a of harmaline (9.55, **2**). Higher pK_a values (more basic) should correlate to higher affinities for the receptor if the proposed hypothesis is valid.

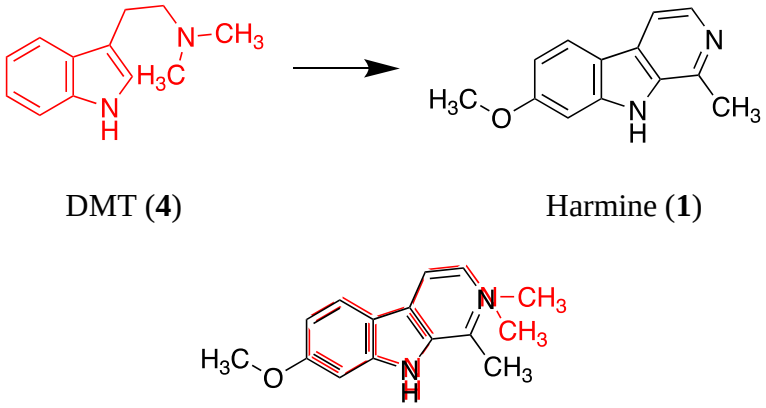
The next area of interest in terms of the comparable structural features of these compounds was their planarity. With the harmala alkaloids varying only in the degree of saturation of their pyridine ring, it was proposed that the flexibility of the compound in the binding pocket may have some effect on its ability to bind. Harmine (**1**), being a completely aromatic compound, should have almost no flexibility, while harmaline (**2**) and THH (**3**) would have increased flexibility due to the increased saturation of their pyridine ring. Since all four components of ayahuasca share a completely planar indole ring system, comparison of their flexibilities should give at least a relatively consistent pattern of binding affinity if it is at all involved in the interaction with the receptor.

Although the comparison between DMT (**4**) and the harmala alkaloids is not a perfect evaluation due to the differences in connectivity and the additional methoxy substituent present on the harmala alkaloids, the determination of the effect of basicity and flexibility of the harmala alkaloids at the 5-HT_{2A} receptor could theoretically be valid for structurally similar series of compounds and their interactions with the receptor. Hence, the basicity and planarity of DMT (**4**) will also be examined. The results of this study will at the very least begin to determine what structural features are beneficial for interactions between β-carbolines and the 5-HT_{2A} receptor.

B. Specific Aim 2: To determine the possible binding modes of DMT and the harmala alkaloids at the 5-HT_{2A} receptor using energy calculations, molecular modeling, and SAR.

Since the β-carbolines share a common indole substructure with tryptamines, and psychoactive tryptamines have had thorough structure-activity relationship (SAR) studies conducted, it is of interest to determine if their relative binding poses and interactions within the receptor mimic those of tryptamines.[198] If the basicity and planarity of the active constituents of ayahuasca do not correlate with their binding affinity, the difference must be the result of some other unconsidered property, or alternatively it must be due to interactions or clashes with the receptor binding pocket.

This is not the first time that the consideration of the relative binding poses between β-carbolines and tryptamines has been examined; Glennon et al.[199,200] previously proposed that due to the common indolealkylamine structure shared by β-carbolines and DMT (**4**), the alkylamine substituent of DMT might be rotated *in vivo* in order to bind to the receptor, in a way that mimics the orientation of the β-carboline scaffold (Figure 13). This comparative binding mode between DMT (**4**) and the β-carboline scaffold will be referred to as Overlay 1. Due to the limitations of molecular modeling at the time Overlay 1 was proposed in 1981, the energetic favorability of this conformation could not be determined. However, since Overlay 1 was published, the advancements in computational chemistry allow for the fairly simple comparison of the DMT (**4**) structure shown in Overlay 1, to its calculated lowest energy state.

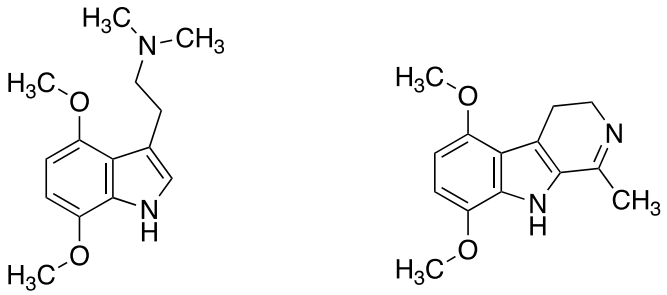


DMT (**4**) Harmine (**1**)

Overlay 1

Figure 13. Possible comparative binding pose of DMT (red, **4**), which mimics the β-carboline scaffold (black) as proposed by Glennon et al.[199]

When this relationship was originally proposed, Glennon et al.[199] made the prediction that although this relative binding pose would be very intuitive due to the high degree of similarity between the structures, it would be highly unlikely that the contortion of the alkylamine portion of the DMT (**4**) molecule would be energetically favorable, and therefore it was very unlikely to occur. This hypothetical comparable binding mode could be experimentally validated using the Portoghese hypothesis,[201] which assumes that if two series of compounds display similar poses within the receptor binding pocket, substituting these compounds with identical substituents in corresponding positions will produce similar changes in affinity for the receptor. With this in mind, Glennon et al.[199] synthesized a DMT analogue (4,7-dimethyoxy-*N*,*N*-dimethyltryptamine, **31**) and a β-carboline (5,8-dimethoxyharmalan, **32**) with identical substitutions on the indole benzenoid substructure, to determine if Overlay 1 is the correct binding mode between the two classes of compounds (Figure 14*). If Overlay 1 is the correct orientation, similar increases/decreases in affinity from the unsubstituted parent compounds should be seen* (Table 7).



4,7-Dimethyoxy-*N*,*N*-dimethyltryptamine (**31**) 5,8-Dimethoxyharmalan (**32**)

Figure 14. DMT analogue 4,7-dimethyoxy-*N*,*N*-dimethyltryptamine (**31**) and β-carboline analogue 5,8-dimethoxyharmalan (**32**).

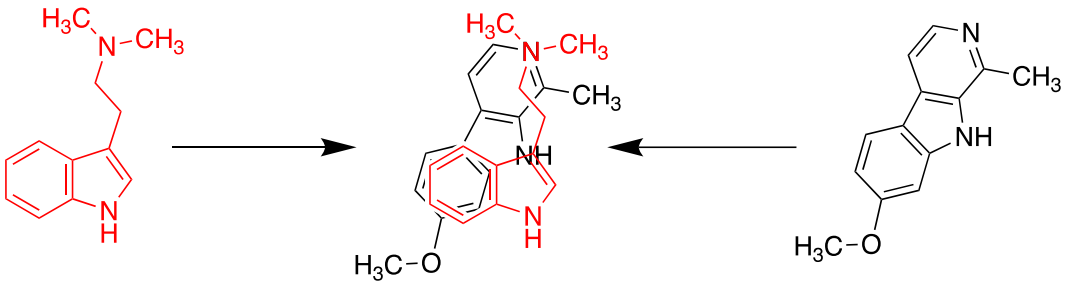
Table 7. Comparison of binding affinities at 5-HT_{2A} receptor of substituted DMT and β-carboline analogs 4,7-dimethyoxy-*N*,*N*-dimethyltryptamine (**31**) and 5,8-dimethoxyharmalan (**32**) to unsubstituted parent compounds DMT (**4**) and harmalan.[199]

Compound	K_i (nM)	Change in binding affinity
DMT	660	
4,7-Dimethyoxy-*N*,*N*-dimethyltryptamine	1,180	~2x lower affinity
Harmalan	1,200	~6x greater affinity
5,8-Dimethoxyharmalan	210	

As shown in Table 7, the changes in binding affinity observed when substituting the indole ring of both compounds with methoxy groups at corresponding positions were not at all parallel to one another. This is strongly suggestive that the relative binding poses shown in Overlay 1 are not the correct orientation for these two sets of compounds, although there is more work that can be done to either validate or refute this finding.

A second proposed relationship, one which was not as intuitive as Overlay 1, was also proposed by Glennon et al.[200] (Figure 15). Assuming that the most energetically favorable orientation of the DMT (**4**) structure would have the alkylamine portion of the molecule extended

upwards and away from the indole moiety, a second set of relative binding poses can be hypothesized, which seemingly retains a degree of similarity between the structures in the overlapping of the benzenoid rings of their indole moiety, and identical positioning of the basic nitrogen.



DMT (**4**) **Overlay 2** Harmaline (**2**)

Figure 15. Second proposed relative binding mode between harmine (black, **1**) and DMT (red, **4**).[200]

As with Overlay 1, molecular modeling was not as readily available at the time that this comparative binding mode was proposed, which limited the degree to which this comparison could be validated. Although the benzenoid rings of both sets of compounds are not as cleanly aligned as they are in Overlay 1, the positions of each ring do seem to be close enough that perhaps the binding relationship can be supported or refuted in a similar manner as Overlay 1. Since the publication of this relative binding mode, studies have been conducted synthesizing substituted β-carboline compounds, which would need to be compared to existing data of substituted DMT (**4**) analogs in search of a relationship.

While the comparison of the harmala alkaloids' binding poses in comparison to DMT (**4**) is an important goal in the differentiation of these two series of compounds, the comparison would be nearly useless without the understanding of whether the harmala alkaloids even bind similarly to one another. Without that knowledge, the comparison between DMT (**4**) and harmine (**1**), for

example, may support Overlay 1, while comparison of DMT and harmaline (**2**) may support Overlay 2. In 2000, Glennon et al.[176] conducted a study where analogs of harmine (**1**), harmaline (**2**), and THH (**3**) which had methoxy-substituents at each point of the benzenoid ring were synthesized. Using the corresponding binding affinities for the 5-HT_{2A} receptor, the relationship between the substitution of these sets of compounds at each position should allow us to validate whether they are binding in a similar or contrasting manner.

The only other structural difference between the β-carboline scaffold and the harmala alkaloids, in addition to the methoxy substitution of the benzenoid ring, is the 1-position methyl group shared by all three compounds. It is of interest to identify if this methyl substituent has any effect on the binding of the harmala alkaloids at the 5-HT_{2A} receptor, as the presence of this methyl group is responsible for the optical isomers in the fully saturated THH (**3**), and any analogs which would be made of that compound. The validation of the effect of the methyl substitution on binding affinity will be carried out in a similar manner to the correlation study performed to determine the relative binding modes of the harmala alkaloids in relation to one another, although it had already been proposed to have little effect on binding affinity for the receptor.[202]

In addition to the proposed correlation studies used to determine the binding poses of ayahuasca constituents, molecular modeling provides a convenient 3-D representation of interactions between of the active components and the 5-HT_{2A} receptor. In theory, the results of the correlation studies to determine the effect of basicity, planarity, or substitution of the indolealkylamine moiety should be predictive of relative binding score obtained from docking these compounds at the 5-HT_{2A} receptor crystal structure. Several crystal structures have been published recently, likely due to the increased interest in this receptor for its therapeutic potential. These crystal structures will be employed in the docking of our ligands, as there are structures co-

crystallized with antipsychotic antagonists (PDB: 6A93, 6A94) as well as hallucinogenic agonists (PDB: 6WHA, 6WGT, 7WC4-7).[203–205] Validation of our molecular modeling methodology will be gained through evaluation of GOLD (Genetic Optimization of Ligand Docking) score, HINT (Hydropathic INTeraction) score, as well re-docking of the co-crystallization ligand and assessment of the resulting binding poses in relation to the published pose of the co-crystallization ligand in the binding pocket.

C. Specific Aim 3: To synthesize DMT analogs with substituents corresponding to previously studied β-carbolines in order to make statistically significant comparisons.

While some data exist for the similarly substituted β-carbolines, which makes correlation studies for determining their binding modes relative to one another possible, there is a lack of data for substituted DMT (**4**) analogs that could be compared to their β-carboline counterpart. For this reason, any correlation found between DMT (**4**) and β-carboline binding affinities does not meet the standards for significance ($n \geq 6$). In a survey of the literature, the only comparable DMT (**4**) analogs and β-carboline analogs available for comparison would be methoxy-substituted on their respective benzenoid rings (Figure 16). In light of the lack of known comparable DMT (**4**) analogs that could provide data points to validate the correlation studies spoken of previously, we propose the synthesis of DMT analogs that would act as compounds of comparison for already reported and tested β-carboline analogs. The most useful set of data for comparison is a study by Grella et al.[202] in which analogs are substituted with an electron-donating methoxy group or electron-withdrawing bromo group at the four open positions of the benzenoid ring (Figure 16).

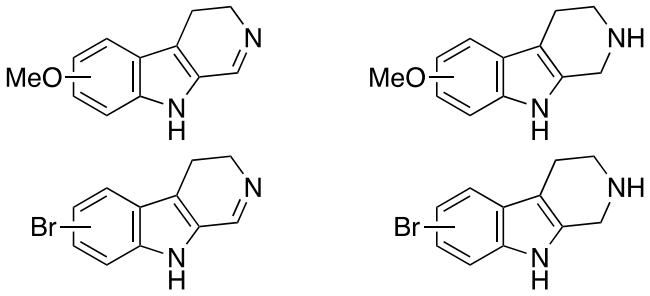
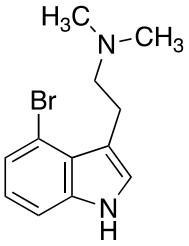


Figure 16. Dihydro and tetrahydro-β-carboline compounds synthesized and examined for binding affinity at 5-HT_{2A} receptors by Grella et al.[202]

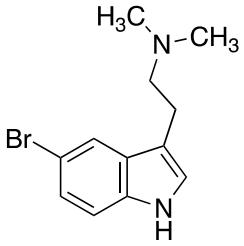
Although this is the largest group of β-carboline analogs that have been examined for binding affinity at 5-HT_{2A} receptors, there are a few issues to be discussed when trying to draw conclusions as to the relative binding poses of DMT (**4**) vs. the harmala alkaloids. There is no 1-methyl position substituent, which was removed from the synthesized compounds due to purported evidence that the 1-methyl group does not have a significant effect on the binding,[176] and the causation of optical isomers of THH (**3**) when substituted at the 1-position. Before making any comparison between the harmala (1-methyl substituted) alkaloids and the results of correlation studies using the data from the 1-*des*-methyl compounds, the results of the correlation study mentioned previously in which the presence of the 1-methyl group on binding affinity at the 5-HT_{2A} receptor would have to be carried out. If the presence of the 1-methyl group showed to be insignificant in correlation studies between 1-substituted and 1-unsubstituted, a valid comparison might be made.

A further issue with the use of the set of β-carbolines synthesized by Glennon et al. is the removal of the fully unsaturated 1-*des*-methyl harmine analogs from consideration, which is troubling in that it removes a group of compounds that are related to the most abundant harmala alkaloid that is found in ayahuasca, and one that has been shown to have intriguing antidepressant

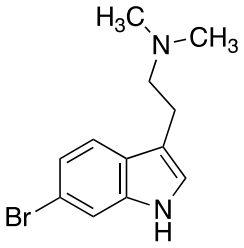
and anxiolytic effects, at least in animal models. The fully unsaturated bromo and methoxy-substituted group of compounds was not examined because fully unsaturated β-carbolines are the most optimal inhibitors of MAO-A compared to the more saturated series of compounds.[181,184] As discussed previously, there are a multitude of reasons for why MAO-A inhibition can be a possibly undesired activity expressed by a compound, but it would be helpful to have these unsaturated analogs for comparison.



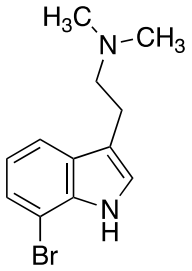
4-Bromo-*N*,*N*-DMT (**33**)



5-Bromo-*N*,*N*-DMT (**34**)



6-Bromo-*N*,*N*-DMT (**35**)



7-Bromo-*N*,*N*-DMT(**36**)

Figure 17. Proposed DMT analogs to be synthesized in order to give statistical significance to correlation studies comparing the binding poses of DMT (**4**) and β-carbolines at the 5-HT_{2A} receptor: 4-bromo-*N*,*N*-DMT (**33**), 5-bromo-*N*,*N*-DMT (**34**), 6-bromo-*N*,*N*-DMT (**35**), and 7-bromo-*N*,*N*-DMT (**36**).

Once these questions about the validity of the comparison between the β-carboline analogs and the harmala alkaloids have been answered, the final goal of this study is to synthesize DMT (**4**) analogs with bromo substituents at each position of the indole benzenoid ring, for direct

comparison to the bromo-substituted β-carboline analogs discussed above, that can be included in the correlation studies between the two sets of compounds and bring the number of compounds being compared into statistical significance (Figure 17). Once synthesized, binding experiments will be conducted for each compound at 5-HT_{2A} receptors. The function of these compounds at the 5-HT_{2A} receptor will also be examined, to determine whether they are acting as agonists or antagonists in a general assay for GPCR activation.

In summary, the approach for the Specific Aims of this investigation are as follows:

Specific Aim 1:

a. Examine basicity of major ayahuasca constituents

b. Examine planarity of harmala alkaloids in comparison to DMT (**4**)

Specific Aim 2:

a. Determine possible binding modes of DMT (**4**) and the harmala alkaloids at the 5-HT_{2A} receptor using:

    a. Energy calculations

    b. Molecular modeling

    c. Structure-Affinity Relationships

Specific Aim 3:

a. Synthesize analogs of DMT (**4**) and determine their binding affinities and function at the 5-HT_{2A} receptor for comparison with previously studied β-carboline analogs with corresponding substituents

IV. Results and Discussion

Specific Aim 1: To determine if the binding affinity at 5-HT_{2A} receptors of the structurally similar constituents of ayahuasca are related to either the strength of their basic amine, or their planarity.

A. Basicity Studies

In order to examine the effect of the basicity of each compound, previously determined literature values were used. The known pK_a of each amine and their corresponding affinities are shown below (Table 8).[176,206]

Table 8. Basicity (pK_a) of the terminal amine of each active component of ayahuasca as determined by Douglas et al.,[206] and binding affinity at 5-HT_{2A} receptors as determined by Glennon et al.[176]

Compound	pK_a	5-HT_{2A} K_i (nM)
Harmine (**1**)	7.45	397
Harmaline (**2**)	9.55	5,010
THH (**3**, R)	Not measured*	>10,000
THH (**3**, S)	Not measured*	5,000
DMT (**4**)	8.68	323

*****Due to the saturation in the C ring of the β-carbolines, it was assumed that THH would be more basic (i.e., > 9.55) than harmine or harmaline.

An inverse relationship between the basicity and the binding affinity was found, with the presumed most basic of the β-carbolines (THH, **3**) having the lowest affinity for the receptor, and the least basic (harmine, **1**) having the highest binding affinity. Although it is harder to draw a comparison between the cyclic harmala alkaloids and tryptamines such as DMT (**4**) in this study,

DMT has a pK_a value roughly halfway between that of harmine (**1**) and harmaline (**2**), yet has higher affinity for the receptor than either of those compounds. In conclusion, there does not appear to be a relationship between the basicity of these compounds and their affinity for the 5-HT_{2A} receptor. The previously mentioned "anchoring" interaction with ASP155 may still be the strongest interaction between these ligands and the receptor, but there are likely to be other interactions influencing the magnitude of the binding affinity of these compounds.

B. Planarity Studies

The distance of the basic amine of each molecule from the indole system plane was determined from the most energetically favorable conformation of the molecule by SYBYLx2.1.1 (Figure 18, Table 9).

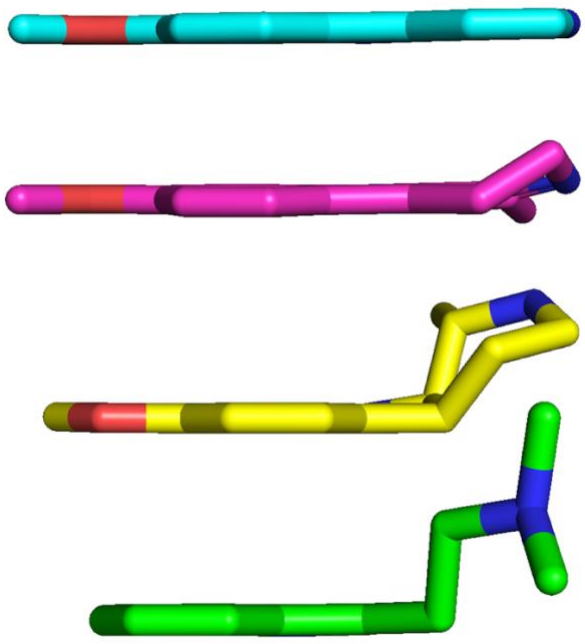


Figure 18. View of harmine (cyan, **1**), harmaline (magenta, **2**), THH (yellow, **3**), and DMT (green, **4**) down the indole plane of each molecule, visualized using PyMOL 2.4.1.

As might have been expected, the amine of **1** is nearly in the plane of the indole ring of the β-carbolines whereas that of **3** is the most out of the plane. Much like the examination of the basicity of the terminal nitrogen of these molecules, the trend observed for the binding affinity of these compounds is consistent between the harmala alkaloids containing nearly identical structural features, but when compared to DMT (**4**) , the trend is no longer consistent. The binding affinity of the harmala alkaloids appears to be positively correlated to the degree of planarity, with the most planar molecule (harmine, **1**) having the highest binding affinity and the least planar molecule (THH, **3**) having the lowest binding affinity. If the structural similarity between DMT (**4**) and the harmala alkaloids resulted in similar binding positions to one another, one would expect that the high degree of flexibility between DMT (**4**) and its relatively high binding affinity at the 5-HT_{2A} receptor would be reflected in the binding affinity of the most similarly flexible harmala alkaloid, THH (**3**).

Table 9. Distance of side chain amine of each molecule from conserved indole ring plane (this study), and binding affinity at 5-HT_{2A} receptors as determined by Glennon et al.[176]

Compound	Distance	5-HT_{2A} K_i (nM)
Harmine (**1**)	0.002 Å	397
Harmaline (**2**)	0.304 Å	5,010
THH (**3**, R)	1.918 Å	>10,000
DMT (**4**)	1.581 Å	323

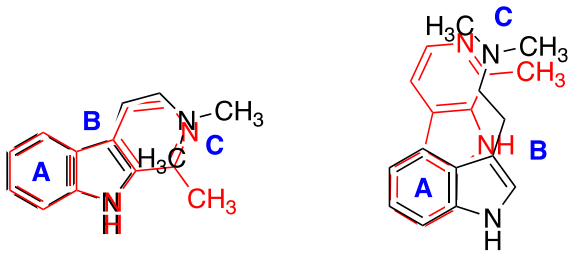
The lack of a trend in the binding affinity between these two very similar compounds suggests that the binding poses of the harmala alkaloids and DMT (**4**) are not similar enough for ligand flexibility to be a factor. The distance of the side chain amine from the conserved indole plane is not a complete description of the degree of ligand flexibility in this case, as the basic amine

of DMT (**4**) has a degree of rotation about the alpha and beta carbons that is unable to be replicated by the cyclized THH (**3**) or other harmala alkaloids.

Specific Aim 2: To determine the possible binding modes of DMT and the harmala alkaloids at the 5-HT$_{2A}$ receptor using energy calculations, molecular modeling, and SAR.

A. Alignment Studies

With the knowledge that the basicity of the side chain amine and the flexibility of these compounds are unlikely to be contributing to similar binding poses between the harmala alkaloids and DMT (**4**), the degree of similarity between the compounds in the relative binding poses proposed by Glennon et al.[200] (Figure 19) were examined. It is probable that with increasing similarity between compounds, that their binding poses at a receptor will be similar.



Overlay 1 **Overlay 2**

Figure 19. Both relative binding poses proposed by Glennon et al.[200] between β-carbolines (e.g. harmine, black, **1**) and DMT (red, **4**).

Using SYBYLx2.1.1, both of the above relative binding poses were examined. For Overlay 1, harmine (**1**) and DMT (**4**) were aligned using the centroid of both substituted benzenoid rings (**A**) of their respective indole substructures, the indole 3-position carbon atom (**B**), and their basic nitrogen atom (**C**). For Overlay 2, the compounds were aligned using the same benzenoid centroid

(**A**), the 3-position carbon of DMT (**4**) and the indole nitrogen atom of harmine (**1**) (**B**), and their basic nitrogens (**C**). The RMS of these overlays is the average distance between the previously mentioned superimposed atoms (Table 10).

Table 10. RMS of Overlay 1 and Overlay 2 alignments between harmine (**1**) and DMT (**4**).

Overlay	RMS
1	0.039
2	0.228

Predictably, the RMS of Overlay 1 shows that DMT (**4**) is almost perfectly superimposed with the β-carboline structure. The alignment for Overlay 2 was obtained using the energy-minimized conformation of both structures. The energy of the constituents of ayahuasca (Table 11) show that the conformation of DMT (**4**) in Overlay 1 is highly energetically unfavorable and unlikely to occur. From these energies, another comparison can also be drawn between the β-carbolines and DMT (**4**). If the energy of the molecule is a significant contributor to the binding affinity of these ligands, the results do not favor Overlay 1.

Table 11. Protonated ayahuasca constituents minimized conformation energy (kcal/mol) as calculated by SYBYLx2.1.1.

Compound	Energy (kcal/mol)
DMT (**4**)	30.1
Harmine (**1**)	23.0
Harmaline (**2**)	24.0
THH (**3**, R)	29.0
β-carboline conformed DMT	455.4

B. Docking Studies

1. Docking of Harmala Alkaloids and DMT (**4**)

The existence of multiple crystal structures of the 5-HT_{2A} receptor and extensive dockings of 5-HT_{2A} ligands published in the literature provided the basis for the docking studies presented in this thesis. When this research project began, only two crystal structures of the 5-HT_{2A} receptor were reported, crystallized with antagonists (risperidone, **24**, PDB ID: 6A93; and zotepine, PDB ID: 6A94). Approximately one year later, two additional crystal structures of the 5-HT_{2A} receptor were published, bound to agonists (25-CN-NBOH, PDB ID: 6WHA; and LSD, **6**, PDB ID: 6WGT). For the majority of the time spent on this project, these were the only available crystal structures.

Table 12. Published crystal structures of the human 5-HT_{2A} receptor.

PDB ID	Co-crystallization Ligand	Resolution (Å)	Year
6A93	Risperidone	2.90	2019[203]
6A94	Zotepine	2.90	2019[203]
6WHA	25-CN-NBOH	3.36	2020[203]
6WGT	LSD	3.40	2020[203]
7WC4	Serotonin	3.20	2022[205]
7WC5	Psilocin	3.20	2022[205]
7WC6	LSD	2.60	2022[205]
7WC7	Lisuride	2.60	2022[205]

A list of all known crystal structures of the human 5-HT_{2A} receptor is shown in Table 12, all of which have been published within the last three years. A mix of both agonists (LSD, psilocin) and antagonists (risperidone, zotepine) alike, along with the crystal structure of the endogenous

ligand serotonin, provide both agonist and antagonist bound protein structures to be employed for docking studies.

Attempts to dock the harmala alkaloids to a model generated using PDB ID: 6WHA resulted in relatively low-scoring (PLP) solutions, in spite of all attempts to optimize docking parameters (see Appendix A). In addition to their poor scoring, none of the examined docked harmala alkaloids produced relative binding poses to DMT (**4**) which resembled either Overlay 1 or Overlay 2. The diverse docking option in GOLD Suite 5.6 was used to further explore the fit of the ligands within the binding pocket, and produced some poses which resembled Overlay 2 (Appendix A). However, none of these poses scored highly using the PLP scoring function. Hydropathic INTeraction analysis on the Overlay 2-like protein-ligand complex also produced poorly-scoring interactions between the ligands and specific residues in the binding pocket, as well as poor total scores.

The 5-HT_{2A} crystal structures PDB ID: 7WC4-7WC7 differed from the previously published crystal structures of the receptor due to the inclusion of monoolein, a lipid used in the crystallization of the protein, which interacts with key residues of the binding pocket. The presence of this lipid cofactor was shown to shift the binding pose of serotonin (**7**) and psilocin away from what was termed the orthosteric binding pocket, and particularly away from SER242, a residue which was suggested to form an interaction with the indole nitrogen atom of serotonin-like compounds.[128,203] The presence of lipid-receptor interactions had been proposed previously, due to the observed effect of the endogenous fatty acid oleamide on serotonergic functions.[207] Monoolein was also shown to have partial G-protein agonism at 5-HT_{2A} via bioluminescent resonance energy transfer assays, which was blocked by the selective 5-HT_{2A} antagonist

volinanserin.[205] All of these observations strongly suggest that lipids may play role in the binding of 5-HT_{2A} ligands to the receptor.

Interestingly, instead of interacting with the SER242 residue, the indole rings of both serotonin (**7**) and psilocin were observed to interact with a cleft of hydrophobic residues from extracellular loop 2, and transmembrane helices 3, 6, and 7, which was termed the extended binding pocket (EBP).[205] The anchoring interaction between the ASP155 residue and the basic nitrogen atom of both ligands was consistent with previously reported crystal structures, but the monoolein occupying the "lower" part of the binding pocket appeared to push the ligands to a different section of the binding pocket from what was previously reported in prior crystal structures. While the crystal structure showed both serotonin and psilocin in this new binding mode, mutation of key residues of the binding pocket (SER239, SER242) associated with ligand interactions with the previous crystal structures published of agonist-bound 5-HT_{2A} receptor found reduced agonist activity as well as reduced binding affinity for the receptor.[205] This finding suggests that certain agonists of 5-HT_{2A} receptors may have multiple different binding modes that can be associated with different downstream signaling pathways. In order to validate our docking model (i.e., 7WC5 crystal structure after staged minimization of the protein and its hydrogens) psilocin was re-docked and a similar pose was obtained to that which was published.[204]

With these observations in mind, a model of the 5-HT_{2A} receptor based on crystal structure PDB ID: 7WC5 was used for computational docking of harmine (**1**), harmaline (**2**), THH (**3**), and DMT (**4**). The monoolein lipid was kept in the binding pocket of the receptor, and all other non-protein atoms were removed. Staged minimization of monoolein was performed to assess its flexibility, and the results showed no change (weighted root-mean-square deviation (RMSD): 0.000) from the reported pose of the lipid in the crystal structure. With the lipid structure present

in place in the binding pocket of the receptor, harmine (**1**), harmaline (**2**), tetrahydroharmine (**3**), and DMT (**4**) were docked using GOLD Suite 5.6 (Figure 20). All shown docking results (Figures 20-24, 1A-3A) retain a distance between the basic nitrogen atoms of the ligands and the ASP155 oxygen atoms within the commonly accepted hydrogen bond distance of 2.5-3.5 Å.

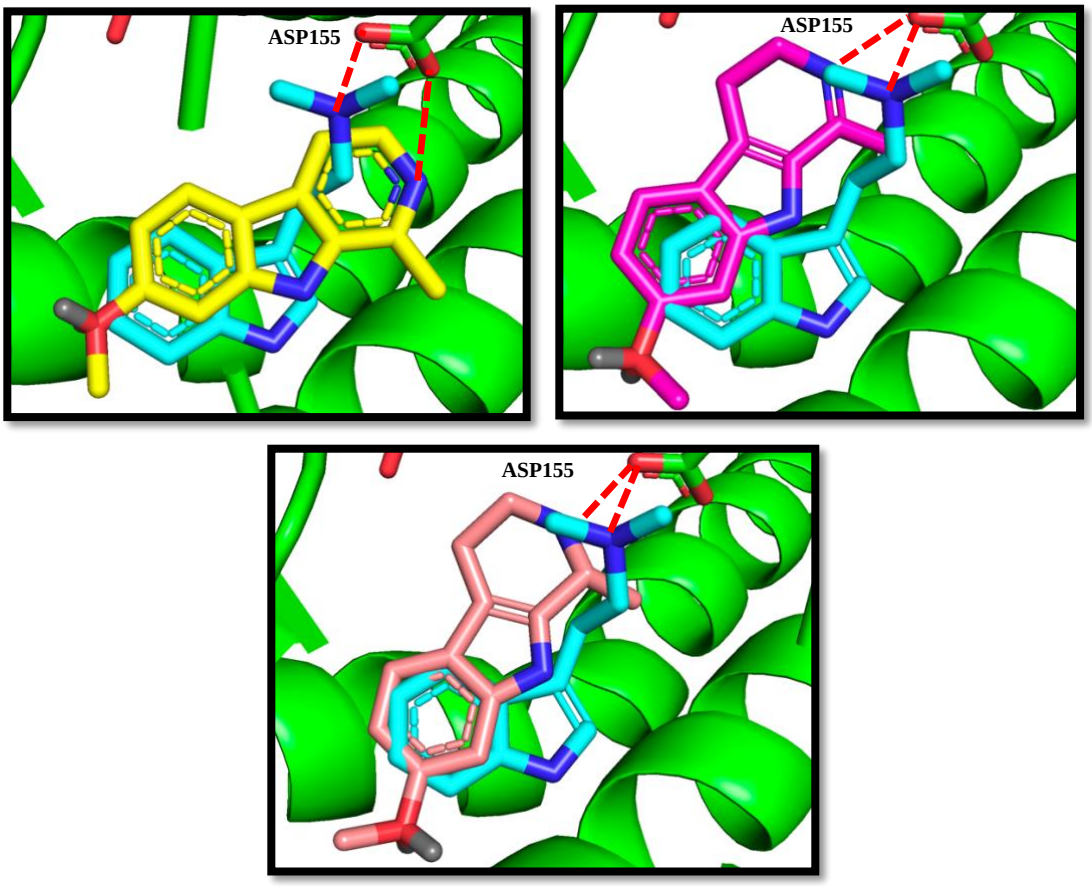


Figure 20. Relative poses of harmine (**1**, yellow, capped sticks), harmaline (**2**, pink, capped sticks), THH (**3**, salmon, capped sticks) with DMT (**4**, cyan, capped sticks) in the binding pocket of the model of the 5-HT_{2A} receptor crystal structure (PDB ID: 7WC5, green, ribbons) which resemble Overlay 2 (Figure 15). Hydrogen bonds are shown as red dashed lines.

The relative binding poses between the harmala alkaloids and DMT (**4**) that resembled Overlay 2 (Figure 20) were supported by high PLP scores, which assign a hydrogen bonding

potential or a lipophilic potential to each heavy atom of the ligand-protein interaction and scores each interaction accordingly. THH (**3**) and harmaline (**2**) most resembled the proposed Overlay 2, while harmine's (**1**) relative binding pose was slightly less similar. The lack of binding data for harmine analogs at 5-HT_{2A} receptors means that this will likely be the only "experimental" determination of the relative binding pose of harmine that can be included in this thesis.

Table 13. Significant (>|100|) Hydropathic INTeraction Scores between DMT (**4**), harmine (**1**), harmaline (**2**), and THH (**3**), and residues of our docking model (PDB ID: 7WC5).

Compound	Ligand Atom	Residue	Interaction Type	Score	HINT Score
DMT	Indole Nitrogen	ASN363	Hydrogen Bond	292	987
	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	835	
	Basic Nitrogen	ASP155 Oxygen 2	Acid/Base	177	
	Methyl Carbon	ASP155 Oxygen 2	Hydrophobic/Polar	-270	
	Methyl Carbon	ASP155 Oxygen 1	Hydrophobic/Polar	-184	
Harmine	Indole Nitrogen	ASN363	Hydrogen Bond	354	476
	C1 Methyl Carbon	ASN363	Hydrophobic/Polar	-103	
Harmaline	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	350	-219
	C1 Carbon	ASP155 Oxygen 1	Acid/Base	149	
	C1 Methyl Carbon	ASP155 Oxygen 1	Hydrophobic/Polar	-258	
	C1 Methyl Carbon	Asp155 Oxygen 2	Hydrophobic/Polar	-229	
	C1 Methyl Carbon	TYR370	Hydrophobic/Polar	-107	
	7-OMe Methyl	ASN363	Hydrophobic/Polar	-176	
THH	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	1366	1671
	Basic Nitrogen	ASP155 Oxygen 2	Acid/Base	359	
	C1 Methyl Carbon	ASP155 Oxygen 1	Hydrophobic/Polar	-142	
	C1 Methyl Carbon	ASP155 Oxygen 2	Hydrophobic/Polar	-123	

The binding affinities of harmala alkaloids (Table 4) suggest that harmine (**1**) should have the strongest interactions with the binding pocket of the receptor, followed by harmaline (**2**), and then THH (**3**). The opposite was seen with the binding poses resembling Overlay 2, with harmine having fewer positive interactions, as well as a lower total HINT score than THH (**3**) which had the most positive interactions, and the highest total HINT score. It is interesting to note that the negative interactions seen between the C1 methyl carbon and ASP155 residue do not seem to be reflected in the experimental binding affinity of the β-carbolines, as the binding affinities of synthesized 1-methyl and 1-*des*-methyl β-carbolines were comparable to one another, and in many cases the 1-methyl counterpart had higher affinity for the receptor.[176,202] In these cases, the overall HINT scores of these compounds do not seem to be indicative of their binding affinities to the protein.

There are a few different possibilities as to why this could be the case, the first of which is that perhaps the harmala alkaloids and DMT have other binding poses which are likely to occur, which do not overlap with one another. A second possibility, is that the differences between the receptors used in the previous binding affinity study of these compounds (cloned rat 5-HT_{2A}) and the human receptor from which the crystal structure was generated, are stark enough that the rat receptor binding affinities of the compounds do not necessarily reflect their interactions with the human receptor. It seems unlikely, however, that the receptors are different enough (91.3% identity calculated using UniProt) to completely flip the trend in binding affinity of a set of similar compounds.

Analysis of a second group of binding poses (Figure 21) between DMT (**4**) and harmine (**1**), as well as DMT (**4**) and harmaline (**2**), were also examined. When clustering the docking results (n = 50, 1.5 Å clusters), both compounds produced a group of results which were in an

entirely different part of the binding pocket than those shown previously (Figure 20). While this second group of binding poses produced the highest PLP score for each of the respective compounds in the docking series, they were not in the same area of the binding pocket that the co-crystallization ligand psilocin was occupying, nor did they align with the docked structure of DMT (**4**). HINT analysis did not produce scores which showed significant additional interactions from the Overlay 2 binding poses (Table 14), with the most significant interactions once again being between the basic nitrogen atom of these compounds and the anchoring ASP155 residue. No second group of binding poses showing a significant change in the positioning of THH (**3**) in the binding pocket was seen.

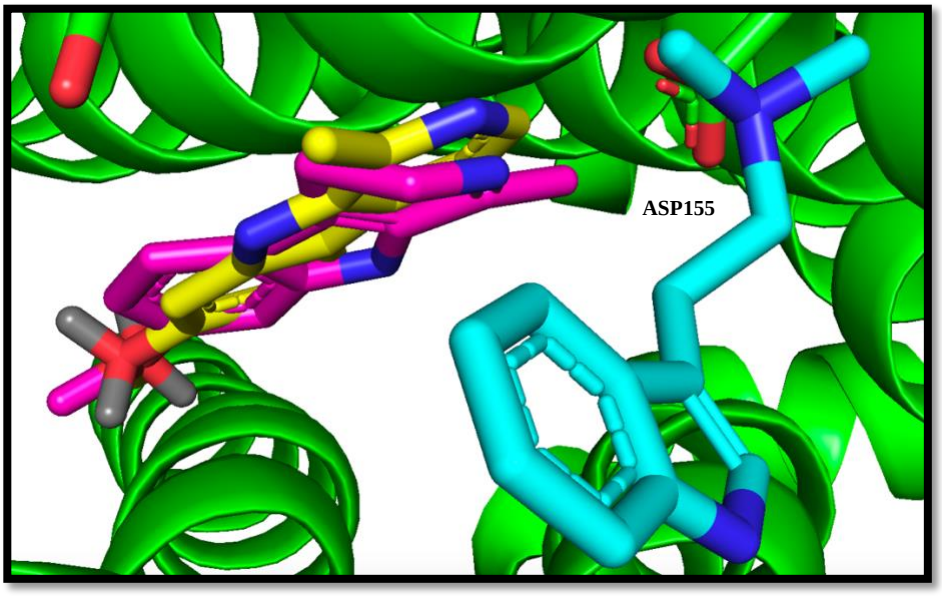


Figure 21. Non-Overlay 2-like relative binding modes of harmine (**1**, yellow, capped sticks) and harmaline (**2**, pink, capped sticks) in relation to DMT (**4**, cyan, capped sticks) in the binding pocket of our docking model (PDB ID: 7WC5, green, ribbon).

In summary, there are numerous unanswered questions about the docking results which have been discussed herein. While the newly published crystal structures of 5-HT_{2A}[205] include the presence of a lipid structure which is docked into the binding pocket of the receptor, there is no

real data supporting the lipid-occupied binding pocket being the natural state of the receptor, even though there is evidence of these lipids having effects on the serotonergic system. Within the paper that these structures were published, mutagenesis studies confirmed that the residues purported to be blocked by the inclusion of the lipid in the binding pocket of the receptor were also influential in the binding of the compounds examined.

Table 14. Significant (>|100|) Hydropathic INTeraction Scores between non-Overlay 2-like poses of harmine (**1**) and harmaline (**2**), and residues of our docking model (PDB ID: 7WC5).

Compound	Ligand Atom	Residue	Interaction Type	Score	HINT Score
Harmine	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	470	521
	Basic Nitrogen	ASP155 Oxygen 2	Hydrogen Bond	123	
	C1 Methyl Carbon	ASN363	Hydrophobic/Polar	-176	
Harmaline	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	357	229
	C1 Carbon	ASP155 Oxygen 1	Acid/Base	162	
	C1 Methyl Carbon	ASP155 Oxygen 1	Hydrophobic/Polar	-293	
	C1 Methyl Carbon	ASP155 Oxygen 2	Hydrophobic/Polar	-296	

With the non-lipid occupied structures, no consistent Overlay 2-like conformation was seen, while Overlay 2-like binding relationships were seen between the harmala alkaloids and DMT (**4**) with the lipid-occupied structures. While the presence of Overlay 2-like relative binding modes seemingly supports our hypothesis, as these poses were "naturally" obtained with no restrictive constraint parameters between the ligands and receptor, the HINT scoring of these poses did not have many significant positive interactions between the receptor and the ligands which would explain their preference for that specific part of the binding pocket. The magnitude of the HINT scores of the harmala alkaloids was also inversely proportional to their experimentally determined binding affinity at rat 5-HT_{2A} receptors. With all of these factors taken into

consideration, it still seems as though Overlay 2 would be supported by our docking model, especially for harmaline (**2**) and tetrahydroharmine (**3**), however there appears to be other factors at play that are not accurately described by the docking, scoring software, or both.

2. Docking of substituted β-Carbolines

The docking model of the PDB ID: 7WC5 crystal structure was used to dock substituted β-carboline compounds synthesized by Glennon et al.[202] (Table 17, see Experimental section for full parameters). If the binding poses produced by the harmala alkaloids in the previous docking study are genuine, then the substituted analogs of these compounds should theoretically bind in a similar manner as well. In order to test this hypothesis, the compounds synthesized by Glennon et al.[202] (Table 17) were built in SYBYLx2.1.1 in the same manner as the harmala coumpounds and DMT (**4**) and docked to the same PDB ID: 7WC5 crystal structure (Figure 22, Figure 23).

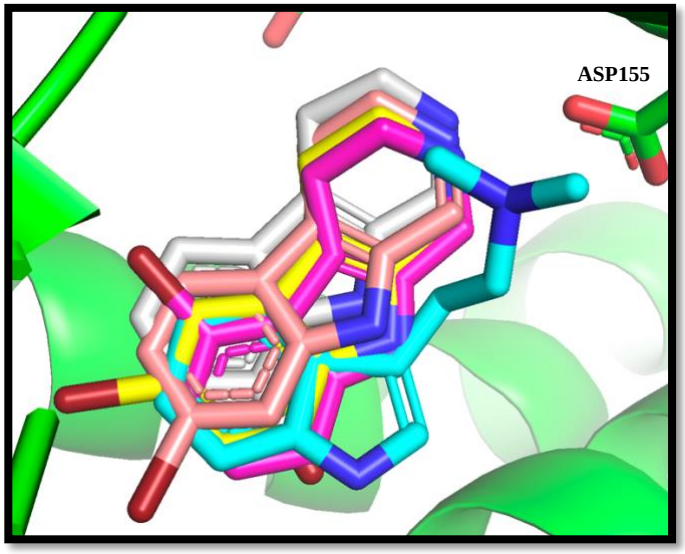


Figure 22. Relative poses of DMT (**4**, cyan, capped sticks) and dihydro β-carbolines (i.e., 5-bromo-3,4-dihydro-β-carboline (pink, capped sticks), 6-bromo-3,4-dihydro-β-carboline (yellow, capped sticks), 7-bromo-3,4-dihydro-β-carboline (salmon, capped sticks), and 8-bromo-3,4-dihydro-β-carboline (white, capped sticks)) in the binding pocket of the model of the 5-HT_{2A} receptor crystal structure (PDB ID: 7WC5, green, ribbons) which resemble Overlay 2 (Figure 15).

In the docking studies of the previously synthesized β-carboline compounds, the bromo-substituted dihydro β-carboline analogs bind with similar relative binding poses to DMT (**4**, Figure 22) which were nearly identical to the relative binding pose of their harmala counterpart, harmaline (**2**, Figure 20). The bromo-substituted tetrahydro β-carboline analogs, however, seemed to take an Overlay 2-like pose (Figure 23), where the molecule is flipped in a manner where the indole nitrogen is facing 180° from its position in Overlay 2. This trend was consistent between all fully saturated and brominated β-carboline compounds, suggesting that the lack of a double bond in the pyridyl moeity of the compounds is causing this flip in binding orientation.

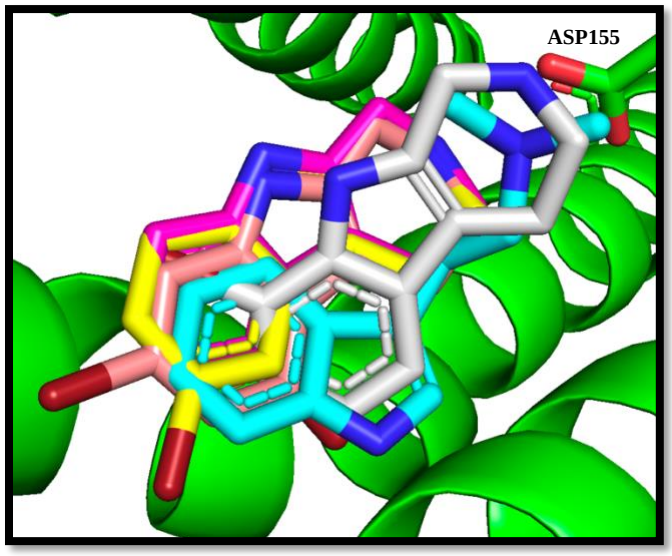


Figure 23. Relative poses of DMT (**4**, cyan, capped sticks) and tetrahydro β-carbolines (i.e., 5-bromo tetrahydro-β-carboline (pink, capped stickse), 6-bromo tetrahydro-β-carboline (yellowd capped sticks), 7-bromo tetrahydro-β-carboline (salmon, capped sticks), and 8-bromo tetrahydro-β-carboline (white, capped sticks)) in the binding pocket of the model of the 5-HT_{2A} receptor crystal structure (PDB ID: 7WC5, green, ribbons).

For the methoxy-substituted β-carboline analogs, no relative binding modes were seen between either the dihydro nor tetrahydro sets of compounds and DMT (**4**). It was not immediately clear why this was the case, as the harmala alkaloids docked previously (Figure 20) have methoxy-

substituents on their benzene moeity and showed Overlay 2-like relative binding poses. Perhaps the lack of a 1-position methyl group, which is present in the naturally occuring harmala alkaloids but missing in the analogs synthesized by Glennon et al.,[202] is necessary for these compounds to assume the Overlay 2-like binding mode of the methoxy-substituted compounds, but not the bromo-substituted compounds. Another possibility is that these compounds differ in functional activity, and that the relative binding modes of these compounds with DMT is influenced by whether these compounds are agonists, or antagonists. As functional data are not available for any of the synthesized β-carboline analogs (Table 17), that comparison can not currently be made.

In regards to the differences between the bromo substituted dihydro and tetrahydro β-carboline compounds, one would expect that the either the indole nitrogen atom, or the bromo substituted benzene moeity would be playing a role in the orientation of the molecules. It seems likely that one of those two factors would need to be making some important interaction to flip the scaffold of these compounds, but HINT scoring of comparably positioned brominated compounds (i.e., 5-bromo dihydro-β-carboline and 8-bromo tetrahydro-β-carboline) found no considerably favorable interactions from neither the indole nitrogen atom, nor the bromine atom, which would help explain this phenomenon (Table 15). In fact, the flipping of the scaffold seen in the tetrahydro set of compounds seemed to increase the number of unfavorably scored interactions between the compound and binding pocket, compared to the same atoms on the dihydro set of compounds. One variable that was consistant between the HINT scores of all compounds, was the strength of the interactions between the basic nitrogens of the ligands and the ASP155 residue (Tables 13, 14, 15). The strength of this interaction was by far the highest for the fully saturated tetrahydro analogs (Tables 13,15), yet THH (**3**) has a binding affinity greater than 10,000 nM (Table 4). If the experimentally determined binding affinity for this compound is correct, then there must be an

interaction which was either not found by the docking, or not adequately described by the scoring software.

Table 15. Significant (>|100|) Hydropathic INTeraction Scores between comparably positioned brominated compounds 5-bromo dihydro-β-carboline (DHBC) and 8-bromo tetrahydro-β-carboline (THBC).

Compound	Ligand Atom	Residue	Interaction Type	Score	HINT Score
5-Br	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	166	999
DHBC	C1 Carbon	ASP155 Oxygen 1	Acid/Base	266	
	C1 Carbon	ASP155 Oxygen 2	Acid/Base	233	
	Bromine	ASN343	Base/Base	-162	
8-Br	Basic Nitrogen	ASP155 Oxygen 1	Hydrogen Bond	1283	1586
THBC	Basic Nitrogen	ASP155 Oxygen 2	Hydrogen Bond	539	
	Bromine	ASN343	Base/Base	-180	

The docking of the analogs of the dihydro and tetrahydro β-carboline compounds presents some support for the idea that Overlay 2, at least in part, explains the relative binding modes of these compounds to DMT. For bromo substituted dihydro β-carbolines, this relationship appears solid, while bromo substituted tetrahydro β-carbolines seem to have a completely different relationship altogether. Methoxy substituted analogs seem to have little to no relationship to the observed binding pose of DMT at all, although the reasoning for this is yet to be determined. The interactions of these different compounds with the receptor seem to be consistent between a set of compounds with similar saturation of their pyridyl moeity, and substitution of their benzene ring with an identical functional group, but not between sets of compounds with differences in either of these two areas. If that is the case, finding a consistent binding mode between sets of β-carboline analogs and DMT may be more complicated then one simple Overlay, if a relative binding mode exists at all.

3. Docking of substituted *N*,*N*-dimethyltryptamines

Substituted bromo-DMT analogs synthesized for this thesis and methoxy-substituted DMT analogs synthsized by Glennon et al.[176] were docked to the docking model of the 5-HT_{2A} receptor (PDB ID: 7WC5), using identical parameters to the above docking studies (see Experimental section).

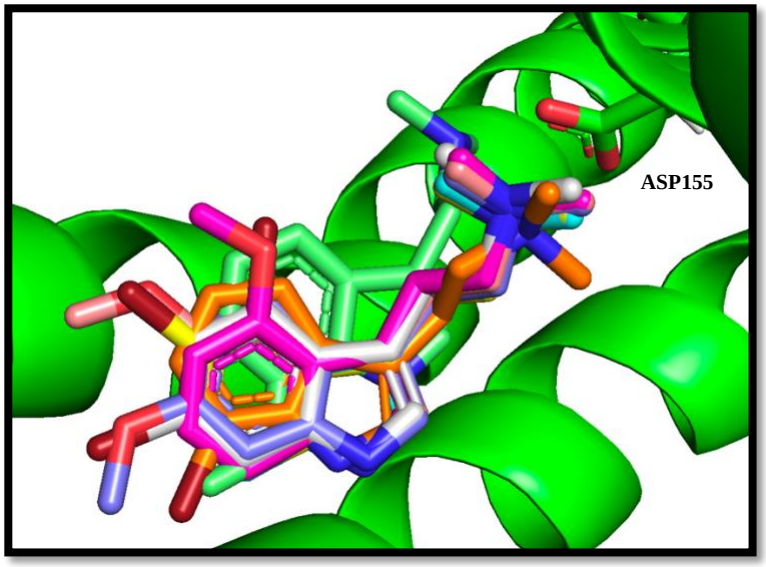


Figure 24. Bromo-substituted (i.e., 4-Br DMT (cyan, capped sticks), 5-Br DMT (yellow, capped sticks), 6-Br DMT (white, capped sticks), and 7-Br DMT (orange, capped sticks)) and methoxy-substituted (i.e., 4-OMe DMT (pink, capped sticks), 5-OMe DMT (salmon, capped sticks), 6-OMe DMT (purple, capped sticks), and 7-OMe DMT (green, capped sticks)) analogs of DMT (**4**) docked to the model of the 5-HT_{2A} receptor (PDB ID: 7WC5, green, ribbons).

As one would expect for similarly substituted compounds, the DMT (**4**) analogues had almost perfect alignments with one another in the binding pocket of the 5-HT_{2A} receptor (Figure 24). The relationship between the binding poses of the compounds in relation to one another is significant, because in order to attempt the correlations discussed in the next section of this thesis between β-carboline and DMT analogs, the compounds within either class are assumed to be

binding similarly to one another. The bromo and methoxy substituents of the benzenoid moeity of the indole ring are aligned nearly perfectly with one another, showing that electron withdrawing and electron donating properties of the substituents do not have an influence on the orientation of these compounds in the binding pocket. Interestingly, the 7-substituted analogs (7-Br DMT and 7-OMe DMT) were both slightly misaligned in regards to the positioning of their indole rings to the other DMT analogs. A possible steric clash is likely the cause for this movement in binding pose, but the relative binding poses of the substituted DMT analogs were similar enough to be employed in correlation studies with similarly substituted β-carbolines.

C. Correlation Studies

In order to further investigate the likelihood of Overlay 1 and Overlay 2, analysis of previously synthesized β-carboline and DMT (**4**) analogs and their respective binding affinities at 5-HT_{2A} receptors were considered. Multiple studies had been published by Glennon et al.[176,202,208] reporting the binding affinities of analogs of β-carbolines and DMT (**4**) which can be used to further examine the binding relationship between these two sets of compounds using the principle of the Portoghese hypothesis.[201]

The first study which was examined was conducted by Glennon et al. in 2000;[176] reporting the binding affinities of harmine (**1**), harmaline (**2**), and THH (**3**) analogs with the methoxy substituent moved about the six-membered benzenoid ring. Due to multiple fully unsaturated harmine (**1**) analogs having binding affinities greater than 10,000 nM, a comparison could only be made between the dihydro and tetrahydro compounds. If the binding affinities of the correspondingly substituted analogs of harmaline (**2**) and THH (**3**) (Figure 25, Table 16) result in

a significant linear correlation, then it is likely that they are binding in a similar manner at the 5-HT_{2A} receptor.

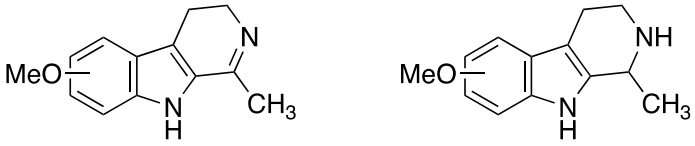
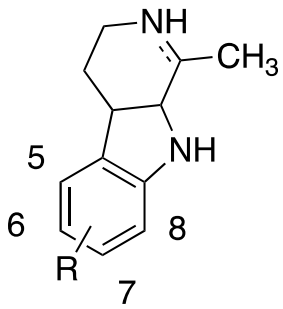


Figure 25. Methoxy-substituted harmaline (**2**) and THH (**3**) analogs prepared by Glennon et al.[176] to be used in correlation studies.

Binding affinities used in the following correlation studies were converted to pK_i values and then subsequently plotted against the pK_i value for the corresponding analog using Prism V9.2.0 software, which was also used to calculate the sample correlation coefficient (r) (GraphPad Software, San Diego, CA, USA).

Table 16. Dihydro and tetrahydro β-carboline analogs synthesized by Glennon et al.[176] and their corresponding binding affinities for the 5-HT_{2A} receptor.



R	Dihydro Series K_i (nM)	Tetrahydro Series K_i (nM)
-H	990	1,430
5-OMe	86	237
6-OMe	4,220	4,360
7-OMe	5,010	5,000 (S)
8-OMe	1,560	770

A good correlation between the fully and partially saturated analogs was found by plotting the pK_i values of the corresponding methoxy-substituted dihydro and tetrahydro β-carbolines against one another (r = 0.940, Figure 26). While these data are representative of only the relationship between the dihydro and tetrahydro β-carbolines, and is not statistically significant

(n = 5); the strength of the correlation suggests that the relative binding poses of β-carbolines could be similar to one another.

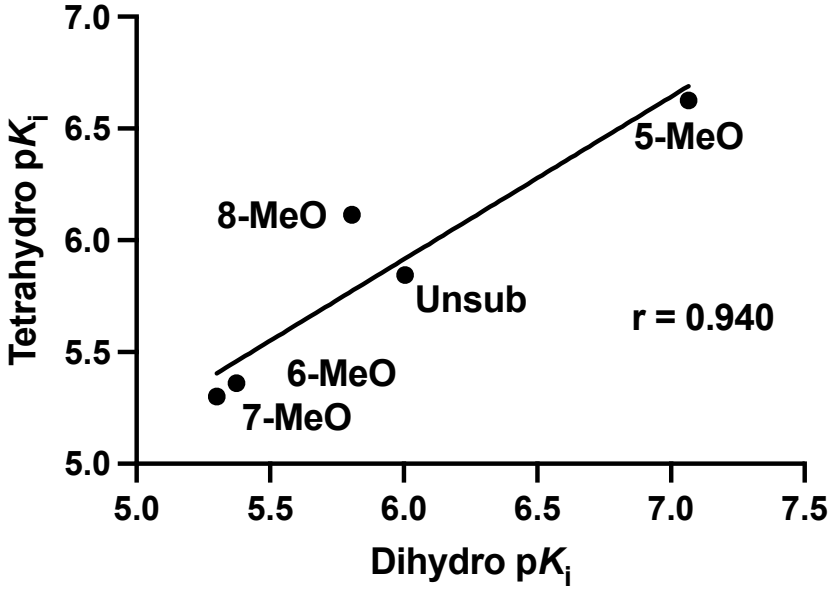


Figure 26. Correlation between pK_i of binding affinities of methoxy-substituted dihydro and tetrahydro β-carboline analogs (n = 5) at the 5-HT_{2A} receptor.

A second study examining the binding affinities of dihydro and tetrahydro β-carboline analogs was completed three years later, also by Glennon et al.,[202] which published the binding affinities of *des*-1-methyl substituted β-carbolines. The 1-position methyl group, which was demonstrated to have little effect on the binding affinity of the compounds, created the presence of optical isomers in the fully saturated group of compounds, which led to its removal. In addition to electron-donating methoxy-substituted analogs, electron-withdrawing bromo-substituted 1-*des*-methyl anagloues of both dihydro and tetrahydro β-carboline were synthesized (Figure 27) and tested at the 5-HT_{2A} receptor (Table 17). The fully unsaturated analogs were not synthesized due to their high activity at MAO-A.[184]

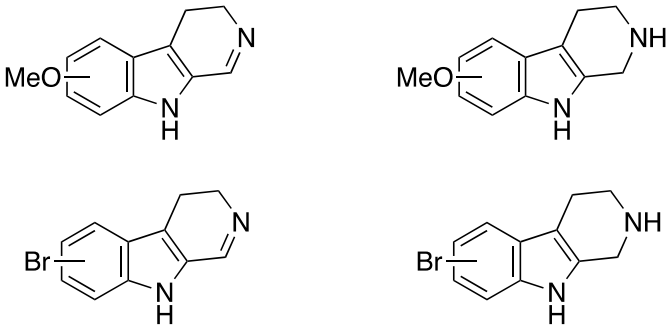
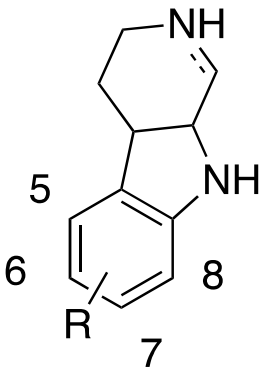


Figure 27. Methoxy and bromo-substituted 1-*des*-methyl β-carboline analogs prepared by Glennon et al.[202] to be used in correlation studies.

The correlation between 1-*des*-methyl methoxy and bromo-substituted β-carboline analogs was substantial (r = 0.873), indicating again that dihydro and tetrahydro β-carbolines bind similarly to one another in the binding pocket of the 5-HT_{2A} receptor. While the original correlation between the methoxy-substituted 1-methyl analogs (Figure 26) did not contain enough data points to be statistically significant (n = 5), the comparison between the 1-*des*-methyl analogs had enough data points (n = 8) to reach statistical significance (Figure 28). This further supports that for at least the dihydro and tetrahydro β-carbolines, the binding poses are very likely to be similar due to the similar changes to binding affinity produced by identical substitution patterns of the dihydro and tetrahdyro β-carboline structure. No conclusions can be drawn about the relationship between these compounds and fully unsaturated compounds, due to their lack of binding affinity for the 5-HT_{2A} receptor.

Table 17. 1-*Des*-methyl dihydro and tetrahydro β-carboline analogs synthesized by Glennon et al.[202] and their corresponding binding affinities for the 5-HT_{2A} receptor.



R	Dihydro Series K_i (nM)	Tetrahydro Series K_i (nM)
-H	2,560	3,800
5-OMe	470	130
6-OMe	2,370	4,780
7-OMe*	>10,000	4,620
8-OMe	3,240	640
5-Br	390	180
6-Br	1.720	500
7-Br	1,330	240
8-Br	110	22

*Not included in Figure 28

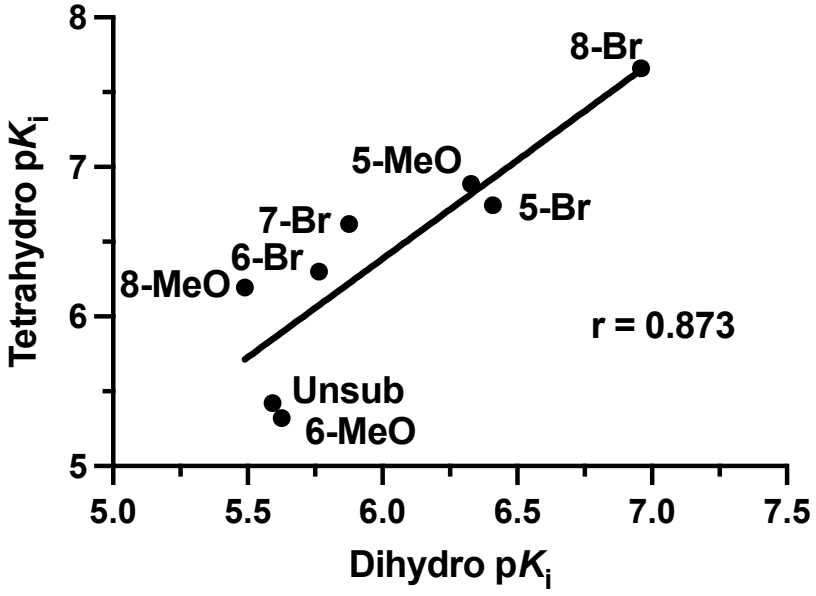


Figure 28. Correlation between the pK_i of binding affinities of methoxy and bromo-substituted 1-*des*-methyl dihydro and tetrahydro β-carboline analogs (n = 8) at the 5-HT_{2A} receptor.

Although the aforementioned paper discussed the absence of a 1-methyl substituent not having a significant effect on the binding affinity of β-carbolines, there was no corresponding correlation study reported. Using the methoxy-substituted 1-methyl and 1-*des*-methyl dihydro and tetrahydro data from Tables 14 and 15, an additional correlation study was completed (Figure 29).

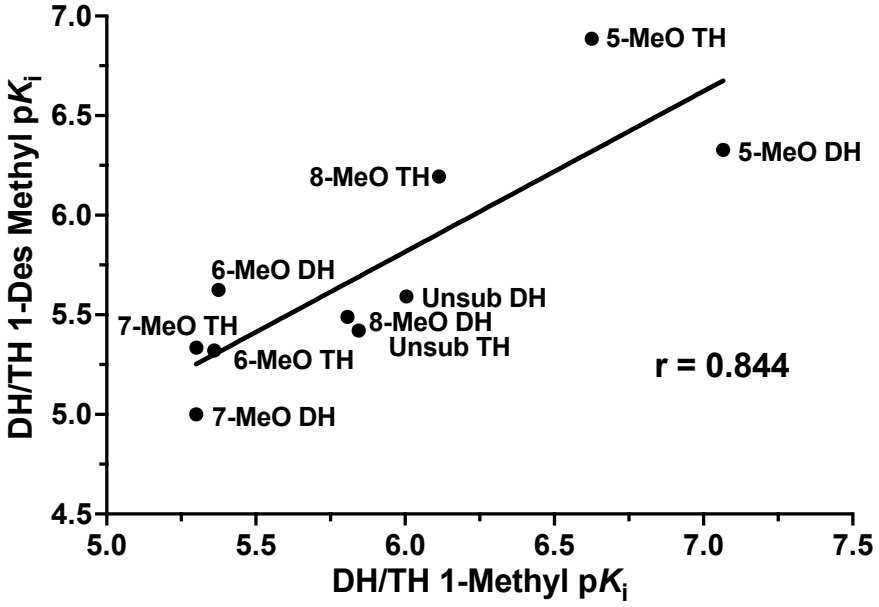
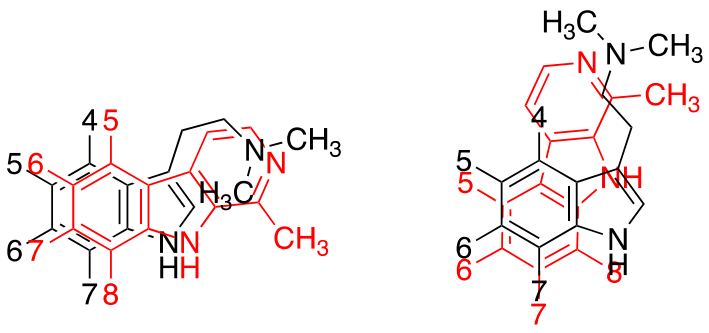


Figure 29. Correlation between pK_i of binding affinities of methoxy substituted 1-methyl and 1-*des*-methyl dihydro (DH) and tetrahydro (TH) β-carboline analogs (n = 10) at the 5-HT_{2A} receptor.

The correlation is strong (r = 0.844) between similarly subsituted 1-methyl and 1-*des*-methyl β-carboline analogs, suggesting that these compounds are occupying similar spaces in the binding pocket. The removal of the methyl substituent also seemed to have little effect on the magnitude of the binding interaction with the receptor, although in most cases the corresponding 1-methyl substituted compound displays slightly better affinity (Figure 29). This comparison also contained enough compounds (n = 10) to reach statistical significance.



Overlay 1 **Overlay 2**

Figure 30. Benzenoid position comparison between DMT (black, **4**) and β-carboline (red) compounds in Overlay 1 and Overlay 2.

Altogether, the correlation studies shown above suggest that the binding poses of β-carbolines should be similar to one another in the binding pocket of the 5-HT_{2A} receptor. With the evidence that β-carbolines bind similarly to one another, the data of previously synthesized β-carboline analogs which have been analyzed at the 5-HT_{2A} receptor become usable as data points for a statistical comparison between the relative binding mode between β-carbolines and DMT (**4**). The basis of the following comparison studies comes from the methoxy-substituted DMT (**4**) analogs that have been prepared and had their binding affinities determined by Glennon et al.[176] Using only previously reported binding affinities of DMT (**4**) and β-carboline analogs, non-significant correlations can be made to examine the validity of Overlay 1 and Overlay 2. For clarity, the positions of each overlay which are to be compared to one another are shown below (Figure 30, Table 18).

It can be clearly seen that for both comparisons, the benzenoid ring positions of both groups are at least partially overlayed with one another. For Overlay 1, there is a very simple direct comparison with the four positions of the benzenoid moiety in complete alignment with one another. For Overlay 2, the comparison becomes more complex, with three of the benzenoid ring

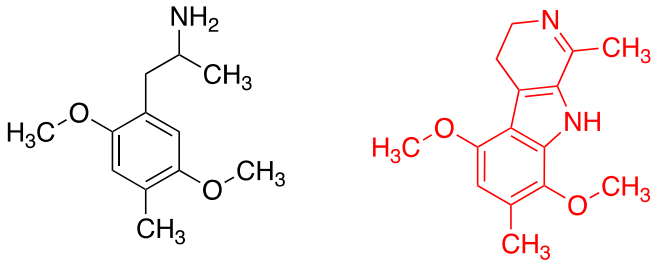
positions overlayed with one another, but the four position DMT (**4**) and the 8 position of the β-carboline scaffold having non-identical counterparts. The 4-position of DMT (**4**) seems to be irreplicable when it comes to the β-carboline structure due to its superimposition of a fused ring, although substitution of the eight position of β-carboline could possibly have a relationship to the area occupied by the indolic nitrogen atom.

Table 18. Benzenoid position substitution comparison between tryptamine and β-carboline compounds in Overlay 1 and Overlay 2.

Tryptamine	Overlay 1 β-carboline	Overlay 2 β-carboline
4	5	n/a
5	6	5
6	7	6
7	8	7
Unsubstituted	Unsubstituted	n/a

As previously shown by Glennon and co-workers,[199] the subsitition of tryptamines and β-carbolines in identical positions in the manner of Overlay 1 produces opposite changes in binding affinity (Table 7). Although Overlay 2 was proposed in that same paper, there was no analysis of the affinities of β-carboline and DMT (**4**) analogs substituted in manners consistent with Overlay 2 to confirm or refute this proposed binding mode. It was suggested by the authors that while β-carbolines and DMT (**4**) were unlikely to share a similar binding pose, the synthesized β-carboline analogue 5,8-dimethoxyharmalan (**32**) had a substitution pattern mimicking that of phenylisopropylamines such as DOM (**37**, Figure 31). The addition of a 7-position methyl group to 5,8-dimethoxyharmalan to yield a DOM-mimicking structure (**38**) increased the binding affinity

(210 nM to 98 nM), but not in the same order of magnitude that substition of 2,5-dimethoxyphenyl-2-aminopropane with a methyl group at the 4 position (5,200 nM to 100 nM).



DOM (**37**) 5,8-Dimethoxy-7-methylharmalan (**38**)

Figure 31. DOM (**37**) and 5,8-dimethoxy-7-methylharmalan (**38**), two similarly substituted compounds with high binding affinity for the 5-HT_{2A} receptor.

While the similarity between the structures of the substituted β-carboline to DOM (**37**) was noted in this study, no direct comparison between the structure of DMT (**4**) and 5,8-dimethoxyharmalan (**32**) was made, aside from the refutation of Overlay 1. It is possible that the similarities between the above structure of 5,8-dimethoxyharmalan (**32**) and tryptamines such as DMT (**4**) were not completely apparent at the time. From the binding data of different β-carboline analogs that have been published by Glennon et al.[176,202] (Table 16, Table 17) it is evident that subsitution of the 5- and 8-positions of the β-carboline scaffold are most associated with improvements in the binding affinity of these compounds for 5-HT_{2A} receptors.

Looking at Overlay 2, the 8 position of the β-carboline, as previously mentioned, would occupy the same space of the indole nitrogen atom of the tryptamine structure. Substitution at this position with a bioisosteric group could produce higher binding affinity at the 5-HT_{2A} receptor than unsubsituted β-carbolines, as the indolic nitrogen atom of DMT (**4**) and other tryptamines has been demonstrated to make crucial interactions in the binding pocket of the receptor.[128] The 5 position of the β-carboline scaffold would be aligned with the 5 position of DMT (**4**), which has

been shown to increase the binding affinity for 5-HT_{2A} receptors.[176,209,210] In both cases, substitution of these positions is not necessarily in disagreement with a binding pose which is related to that of the tryptamines, it just happens to be in a manner that is not as immediately obvious as Overlay 1.

The synthesis of bromo-subsituted DMT (**4**) analogs (Figure 17) is the simplest way to add validity to any comparison between the tryptamines and β-carbolines. Since the largest set of binding data at 5-HT_{2A} receptors for compounds containing the β-carboline structure comes from the study examining methoxy and bromo substituted 1-*des*-methyl β-carbolines, and data exist for the methoxy substituted DMT (**4**) analogs, the synthesis of these four compounds would allow for statistically significant comparisons between the two sets of compounds ($n > 6$).

The lack of fully aromatic harmine (**1**) analogs and the absence of the 1-methyl position substituent might raise questions about the soundness of this study when making comparison to the three naturally occuring compounds present in ayahuasca, but the correlation studies presented above have attempted to show that these differences are theoretically inconsequential. Ideally, there would be fully aromatic and 1-methyl substituted β-carboline analogs with identical subsituents on their respective benzenoid rings, but the span of that sort of study is beyond the scope of this thesis.

Once the bromo-DMT analogs had been synthesized (see next section) and assayed for binding at the human 5-HT_{2A} recptor, new correlations were conducted, using the β-carboline binding data from Glennon et al.[202] (Table 17), methoxy-substituted tryptamine data from Glennon et al.[176] (Table 19) and bromo-substituted tryptamine data from this thesis (Table 19).

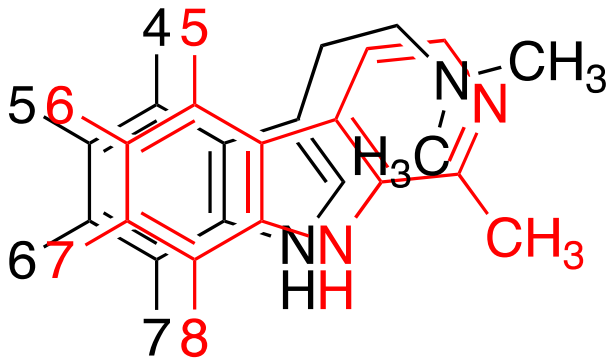
Table 19. Methoxy-substituted DMT analogs from Glennon et al.,[174] and bromo-substituted DMT analogs synthesized and analyzed for binding affinity for this thesis.[+]

Compound	K_i (nM)
DMT*	660
4-OMe DMT*	180
5-OMe DMT*	280
6-OMe DMT*	4,190
7-OMe DMT*	9,200
DMT^	168
4-Br DMT^	62
5-Br DMT^	46
6-Br DMT^	814
7-Br DMT^	353

+See Table 22 for detailed binding data.
* Data from [^{3}H]ketanserin-labeled rat 5-HT$_{2A}$ receptors in NIH-3T3 cells.
^Data from [^{3}H]ketanserin-labeled cloned human 5-HT$_{2A}$ receptors in HEK-293 cells.

The correlation of the pK_i values of the OMe-DMT analogs and Br-DMT analogs resulted in a very strong linear correlation (r = 0.907), which was supported by docking poses observed for both sets of compounds in our 5-HT$_{2A}$ receptor model (Figure 24).

Table 20. Points of comparison for Overlay 1 between OMe and Br-substituted DMT (**4**, black) and β-carboline (red) analogs.



DMT	Dihydro β-carboline	Point
Unsub	Unsub	A
4-OMe	5-OMe	B
5-OMe	6-OMe	C
7-OMe	8-OMe	D
4-Br	5-Br	E
5-Br	6-Br	F
6-Br	7-Br	G
7-Br	8-Br	H

DMT	Tetrahydro β-carboline	Point
Unsub	Unsub	I
4-OMe	5-OMe	J
5-OMe	6-OMe	K
6-OMe	7-OMe	L
7-OMe	8-OMe	M
4-Br	5-Br	N
5-Br	6-Br	O
6-Br	7-Br	P
7-Br	8-Br	Q

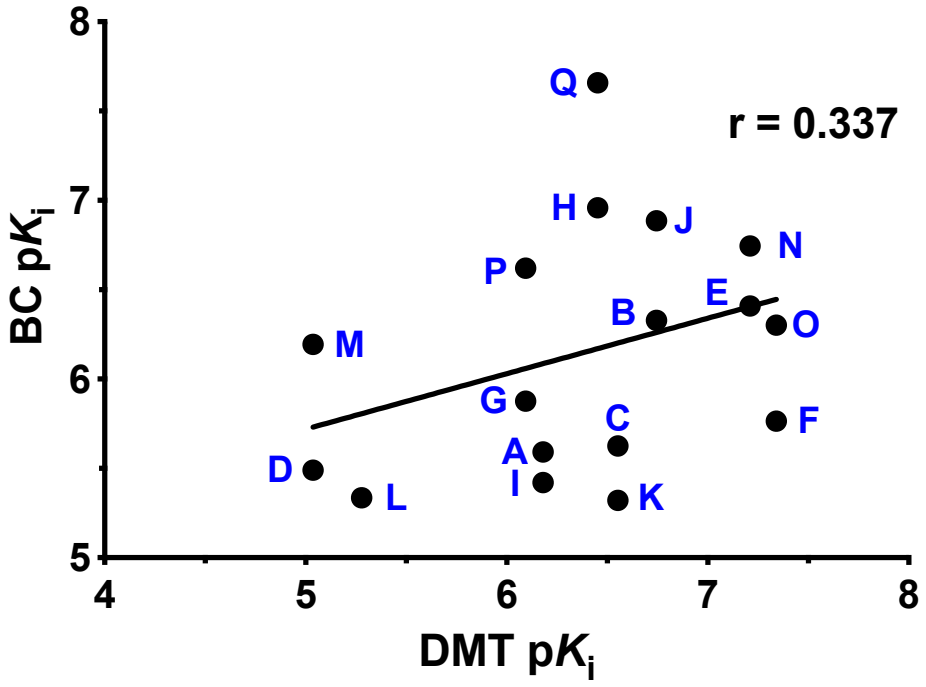
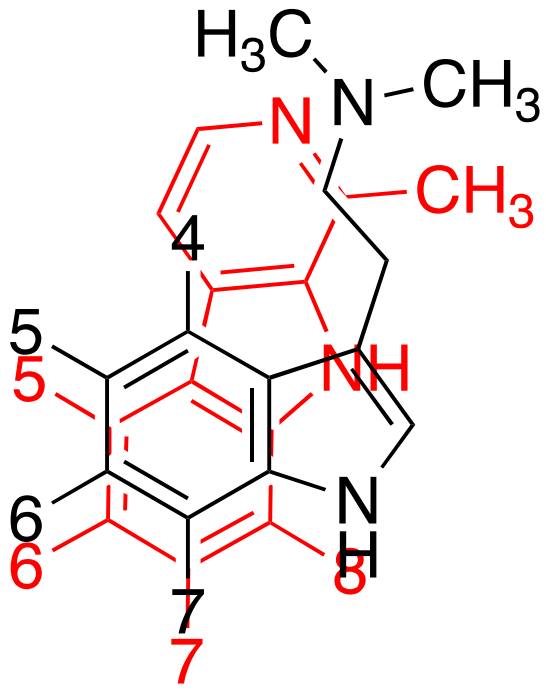


Figure 32. Overlay 1 comparison between similarly substituted DMT and β-carboline analogs (n = 17, Table 20).

As can be seen by the above correlation (Figure 32) and legend (Table 20), Overlay 1 does not provide a very strong linear correlation between the analogs of DMT and β-carboline. This was as expected, dating back to the original paper by Glennon et al.,[199] suggesting that this binding relationship would be unfavorable. This is the first correlation study of statistical significance that has examined this relationship, and supports the original hypothesis that this relative binding pose between these sets of compounds is unlikely to occur. It is worth once again mentioning that these correlations of compounds do not include fully aromatic harmine analogs. Individually, the comparison of dihydro and tetrahydro β-carboline sets of compounds with their DMT counterparts produced correlation coefficients of 0.400 and 0.356, respectively.

Table 21. Points of comparison for Overlay 2 between OMe and Br-substituted DMT (**4**, black) and β-carboline (red) analogs.



DMT	Dihydro β-carboline	Point
5-OMe	5-OMe	A
6-OMe	6-OMe	B
5-Br	5-Br	C
6-Br	6-Br	D
7-Br	7-Br	E
DMT	Tetrahydro β-carboline	Point
5-OMe	5-OMe	F
6-OMe	6-OMe	G
7-OMe	7-OMe	H
5-Br	5-Br	I
6-Br	6-Br	J
7-Br	7-Br	K

Although there are not nearly as many points of comparison (Table 21) for Overlay 2 as there are for Overlay 1, due to the imperfect alignment of their benzenoid moieties, there are still enough points between the β-carbolines and DMT analogs to make a statistically significant correlation (n = 11). The correlation obtained (Figure 33) from the comparison of both the DMT and β-carbolines was strongly linear (r = 0.830), especially in comparison to Overlay 1 (r = 0.337). Individually, the dihydro and tetrahydro sets of compounds had correlation coefficients with their DMT counterparts of 0.449 and 0.913, respectively.

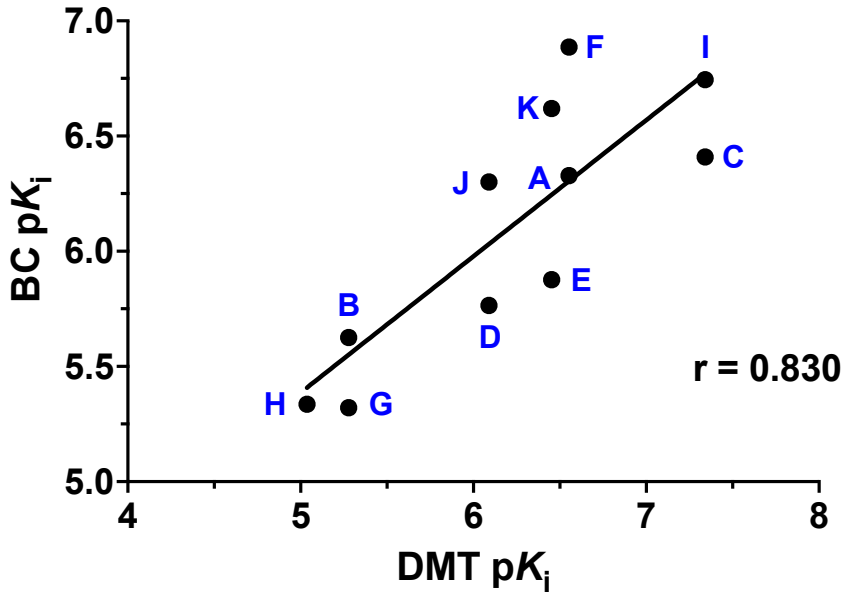


Figure 33. Overlay 2 comparison between similarly substituted DMT and β-carboline analogs (n = 11, Table 21).

While the linear correlation between the β-carbolines and DMT (**4**) was much stronger for Overlay 2 than Overlay 1, they are not necessarily in agreement with the docking studies mentioned previously. In contrast to the docking studies, which showed the bromo-substituted dihydro β-carboline analogs mimicking Overlay 2 nearly perfectly (Figure 22), the correlation coefficient for this set of compounds is not as strongly supportive of this Overlay in regards to these compounds (r = 0.613, n = 3). On the other hand, the bromo-substituted tetrahdyro β-carboline analogs were shown in a flipped Overlay 2-like conformation (Figure 23), while producing a very strong correlation coefficient with DMT analogs in Overlay 2 (r = 0.913, n = 6). However, when the points of comparison for tetrahydro β-carboline are flipped to match the relationship seen from the docking results (5-position of DMT to 8-position of β-carboline, 6-position of DMT to 7-position of β-carboline, and 7 position of DMT to 6-position of β-carboline), a similarly strong correlation between the two sets of compounds was observed (r = 0.849, n = 6).

The results of this correlation study indicate two things: that Overlay 2 is more likely to be the correct relative binding mode of these compounds compared to Overlay 1, and that for tetrahydro β-carbolines, an Overlay 2 or Overlay 2-adjacent binding mode is supported by both docking and correlation studies. For the dihydro set of compounds, docking was almost perfectly in agreement with the proposed Overlay 2 alignment, but the correlation of the dihydro β-carboline analogs to DMT was relatively poor ($r = 0.449$). If the results of the docking were correct, and the Overlay 2-like relationships for both the dihydro and tetrahydro β-carboline analogs were only seen with bromo-substituted analogs, and not the methoxy-substituted analogs, then the relatively low linear correlation for the dihydro set of compounds would be justified. The strong linear correlations for the tetrahydro set of both bromo and methoxy-substituted compounds seems to refute the validity of the docking model, as the methoxy-substituted analogs of tetrahydro β-carboline which did not at all resemble Overlay 2 in the docking results shown make up half of the data points in this very linear relationship. If methoxy-substituted tetrahydro β-carbolines truly had no connection to the binding mode of DMT (**4**), it would be a remarkable coincidence for them to produce strong linear correlations with similarly substituted methoxy-DMT analogs.

Specific Aim 3: Synthesis of DMT analogs with substituents corresponding to previously studied β-carbolines in order to make statistically significant comparisons.

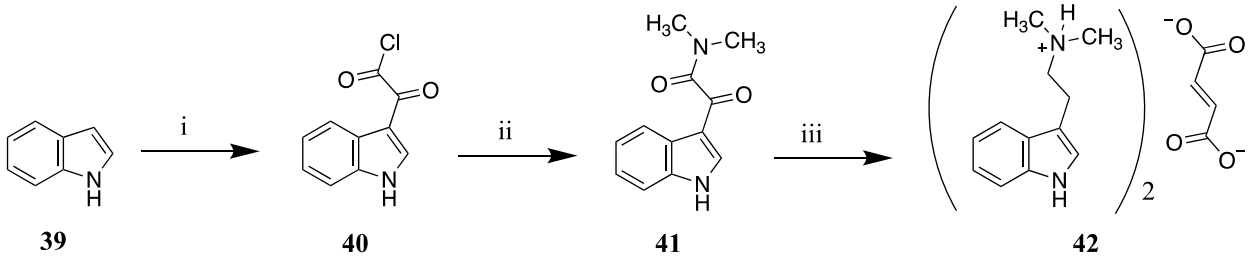
A. Synthesis of DMT Analogs

1. Synthesis of *N*,*N*-DMT Hemifumarate (**42**)

The first compound to be synthesized was the salt of the unsubstituted parent compound, DMT (**4**). In order to validate the binding affinities of the following analogs of DMT (**4**), it was

determined that DMT (**4**) would be synthesized in order to have a compound which has been thoroughly analyzed for binding at 5-HT_{2A} receptors in the literature. Optimization of the Speeter-Anthony tryptamine synthesis in relation to DMT (**4**) had been published recently by Cozzi and Daley,[211] substituting the standard lithium aluminum hydride reduction with alane (aluminum hydride, AlH_3) modifying a method used by Glennon et al.[212] for the synthesis of 7-bromo-*N*,*N*-dimethyltryptamine. The Speeter-Anthony [213] synthesis of DMT (**4**) involves the reaction of indole (**39**) with oxalyl chloride to yield indol-3-ylglyoxyl chloride (**40**). The resulting acid chloride is then reacted with dimethylamine to produce indol-3-ylglyoxyl amide (**41)** and reduced with lithium aluminum hydride to form the resulting crude freebase, and then fumaric acid in acetone to produce DMT hemifumarate (**42**, Scheme 1).

Scheme 1.[a] Speeter-Anthony synthesis of *N*,*N*-DMT Hemifumarate (**42**).



39 **40** **41** **42**

[a]Reagents and conditions: i. 1) ClCOCOCl, N_2/Et_2O, 0°C 10 min, 2) N_2/Et_2O/THF, rt 20 min; ii. $(CH_3)_2NH$ (40%, aqueous), stirring 48 h rt; iii. 1) $LiAlH_4/Et_2O$, reflux 6 h, 2) fumaric acid/acetone

Although lithium aluminum hydride was used in our synthesis of DMT (**4**), the reduction of glyoxylamides using alane was intriguing for its decreased time of reaction (5 h from 15-120 h reported by Cozzi and Daley)[211] and for its selectivity in relation to cycloalkyl halides.[214] For the bromo-substituted analogs of DMT, this seemed an ideal reducing agent for avoiding the removal of the bromine atom from the aromatic ring system. The first attempt to synthesize DMT (**4**)

yielded the pure hemifumarate product, with 86% and 54% yield for the first and second step, respectively. The reduction with lithium aluminum hydride produced a low yield of the product, with only 16% of the theoretical amount recovered.

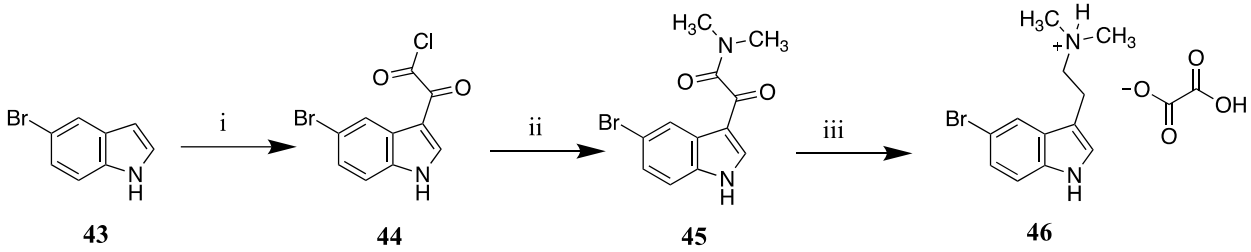
2. Synthesis of 5-Bromo-*N*,*N*-DMT Oxalate (**46**)

The next compound undertaken was 5-bromo-*N*,*N*-dimethyltryptamine oxalate (**46**), which is the most studied bromo-substituted DMT analogue that has been made due to its occurrence as a natural product of certain species of sponges.[215,216] This compound has also been shown to have sedative-like properties in animal models, while the 5,6-dibromo-*N*,*N*-dimethyltryptamine compound was shown to have antidepressant-like effects. This compound had been previously synthesized using the Speeter-Anthony pathway, as well as with nitro olefination of the bromo-substituted indole, followed by reduction with lithium aluminum hydride and Eschweiler-Clarke reductive methylation of the resulting *N*,*N*-unsubstituted tryptamine.[216,217] Due to the familiarity with the Speeter-Anthony pathway, it was once again used for the synthesis of 5-bromo-*N*,*N*-dimethyltryptamine (Scheme 2).

The reaction of commercially available 5-bromoindole (**43**) with oxalyl chloride and consequently with dimethylamine occurred as expected, with yields of 47% and 80% respectively. Initial attempts at reduction of the 5-bromoindol-3-yl-*N*,*N*-dimethylglyoxylamide (**45**) with both lithium aluminum hydride and diborane yielded significant amounts of de-brominated DMT (**4**), which was unable to be separated from the product via column chromatography or by distillation. Eventually, alane was used due to its previous success in reducing the unsubstituted indol-3-yl-*N*,*N*-dimethylglyoxylamide (**41**) by Cozzi and Daley, as well as the 7-bromoindol-3-yl-*N*,*N*-dimethylglyoxylamide (**58**) made by Glennon et al.[212]

Unlike the methods of Cozzi and Glennon, which used 100% sulfuric acid to generate alane from lithium aluminum hydride in ether, the procedure of Finholt et al.[218] was applied, which generates alane *in situ* from lithium aluminum hydride and aluminum chloride. With alane, no de-brominated product was seen when subjected to thin-layer chromatography (TLC) in comparison to the reductions with diborane and lithium aluminum hydride. For the brominated analogs of DMT (**4**), the oxalate salt was prepared by dissolving the freebase in anhydrous ether, and then adding an equimolar equivalent of oxalic acid dissolved in anhydrous ether. The identity of the mono-oxalate product was confirmed by CHN analysis. The oxalate salt was formed more readily than the fumarate salt for the brominated compounds.

Scheme 2.[a] Speeter-Anthony synthesis of 5-bromo-*N*,*N*-DMT Oxalate (**46**).



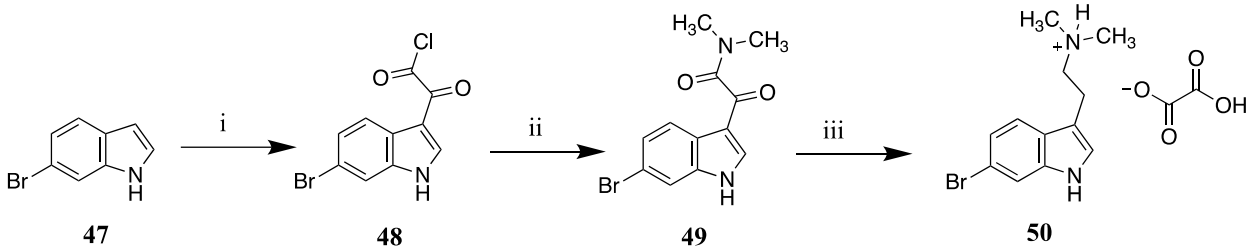
[a]Reagents and conditions: i. 1) ClCOCOCl, N_2/Et_2O, 0°C 30 min, 2) N_2/Et_2O, rt 1 h; ii. $(CH_3)_2NH$ (40%, aqueous), stirring overnight rt; iii. 1) AlH_3/Et_2O, reflux 1 h, 2) Oxalic acid/Et_2O

3. Synthesis of 6-Bromo-*N*,*N*-DMT Oxalate (**50**)

6-Bromo-*N*,*N*-dimethyltryptamine oxalate (**50**) was prepared in an identical manner to its 5-substituted counterpart via the Speeter-Anthony tryptamine synthesis (Scheme 3). The commercially available 6-bromoindole (**47**) was converted to 6-bromoindol-3-ylgloxyl chloride (**48**), but due to observed degradation of the acid chloride product when exposed to air with the unsubstituted and 5-substituted acid chlorides, the filtering and weighing of the reactive intermediate was skipped entirely. Instead, the ether used in the reaction between the substituted

indole and oxalyl chloride was removed under reduced pressure, and then the aqueous dimethylamine solution was added directly to the residue. This change seemed to increase the yield of the initial two steps of the reaction, as the 6-bromoindol-3-yl-*N*,*N*-dimethylglyoxylamide (**49**) was obtained in a 52% yield from the corresponding indole, in comparison to a 38% overall yield from 5-bromoindole (**43**) to 5-bromoindol-3-yl-*N*,*N*-dimethylglyoxylamide (**45**). The reduction with alane once again produced no noticeable amount of the de-brominated DMT (**4**) product when subjected to TLC. The oxalate salt of 6-bromo DMT was prepared in the same manner as 5-bromo-*N*,*N*-DMT oxalate (**46**).

Scheme 3.[a] Speeter-Anthony synthesis of 6-Bromo-*N*,*N*-DMT Oxalate (**50**).



[a]Reagents and conditions: i. 1) ClCOCOCl, N_2/Et_2O, 0°C 30 min, 2) N_2/Et_2O, rt 1 h; ii. $(CH_3)_2NH$ (40%, aqueous), stirring overnight rt; iii. 1) AlH_3/Et_2O, reflux 1 h, 2) Oxalic acid/Et_2O

4. Synthesis of 4-Bromo-*N*,*N*-DMT Oxalate (**54**)

4-Bromo-*N*,*N*-dimethyltryptamine oxalate (**54**) was the only brominated analogue of DMT (**4**) which had not been previously reported as being synthesized via the Speeter-Anthony pathway. Very few reported syntheses of this compound exist, and the only reasonably simple pathway reported consisted of the nitro olefination of 4-bromoindole (**51**), followed by reduction of the nitro olefin to the tryptamine, and finally Eschweiler-Clarke reductive methylation of the tryptamine to the *N*,*N*-dimethylated product (Scheme 4). It was of interest to investigate whether the Speeter-Anthony synthesis was a viable pathway for synthesis of 4-bromo-*N*,*N*-

dimethyltryptamine oxalate (**54**), as this compound has seemingly not been studied in great detail. Commercially available 4-bromoindole (**51**) was reacted with oxalyl chloride and then taken to the next step of the reaction without purification. The bright color of a glyoxyl chloride intermediate was absent from this reaction, but it was taken to the next step to avoid degradation if the acid chloride was indeed forming.

Addition of dimethylamine solution to indol-3-ylgloxyl chlorides had previously produced a white precipitate almost immediately, which increased in density over time. No such precipitate was produced from what should have been the 4-bromoindol-3-ylgloxyl chloride, even after allowing the reaction to sit overnight. TLC analysis of the reaction mixture did not show a spot which had a similar Rf to the previously examined bromo-substituted indol-3-ylgloxyl amides, and removal of the solvent yielded a brown oil in place of the expected ivory colored solid. The brown oil obtained from this reaction was analyzed by NMR, and did not contain two distinct signals corresponding to the two methyl groups attached to the amide. This reaction pathway was tried three separate times with modification of reaction length and temperature, with a lack of amide formation each time.

It is possible that the steric bulk of a bromine atom at the 4-position is too significant for the addition of oxalyl chloride to the 3-position of the indole ring, or that the electron withdrawing character of a bromo substituent at this position is strong enough to de-activate the double bond between the 2 and 3 position of the indole. The 4 position of tryptamines has been successfully substituted in a variety of ways, with naturally occurring compounds such as psilocin and psilocybin (4-hydroxyl-DMT and 4-phosphoryloxy-DMT, respectively), and convolutindole A (2,4,6-tribromo-1,7-dimethoxy-*N*,*N*-DMT) as well as designer-drug DMT analogs such as 4-AcO-DMT (4-acetoxy-DMT) and 4-OMe-MiPT (4-methoxy-*N*-methyl-*N*-isopropyltryptamine).[219]

Convolutindole A is the only known naturally occurring 4-bromo-subsituted DMT analogue, but the natural synthetic pathway for this compound is unlikely to be comparable to the reaction conditions used in the Speeter-Anthony tryptamine synthesis.

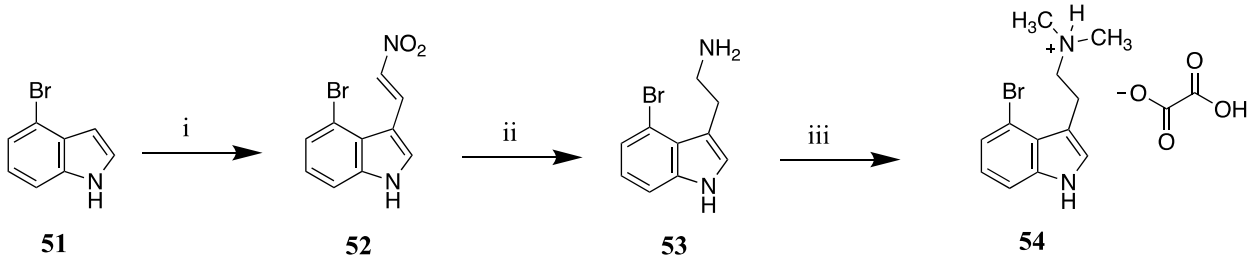
The previously mentioned nitro olefination of 4-bromoindole (**51**) was reported by Olsen et al.[216], along with the 5, 6, and 7-bromo counterparts. The reported synthetic pathway used 1,2-dimethylamino-2-nitroethylene with trifluoroacetic acid to introduce the nitroethenyl moiety to the 3-position of the 4-bromoindole starting material, followed by two separate reductions. First, the double bond of the nitroethenyl moiety was reduced using sodium borohydride, followed by the reduction of the resulting nitroethyl compound to the tryptamine using lithium aluminum hydride. The 4-bromotryptamine (**53**) was then dimethylated by Eschweiler-Clarke reductive methylation to yield the 4-bromo-*N*,*N*-dimethyltryptamine.

A procedure published by Kalinin et al.[220] found that the reduction of both the double bond and the nitro group to 4-bromotryptamine (**53**) could be completed in one step with lithium aluminum hydride, with greater yield (80%) vs. the two-step reduction (43% and 16%) published by Olsen et al.[216] This procedure was substituted for the two reduction steps of the procedure published by Olsen et al.,[216] and was the only modification made.

The nitro olefination of commercially available 4-bromoindole (**51**) was successfully completed with an 85% yield (52% literature[216]), and the following single reduction step produced 4-bromotryptamine (**53**) with a 57% yield (80% literature[220]) (Scheme 4). Once again, the removal of the bromine atom by lithium aluminum hydride was a concern with this reaction, but the equivalents of lithium aluminum hydride were low enough and reaction conditions mild enough that no debrominated product was seen via TLC analysis or NMR. Eschweiler-Clarke reductive methylation produced 100% yield (84% literature)[216] of the 4-bromo-*N*,*N*-DMT product, which

was converted to 4-bromo-*N*,*N*-DMT oxalate (**54**) in the same manner as 5-bromo-*N*,*N*-DMT oxalate (**46**) and 6-bromo-*N*,*N*-DMT oxalate (**50**).

Scheme 4.[a] Synthesis of 4-bromo-*N*,*N*-DMT Oxalate (**54**).



51 **52** **53** **54**

[a]Reagents and conditions: i. N_2/trifluoroacetic acid, rt 50 min; ii. 1) N_2/THF, $LiAlH_4$, reflux 4 h, 2) Stir 18 h rt; iii. 1)N_2/MeOH, CH_3COOH, $NaCNBH_3$, 24 h rt 2) Oxalic acid/Et_2O

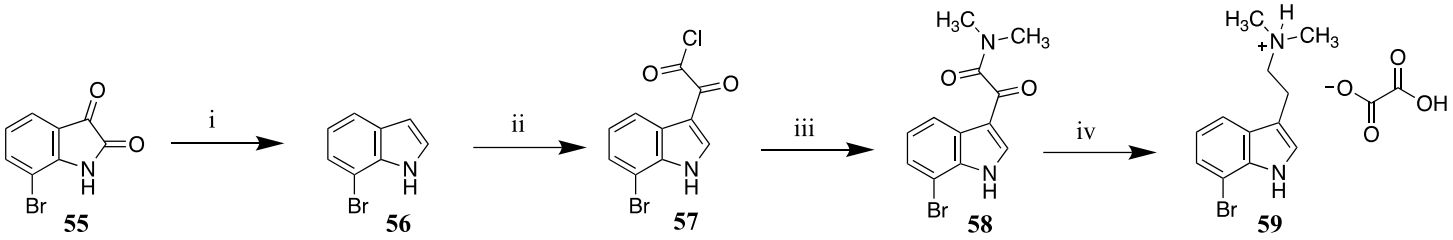
5. Synthesis of 7-Bromo-*N*,*N*-DMT Oxalate (**59**)

Unlike the previous compounds, the indole starting material for 7-bromo-*N*,*N*-DMT oxalate (**59**) was not readily available. The first published synthesis of 7-bromo-*N*,*N*-DMT oxalate (**59**) by Glennon et al.[212] used 7-bromoindole (**56**) made from a reaction between 2-bromoaniline, boron trichloride, and chloroacetonitrile to yield 1-(2-bromophenyl)-2-chloroethan-1-one, which was subsequently cyclized by reduction with sodium borohydride. Due to natural product supply chain issues brought about by Covid-19, boron trichloride was not available.

7-Bromoisatin (7-bromoindole-2,3-dione, **55**) is an inexpensive and readily available starting material for 7-bromoindole (**56**), which has been shown to be reduced under relatively mild conditions with good yield (74%).[221] Sodium borohydride was used to reduce commercially available 7-bromoisatin (**55**), although the yields obtained from this procedure were poor in comparison (15%). A large portion of the reactants polymerized to form 7,7'-dibromoindigo, a deeply purple substance related to Tyrian purple dye, which was immediately evident when isolating the 7-bromoindole product via flash chromatography. Despite the low yield of the first step of the reaction, enough 7-bromoindole (**56**) was recovered to run the Speeter-Anthony

synthesis (Scheme 5), with the amide being produced in a 24% yield from the indole (21% literature[212]).

Scheme 5.[a] Speeter-Anthony synthesis of 7-Bromo-*N*,*N*-DMT Oxalate (**59**).



[a]Reagents and conditions: i.THF,BF_3 diethyl etherate, -5 °C, $NaBH_4$; ii. 1) ClCOCOCl, N_2/Et_2O, 0°C 30 min, 2) N_2/Et_2O, rt 1.5 h; iii. $(CH_3)_2NH$ (40%, aqueous), stirring overnight rt; iv. 1) AlH_3/Et_2O, reflux 1 h, 2) oxalic acid/Et_2O

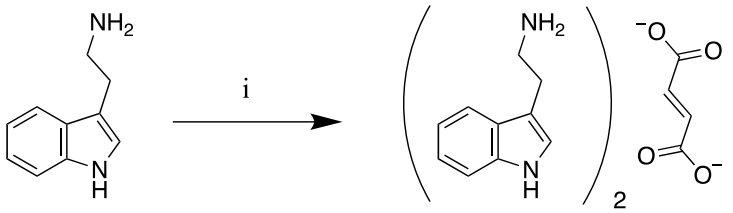
It was not immediately evident why the yield for the production of the amide was lower than for the 5 and 6-bromo substituted analogs, but the electron withdrawing character of the bromine in the 7 position of the indole ring could be deactivating the 2,3-position double bond where the reaction with oxalyl chloride occurs. Reduction of 7-bromo-*N*,*N*-dimethylglyoxylamide (**58**) to the amine was done once again with alane, produced *in situ* from lithium aluminum hydride and aluminum chloride and converted to the oxalate salt with a 54% yield (77 % literature[212]).

6. Synthesis of Tryptamine Hemifumarate (**61**)

Tryptamine hemifumarate (**61**) was synthesized to serve as a point of comparison for DMT hemifumarate (**42**) in binding affinity studies, as the hemifumarate salt of unmethylated tryptamine has never been reported in the literature. Since the hemifumarate salt is the preferred form of DMT used in human clinical trials,[211] it would be useful to have information on the physical properties of the unmethylated tryptamine salt (**61**). It was also used as a standard when synthesizing 4-bromotryptamine (**53**), to ensure that the removal of the 4-position bromine atom had not occurred.

Commercially acquired tryptamine (Sigma Aldrich, **60**) was treated with fumaric acid-saturated acetone in the same manner as DMT (**4**), and recrystallized four times to yield a pure white solid which was identified as the hemifumarate salt of tryptamine by CHN analysis (Scheme 6).

Scheme 6.[a] Synthesis of tryptamine Hemifumarate (**61**) from tryptamine (**60**).



60 **61**

[a]Reagents and conditions: i. Fumaric acid/acetone

B. Binding Studies

All synthesized compounds were evaluated for binding at the human 5-HT_{2A} receptor stably expressed in HEK-293 cells (full description of assay found in experimental section). DMT (**4**) had a comparable binding affinity to the most recently published K_i value for the same drug at cloned human 5-HT_{2A} receptors using [^{3}H]ketanserin as a radioligand (168 nM vs. 175 nM[222]). The 4- and 5-Br DMT analogues both displayed improved binding affinities over the unsubstituted parent compound (Table 22), which is in line with the improvements seen in binding affinity for 4- and 5-substituted DMT analogs such as psilocin and 5-OMe DMT.[209,223] It was also observed that 7-Br DMT had a relatively high affinity for the 5-HT_{2A} receptor (353 nM), considering that the 7-OMe DMT synthesized by Glennon et al. binds with an affinity of 5,440 nM at rat 5-HT_{2A} receptors, but the data was consistent with values for binding affinity published in a previous paper by Glennon et al.[176] This previous study included 7-OMe DMT, 7-OH DMT, and 7 Br-DMT, which had affinities of 5,400 nM, >10,000 nM, and 170 nM at rat 5-HT_{2A} receptors, respectively.[224] In a previously published paper, substituting the DMT structure at the 7-position

with methyl, ethyl, and bromo groups all increased affinity for rat stomach fundus preparations containing serotonin receptor populations.[212] It appears as if strong electron-donating character of substituents at the indole 7-position have a negative effect on the binding affinity of these compounds at 5-HT_2 receptor populations. The binding affinity of 6-Br DMT was roughly in line with what was expected from the previously published data on 6-substituted DMT analogues, with the corresponding 6-OMe DMT (3,960 nM) from a study by Glennon et al.[176] and another study in which 6-OMe DMT and 6-OMe-1-methyl-*N*,*N*-dimethyltryptamine had affinities at rat 5-HT_2 receptor populations of 7,300 nM and 2,500 nM, respectively. It seems that the indole substitution positions for which binding affinity is most improved for serotonin receptors follows a pattern: 4≈ 5 > unsubstituted > 7 > 6.

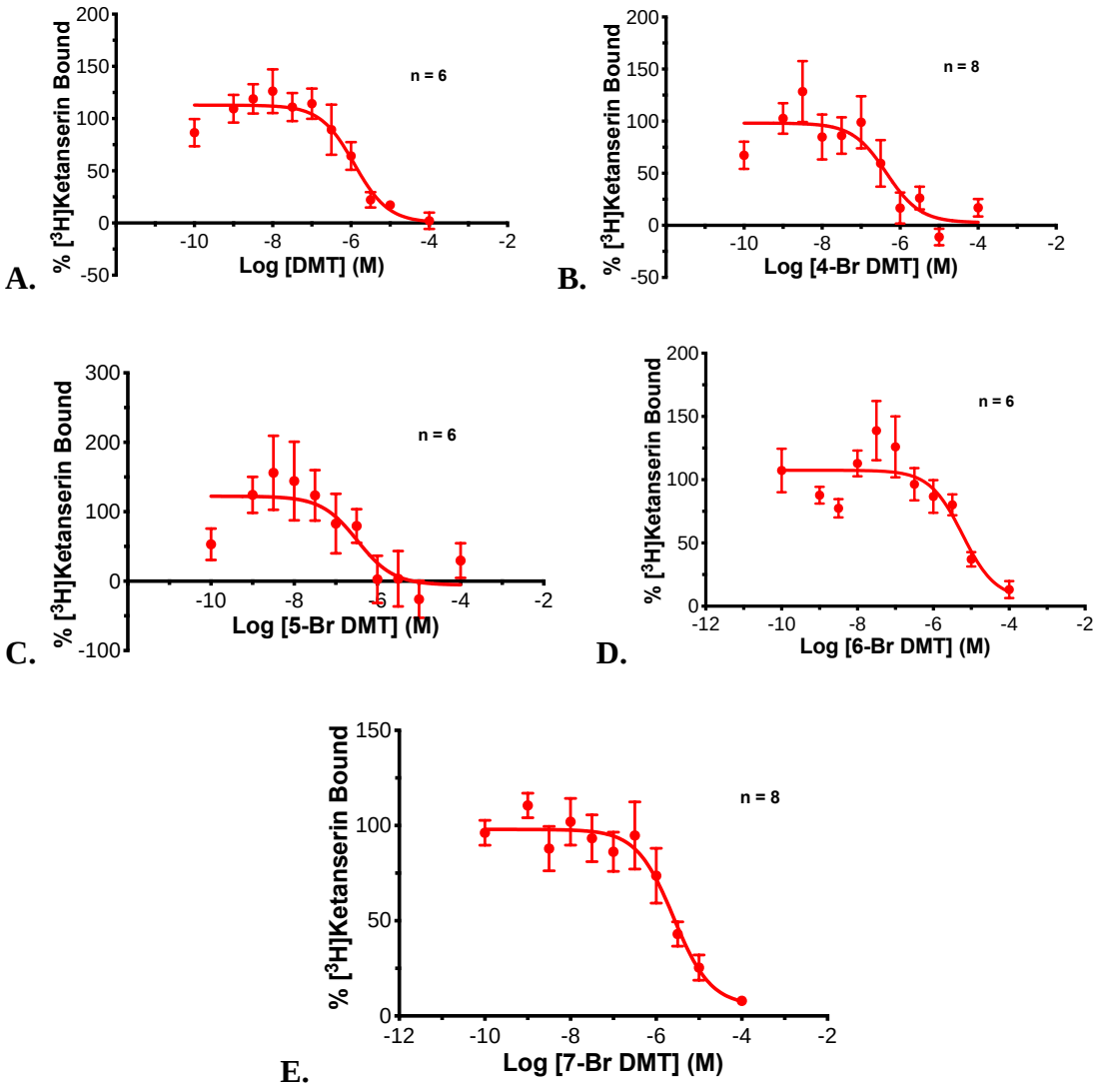


Figure 34. Processed one-site non-linear regression binding curves of **A.** DMT hemifumarate (**42**), **B.** 4-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**54**) **C.** 5-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**46**), **D.** 6-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**50**), and **E.** 7-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**59**). K_i values are shown in Table 22.

Table 22. Binding affinities (pK_i ± SEM and K_i) of DMT (**4**) and brominated DMT analogs.

Compound	pK_i ± SEM	K_i (nM)
DMT hemifumarate (**42**)	6.775 ± 0.207	168
4-Br DMT hydrogen oxalate (**54**)	7.211 ± 0.307	62
5-Br DMT hydrogen oxalate (**46**)	7.340 ± 0.456	46
6-Br DMT hydrogen oxalate (**50**)	6.089 ± 0.262	814
7-Br DMT hydrogen oxalate (**59**)	6.452 ± 0.192	353

C. Functional Studies

With the lack of data for functional activity of the harmala alkaloids at the 5-HT_{2A} receptor, it was of interest to use a basic calcium mobilization assay to determine possible agonism or antagonism. Calcium mobilization is a shared characteristic of agonist action at many GPCRs including the 5-HT_{2A} receptor,[101] and therefore determining the calcium mobilization of these compounds with cells expressing this receptor should be indicative of agonist activity.

Harmine (**1**), harmaline (**2**), and THH (**3**) were all tested using a calcium mobilization assay using cells stably expressing the 5-HT_{2A} receptor. Both harmine (**1**) and harmaline (**2**) produced no flourescence, indicating that no calcium was being released by these compounds. This is consistent with the data obtained previously for these compounds[175,176] which showed that they had no activity in a PI hydrolysis assay (not shown). Neither compound was seen to antagonize the effects of serotonin (**7**) in this assay either, meaning that they seem to be devoid of antagonistic acitivty as well. THH (**3**), however, showed antagonist activity in this assasy, with an IC_{50} of roughly 48,000 nM (Figure 35). This is the first indication that any of the harmala alkaloids have any activity, agonist or antagonist, at the 5-HT_{2A} receptor. The miniscule amount of human reports of psychoactivity of THH (**3**) and the observed antagonistic effect of this compound on the receptor

bring further into question the validity of the human data on this compound, as this would be the first ligand with antagonist activity at the 5-HT_{2A} receptor which was also reported to be hallucinogenic. Even though this compound has antagonist-like activity in this assay, its potency is incredibly weak, suggesting that at the concentrations that it is found at physiologically after ingestion of ayahuasca, it is unlikely to be exerting much of an effect on the receptor population. The effect of 5-HT_{2A} antagonists on antidepressant action has been studied,[111] but once again the compounds used in that analysis had antagonist potencies much greater than that which was measured for THH (**3**). There still remains the possibility that THH (**3**) acts as a selective agonist of a different downstreams signaling pathway at the 5-HT_{2A} receptor, but the poor affinity of this compound for the receptor brings into question just how much of an affect it could be causing through 5-HT_{2A} signaling.

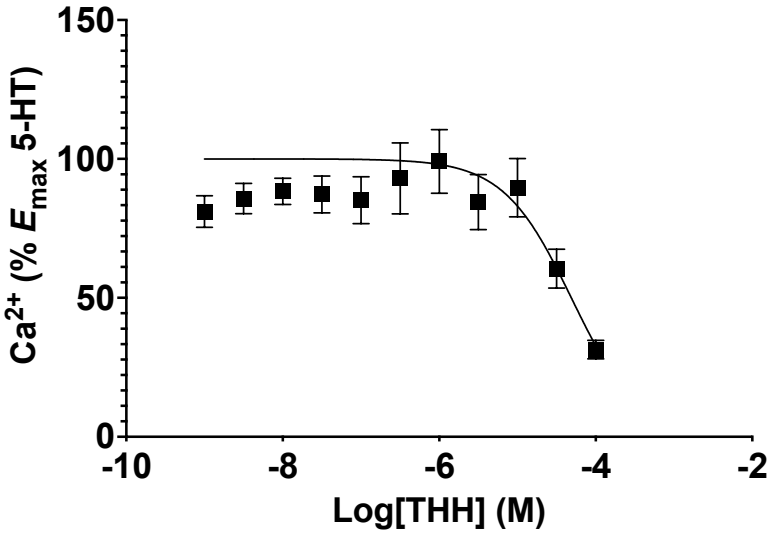


Figure 35. Ca^{2+} mobilization of THH (**3**) as a percentage of the Ca^{2+} mobilization of the endogenous ligand serotonin (**7**).

Once the binding affinity of the brominated DMT analogs for the human 5-HT_{2A} receptor had been determined, it was of interest to examine their functional activity. Since these compounds

are structural derivatives of the partial agonist DMT (**4**), and some of them have better binding affinity for the receptor, it was expected that they would possibly have some functional activity. All four analogs and DMT were assayed using a calcium mobilization assay, and compared to the calcium mobilization of the endogenous ligand serotonin (**7**). None of the synthesized compounds were potent in producing a calcium mobilization effect. The unsubstituted parent compound DMT produced an EC_{50} for calcium mobilization of 5,345 nM, and an E_{max} of 16% of the observed serotonin response (Figure 36), which combined with the knowledge that DMT is a full agonist in arachidonic acid signaling[225] (Max 5-HT effect = 93%, EC_{50} = 260 nM) and a partial agonist in IP_3 signaling[180,225] (Max 5-HT effect = 39%, EC_{50} = 269 nM), suggest that DMT is a nonspecific partial agonist of the receptor via multiple different types of downstream signaling pathways. No functional data at the human 5-HT_{2A} receptor exists for any of the other synthesized DMT analogs. No other analogs of DMT had a potency of <10,000 nM in the calcium mobilizaiton assay, however, calcium mobilization with an E_{max} of 55% of the effect of serotonin was observed for 7-Br DMT (Figure 37), suggesting that at high concentrations this compound could be acting as a partial agonist. For 4-Br DMT, 5-Br DMT, and 6-Br DMT, a fluorescence response was seen at a concentration of 100 μM, but this response might be an artefact produced by off-target receptor populations, such as calcium channels (Figure 38). In order to rule this out as a response caused by activation of the 5-HT_{2A} receptor, running this assay with a cell population not expressing the 5-HT_{2A} receptor would need to be conducted to see if a similar response was observed. These studies are currently ongoing.

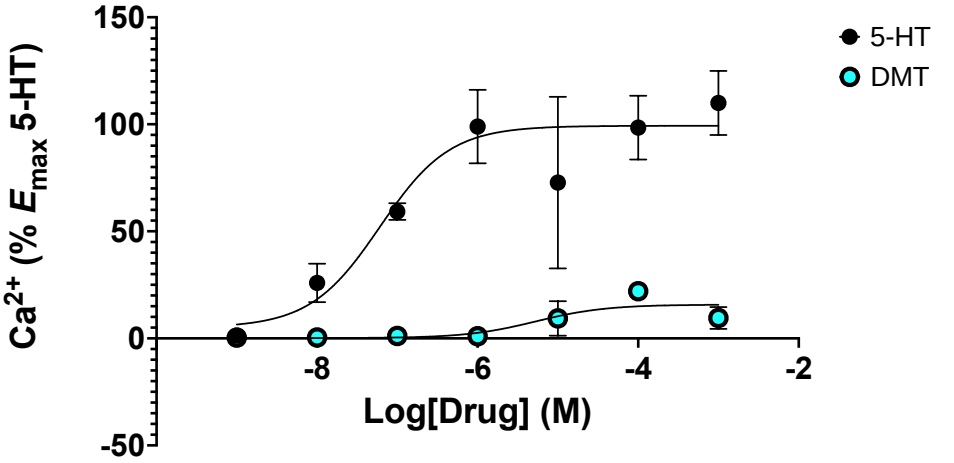


Figure 36. Ca^{2+} mobilization of DMT hemifumarate (**42**) as a percentage of the Ca^{2+} mobilization of the endogenous ligand serotonin (**7**).

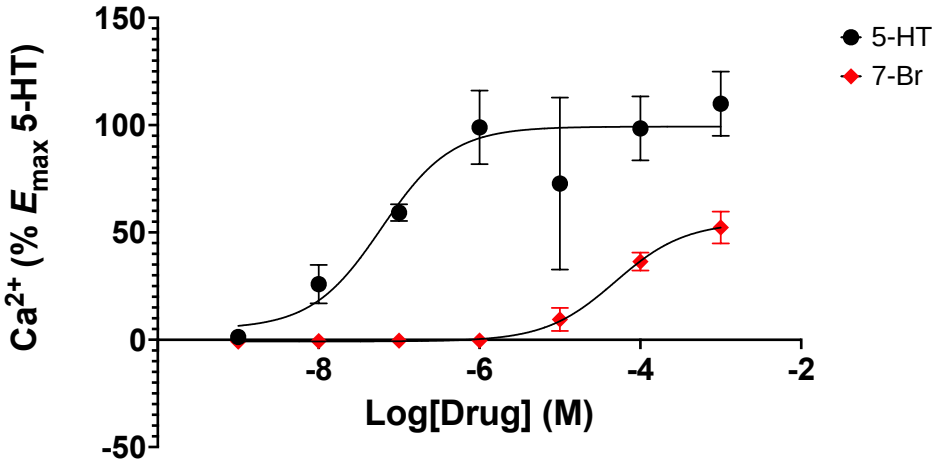


Figure 37. Ca^{2+} mobilization of 7-Br DMT hydrogen oxalate (**59**) as a percentage of the Ca^{2+} mobilization of the endogenous ligand serotonin (**7**).

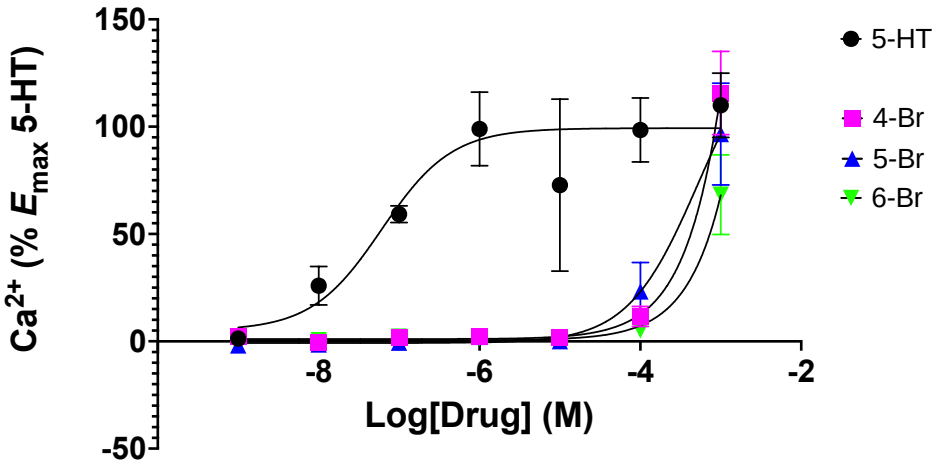


Figure 38. Ca^{2+} mobilization of 4-Br DMT hydrogen oxalate (**54**), 5-Br DMT hydrogen oxalate (**46**), and 6-Br DMT hydrogen oxalate (**50**) as a percentage of the Ca^{2+} mobilization of the endogenous ligand serotonin (**7**).

Further experiments would need to be conducted to determine whether these compounds are antagonists, or if they are inverse agonists. Full spectrum downstream signaling assays spanning multiple different pathways would be needed to determine if the Br-analogs of DMT are agonists at other signaling cascades, but inactive in calcium mobilization. It is still possible that these compounds could be agonists towards arachidonic acid release, or inositol triphosphate accumulation, or even beta-arrestin. A lack of activity at the 5-HT_{2A} receptor would be an unexpected result, given that most DMT (**4**) analogs such as psilocin and 5-OMe DMT are agonists at the receptor.[224,226]

V. Conclusions

The original goal of this thesis was to determine the relative binding modes of DMT (**4**), and the harmala alkaloids harmine (**1**), harmaline (**2**), and tetrahydroharmine (**3**). The antidepressant effects of the combination of these compounds into a brew called ayahuasca has not been attributed to any single component of the mixture, and all four components have similar structural features. Without clinical trials of each individual compound in humans, narrowing the effects down to a particular chemical constituent of ayahuasca is nearly impossible. However, there has been an increase in the evidence linking these compounds, as well as other psychoactive substances, to antidepressant effects through the 5-HT_{2A} serotonin receptor. Furthering our understanding of this receptor allows for the possibility of determining which structural features of both the membrane protein and the ligands interacting with it, are responsible for these effects.

The affinities and functional activity of each of the individual components of ayahuasca for the 5-HT_{2A} receptor have been examined, as well as their physiological activity in animal models of human depression. Three out of four of the compounds found in ayahuasca have been shown to be antidepressant-like in animal models of depression,[227,228] and all four have been shown to be hallucinogenic, although with varying potencies and validities.[172] Parsing the antidepressant effects from the hallucinogenic (and therefore 5-HT_{2A} agonist effects) seems to be a fruitless endeavor without human clinical trials, but none of the harmala alkaloids have been shown to be agonists of the 5-HT_{2A} receptor via any examined downstream signaling assay.[175,176] It appears as though these reportedly hallucinogenic compounds may be some of the only ones that have been examined that do not produce 5-HT_{2A} agonism. But, this remains to be determined.

We were able to determine, through examination of both the basicity of the terminal amine, and the planarity of each of the components of ayahuasca, that neither explains the binding affinity

of these compounds at the 5-HT_{2A} receptor, and concluded that the affinity must be due to other physical interactions between the ligands and receptor. The only structural feature for which affinity was correlated was the planarity of the harmala alkaloids, but the planarity of DMT was the lowest of all compounds, and has similar planarity to the lowest affinity harmala alkaloid, THH (**3**). From this study, it did not seem likely that the harmala alkaloids, nor DMT (**4**), bound in a similar manner.

To confirm or refute that observation, correlation studies between the binding affinities of previously synthesized β-carboline and DMT (**4**) analogs at the 5-HT_{2A} receptor were conducted. The first correlation, to determine whether β-carbolines bind similarly to one another, showed that the 5-HT_{2A} binding affinities of similarly substituted dihydro and tetrahydro β-carbolines were strongly linearly correlated, suggesting that they do bind similarly to one another at this receptor. Once the hypothesis that β-carbolines bind similarly to one another had been supported, comparisons to the binding pose of DMT at this receptor were made. The previously proposed relative binding modes Overlay 1 and Overlay 2 were examined using molecular modeling, docking, and correlation studies. Overlay 1 (Figure 13) was not supported by any of these methods, while Overlay 2 (Figure 15) had strong support in all three. Overlay 2 was more energetically favorable, was replicated by the docking results of the harmala alkaloids and DMT (**4**), and had stronger linear correlations between similarly substituted β-carboline and DMT (**4**) analogs. All of these observations point to Overlay 2 being the more correct relative binding mode between these two sets of compounds, although docking of certain β-carboline analogs produced non-Overlay 2-like poses in relation to DMT (**4**). Once these compounds had been docked, HINT scoring allowed us to quanitfy interactions between specific residues in the binding pocket of the receptor, and atoms on the ligand. If biased agonism at the 5-HT_{2A} receptor is predicated on interactions with

certain residues in the binding pocket of the receptor, it is possible that docking and scoring with programs like HINT will allow for screening of potentially selective agonist ligands, although that cannot be determined for the compounds examined in this paper at this time. Site-specific mutagenesis of residues within the binding pocket and full spectrum downstream signaling assays of ligands will need to be conducted in the future to identify these residues.

Since the functional activity of the individual components of ayahuasca at the 5-HT_{2A} receptor was mostly unknown outside of a phosphatidyl inositol (PI) hydrolysis assay, we examined each of these compounds using a calcium mobilization assay, which is commonly used to determine agonist activity at G-protein coupled receptors such as the 5-HT_{2A} receptor. In the PI hydrolysis assay, both harmine (**1**) and harmaline (**2**) were shown to be inactive, while DMT (**4**) was shown to be an agonist. THH (**3**) had not been examined in any functional activity assay at the 5-HT_{2A} receptor. In the calcium mobilization assay, DMT (**4**) was shown once again to be an agonist, but both harmine (**1**) and harmaline (**2**) were once again inactive. THH (**3**) antagonized the effect of serotonin (**7**), showing for the first time that this compound seems to be an antagonist of the 5-HT_{2A} receptor, although it was not particularly potent. Due to the questionable nature of the hallucinogenic activity of these compounds in humans, it still remains unclear whether these compounds are producing a hallucinogenic or hallucinogen-related response through the 5-HT_{2A} receptor. The addition of calcium mobilization data makes it clear that if these compounds are working through the 5-HT_{2A} receptor, their mechanism of action is unique to any known hallucinogenic compound.

In summary, we have made significant strides in the understanding of how the chemical constituents of ayahuasca are interacting with the 5-HT_{2A} receptor, which is thought to be responsible for both the hallucinogenic and antidepressant effects of ayahausca. We have identified

the likely relative binding modes of certain β-carbolines to DMT, and supported them using molecular modeling and correlation studies of synthesized and assayed compounds of both classes. We have also tested all of the major psychoactive constituents of ayahuasca for functional activity in a general assay for GPCR activation, for which DMT (**4**) was an agonist, harmine (**1**) and harmaline (**2**) were inactive, and THH (**3**) was a weak antagonist. With the currently available information on the harmala alkaloids, it appears unlikely that they act as classical hallucinogens through activation of the 5-HT_{2A} receptor and activation of a calcium-releasing mechanism. However, with data on the structural similarity and binding poses of β-carbolines, it may be possible to synthesize analogs of this class of compounds which are more selective for 5-HT_{2A} serotonin receptor down-stream second messenger systems, and which may produce either agonist or antagonist activity as well. At this time, determining which of the ayahuasca components is responsible for its antidepressant effect, as well as the mechanism underlining this action cannot be determined without additional data. Examination of the compounds in both arachidonic acid and β-arrestin signaling assays would give a more complete picture of the effects of ayahuasca on the 5-HT_{2A} receptor, and might answer whether this receptor is responsible for the therapeutic benefits of this beverage.

VI. Experimental

A. Alignment and Docking

All compounds used in docking studies were first sketched in SYBYLx2.1.1 (Tripos International) and then minimized using the Tripos Force Field and Gasteiger-Hückel charges, with non-bonded interactions cut off at 8 Å, a dielectric constant of 4.00 D/Å, and a termination gradient of 0.02 kcal/(mol*Å). Alignment studies were conducted using the native alignment suite of SYBYLx2.1.1. and fitting 3 corresponding atoms of each ligand based on which Overlay was being employed. Protein crystal structure files were prepared by removing co-crystallization factors such as the co-crystallization ligand, lipids, and metal ions, and then adding hydrogen atoms, which were optimized by a 10,000-step staged minimization using the same parameters as the ligand minimization mentioned above. The co-crystallization ligand was re-docked to the prepared crystal structure to determine its viability as a docking model, by comparing the resulting docking poses to the published pose of the co-crystallization ligand in the binding pocket of the crystal structure.

Docking of ayahuasca constituents and ayahuasca constituent analogs to previously published crystal structures was done using GOLD suite 5.6 (Cambridge Crystallographic Data Centre, Cambridge, UK) and scored using the default pairwise additive piecewise linear potential (ChemPLP) score, which assigns a hydrogen bonding potential or a lipophilic potential to each heavy atom of the ligand-protein interaction and scores each interaction accordingly. The binding site was defined as a spherical region of radius 8 Å from the delta carbon atom of ASP155. All docked ligands were constrained so that their basic nitrogen atom was no further than 3.5 Å from the ASP155 oxygen atom which was most exposed to the binding pocket. Further constraints were tested, including hydrogen bond donor and acceptor constraints, and aromatic ring center

constraints based on the location of the aromatic ring of the co-crystallization ligand, but these produced inconsistent and poorly scoring results, so they were discarded. Fifty docking solutions were produced per ligand per docking. Docking solutions were clustered using an RMSD of 1.5 Å when necessary. Docked protein-ligand complexes of interest were merged, and then optimized using a staged minimization using the same parameters mentioned above.

Quantification of the interactions of binding poses was conducted using HINT analysis[229] in SYBYL 8.1 to identify specific atom-residue interactions. HINT scoring assigns a hydropathic value to each atom in an interaction based on the calculated octanol/water partition coefficient, and then classify each interaction based on hydrophobic or hydrophilic character. The magnitude of the score of each interaction is indicative of its predicted relative strength. The docked ligand was extracted from the protein and then partitioned using the calculate function, while the protein was partitioned using the dictionary function, using default settings.

B. Synthesis

All compounds synthesized herein were characterized using a combination of melting point, proton nuclear magnetic resonance (^{1}H NMR), infrared (IR), and elemental (CHN) analysis. All melting points were taken on a Thomas-Hoover melting apparatus in glass walled capillary tubes and are uncorrected. ^{1}H NMR spectra were obtained using a Bruker Ultrashield™ 400 Plus, and peak positions are reported in parts per million (δ) downfield from that of tetramethylsilane (TMS), followed by splitting pattern of the peak (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (*J*, Hz), and integration. All CHN analyses were performed by Atlantic Microlab Inc. (Norcross, GA) for the indicated elements and all results were within 0.4% of the calculated values. CHN analysis was done only for compounds which

had not been previously reported in the literature. Reactions were monitored by thin-layer chromatography on silica gel GHLF plates (250 μ, 2.5 x 10 cm; Analtech Inc. Newark, DE). All processed ^{1}H NMR spectra of final products can be found in Appendix B. Starting material was purchased from Sigma-Aldrich (St. Louis, MO), Synthonix (Wake Forest, NC), or GoldBio (St. Louis, MO). Anhydrous solvents were purchased from Sigma Aldrich (St. Louis, MO).

Indol-3-ylglyoxyl chloride (40)

The procedure of Neumeyer et al.[230] was utilized in the synthesis of **40**. A solution of oxalyl chloride (1.26 g, 10 mmol) in anhydrous Et_2O (5 mL) was added in a dropwise manner to a stirred solution of indole (**39**, 1.17 g, 10 mmol) in anhydrous Et_2O (15 mL) under a N_2 atmosphere at 0 °C (ice-bath). The reaction mixture was stirred at 0 °C for 10 min, and then removed from the ice bath and allowed to stir at room temperature for 20 min. The bright yellow precipitate was collected by filtration and washed with cold Et_2O and air dried (1.79 g, 86% yield) to be immediately used in the synthesis of **41**. mp 124 °C (lit.[230] mp 128 °C).

Indol-3-yl-*N*,*N*-dimethylglyoxylamide (41)

The procedure of Heinzelman et al.[231] was utilized in the synthesis of **41**. Indole-3-ylglyoxyl chloride (**40**, 1.79 g, 8.62 mmol) was added to a stirred solution of dimethylamine (40%) in H_2O. The solution was allowed to stir for 48 h at room temperature, during which a white precipitate was formed. The precipitate was collected by filtration, washed with cold H_2O, and the filtrate concentrated to produce further precipitate. Filtration was repeated two times, for a total of three samples of white solid. The three samples of solid were determined to be homogenous and

identical by TLC (CH_3Cl/MeOH 9:1) and were combined for a total yield of 0.88 g of the crude product (54%). mp 154-160 °C (lit.[231] mp 159-160°C).

***N,N*-Dimethyltryptamine Hemifumarate (42)**

A modification of the procedures of Glennon et al.[212] and Cozzi et al.[211] was utilized in the synthesis of **42**. A solution of **41** (0.759 g, 4 mmol) in anhydrous tetrahydrofuran (THF, 10 mL) was added in a dropwise manner at 0 °C (ice-bath) to a stirred solution of $LiAlH_4$ (0.607 g, 16 mmol) in anhydrous Et_2O (15 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 6 h, cooled to room temperature, quenched by addition of H_2O (0.6 mL), NaOH (15%, 0.6 mL), then H_2O (1.8 mL). After quenching, the reaction mixture was then filtered through a pad of celite, and washed with portions of Et_2O (3 x 20 mL). The organic portion was then dried ($MgSO_4$), and evaporated under reduced pressure to yield a tan-colored oil. Crystallization of this oil from petroleum ether produced two fractions of off-white residue. These residues were found to be homogenous by TLC (CH_3Cl/MeOH 9:1) and melting point (38-40 °C) and were combined.

A saturated fumaric acid solution in anhydrous acetone was added in a dropwise manner to the combined residue solution in acetone (15 mL) and the stirred reaction mixture became cloudy immediately. The precipitate was collected by filtration to yield a white solid which upon drying afforded 0.134 g (16%) of **42** as a white solid: mp 146-147 °C (lit.[211] mp 148.3 °C); ^{1}H NMR (DMSO-d_6) δ: 2.54 (s, 6H, $N(CH_3)_2$), 2.94 (s, 4H, CH_2), 6.52 (s, 4H, CHCH, fumarate), 6.94 (t, 1H, J = 7.5 Hz, ArH), 7.02 (t, 1H, J = 7.6 Hz, ArH), 7.13 (s, 1H, ArH), 7.30 (d, 1H, J = 8.0 Hz, ArH), 7.53 (d, 1H, J = 8.9 Hz, ArH), 10.83 (s, 1H, indole NH).

5-Bromoindol-3-ylgloxyl chloride (44)

The procedures of Tymiak et al.[217] were utilized in the synthesis of **44-46**. A solution of oxalyl chloride (1.26 g, 10 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred solution of 5-bromoindole (**43**, 1.96 g, 10 mmol) in anhydrous THF (10 mL) under a N_2 atmosphere at 0 °C (ice-salt bath). The reaction mixture was stirred at 0 °C for 30 min, and then removed from the ice bath and allowed to stir at room temperature for 1 h. The bright yellow precipitate was collected by filtration and washed with cold THF and air dried (1.35 g, 47% yield) to be immediately used in the synthesis of **45**.

5-Bromoindol-3-yl-*N*,*N*-dimethylglyoxylamide (45)

Compound **44** (1.35 g, 4.7 mmol) was added to a stirred solution of dimethylamine (40%) in H_2O (20 mL). The solution was allowed to stir overnight at room temperature, during which a tan precipitate was formed. The solvent was reduced and the reaction mixture was resuspended in H_2O (50 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The solvent was reduced and the precipitate was dried under high-vacuum to yield 1.39 g of crude solid product. The product was recrystallized from MeOH which afforded 1.11 g of pure **45** (80% yield). mp 202-204 °C (lit.[217] mp 201-203 °C); ^{1}H NMR (DMSO-d_6) δ: 2.92 (s, 3H, N(CH_3)), 2.99 (s, 3H, N(CH_3)), 7.42 (d, 1H, J = 8.6 Hz, ArH), 7.51 (d, 1H, J = 8.6 Hz, ArH), 8.17 (s, 1H, ArH), 8.24 (s, 1H, ArH), 12.45 (s, 1H, indole NH).

5-Bromo-*N*,*N*-dimethyltryptamine Hydrogen Oxalate (46)

A stirred solution of $LiAlH_4$ (0.51 g, 13.5 mmol) in 15 mL anhydrous Et_2O was cooled to 0 °C (ice bath) and freshly sublimed $AlCl_3$ (0.13 g, 4.5 mmol) was added in portions to produce a

solution of AlH_3 over precipitated LiCl. A solution of **45** (0.44 g, 1.5 mmol) in anhydrous Et_2O, (5 mL) was added in a dropwise manner at 0 °C (ice-salt bath) to the stirred solution of AlH_3 in anhydrous Et_2O under an N_2 atmosphere. The reaction mixture was allowed to stir for 30 min at 0 °C and then heated at reflux for 1 h. After 1 h, TLC analysis showed consumption of the starting material, and the reaction was cooled to room temperature, quenched by addition of sodium sulfate decahydrate until the formation of hydrogen gas ceased, and then filtered through celite. The filter cake was re-suspended in Et_2O and then re-filtered twice. The filtrate was dried ($MgSO_4$), and evaporated under reduced pressure to yield a yellow-colored oil. A saturated solution of oxalic acid in anhydrous Et_2O was added to a solution of the free base in anhydrous Et_2O (5 mL) to form a yellow precipitate which was filtered and washed with anhydrous Et_2O to yield 0.20 g of the crude product. The crude product was crystallized from *i*-PrOH and then recrystallized twice from MeOH to afford 0.06 g (12% yield) of pure **46**. mp 191-193°C; ^{1}H NMR (DMSO-d_6) δ: 2.84 (s, 6H, $N(CH_3)_2$), 3.08 (t, 2H, J = 9.4 Hz, CH_2), 3.27 (t, 2H, J = 7.6 Hz, CH_2), 7.20 (d, 1H, J = 8.6 Hz, ArH), 7.30 (s, 1H, ArH), 7.35 (d, 1H, J = 8.6 Hz, ArH), 7.81 (s, 1H, ArH), 11.24 (s, 1H, indole NH); Anal. Calc'd for ($C_{12}H_{16}BrN_2^+ \cdot C_2HO_4^-$) C, 47.08; H, 4.80; N, 7.84. Found: C, 46.99; H, 4.86; N, 7.70.

6-Bromoindol-3-ylgloxyl chloride (48)

The procedures of Tymiak et al.[217] were utilized in the synthesis of **48-50**. A solution of oxalyl chloride (1.26 g, 10 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred solution of 6-bromoindole (**47**, 1.96 g, 10 mmol) in anhydrous THF (15 mL) under a N_2 atmosphere at 0 °C (ice-salt bath). The reaction mixture was stirred at 0 °C for 30 min, and then removed from the ice bath and allowed to stir at room temperature for 3 h. The solvent was

removed under reduced pressure to yield a bright orange solid which was used immediately without further characterization in the synthesis of **49** to prevent degradation.

6-Bromoindol-3-yl-*N,N*-dimethylglyoxylamide (49)

The crude **48** was added to a stirred solution of dimethylamine (40%) in H_2O (20 mL). The solution was allowed to stir overnight at room temperature. The solvent was reduced under vacuum and the reaction mixture was extracted with CH_2Cl_2 (3 x 25 mL). The solvent was evaporated under reduced pressure and the residue was dried under high-vacuum to yield a tan solid crude product. The product was recrystallized from MeOH which afforded 1.52 g of **49** (52% yield of from 6-bromoindole). mp 240°C; ^{1}H NMR (DMSO-d_6) δ: 2.94 (s, 3H, N(CH_3)), 3.02 (s, 3H, N(CH_3)), 7.43 (d, 1H, J = 6.8 Hz, ArH), 7.75 (s, 1H, ArH), 8.07 (d, 1H, J = 8.4 ArH), 8.17 (s, 1H, ArH), 12.21 (s, 1H, indole NH).

6-Bromo-*N,N*-dimethyltryptamine Hydrogen Oxalate (50)

A stirred solution of $LiAlH_4$ (1.70 g, 45 mmol) in 30 mL anhydrous Et_2O was cooled to 0 °C (ice bath) and freshly sublimed $AlCl_3$ (0.44 g, 15 mmol) was added in portions to produce a solution of AlH_3 over precipitated LiCl. **49** (1.47 g, 5 mmol in anhydrous Et_2O, 45 mL) was added in a dropwise manner at 0 °C (ice-bath) to the stirred solution of AlH_3 in anhydrous Et_2O under an N_2 atmosphere. The reaction mixture was allowed to stir for 30 min at 0 °C and then heated at reflux for 1 h. After 1 h, TLC analysis showed consumption of the starting material, and the reaction mixture was cooled to room temperature, quenched by addition of Glauber's salt until the formation of H_2 gas ceased, and then filtered through celite. The filter cake was re-suspended in Et_2O and then re-filtered twice. The filtrate was dried ($MgSO_4$), and evaporated under reduced

pressure to yield a yellow-colored oil. A saturated solution of oxalic acid in anhydrous Et_2O was added to a solution of the free base in anhydrous Et_2O (5 mL) to form a tan precipitate which was filtered and washed with further anhydrous Et_2O to yield 0.60 g of the crude product. The crude product was recrystallized twice with *i*-PrOH which afforded 0.25 g of pure **50** as a fine ivory solid. mp 210-212 °C; ^{1}H NMR (DMSO-d_6) δ: 2.83 (s, 6H, $N(CH_3)_2$), 3.08 (t, 2H, *J* = 8.9 Hz, CH_2), 3.28 (t, 2H, *J* = 7.4 Hz, CH_2), 7.17 (d, 1H, *J* = 8.3 Hz, ArH), 7.30 (s, 1H, ArH), 7.59 (s, 1H, ArH), 7.61 (d, 1H, *J* = 8.5 Hz, ArH), 11.18 (s, 1H, indole NH); Anal. Calc'd for ($C_{12}H_{16}BrN_2^+ \cdot C_2HO_4^-$) C, 47.08; H, 4.80; N, 7.84. Found: C, 47.14; H, 4.87; N, 7.79.

4-Bromo-3-(2-nitroethenyl)-indole (52)

The procedure of Olsen et al.[216] was utilized in the synthesis of **52**. A mixture of 4-bromoindole (**51**, 2.0 g, 10.2 mmol) and 1-dimethylamino-2-nitroethylene (1.18 g, 10.2 mmol) was treated with trifluoroacetic acid (20 mL) in a dropwise manner at room temperature under an N_2 atmosphere. The reaction mixture was allowed to stir vigorously for 50 min at room temperature, and then was quenched by the addition of saturated aqueous $NaHCO_3$ until a pH of ~9. The resulting slurry was extracted first with $CHCl_3$ (3x50 mL), then with EtOAc (3x50 mL). The combined organic portion was washed with H_2O (50 mL), dried (Na_2SO_4), and then evaporated under reduced pressure to yield an amorphous red/brown solid. This solid was triturated with toluene (150 mL) and filtered to yield 2.31 g (85%) of **52** as a brown solid. mp 219-220 °C (lit.[232] mp >200 °C decomposition); ^{1}H NMR (DMSO-d_6) δ: 7.16 (t, 1H, *J* = 3.88 Hz, ArH), 7.43 (d, 1H, *J* = 9.4 Hz, CH_2), 7.56 (t, 2H, *J* = 7.6 Hz, CH_2), 8.13 (d, 1H, *J* = 13.2 Hz, ArH), 8.56 (d, 1H, *J* = 2.9, ArH), 9.13 (d, 1H, *J* = 13.3 Hz, ArH), 12.60 (s, 1H, indole NH).

4-Bromotryptamine (53)

The procedure of Muratore et al.[233] was utilized for the synthesis of **53**. A solution of **52** (1.00 g, 3.74 mmol) in anhydrous THF (10 mL) was added in a dropwise manner at 0 °C (ice-bath) to a stirred slurry of $LiAlH_4$ (0.810 g, 21.34 mmol) in anhydrous THF (10 mL) under an N_2 atmosphere. The stirred reaction mixture was heated at reflux for 4 h, then cooled to room temperature, and allowed to stir for a further 18 h. After 18 h, the reaction was cooled to 0 °C (ice-bath), and quenched by the slow and careful addition of ice-cold H_2O (50 mL). Et_2O (50 mL) was added, and the mixture was filtered through a celite pad. The aqueous layer was extracted with Et_2O (2 x 20 mL), and the combined organic layer was washed with HCl (2N, 3x30 mL). The combined aqueous extract was basified with aqueous NaOH (2N, to pH 9) and then extracted with Et_2O (3 x 25 mL). The organic extracts were combined, washed with brine (20 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.51 g (57%) of **53** as a brown solid. mp 95-110 °C (lit.[233] mp 113-115 °C); ^{1}H NMR (DMSO-d_6) δ: 1.47 (s, 2H, Basic NH_2), 2.85 (t, 2H, J = 7.3 Hz, CH_2), 2.99 (t, 2H, J = 7.1 Hz, CH_2), 6.96 (t, 1H, J = 7.8, ArH), 7.16 (d, 1H, J = 7.5 ArH), 7.25 (s, 1H, J = 13.3 Hz, ArH), 7.37 (s, 1H, J = 13.1 Hz, ArH), 11.18 (s, 1H, indole NH).

4-Bromo-*N*,*N*-dimethyltryptamine Hydrogen Oxalate (54)

The procedure Olsen et al.[216] was utilized in the synthesis of **54**. Glacial acetic acid (0.249 mL, 4.35 mmol) was added to a stirred solution of **53** (0.26 g, 1.1 mmol) in anhydrous MeOH (10 mL), followed by $NaCNBH_3$ (0.14 g, 2.19 mmol) under an N_2 atmosphere at 0 °C (ice-bath). A solution of formaldehyde (37%, stabilized by 12% MeOH, 0.20 mL, 2.6 mmol) in anhydrous MeOH (5 mL) was then added in a dropwise manner over 20 min. After the addition was

completed, the reaction mixture was allowed to warm to room temperature and stir for 24 h. After 24 h, the reaction mixture was cooled to 0 °C (ice-bath), and quenched by the addition of aqueous Na_2CO_3 (2N, to pH 9), and the organic solvents were evaporated under reduced pressure. The resulting residue was partitioned between $CHCl_3$ (3 x 20 mL) and H_2O (20 mL). The organic layers were combined, washed with H_2O (20 mL), brine (20 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 0.29 g (100%) of a brown solid (mp 160 °C). A saturated solution of oxalic acid in anhydrous Et_2O was added to a solution of 0.15 g the free base in anhydrous Et_2O (5 mL) to form a tan precipitate which was filtered and washed with anhydrous Et_2O to yield 0.18 g (94%) of the crude product. The crude product was recrystallized twice with EtOH to afford 0.13 g of pure **54** as a fine tan solid. mp 168-170 °C; ^{1}H NMR (CD_3OD-*d*$_4$) δ: 2.26 (s, 6H, $N(CH_3)_2$), 2.59 (t, 2H, *J* = 5.6 Hz, CH_2), 3.07 (t, 2H, *J* = 8.0 Hz, CH_2), 6.83 (t, 1H, *J* = 7.9, ArH), 7.04 (s, 1H, ArH), 7.05 (d, 1H, *J* = 8.3 Hz, ArH), 7.21 (d, 1H, *J* = 8.0 Hz, ArH); Anal. Calc'd for ($C_{12}H_{16}BrN_2^+ \cdot C_2HO_4^-$) C, 47.08; H, 4.80; N, 7.84. Found: C, 47.26; H, 5.07; N, 7.64.

7-Bromoindole (56)

The procedure for synthesizing **56** from 7-bromoisatin (**55**) was obtained from patent CN105732462A.[221] Compound **55** (3.5 g, 15.4 mmol) and $NaBH_4$ (2.33 g, 61.6 mmol) were added to a dry round bottom flask, and cooled to below -10 °C (ice-salt bath). Freshly distilled dry THF (25 mL) was added in a dropwise manner, followed by boron trifluoride diethyl etherate (4.91 g, 4.27 mL, 35 mmol) in the same manner. The reaction mixture was maintained below -5 °C for 24 h, then quenched with saturated NH_4Cl solution (60 mL), and extracted with EtOAc (3 x 75 mL). The combined organic layers were dried (Na_2SO_4) and solvent was removed under

reduced pressure to yield 3.78 g of a yellow residue. This residue was subjected to flash chromatography (100% hexane) to yield 1.38 g of an off-white oily solid. Recrystallization of this material from hexane yielded 0.47 g of **56** as a white solid (15% yield). mp 38-40 °C (lit.[212] mp 42-43 °C); ^{1}H NMR (DMSO-d_6) δ: 6.57 (d, 1H, ArH, J = 1.6 Hz), 6.95 (t, 1H, ArH, J = 7.6 Hz), 7.30 (d, 1H, J = 7.6 Hz, ArH), 7.41 (s, 1H, ArH), 7.57 (d, 1H, J = 7.8 ArH), 11.21 (s, 1H, indole NH).

7-Bromoindol-3-ylgloxyl chloride (57)

The procedure of Glennon et al.[212] was utilized for the synthesis of **57-59**. A solution of oxalyl chloride (0.91 g, 7.2 mmol) in anhydrous THF (2 mL) was added in a dropwise manner to a stirred solution of 7-bromoindole (**56**, 0.46 g, 2.3 mmol) in anhydrous THF (12 mL) under a N_2 atmosphere at 0 °C (ice-salt bath). The reaction mixture was stirred at 0 °C for 30 min, and then removed from the ice bath and allowed to stir at room temperature for 1.5 h. The solvent was removed under reduced pressure to yield an orange solid which was used immediately without further characterization in the synthesis of **58** to prevent degradation.

7-Bromoindol-3-yl-*N*,*N*-dimethylglyoxylamide (58)

The crude **57** was added to a stirred solution of dimethylamine (40%) in H_2O (15 mL). The solution was allowed to stir overnight at room temperature. The solvent was reduced under vacuum and the reaction mixture was extracted with EtOAc (3 x 25 mL). The solvent was evaporated under reduced pressure and the residue was dried on the high-vacuum to yield a fluffy off-white crude product. The product was recrystallized from MeOH which afforded 0.17 g of **58** (25% overall yield). mp 220-221 °C (lit.[212] mp 220-221 °C); ^{1}H NMR (DMSO-d_6) δ:

2.91 (s, 3H, N(CH_3)), 2.98 (s, 3H, N(CH_3)), 7.21 (t, 1H, *J* = 7.6 Hz, ArH), 7.51 (d, 1H, ArH, *J* = 7.6), 8.10 (d, 1H, *J* = 3.5 ArH), 8.12 (s, 1H, ArH).

7-Bromo-*N,N*-dimethyltryptamine Hydrogen Oxalate (59)

A stirred solution of $LiAlH_4$ (0.17 g, 4.5 mmol) in 5 mL anhydrous Et_2O was cooled to 0 °C (ice bath) and freshly sublimed $AlCl_3$ (0.20 g, 1.5 mmol) was added in portions to produce a solution of AlH_3 over precipitated LiCl. A solution of **58** (0.15 g, 0.5 mmol) in anhydrous Et_2O (5 mL) was added in a dropwise manner at 0 °C (ice-bath) to the stirred solution of AlH_3 in anhydrous Et_2O under an N_2 atmosphere. The reaction mixture was allowed to stir for 30 min at 0 °C and then heated at reflux for 1.5 h. After 1 h, TLC showed consumption of the starting material, and the reaction mixture was cooled to room temperature, quenched by addition of Glauber's salt until the formation of H_2 gas ceased, and then filtered through celite. The filter cake was re-suspended in Et_2O and then re-filtered twice. The filtrate was dried ($MgSO_4$), and evaporated under reduced pressure to yield a clear oil (0.07 g). An equimolar amount of oxalic acid in anhydrous Et_2O was added to the free base to form a white solid which was filtered and washed with further anhydrous Et_2O to yield 0.08 g of the crude product. The crude product was recrystallized with MeOH which afforded 0.07 g of pure **59** as a fine white solid (43% yield). mp 176-178°C (lit.[212] mp 176-177 °C); ^{1}H NMR (MeOD-d_4) δ: 2.96 (s, 6H, N(CH_3)$_2$), 3.23 (t, 2H, *J* = 8.2 Hz, CH_2), 3.47 (t, 2H, *J* = 7.3 Hz, CH_2), 7.01 (t, 1H, *J* = 7.8 Hz, ArH), 7.32 (s, 1H, ArH), 7.35 (d, 1H, *J* = 8.4 Hz, ArH), 7.62 (d, 1H, *J* = 7.8 Hz, ArH).

Tryptamine Hemifumarate (61)

Tryptamine freebase (**60**, 0.865 g) was dissolved in acetone with stirring at room temperature. A saturated solution of fumaric acid in acetone was added to the solution of tryptamine in a dropwise

manner, which immediately produced a white precipitate. The addition of saturated fumaric acid solution was continued until no additional precipitate was formed. The mixture was filtered to yield a flakey off-white solid (0.82 g, 70%, mp. 182 °C) This solid was recrystallized with MeOH/EtOH four times to obtain a white solid (0.21 g) with a constant melting point (196-200 °C). Calc'd for hemifumarate ($C_{24}H_{28}N_4O_4$) C, 66.04; H, 6.47; N, 12.84. Found: C, 65.75; H, 6.51; N, 12.94.

C. Binding

Human embryonic kidney 293 (HEK-293) cells stably expressing the human 5-HT_{2A} receptor were passaged into 150 mm cell culture plates and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37° C in a 5% CO_2-humidified atmosphere until approximately 90% confluent. Once grown, these cells were scraped from the plate with chilled phosphate buffered saline and centrifuged at 4° C at 3000 RPM. The supernatant liquid was then removed by vacuum and the resulting pellet was stored at -80° C. On the day of the assay, the HEK-293 cell pellet was resuspended in Tris HCl buffer (50 mM, pH 7.4) with sucrose (0.25 M) and manually homogenized using a Teflon-glass grinder at 1500 rpm (50 up-and-down strokes). The resulting homogenate was then centrifuged at 1000 xg for 5 minutes at 4 °C. The supernatant was then decanted and centrifuged at 40,000 xg for 15 minutes at 4 °C. The pellet was then washed twice with homogenization buffer and centrifuged in an identical manner. The pellet was then resuspended in 1 mL of Tris buffer and stored on ice. Protein concentration was determined using the Pierce™ Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific). Protein concentration values were determined using simple linear regression of protein

concentration standards. The protein samples were diluted in order to deliver 38 μg of protein in 160 μL of Tris buffer per active well.

Nonspecific protein binding was determined as an average of four active wells using 10 μM methysurgide. Baseline ketanserin binding was determined as an average of four active wells containing protein and 5 nM of [^{3}H]ketanserin. Competition curves were obtained by incubating the compound of interest (10^{-10} to 10^{-4} M; 11 concentrations) in Tris buffer containing 5 nM of [^{3}H]ketanserin with protein at 37 °C for 1 hour. The incubation was terminated using incubation buffer, which separated free ligand from bound ligand via GF/C glass fiber filters using a microbeta filtermat-96 harvester (PerkinElmer). The filter was then rinsed twice with buffer, dried in an oven at 50 °C for 1 hour, placed into a filter bag with 6 mL of scintillation fluid, and then heat sealed. The filter was then placed into a MicroBeta2 detector (PerkinElmer) and examined using scintillation spectrometry. The resulting scintillation counts were examined using nonlinear single binding site curve fitting software and then plotted (Figure 34, Table 22, GraphPad Prism). All binding experiments of synthesized compounds were done in the lab of Dr. Gonzalez-Maeso in the VCU Department of Physiology and Biophysics.

D. Ca^{2+} Mobilization Assay

Functional signaling was assessed by measuring Ca^{2+} release in the presence of serotonin and synthesized compounds (1mM, 100μM, 10μM, 1μM, 100nM, 10nM, 1nM, 0nM) in cells stably transfected with 5-HT_{2A} receptor. The day before the assay, approximately 6,000-8,000 cells/50 μL were seeded in poly-D-lysine coated (1mg/ml) 96-well black plates (Greiner 781091) to achieve 60-70% confluency. The cells were incubated with 50μl of 3 μM Fluo 4 Direct (Thermo Fisher Scientific; CN: F10471) in imaging solution (5 mM KCl, 0.4 mM KH_2PO_4, 138

mM NaCl, 0.3 mM Na_2HPO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM glucose, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4) supplemented with pluronic acid (10% solution in DMSO) for 1 h at 37 °C, as described in the manufacturer's instructions. Changes in fluorescence due to intracellular Ca^{2+} mobilization were measured using a FlexStation® plate reader (Molecular Devices) with an λ_{ex} of 494 nm and an λ_{em} of 525 nm. Baselines were recorded every 2 s for 30 s. Serotonin or synthesized compound were added at 30 s, followed by reading every 2 s for a total of 180 s. The fluorescence was a measure of intracellular calcium mobilization and was normalized to basal fluorescence using SoftMax Pro (Molecular Devices, Wokingham, UK). Functional data was analyzed by nonlinear regression to generate concentration-response curves and EC_{50} values by Prism V9.2.0 software (GraphPad Software, San Diego, CA, USA). All functional experiments were done by Dr. Jason Younkin in the lab of Dr. Gonzalez-Maeso in the VCU Department of Physiology and Biophysics.

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Appendix A.

3D Modeling Results Employing PDB ID: 6WHA

Prior to the publication of the most recent crystal structures of the 5-HT_{2A} receptor, PDB IDs: 7WC4-7WC7, docking experiments were conducted utilizing the PDB ID: 6WHA crystal structure as a template. In the discussion of our results in the docking section of this thesis, it was noted that the results of the previous docking experiments were not as supportive of Overlay 2 as the ones which were obtained with the newer crystal structure PDB ID: 7WC5, which contained an oleamide lipid in the binding pocket of the docking model. The results of docking the harmala alkaloids and DMT to the model of the 5-HT_{2A} receptor generated using crystal structure PDB ID: 6WHA (crystallized with the agonist 25-CN-NBOH) are shown below (Figure 1A-3A).

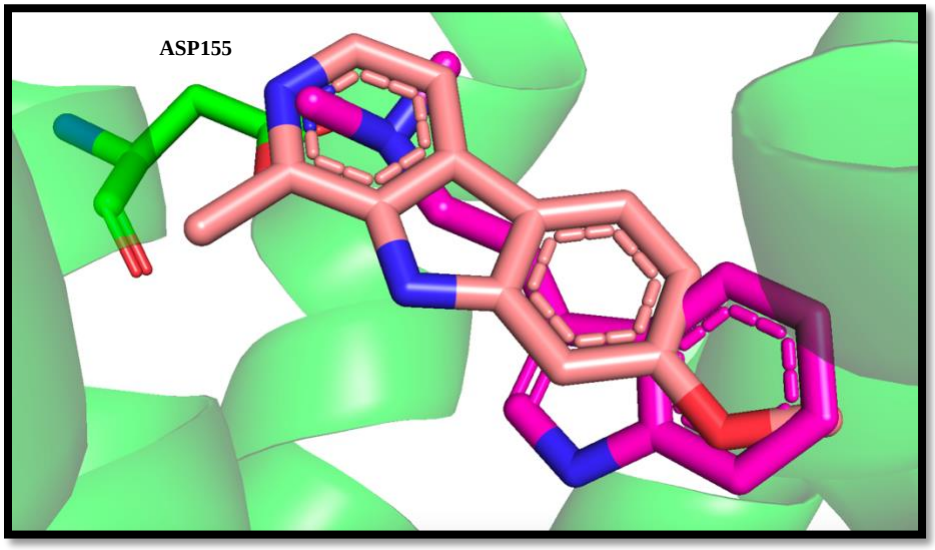


Figure 1A. Relative binding modes of DMT (**4**, pink, capped sticks) and harmine (**1**, salmon, capped sticks) bound to the docking model (PDB ID: 6WHA, green, ribbons).

It is worth noting that the poses shown were not the highest scoring of the docking results in any case, and frequently were not part of the most populated cluster of docking results either.

Selection of poses which most resembled Overlay 2 was done manually through examination of each of 100 solutions generated by the GOLD docking software.

The binding pose of harmine (Figure 1A) was similar to that which was seen with the discussed docking results of the PDB ID: 7WC5 docking model (Figure 20), and not completely in disagreement with the proposed Overlay 2. However, the benzenoid moiety of both compounds are not aligned with one another, which strays from the proposed relationship in Overlay 2. Since there were no alignment studies done for the fully unsaturated harmine (**1**) analogs for this thesis which would give support or refute this binding mode, analysis of the binding poses of harmaline (**2**) and THH (**3**), for which correlation studies have been done, is essential.

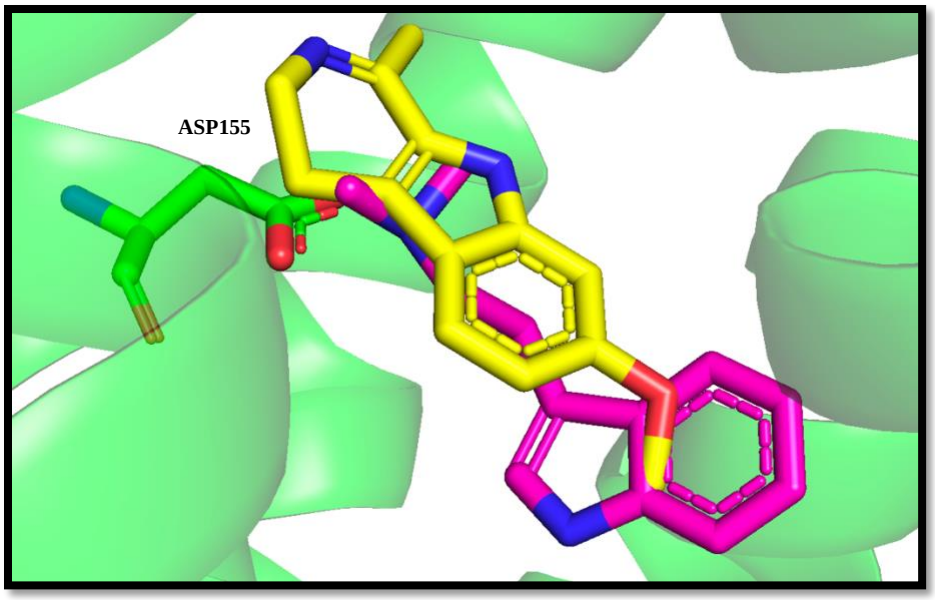


Figure 2A. Relative binding modes of DMT (**4**, pink, capped sticks) and harmaline (**2**, yellow, capped sticks) bound to the docking model (PDB ID: 6WHA, green, ribbons).

Harmaline (**2**) had an even more distinct binding pose (Figure 2A) in comparison to harmine (**1**), which did not mimick Overlay 2 at all. The tricyclic structure of the compound was

flipped upside-down, not unlike the results of the susbstituted tetrahydro β-carbolines discussed in the docking section of this thesis (Figure 23). It is clear that if Overlay 2 is the correct relationship between these two sets of compounds, which is a hypothesis that is supported by the correlation studies of both the dihydro and tetrahydro β-carbolines, that the benzenoid moieties of both classes of compounds are probably aligned. From this docking result, that is clearly not the case, and would go against the decent correlation which was found between the β-carboline analogs and DMT (**4**) analogs for Overlay 2 (r = 0.830, Figure 33), although the dihydro β-carbolines had a significantly worse correlation (r = 0.449) than the tetrahydro β-carbolines (r = 0.913).

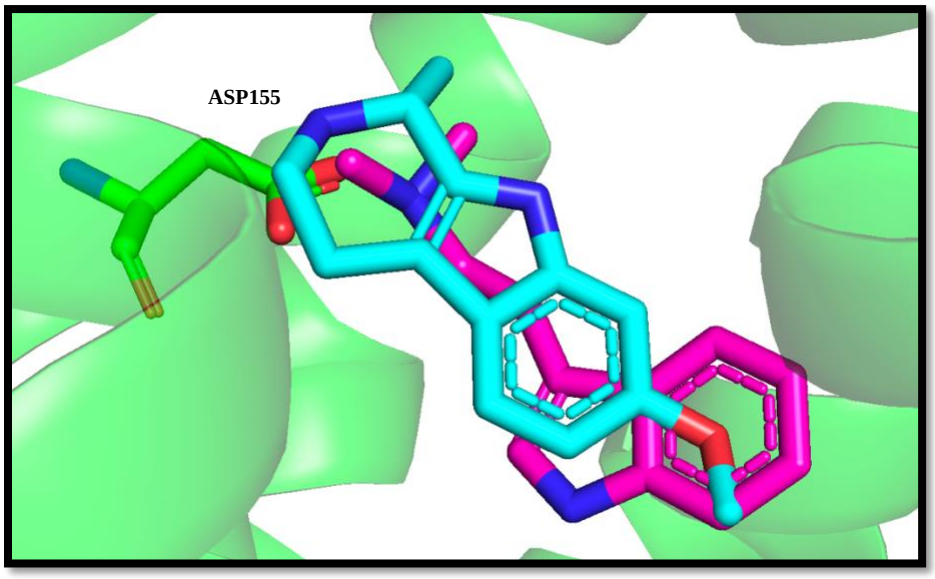


Figure 3A. Relative binding modes of DMT (**4**, pink, capped sticks) and THH (**3**, cyan, capped sticks) bound to the docking model (PDB ID: 6WHA, green, ribbons).

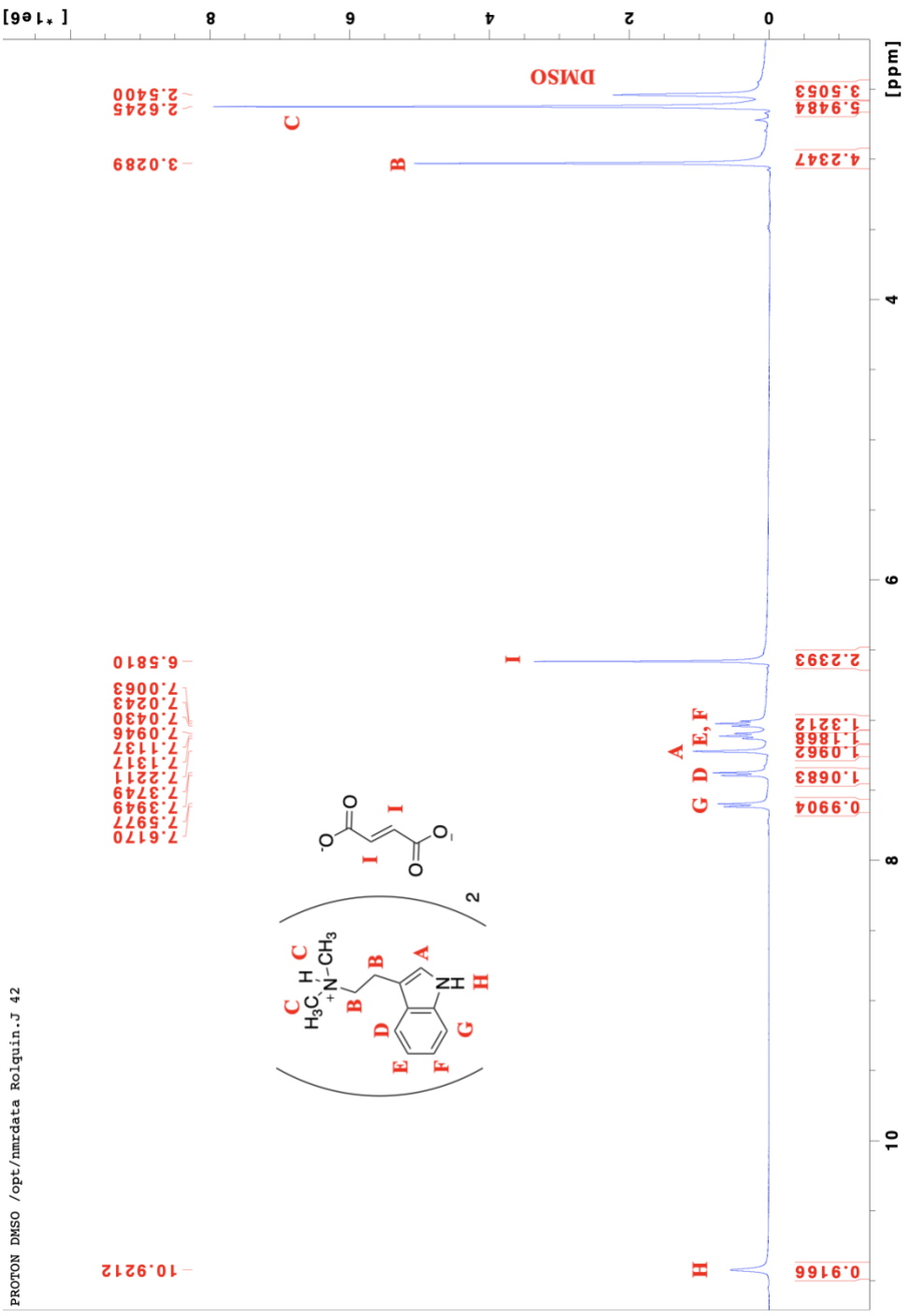
The docking results of THH (**3**, Figure 3A) were nearly identical to those of harmaline (**2**), with the tricyclic ring structure flipped upside-down in comparison to Overlay 2. The docking results for this compound are even more unexplainable than those of harmaline (**2**), as the correlation experiment comparing the analogs of tetrahydro β-carboline and DMT was nearly

perfect (r = 0.913). For the correlation result and docking result shown above to both be correct, it would be an incredible coincidence. The chances of tetrahydro β-carbolines and DMT analogs producing nearly identical changes in their binding affinity for the receptor when similarly substituted, but not have their benzenoid moieties aligned when bound to the receptor, is extremely unlikely to say the least.

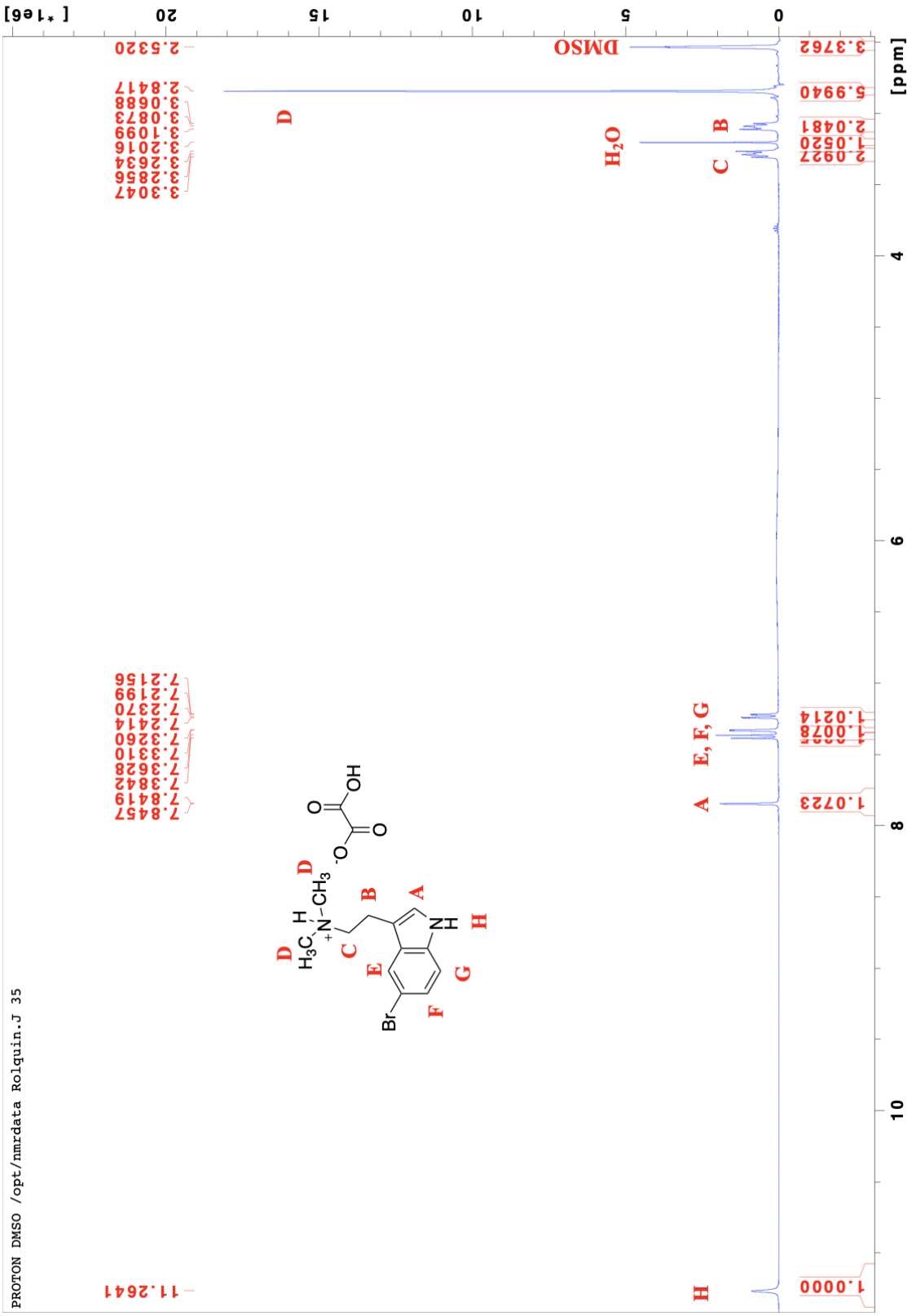
We felt that the results of our correlation studies were much better explained by the docking poses obtained from the docking model of PDB ID: 7WC5, where the Overlay 2-like poses were among the highest scoring docking results. The high scoring of the β-carbolines in the PDB ID: 7WC5 docking model were also supported by HINT scoring, whereas the ones shown in this appendix scored poorly in both the docking software as well as post-docking HINT analysis. It is possible that we were not able to find the optimal constraints to dock these ligands to the receptor, but the number of different docking parameters and constraints tried without obtaining results which were in agreement with the biological data provides support that the docking results shown in the main body of this thesis is a superior docking model compared to that which was obtained using the 5-HT_{2A} receptor model generated from X-ray crystal structure PDB ID: 6WHA as a template.

Appendix B.

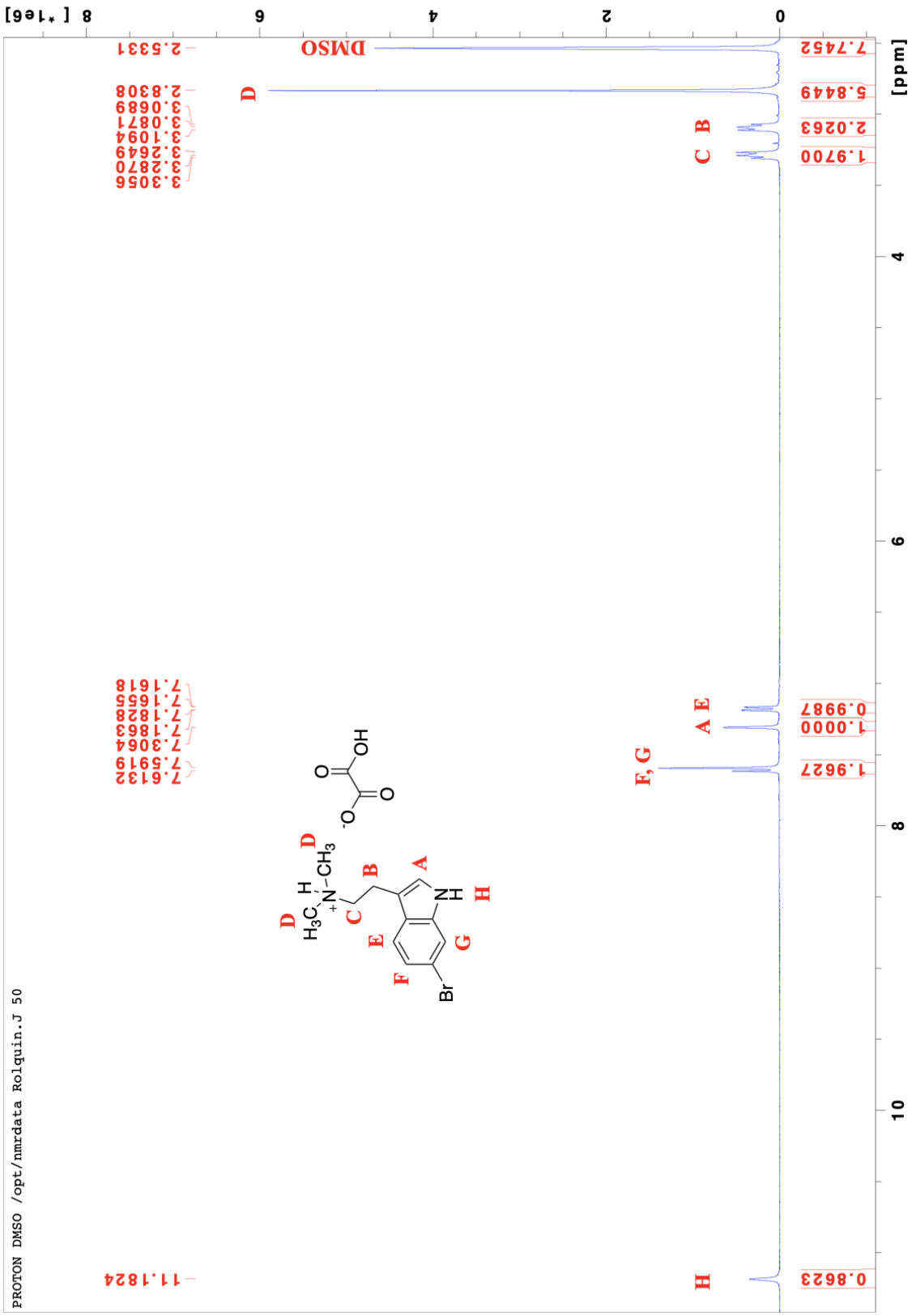
NMR Spectra of Target Compounds 42, 46, 50, 54, 59



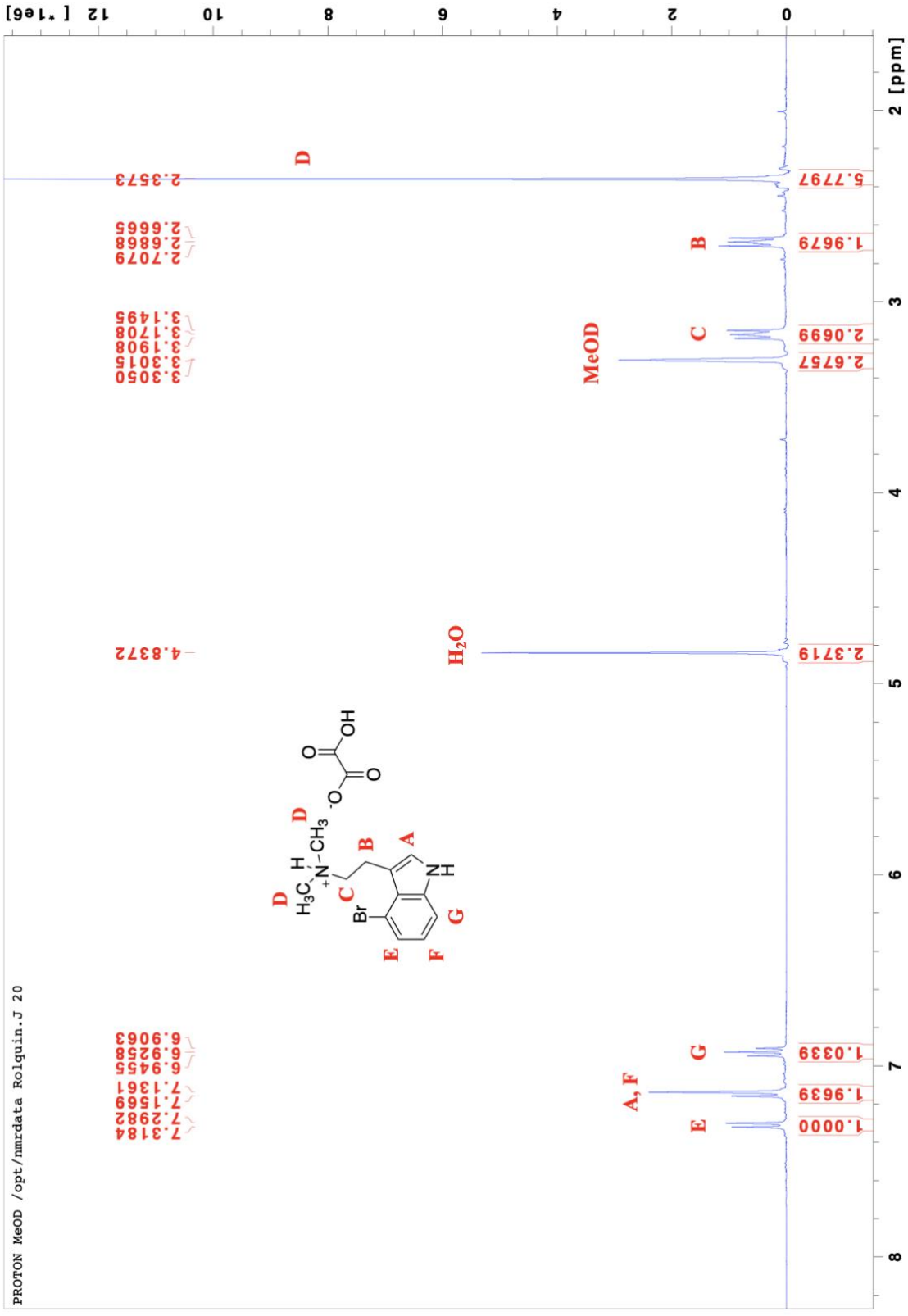
^{1}H NMR spectrum of *N*,*N*-dimethyltryptamine hemifumarate (**42**).



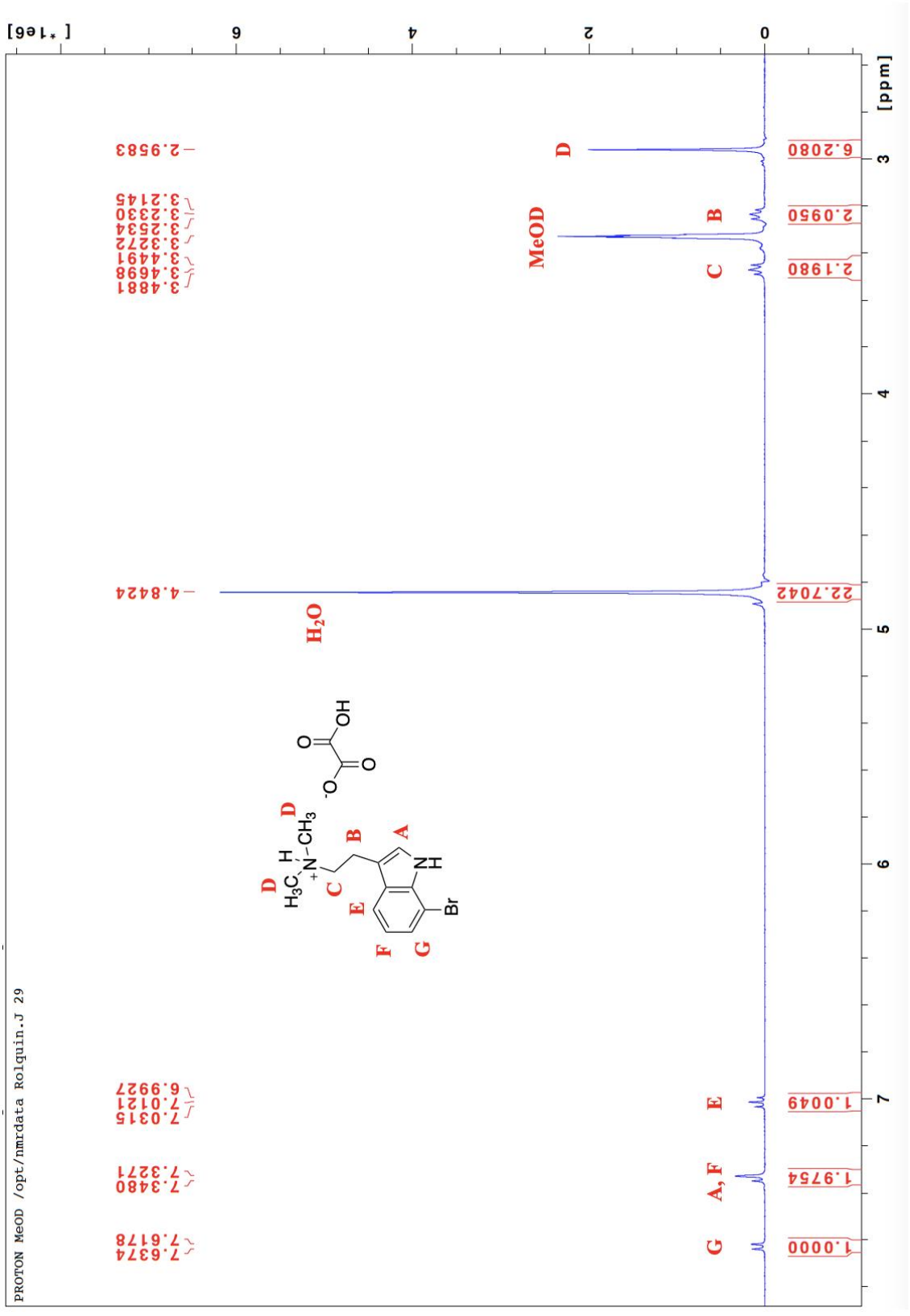
^{1}H NMR spectrum of 5-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**46**).



^{1}H NMR spectrum of 6-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**50**).



^{1}H NMR spectrum of 4-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**54**).



[1]HNMR spectrum of 7-bromo-*N*,*N*-dimethyltryptamine hydrogen oxalate (**59**).

Vita

Jeremy Derham Rolquin was born to James Edward Rolquin and Monica Derham Rolquin February 16, 1996 in Wilmington, North Carolina. He received his Bachelor of Science in Chemistry from the University of North Carolina at Wilmington in December 2018. After completing his bachelor degree, he subsequently enrolled in Virginia Commonwealth University's School of Pharmacy Medicinal Chemistry graduate program.