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On the Binding of N1-substituted Tryptamines at h5-HT6 Receptors

Abner Nyamwaro Nyandege
Virginia Commonwealth University

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ON THE BINDING OF N₁-SUBSTITUTED TRYPTAMINES AT h5-HT₆ RECEPTORS

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

By

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# Table of Contents

Acknowledgement........................................................................................................... ii  
List of Tables...................................................................................................................... vi  
List of Figures..................................................................................................................... vii  
List of Schemes.................................................................................................................. x  
List of Abbreviations......................................................................................................... xi  
Abstract............................................................................................................................... xiv  
I. Introduction....................................................................................................................... 1  
II. Background....................................................................................................................... 5  
   1 Neuropsychiatric disorders............................................................................................ 5  
      1.1 Depression................................................................................................................. 5  
      1.2 Psychosis.................................................................................................................. 7  
      1.3 Cognition.................................................................................................................. 7  
   2 Serotonin and Serotonin receptors................................................................................. 9  
      2.1 Serotonin................................................................................................................ 9  
      2.2 Classification......................................................................................................... 10  
      2.3 Structure and Pharmacology................................................................................. 11  
   3 5-HT₆ receptors.............................................................................................................. 15  
      3.1 Structure............................................................................................................... 15  
      3.2 Distribution.......................................................................................................... 16  
      3.3 Signal transduction............................................................................................. 16  
      3.4 Site directed-mutagenesis.................................................................................... 17  
      3.5 Radioligands......................................................................................................... 18  
      3.6 Clinical significance............................................................................................ 19  
      3.7 5-HT₆-selective agents....................................................................................... 20
## III. Specific aims and rationale

A. Specific aims

B. Hypotheses

C. Approach

D. QSAR (Hansch relationships)

E. Selection of benzyl substituents

## IV. Results and Discussion

A. Synthesis

1. Synthesis of $N,N$-dimethyltryptamine (DMT)

2. Synthesis of $N_1$-benzyltryptamines

3. Synthesis of $N_1$-benzenesulfonyletryptamines

4. Synthesis of alkylsulfonyletryptamines

B. Radioligand binding studies

## V. Conclusions

## VI. Experimentals

A. Chemistry

$N,N$-Dimethyl-2-[$1-(4$-methoxybenzenesulfonyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Oxalate ($31c$)

$N,N$-Dimethyl-2-[$1-(p$-toluenesulfonyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Oxalate ($31d$)

$N,N$-Dimethyl-2-[$1-(4$-trifluoromethylbenzenesulfonyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Oxalate ($31e$)

$N,N$-Dimethyl-2-[$1-(4$-chlorobenzenesulfonyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Oxalate ($31f$)

$N,N$-Dimethyl-2-[$1-(4$-aminobenzenesulfonyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Hydrochloride ($33b$)

$N,N$-Dimethyl-2-[$1-(4$-methoxybenzyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Oxalate ($33c$)

$N,N$-Dimethyl-2-[$1-(4$-methylbenzyl)$-1H$-indol$-3$-yl$]-1$-aminoethane Oxalate ($33d$)
$N,N$-Dimethyl-2-[1-(4-trifluorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33e)..................................................................................................................89
$N,N$-Dimethyl-2-[1-(4-chlorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33f)..................................................................................................................89
$N,N$-Dimethyl-2-[1-(3-chlorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33g)..................................................................................................................90
$N,N$-Dimethyl-2-[1-(3,4-dichlorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33h)..................................................................................................................91
$N,N$-Dimethyl-2-[1-(isopropylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78a)...................................................................................................................91
$N,N$-Dimethyl-2-[1-(n-propylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78b)...................................................................................................................92
$N,N$-Dimethyl-2-[1-(n-butylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78c)...................................................................................................................92
$N,N$-Dimethyl-2-[1-(n-amylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78d)...................................................................................................................94
$N,N$-Dimethyl-2-[1-(n-octylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78e)...................................................................................................................94
$N,N$-Dimethyl-2-[1-(cyclohexylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78f)...................................................................................................................95
$N,N$-Dimethyltryptamine (82)..........................................................................................96
1-(4-Nitrobenzyl)-1H-indole (83)......................................................................................97
1-(4-Aminobenzyl)-1H-indole (84)...................................................................................98
1-[(4-Dibenzylaminophenyl)methyl]-1H-indole (85).........................................................98
1-[(4-Dibenzylaminophenyl)methyl]-1H-indole-3-carboxaldehyde (86)...............................99
$N,N$-Dibenzyl-4-([3-[2-nitrovinyl]-1H-indol-yl]methyl)aniline (87).....................................99
$n$-Amylsulfonylchloride (90)..........................................................................................100
Cyclohexylsulfonyl chloride (93)......................................................................................100

B. Binding Assay.............................................................................................................101

VII. List of references......................................................................................................102
Vita...................................................................................................................................120
List of Tables

Table 1: Classification and nomenclature for the various populations of 5-HT receptors ................................................................. 11
Table 2: 5-HT_{6} receptor affinities of some simple N_{1}-substituted derivatives .......... 25
Table 3: 5-HT_{6} serotonin receptor binding of N_{1}-(arylsulfonyl)-N,N-dimethyl-tryptamine derivatives ......................................................... 26
Table 4: 5-HT_{6} receptor binding of benzenesulfonyltryptamine analogs .......... 28
Table 5: Binding affinities for h5-HT_{6} receptor ligands ........................................ 32
Table 6: Affinities of some ligands at h5-HT_{6} receptors ........................................ 38
Table 7: Lipophilicities (π values) of substituents to be examined ...................... 42
Table 8: 5-HT_{6} receptor binding affinities of alkylsulfonyltryptamines 78 ............ 58
Table 9: Electronegativities (σ values) of alkyl substituents ................................. 63
Table 10: 5-HT_{6} receptor binding affinities of benzenesulfonyltryptamines 31 relative to their binding counterparts 33 ................................ 65
Table 11: Summary of the physicochemical parameters employed .................... 69
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>The structures of 5-HT (1) and (+)lysergic acid diethylamide (LSD, 2)</td>
<td>10</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Schematic diagram of the general structure of G protein-coupled receptors</td>
<td>12</td>
</tr>
<tr>
<td>Figure 3</td>
<td>5-HTR signaling pathways and effectors</td>
<td>14</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Schematic representation of a human 5-HT6 receptor polypeptide chain</td>
<td>15</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Representative agents that bind at (although not necessarily selectively) 5-HT6 receptors</td>
<td>19</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Influence of structural modification of the binding of 5-HT and simple tryptamines at 5-HT6 receptors</td>
<td>22</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Some representative tryptamine related 5-HT6 receptor ligands</td>
<td>24</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Representative 5-HT6 receptor ligands</td>
<td>27</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Structures of N1-benzenesulfonilygramines 37 and N1-benzenesulfonilskatoles 38</td>
<td>29</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Some representative atypical 5-HT6 receptor ligands</td>
<td>34</td>
</tr>
</tbody>
</table>
Figure 11: Relationship between the 5-HT₆ receptor affinities (pKᵢ values) of 14 N₁-unsubstituted tryptamines and their corresponding N₁-benzene-sulfonyl counterparts ................................................................. 39

Figure 12: The craig plot .............................................................................................................. 46

Figure 13: Topliss scheme for aromatic substituents ............................................................... 46

Figure 14: Relationship between the affinities of 78a-78f and 31a versus π .................. 60

Figure 15: Relationship between the affinities of 78a-78f and 31a versus Eₛ .................. 62

Figure 16: Relationship between the affinities of N₁-benzyl- and N₁-benzene-sulfonyltryptamine analogs 33 and 31, respectively .................................................. 68

Figure 17: Ester hydrolysis reaction and equation used to define the taft steric parameter, Eₛ .............................................................................................................................................. 70

Figure 18: Schematic representation of dimensions of the benzoic acid substituent..... 71

Figure 19: Relationship between the affinities of N₁-benzyltryptamine analogs 33 versus the Eₛ .............................................................................................................................................. 72

Figure 20: Relationship between the affinities of N₁-benzenesulfonyltryptamine analogs 31 versus the Eₛ .............................................................................................................................................. 72

Figure 21: Relationship between the affinities of N₁-benzyltryptamine analogs 33 versus σ .............................................................................................................................................. 73

Figure 22: Relationship between the affinities of N₁-benzenesulfonyltryptamine analogs 31 versus σ .............................................................................................................................................. 74

Figure 23: Relationship between the affinity of N₁-benzyltryptamine analogs 33 versus π .............................................................................................................................................. 75
Figure 24: Relationship between the affinities of N$_1$-benzenesulfonyltryptamine analogs 31 versus $\pi$ ................................................................. 75

Figure 25: Relationship between the affinities of N$_1$-benzyltryptamine analogs 33 versus length ................................................................. 76

Figure 26: Relationship between the affinities of N$_1$-benzyltryptamine analogs 33 versus width ................................................................. 76

Figure 27: Relationship between the affinities of N$_1$-benzenesulfonyltryptamine analogs 31 versus length ................................................................. 77

Figure 28: Relationship between the affinities of N$_1$-benzenesulfonyltryptamine analogs 31 versus width ................................................................. 78
## List of Schemes

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheme 1</td>
<td>50</td>
</tr>
<tr>
<td>Scheme 2</td>
<td>52</td>
</tr>
<tr>
<td>Scheme 3</td>
<td>53</td>
</tr>
<tr>
<td>Scheme 4</td>
<td>54</td>
</tr>
<tr>
<td>Scheme 5</td>
<td>55</td>
</tr>
<tr>
<td>Scheme 6</td>
<td>56</td>
</tr>
<tr>
<td>Scheme 7</td>
<td>56</td>
</tr>
</tbody>
</table>
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Adenylyl cyclase</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AcONH₄</td>
<td>Ammonium acetate</td>
</tr>
<tr>
<td>AOs</td>
<td>Antisense oligonucleotides</td>
</tr>
<tr>
<td>AHP</td>
<td>After-hyperpolarization</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>B</td>
<td>Breadth</td>
</tr>
<tr>
<td>BS/5-OMe DMT</td>
<td>5-Methoxy-N,N-dimethyltryptamine</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic 3', 5'-adenosine monophosphate</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartate</td>
</tr>
<tr>
<td>ca.</td>
<td>Calculated</td>
</tr>
<tr>
<td>cDNA</td>
<td>Cloned deoxyribonucleic acid</td>
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<tr>
<td>CDCl₃</td>
<td>Dueterated chloroform</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>Methylene chloride</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>5-CT</td>
<td>5-Carboxamidotryptamine</td>
</tr>
<tr>
<td>COS-7</td>
<td>African Green Monkey Kidney Fibroblast cells</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DMSO-d₆</td>
<td>Deuterated methyl sulfoxide</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>GPCRs</td>
<td>G-Protein coupled receptors</td>
</tr>
<tr>
<td>GDP</td>
<td>Guanine nucleotide guanosine diphosphate</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanine nucleotide guanosine triphosphate</td>
</tr>
<tr>
<td>Epa</td>
<td>Activated exchange factor</td>
</tr>
<tr>
<td>EPS</td>
<td>Extrapyramidal symptoms</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>EMDT</td>
<td>2-Ethyl-5-methoxy-N,N-dimethyltryptamine</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HEK</td>
<td>Human embryonic kidney cells</td>
</tr>
<tr>
<td>H₂CO</td>
<td>Formaldehyde</td>
</tr>
<tr>
<td>HCO₂NH₄</td>
<td>Ammonium formate</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-Hydroxytryptamine</td>
</tr>
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</table>
5-HTR  5-Hydroxytryptamine receptor
h5-HT$_6$  Human 5-HT$_6$
ih  Hyperpolarization-activated current
IC$_{50}$  Inhibition concentration (half-maximal effect)
EC$_{50}$  Activation concentration (half-maximal effect)
IP3  Inositol triphosphate
JAK  Janus kinase
JEG-3  Human choriocarcinoma cells
K$_i$  Dissociation constant
L  Length
LSD  Lysergic acid diethylamide
LiAlH$_4$  Lithium aluminium hydride
m  Multiplet
MAO  Monoamine oxidase
MAPK  Mitogen-activated protein kinase
Me  Methyl
MeCN  Acetonitrile
MeOH  Methanol
MeNO$_2$  Nitromethane
mL  Milliliter
mmol  Millimolar
mp  Melting point
MWt  Molecular weight
mRNA  Messenger ribonucleic acid
NaOH  Sodium hydroxide
NaH  Sodium hydride
Na$_2$SO$_4$  Sodium sulfate
NaBH$_3$CN  Sodium cyanoborohydride
nM  Nanomolar
NMR  Nuclear magnetic resonance
NOS  Nitric oxide synthase
Pd/C  Palladium on carbon
PI  Phosphatidylinositol
PLC  Phospholipase C
PKA  Protein kinase A
PMDT  5-Methoxy-2-phenyl-\(N,N\)-dimethyltryptamine
QSAR  Quantitative structure-activity-relationships
r  Correlation coefficient
r$^2$  Coefficient of determination
rt  Room temperature
s  Singlet
SnCl$_2$  Tin (II) chloride
SNRIs  Selective norepinephrine reuptake inhibitors
SSRIs  Selective serotonin reuptake inhibitors
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFIR</td>
<td>Structure-affinity-relationships</td>
</tr>
<tr>
<td>S.E.M</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>STAT</td>
<td>Transcription pathway</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>t-BuOK</td>
<td>Potassium tert-butoxide</td>
</tr>
<tr>
<td>TCAs</td>
<td>Tricyclic antidepressants</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>TM</td>
<td>Transmembrane</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>W</td>
<td>Width</td>
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Abstract

ON THE BINDING OF N₁-SUBSTITUTED TRYPTAMINES AT h5-HT₆ SEROTONIN RECEPTORS

By Abner Nyamwaro Nyandege, M.S.

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

Virginia Commonwealth University, 2007

Major Director: Richard A. Glennon, Professor
Department of Medicinal Chemistry

Serotonin was first discovered in the late 1940s as a vasotonic factor and is now considered a principal neurotransmitter in the nervous system. 5-HT₆ receptors are one of the most recently identified members of the serotonin receptor family which consists of seven classes (5-HT₁-5-HT₇). 5-HT₆ receptors are G-protein coupled, positively coupled to an adenylate cyclase second messenger system and are primarily found in the central nervous system (CNS). The exact functional role of 5-HT₆ receptors has not been determined, but is implicated to have possible involvement in certain neuropsychiatric disorders and cognition. To investigate the functional role of these receptors, it is useful to identify 5-HT₆ selective ligands as pharmacological tools.
Our laboratory identified one of the first 5-HT$_6$ receptor antagonists: the arylsulfonamide MS-245 (14a). It has been assumed that a sulfonyl (i.e., SO$_2$) moiety is important for the binding of arylsulfonamides at 5-HT$_6$ receptors. We now have identified benzyl analog 33 (R=H) as a single example of a non-sulfonyl analog that retains affinity. This questions the importance of the SO$_2$ group and whether an aryl moiety or other hydrophobic groups (of equal or greater hydrophobicity) is required for binding. The purpose of the present investigation was to determine if the SO$_2$ and the aryl moieties are required for high affinity binding. N$_1$-Alkylsulfonyl- 78, and N$_1$-benzyl-substituted tryptamines 33 were synthesized and affinities compared with their corresponding N$_1$-benzenesulfonyl-substituted counterparts 31 at h5-HT$_6$ receptors.

None of the alkylsulfonyl or benzyl analogs displayed and/or retained the affinity of the simple benzenesulfonyl tryptamine analog (31a) ($K_i = 4.1$ nM). The results show that an arylsulfonyl group at the tryptamine N$_1$ position is optimal, relative to an alkylsulfonyl group, for 5-HT$_6$ receptor affinity. In a comparative analysis utilizing six pairs of tryptamines, it was found that there was little correspondence ($r^2 = 0.048$) between the 5-HT$_6$ receptor affinities of the examined benzyl and benzenesulfonyl pairs. Current findings indicate that an aryl (or substituted aryl)sulfonyl (rather than benzyl) moiety is optimal for high affinity binding, and further suggest that N$_1$-benzenesulfonyl- and their corresponding N$_1$-benzyltryptamine counterparts bind in a different fashion.
I. Introduction

Increasing evidence and awareness indicate that treatment of neuropsychiatric disorders such as depression, psychosis (e.g., schizophrenia) and cognition is a viable and productive endeavor. Tricyclic antidepressants (TCAs), modern selective reuptake inhibitors, and antipsychotics remain the mainstay in pharmacologic treatment of these disorders. However, the development of neuropsychiatric disorder medications that are effective but free of serious side effects remains a major focus in the field of drug discovery. Therefore, development of safe and effective medications represents a considerable therapeutic opportunity. This process of new drug development has continued to change greatly in the last five decades and is likely to change even more rapidly in the immediate future.

The history of the first major class of neuropsychiatric disorder medications, antipsychotics, began in 1883, when phenothiazine was first synthesized by Bernthesen. However, phenothiazines were not introduced into human pharmacology until the late 1940’s when they were first investigated as possible antihelmintic agents and later as antihistamines. Later, Charpentier (of Rhone-Poulenc Pharmaceuticals) synthesized a new derivative of phenothiazine, chlorpromazine, and in early 1952, French psychiatrists, Delay and Deniker, first reported chlorpromazine’s antipsychotic activity, particularly for the treatment of schizophrenia – a serendipitous discovery.

The discoveries of the antidepressant properties both of tricyclic agents and monoamine oxidase (MAO) inhibitors, first generation antidepressants, were the
fortuitous and unexpected consequences of tangential avenues of research. While investigating the antipsychotic activity of imipramine (which is structurally similar to the phenothiazines), the Swiss clinical researcher Kuhn (1958) found that although it was ineffective in schizophrenia it produced considerable improvements in depressed patients. Similar serendipitous findings contributed to the development of MAO inhibitors when iproniazid, the first of these agents, was found to produce mood elevation and euphoria (as side effects) in tuberculosis patients. It is important to note that this discovery was subsequent to the discovery by Thiele and Holzinger in 1889 of iminodibenzyl. The new generation of antidepressants involves the use of selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs), mixed action agents, and alternative (nontraditional) antidepressants.

The discovery of dopaminergic receptor-blocking capabilities, especially in the mesolimbic dopamine pathway, of conventional antipsychotic drugs led to the dopamine hypothesis of schizophrenia. Conventional antipsychotics were found to be effective in controlling psychotic symptoms, such as hallucinations, delusions and agitation, and in reducing both morbidity and mortality. However, they have significant side effects such as extrapyramidal effects (EPS), weight gain, cardiovascular liabilities and type II diabetes. In the 1960s and 1970s, clozapine, the first of the second generation of antipsychotics, was introduced into clinical practice. It was called an atypical antipsychotic because it produced few, if any, EPS at clinically effective doses in humans. Other examples are known, such as fluperlapine, melperone and thiosperone. Since 1990, this second generation of antipsychotic drugs has led to dramatic shifts in the treatment of major mental illness. It has been postulated that this unique property is due
their affinity for receptors of the serotonergic neurotransmitter system in addition to their effects on dopamine D2 receptors.\textsuperscript{10} Most of the atypical antipsychotics, with the exception of the substituted benzamides such as amisulpride, have, in addition to D2 receptor antagonism or partial agonism, high affinity for 5-hydroxytryptamine (5-HT)\textsubscript{2A} receptors and function as inverse agonists at this receptor.\textsuperscript{11,12} In addition, some, but not all, are 5-HT\textsubscript{2C}, 5-HT\textsubscript{6} and 5-HT\textsubscript{7} receptor antagonists, as well as direct or indirect 5-HT\textsubscript{1A} receptor agonists.\textsuperscript{13,14}

Schizophrenia appears to be associated with decline in general cognitive function at some point during the course of the illness.\textsuperscript{10} One approach to the development of new agents for schizophrenia to improve functional outcomes is focusing on cognitive impairment (for example, attention deficits, working memory deficits, and deficits in executive function).\textsuperscript{15,16} Achievements in treating cognitive impairments may tremendously contribute to treating other components of cognition deficits (for example, memory and learning disorders). Conventional antipsychotics appear to produce little benefit in cognitive function, but atypical antipsychotics may reduce cognitive impairments.\textsuperscript{17} However, in general, conventional antipsychotic drugs, which act on dopamine D\textsubscript{2} and 5-HT\textsubscript{2} serotonin receptors, are limited in their ability to treat cognitive deficits.\textsuperscript{18} Serotonin receptors (specifically 5-HT\textsubscript{6} receptors) have been implicated in cognitive processes such as memory.\textsuperscript{19,20} The 5-HT\textsubscript{6} receptor has a unique pharmacological profile, including a high affinity both for typical, such as chlorpromazine, and atypical, such as clozapine and olanzapine, antipsychotics.\textsuperscript{21} To further investigate the functional role of 5-HT\textsubscript{6} receptors, it is necessary to identify 5-HT\textsubscript{6} selective ligands as pharmacological tools. Much work is ongoing to develop selective
novel agents with unique pharmacological effects that may enhance cognition and other neuropsychiatric disorders. One of the first examples of a 5-HT₆ receptor antagonists was the N₁-arylsulfonyltryptamine MS-245 – an agent developed in our laboratories. The purpose of the present study was to investigate further the binding of Nₑ-substituted tryptamines at h5-HT₆ receptors in order to better understand their binding requirements. This work can lead to further investigation of the functional role of h5-HT₆ receptors geared towards developing an effective and safe treatment for neuropsychiatric and cognitive disorders.
II. Background

1. Neuropsychiatric disorders and cognition.

Since the current drugs for neuropsychiatric disorders and cognition are of moderate safety and efficacy, they are far from ideal. Continued discovery and development efforts, aimed to relieving these shortfalls, is greatly dependent on a clear biomedical understanding of the underlying disease process, and the psychosocial aspects of the illness. As mentioned earlier, these disorders include depression, psychosis (e.g., schizophrenia), and cognition.

1.1 Depression:

The term “depression” can refer to a clinical syndrome, such as a primary or secondary affective disorder, and a symptom in the sense of being severely “sad” or “unhappy”. Furthermore, the term “depression” may be used by a patient to communicate problems of cognition, perception, mobility and behavior as well as affect and mood. This is to be expected given the interdependence of the components which make up the composite mental state. Depression disorders constitute a great burden to society. Disability associated with depression is greater than that reported for other chronic physical conditions such as hypertension, diabetes, arthritis, and back pain. Depression is a common condition that has both emotional and physical aspects. Physical manifestations, common features of depression, are present in up to 80% of depressed patients. The diversity of clinical symptoms argues against an etiology associated with single brain location, lesion type, or neurochemical system. Rather, the
associated impairment of cognitive, motor, somatic, and circadian functions in patients with dysphoria suggest that depression is a composite disorder affecting discrete but functionally interconnected limbic, paralimbic, and neocortical circuits. \(^{29,30}\) Depression may accompany a variety of neurological, psychiatric, and other medical illnesses, and recognition of these comorbid conditions can influence the approach to treatment as well as therapeutic outcome. \(^{31}\) No single neurotransmitter abnormality has been identified that fully explains the pathophysiology of the depressive disorders or the associated constellation of mood, motor, cognitive, and somatic manifestations. \(^{32}\) Changes in norepinephrine, serotonin, dopamine, acetylcholine, opiate, and \(\gamma\)-aminobutyric acid (GABA) systems have all been reported. \(^{33-35}\) However, postulated disturbances in serotonergic (5-HT) and noradrenergic mechanisms have dominated the neurochemical literature on depression for more than 30 years based, in large part, on the consistent observations that most antidepressant drugs affect the synaptic concentrations of these two transmitters. \(^{36,37}\) Further evidence that depression is caused by a functional deficiency of serotonin in the brain has been reported. \(^{25,38-41}\) The early antidepressants include MAO inhibitors and TCAs; they have a delayed onset of action and have side effects such as dryness of the mouth, sweating, blurring of vision and, occasionally, tachycardia. \(^{42}\) Newer treatments are available: both psychologic and pharmacologic, that are safe and effective and that have a high degree of patient acceptability. Perhaps least well known is that depression is frequently a chronic or recurrent condition, and effective management must extend beyond the treatment of an individual episode. \(^{38}\)

**1.2. Psychosis:**
The term “psychosis” has a variety of meanings. Currently, in clinical practice in North America, psychotic means that the person has a biomedical brain disease or a chemical imbalance, requires medication, and cannot be treated with psychotherapy. Psychosis includes less insight and reduced social and occupational function. Schizophrenia is a subset of psychosis. It is widely regarded as the most severe mental illness, and schizophrenia cannot occur without psychosis. Disorders that may or may not have psychotic symptoms as an associated feature include depression as well as several cognitive disorders such as Alzheimer’s dementia. Schizophrenia is characterized by positive and negative symptoms: Positive symptoms of schizophrenia include hallucinations, delusions, thought disorder, and agitation. Negative symptoms are absence of normal attributes; they include reduced drive, social withdrawal, emptiness, and a flat emotional tone. Four decades of data demonstrate consistently and conclusively that antipsychotic medications have only a modest effect on psychotic symptoms. A role for serotonergic systems has been reported.

1.3 Cognition:

Cognition refers to the ‘nature of memories at the highest level analysis, the psychological level’ or ‘is that of the mind by which one becomes aware of the objects of thought and perception. It includes the processes of understanding and reason’. Learning is clearly a fundamental aspect of cognitive development. Learning and memory are an integral part of our existence, yet the concept is only vaguely understood. One major discovery about the brain that contributes directly to our understanding of the cellular and molecular substrates of memory is the development of the “neuron doctrine”
by the Spanish anatomist Santiago Ramon y Cajal. This development won him the Nobel prize in 1906. Conceptual development in cognitive theorizing is a subdivisions of memory: short-term versus long-term memory (1890), encoding versus retrieval, retroactive versus proactive interference, anterograde versus retrograde amnesia, recent versus remote memory, declarative versus non declarative memory, and episodic versus semantic memory. Evidence shows that components of learning and memory can be disrupted in memory profiles of selected clinical populations: chronic alcoholism, unilateral brain damage, drug induced memory deficits (e.g., anticholinergics, benzodiazepines, some anticonvulsant medications), Alzheimer’s disease, Huntington’s disease, affective disorders, schizophrenia, normal aging, and amnesia. Various environmental conditions such as stress, pollutants, and diet can also result in cellular dysfunctioning and consequential problems in learning and memory. Recently, with the rise of modern methods of cognitive science and neuroscience, and their combination, many new and deep insights about the mechanisms of memory have emerged. Today, there is a consensus that neuronal changes associated with learning and memory occur at the level of the synapse. Some of the key structures thought to be involved in memory, in particular the dorsolateral prefrontal cortex and the hippocampus: cerebral cortex, amygdala, cerebellum, thalamus.

The use of selective serotonin (5-hydroxytryptamine, 5-HT) agonists and antagonists has increased our understanding of the role of 5-HT receptors in the processes of learning and memory. In particular 5-HT subtypes serotonin receptors are located in brain structures associated with learning and memory.

2. Serotonin and Serotonin receptors.
2.1 Serotonin:
Serotonin (5-hydroxytryptamine, 5-HT; 1), was first discovered in the late 1940s as a vasotonic factor and is now considered a principal neurotransmitter in the nervous system. As long ago as 1868 it was known that the blood contained a vasoconstrictive substance. This substance, released in serum by platelet breakdown, proved to be a problem to Page in his studies on malignant hypertension so he, together with Green and postdoctoral student Rapport, isolated and characterized this interfering substance, and named it for its vasoconstrictor properties – serotonin. However, during the same time as the studies by Page and colleagues, in Italy, Erspamer and Asero had been investigating a constituent of the gastric and enteric mucosa of mammals, and salivary glands of the octopus. They identified a compound, enteramine, and this substance was later demonstrated to be 5-HT. Gaddum, in 1950, made the observation that 5-HT is present in the brain. In addition, serotonin (5-HT) was detected in numerous plant and animal species. 5-HT has been implicated in a variety of central and peripheral physiologic actions: vasoconstriction and vasodilation, regulation of body temperature, sleep, and hormonal regulation. The structural similarity between 5-HT and the, then, recently discovered hallucinogenic agent (+)lysergic acid diethylamide (LSD, 2; Figure 1) led to speculation that 5-HT might be involved in the mechanism of action of psychoactive substances, and that it might play a seminal role in various mental disorders.
2.2 Classification:

In mammals, seven populations of 5-HT receptors have been identified, 5-HT₁ to 5-HT₇, and are divided into 14 distinct subpopulations (see Table 1). There are six classes of G-protein coupled receptors, namely 5-HT₁, 5-HT₂, 5-HT₄, 5-HT₅, 5-HT₆, and 5-HT₇, but 5-HT₃ receptors belong to the ligand gated ion channel receptor family. Whereas other classes are further subdivided into subclasses, for the most part, the 5-HT₆ and 5-HT₇ classes do not contain subclasses of receptors, and sequence diversity in these classes is provided mainly by alternative mRNA splicing.
### Table 1. Classification and nomenclature for the various populations of 5-HT receptors.\(^{62}\)

<table>
<thead>
<tr>
<th>Populations and Subpopulations</th>
<th>Second messenger System</th>
<th>Currently accepted name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT(_1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT(_1)A</td>
<td>AC(-)</td>
<td>5-HT(_1)A</td>
</tr>
<tr>
<td>5-HT(_1)B</td>
<td>AC(-)</td>
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<tr>
<td>5-HT(_1)D</td>
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<tr>
<td>5-HT(_1)D(\alpha)</td>
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<tr>
<td>5-HT(_1)D(\beta)</td>
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</tr>
<tr>
<td>5-HT(_1)E</td>
<td>AC(-)</td>
<td>h5-HT(_1)D</td>
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<tr>
<td>5-HT(_1)E(\alpha)</td>
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<tr>
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<td>PI</td>
<td>5-HT(_2)C</td>
</tr>
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<tr>
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<td>5-ht(_5)B</td>
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<tr>
<td>5-HT(_7)</td>
<td>AC(+)</td>
<td>5-HT(_7)</td>
</tr>
</tbody>
</table>

*AC = adenylate cyclase, (-):negatively coupled and (+):positively coupled; PI = phospholipase coupled

### 2.3 Structure and Pharmacology:

All the 5-HT receptors, except 5-HT\(_3\) receptors, are members of the typical group A rhodopsin-like G protein-coupled receptor family (GPCR; Figure 2) in that they are predicted to possess seven transmembrane spanning helices, three intracellular loops, an
extracellular amino-terminus, and an intracellular carboxy-terminus. Functionally, the transmembrane regions serve to bind ligands, especially the endogenous ligand serotonin; the intracellular domains couple these receptors to various intracellular functions and, for the most part, the extracellular domains have uncertain functional roles. The true structures of these receptors remain unknown, although crystallization of the bovine rhodopsin receptor provides promise for the solution of the structures of the G-protein-coupled receptors in the near future. In contrast to the G protein-coupled 5-HT receptors which modulate cellular activities via second messenger systems, 5-HT receptors directly activate a 5-HT-gated ion channel that causes the rapid depolarization of neurons. The depolarization mediated by 5-HT receptors is caused by a transient inward current, specifically the opening of a channel for cations.

![Figure 2. Schematic diagram of the general structure of G protein-coupled receptors.](image)

The G-proteins derive their name from the fact that they bind the guanine nucleotide guanosine triphosphate (GTP) and guanosine diphosphate and possess intrinsic GTPase activity. The G proteins exist in cell membranes as heterotrimers composed of single α, β and γ subunits and are associated only loosely with neurotransmitter receptors. The receptors have a diverse array of signaling pathways (Figure 3). It is the interaction of receptor with the G-protein that allows the receptor to modulate the activity of different
effector systems, such as ion channels, phospholipase C and adenylyl cyclase (the enzyme that catalyzes the synthesis of cAMP). In the 5-HT family, the second messengers involved are activation or inhibition of adenylyl cyclase (AC), which controls intracellular cAMP concentrations, and activation of phosphoinositide phospholipase C (PI PLC) to form the second messengers inositol triphosphate (IP3) and diacylglycerol (DAG).\textsuperscript{66,67}

5-HT\textsubscript{6} receptors are included in a family of serotonin receptors coupled to the stimulation of adenylyl cyclase. The form of heterotrimeric G protein involved is called G\textsubscript{s}. Activation of these receptors results in the generation of free G\textsubscript{\alpha} subunits, which bind to and, thereby, directly activate adenylyl cyclase. In addition, free βγ-subunit complexes activate certain subtypes of adenylyl cyclase.\textsuperscript{67}
Figure 3. 5-HTR signaling pathways and effectors (blue 5-HTR signal transduction in neurons, gray signaling linkages only in transfected cell lines, PL phospholipase, ERK extracellular signal-regulated kinase, PK protein kinase, IP3 inositol triphosphate, DAG diacylglycerol, MAPK mitogen-activated protein kinase, NOS nitric oxide synthase, AHP after-hyperpolarization, JAK Janus kinase, STAT transcription pathway, ih hyperpolarization-activated current, Epa activated exchange factor)
3. 5-HT₆ receptors.

3.1 Structure:

The basic structure of the G-protein-coupled 5-HT receptors is similar to that proposed for nearly all of the GPCRs. The 5-HT₆ receptors are integral membrane proteins that possess seven transmembrane (TM)–spanning domains connected by three intracellular loops (termed i1-i3 loops) and three extracellular loop (termed e1-e3 loops) and are positively coupled to an adenylyl cyclase second messenger system. Human 5-HT₆ receptors, a 440 amino acid polypeptide (see Figure 4), display 89% overall sequence homology with the rat and mouse 5-HT₆ receptors and a high homology to other 5-HT receptors. 

![Figure 4](image_url)

**Figure 4.** Schematic representation of a human 5-HT₆ receptor polypeptide chain. 

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3.2 Distribution:

Human 5-HT<sub>6</sub> receptors are nearly exclusively localized in the central nervous system. Expression of 5-HT<sub>6</sub> receptor mRNA has been detected in the striatum, nucleus accumbens, olfactory tubercle, hippocampus and cerebral cortex. Furthermore, antibody, antisense oligonucleotide, and radioligand-binding studies suggest that 5-HT<sub>6</sub> receptors are abundant in several brain regions, including cortical and limbic brain regions, the olfactory tubercle, caudate nucleus, hippocampus, nucleus accumbens, and striatum. Lower levels have been found in the amygdala, hypothalamus, substantia nigra, and cerebral cortex. No 5-HT<sub>6</sub> mRNA has been detected in several human peripheral tissues.

3.3 Signal transduction:

As mentioned earlier, 5-HT<sub>6</sub> receptors are positively coupled to adenylate cyclase. 5-HT<sub>6</sub> receptors increase cellular levels of cAMP when transfected into JEG-3, COS-7, and HEK293 cells. In HEK293 cells, the increases in cAMP levels are mediated primarily through stimulation of AC Type 5 (AC5, a G<sub>s</sub>-sensitive isoform), but not through CaM-regulated AC isoforms, AC1 and AC8. In aggregate, the aforementioned signaling studies strongly support elevation of cAMP through G<sub>s</sub> as the primary signaling linkage of 5-HT<sub>6</sub> receptors. There are four potential regions of the GPCR that could interact with the G-protein; i1, i2, i3, and the cytosolic C-terminal tail. Among them, the most critical regions in determining the receptor/G-protein interactions have been suggested to be i2, i3, or the C-terminal tail. It has been demonstrated that the third intracellular loop region (i3) of the 5-HT<sub>6</sub> receptor is critical for the interaction with Gα<sub>s</sub>. Until selective ligands were developed, exploration of 5-HT<sub>6</sub> pharmacology was largely
dependent on the use of nonselective agents. Stimulation of adenylyl cyclase was employed to identify potential agonists, and antagonists that could be identified by their ability to attenuate 5-HT-induced cAMP formation. In the first such investigation of this kind, certain nonselective agents were characterized as 5-HT₆ agonists with the following rank order of potency: 5-methoxytryptamine (9) > 5-HT (1) > 2-methyl 5-HT (11) > 5-CT (13) >> α-methyltryptamine (4).⁷⁸,⁷⁹ However, owing to their lack of selectivity, they were of limited value for most other pharmacological studies.

### 3.4 Site directed-mutagenesis:

Many non-selective compounds, such as tricyclic antidepressant drugs and a large number of antipsychotic agents, tryptamine, and ergoline derivatives, interact with the 5-HT₆ receptors.²¹,⁸⁰,⁹² Because no selective ligands were available, identification of functional 5-HT₆ receptors in physiological preparations were only tentative. Site-directed mutagenesis has been used to identify residues contributing to the ligand binding site of the 5-HT₆ receptors. Results obtained from site-directed mutagenesis demonstrated that interactions with residues in two characteristic positions of transmembrane region V are important for ligand binding in several bioamines. In the 5-HT₆ receptors, one of these residues is a threonine (Thr196); this residue interacts with the indole nitrogen of N₁-unsubstituted ergolines and tryptamines. Another structural feature is Asp106 in TM III that demonstrates an ionic interaction with the positively charged nitrogen atoms of 5-HT (1) and LSD (2).⁸¹,⁸²
3.5 Radioligands:

Tritiated Ro 63-0563 (3a) was introduced in 1998 as the first radioligand selective for 5-HT\textsubscript{6} receptors.\textsuperscript{83} In rat brain, \(^{3}\text{H}\)Ro 63-0563 was displaced by a variety of ligands with the following rank of order of affinities: methiothepin (8) > LSD (2) > clozapine (5) \(
\approx\) Ro 63-0563 (3a) > ergotamine (7) > 5-HT (1) > mianserin (6).\textsuperscript{83} Tritiated 5-HT, \(^{3}\text{H}\)lysergic acid diethylamide (\(^{3}\text{H}\)LSD), and \(^{125}\text{I}\)-2-iodo LSD also have been used to radiolabel 5-HT\textsubscript{6} receptors. Serotonin (5-HT) binds with moderately high affinity (K\textsubscript{i} = 10-150 nM).\textsuperscript{78} A variety of tryptamine derivatives have been tested and showed a significantly higher affinity when the 5-HT\textsubscript{6} receptors were labeled with \(^{3}\text{H}\)-5-HT, whereas ergoline compounds and several antagonists had higher affinities when \(^{3}\text{H}\)-LSD was used as radioligand.\textsuperscript{75}
Figure 5. Representative agents that bind at (although not necessarily selectively) 5-HT<sub>6</sub> receptors.

3.6 Clinical significance:
The exact clinical significance of 5-HT<sub>6</sub> receptors has not yet been determined. Of interest is that a number of typical and atypical antipsychotic agents and tricyclic antidepressants bind at 5-HT<sub>6</sub> receptors with Ki values in the nM range. For example, lisuride acted as a partial agonist, and amoxipine, clozapine (5) and methiothepin (8) acted as antagonists. Interestingly, furthermore, 5-HT<sub>6</sub> receptors regulate cholinergic and glutamatergic neuronal activity (rather than dopaminergic) and have been proposed as regulators of feeding, cognition, emotion, learning, and memory. Initial in vivo experiments showed that administration of antisense oligonucleotides (AOs), directed at 5-HT<sub>6</sub> receptor mRNA, elicited a behavioral syndrome in rats consisting of yawning, stretching, and chewing, which could be dose dependently blocked by the muscarinic antagonist atropine. This study implies that 5-HT<sub>6</sub> receptors modulate cholinergic
neurotransmission and, hence, that 5-HT\textsubscript{6} receptor antagonists may be useful for the treatment of memory dysfunction. In addition, treatment with AOs significantly inhibited the increase in 5-HT release from the prefrontal cortex produced by conditioned fear stress, suggesting that 5-HT\textsubscript{6} receptors may be involved in certain anxiety disorders.\textsuperscript{85}

\textbf{3.7 5-HT\textsubscript{6}-selective agents:}

Ligands displaying selectivity for 5-HT\textsubscript{6} receptors have become available in increasing numbers only very recently. With an intent to understand structure-affinity relationships (SAFIR), our laboratory developed some of the very first ligands and has continued to identify analogs based on tryptamine as a design template that display a high degree of binding affinity and selectivity for h5-HT\textsubscript{6} receptors. It was not until 1998 that the first 5-HT\textsubscript{6}-selective antagonists were described: Ro 04-6790 (3b) and Ro 63-0563 (3a) (Figure 5) (h5-HT\textsubscript{6} K\textsubscript{i} = 55 and 12 nM, respectively).\textsuperscript{86} This prompted others to quickly report their efforts in this area. Shortly thereafter, compounds (10) (Figure 5),\textsuperscript{87} and MS-245 (14a) (Figure 7)\textsuperscript{88} were described. Interestingly, although they represented independent discoveries, all were identified by random screening methods and all four possessed a sulfonamide moiety. The first nonsulfonamide showing 5-HT\textsubscript{6} antagonist character was 5-methoxy-2-phenyl-N,N-dimethyltryptamine (PMDT; 15).\textsuperscript{88} Compound 3b displayed >100-fold selectivity for [\textsuperscript{3}H]-LSD-labeled 5-HT\textsubscript{6} receptors over other 5-HT receptors and lacked measurable affinity for 23 other receptor populations, whereas 3a showed >100-fold selectivity over a total of 69 other receptor/binding sites. Both behaved as competitive antagonists of 5-HT-induced cAMP accumulation (pA\textsubscript{2} = 6.75 and 7.10, respectively) and lacked agonist or inverse agonist actions. One problem associated with some of these early antagonists was their low penetration of the CNS.\textsuperscript{89}
Particularly germane to the present discussion is the binding of tryptamine derivatives at 5-HT\(_6\) receptors. What follows is an historical narrative of their development. Various indolealkylamines, including the tryptamines, bind with high affinity at 5-HT\(_6\) receptors. However, these analogs typically bind at different populations of 5-HT receptors with little selectivity.\(^{91}\) It has been demonstrated, however, that with appropriate molecular modification tryptamine derivatives can be developed that display enhanced selectivity for different populations of 5-HT receptors.\(^{91}\) This prompted investigation by exploring the structure-affinity relationships for the binding of tryptamines at 5-HT\(_6\) receptors. In 1999 Glennon et al.\(^{92}\) (pioneer of the first indole-based structures, starting from the endogenous ligand 5-HT) reported the results of such an investigation. A summary of the results is shown in Figure 6.\(^{78}\) It was found that \(O\)-methylation of 5-HT (1: \(K_i = 75\) nM) to 5-methoxytryptamine (9, \(K_i = 88\) nM) had little effect on affinity and that removal of the hydroxyl group to give tryptamine (12: \(K_i = 180\) nM) only halved affinity.\(^{92}\) Most other changes led to significant decreases in affinity. For example, lengthening the alkyl chain by one methylene unit, conformational restriction of the side chain as a 1,2,3,4-tetrahydropyrido[3,4-b]indole, replacement of the indolic nitrogen atom with an sp\(^3\)-hybridized carbon atom, and quaternization of the terminal amine all resulted in a dramatic reduction in affinity (i.e., \(K_i > 5000\) nM). On the other hand, \(N\)-monomethylation and \(N,N\)-dimethylation resulted in retention or a slight increase in affinity.\(^{88}\)
Figure 6. Influence of structural modification of the binding of 5-HT and simple tryptamines at 5-HT<sub>6</sub> receptors.  
1. Small alkyl substituents (e.g., methyl, ethyl, n-propyl) at the N<sub>1</sub>-position dramatically decrease affinity but N<sub>1</sub>-benzyl and N-SO<sub>2</sub>Ph enhance affinity.  
2. A methyl group is tolerated at the 2-position, as an ethyl group and a phenyl group.  
3. An ergoline-like extended conformation seems preferred for the binding of N<sub>1</sub>-unsubstituted tryptamines, and introduction of an α-methyl group decreases affinity.  
4. N-monomethyl and N,N-dimethyl substitution slightly increase affinity, N,N-diethyl substitution is tolerated, N,N-di-n-propyl substitution decreases affinity, and quaternization dramatically decreases affinity.  
5. Replacement of the 5-hydroxy group of 5-HT with –H halves affinity, whereas the O-methyl and O-benzyl ethers retain affinity. Of the four possible methoxy derivatives, affinity decreases as 5>4>6>7, and replacement of 5-OCH<sub>3</sub> by 5-SCH<sub>3</sub> enhances affinity.  
6. Conversion to an indane abolishes affinity. N<sub>1</sub>-Benzenesulfonyltryptamine derivatives do not necessarily follow this same SAFIR.

One of the more interesting findings from this study was that 5-HT<sub>6</sub> receptors tolerated an indolic 2-methyl substituent. Until that time, 2-methyl 5-HT (11) had been considered a 5-HT<sub>3</sub>-selective agonist. Compound 11 was shown to bind at 5-HT<sub>6</sub> receptors (K<sub>i</sub> = 46 nM) with 20 times the affinity it displayed for 5-HT<sub>3</sub> receptors. Taking advantage of the finding that 5-HT<sub>3</sub> receptors do not tolerate a 5-methoxy group on the tryptamine ring, 11 was O-methylated to afford a more selective agent (i.e., 5-methoxy-2-methyltryptamine; 5-HT<sub>6</sub> K<sub>i</sub> = 80 nM and 5-HT<sub>3</sub> K<sub>i</sub> >10,000 nM). Subsequent studies showed that other substituents were tolerated at the 2-position, including an ethyl group (EMDT; K<sub>i</sub> = 16 nM; agonist) and a phenyl group (PMDT; K<sub>i</sub> = 20 nM; antagonist). During the synthesis of some of these analogs, N<sub>1</sub>-benzenesulfonyl derivatives were prepared to protect the indolic nitrogen atom as the 2-position substituents were being elaborated. Random
screening of one of these intermediates, BS/5-OMe DMT (MS-245, 14a; $K_i = 2.3$) (Figure 7), revealed that it displayed high affinity for 5-HT$_6$ receptors, and a higher affinity than that of 5-HT (1; $K_i = 78$ nM) itself, although it had been shown that substitution at the tryptamine N$_1$-position by small alkyl groups resulted in decreased affinity.$^{88,92}$ Subsequently 14a was shown to act as a 5-HT$_6$ receptor antagonist ($pA_2 = 8.88$).$^{93}$

In a continued effort to identify selective ligands for 5-HT$_6$ receptors, other selective (sulfonamide-type) ligands were later reported. These included ALX1175 (17) and ALX1161 (18), discovered by the research team of Allelix/NPS Pharmaceuticals during a collaboration with Glennon.$^{94}$ Compound 16 (SB-271046) (Figure 5) was obtained from further SAR studies with 10; it was shown to be a potent, selective, and orally bioavailable 5-HT$_6$ receptor antagonist.$^{87}$
Figure 7. Some representative tryptamine-related 5-HT$_6$ receptor ligands.

Further studies were done to investigate the role of the benzenesulfonyl group. Specifically, the benzenesulfonyl –SO$_2$– moiety of 14a was replaced by a methylene group, a carbonyl group (i.e., 21 and 22 respectively), or was eliminated (i.e., 20) to determine its necessity for, and impact on, 5-HT$_6$ receptor binding. Several other aryl-substituted and indole-substituted analogs were also investigated.\textsuperscript{95} The binding of 19 and 14a at 5-HT$_6$ receptors (Table 2) was re-examined and their affinities ($K_i$ = 77 nM and 2.1 nM, respectively) were nearly identical with what had been previously reported.\textsuperscript{88,93} The affinity of the N$_1$-benzyl analog 21 ($K_i$ = 6.5 nM) was about a third that of 14a, whereas the affinities of the benzoyl derivative 22 ($K_i$ = 18 nM) and N$_1$-phenyl derivative 20 ($K_i$ = 33 nM) were about 10- to 15-fold lower than 14a. Nevertheless, all three compounds (i,e., 20, 21, 22) displayed higher affinity than their N$_1$-unsubstituted parent 19. Although optimal among the substituents examined, it would seem that the benzenesulfonyl –SO$_2$– moiety is not a requirement for binding, and that the benzenesulfonyl group of 14a can be replaced by a benzyl, benzoyl, or phenyl group.\textsuperscript{95}
Table 2. 5-HT$_6$ receptor affinities of some simple N$_1$-substituted derivatives.$^{95}$

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>$R_1$</th>
<th>$K_i$, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>-H</td>
<td>77</td>
</tr>
<tr>
<td>-SO$_2$Phenyl</td>
<td>2.1</td>
</tr>
<tr>
<td>-Phenyl</td>
<td>33</td>
</tr>
<tr>
<td>-CH$_2$Phenyl</td>
<td>6.5</td>
</tr>
<tr>
<td>-C(=O)Phenyl</td>
<td>18</td>
</tr>
</tbody>
</table>

Introduction (Table 3) of an electron withdrawing 4-chloro group (i.e., 23) or electron-donating methoxy substituent (i.e., 24 and 25) had little effect on 5-HT$_6$ receptor affinity. Replacement of the N$_1$-benzenesulfonyl group with sterically larger 2-naphthalenesulfonyl (i.e., 26) or 1-naphthalenesulfonyl (i.e., 27) groups also had little effect. The affinities of these derivatives were not more than four times greater than, or less than, that of 14a.$^{93}$
Table 3. 5-HT₆ serotonin receptor binding of N₁-(arylsulfonyl)-N,N-dimethyltryptamine derivatives.⁹³

<table>
<thead>
<tr>
<th>R</th>
<th>Kᵢ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a Ph</td>
<td>2.3</td>
</tr>
<tr>
<td>23 4-Cl Ph</td>
<td>3.1</td>
</tr>
<tr>
<td>24 4-OMe Ph</td>
<td>8.0</td>
</tr>
<tr>
<td>25 2, 5-diOMe Ph</td>
<td>1.3</td>
</tr>
<tr>
<td>26 2-Naphthyl</td>
<td>1.6</td>
</tr>
<tr>
<td>27 1-Naphthyl</td>
<td>0.9</td>
</tr>
</tbody>
</table>

The conformationally constrained tryptamine 28 (Kᵢ = 168 nM) (Figure 8) binds with modest affinity at 5-HT₆ receptors.⁹² Here too, incorporation of an N₁-benzenesulfonyl substituent resulted in a dramatic increase in affinity (29; Kᵢ = 1.5 nM).⁹⁵ Interestingly, unlike what was seen in the tryptamine series, the N₁-benzyl analogue 30 (Kᵢ = 136 nM) did not display enhanced affinity.⁹⁵
Figure 8. Representative 5-HT₆ receptor ligands.

Replacement of the 5-methoxy group of 14a (Kᵢ = 2.3 nM) with hydrogen had little impact on affinity (i.e., 31a; Kᵢ = 4.1 nM) (Figure 8) and O-demethylation to the hydroxyl analog 32 (Kᵢ = 28 nM) decreased affinity by about 10-fold. Russell et al. later reported similar results for 31a (Kᵢ = 2.9 nM). Apparently, the 5-methoxy group is not a major contributor to binding. Further pharmacophoric studies have revealed that the benzenesulfonyl moiety of 31a can be replaced by a benzyl group (33a; Kᵢ = 6 nM) with retention of antagonist action at 5-HT₆ receptors. Pullagurla et al., in a further investigation, examined closely the terminal amine portion of 14a (Table 4), and the aminoethyl group in particular (Figure 9), to determine their influence on 5-HT₆ receptor binding. With respect to the terminal amine, the secondary amine 34 (Kᵢ = 23 nM) was
found to bind with 10-fold lower affinity than its parent, 14a. The \(N,N\)-dimethyl substituents of 14a could be homologated to \(N,N\)-diethyl (35; \(K_i = 6.2\) nM) with little effect on affinity; however, incorporation of a bulkier benzyl group, as in 36 (\(K_i = 43\) nM), decreased affinity by about 20-fold.\(^9\)

**Table 4.** 5-HT\(_6\) receptor binding of benzenesulfonyltryptamine analogs.\(^9\)

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>R</th>
<th>R'</th>
<th>Z</th>
<th>(K_i) (nM)</th>
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</thead>
<tbody>
<tr>
<td>14a</td>
<td>–OCH(_3)</td>
<td>–CH(_3)</td>
<td>–CH(_3)</td>
<td>–H</td>
<td>2.3</td>
</tr>
<tr>
<td>34</td>
<td>–OCH(_3)</td>
<td>–CH(_3)</td>
<td>–H</td>
<td>–H</td>
<td>23</td>
</tr>
<tr>
<td>35</td>
<td>–OCH(_3)</td>
<td>–C(_2)H(_5)</td>
<td>–C(_2)H(_5)</td>
<td>–H</td>
<td>6.2</td>
</tr>
<tr>
<td>36</td>
<td>–OCH(_3)</td>
<td>–CH(_3)</td>
<td>–CH(_2)Ph</td>
<td>–H</td>
<td>43</td>
</tr>
<tr>
<td>39</td>
<td>–OCH(_3)</td>
<td>–CH(_3)</td>
<td>–CH(_3)</td>
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</tr>
<tr>
<td>40</td>
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<td>–C(_2)H(_5)</td>
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<td>230</td>
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<td>–C(_2)H(_5)</td>
<td>–NH(_2)</td>
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</tr>
<tr>
<td>42</td>
<td>–H</td>
<td>–CH(_3)</td>
<td>–CH(_3)</td>
<td>–NHAc</td>
<td>27</td>
</tr>
<tr>
<td>31b</td>
<td>–H</td>
<td>–CH(_3)</td>
<td>–CH(_3)</td>
<td>–NH(_2)</td>
<td>0.8</td>
</tr>
<tr>
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<td>–C(_2)H(_5)</td>
<td>–NHAc</td>
<td>90</td>
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<tr>
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<td>–H</td>
<td>–C(_2)H(_5)</td>
<td>–C(_2)H(_5)</td>
<td>–NH(_2)</td>
<td>0.6</td>
</tr>
<tr>
<td>45</td>
<td>–OCH(_3)</td>
<td>–CH(_3)</td>
<td>–CH(_2)Ph</td>
<td>–NH(_2)</td>
<td>3.0</td>
</tr>
<tr>
<td>46</td>
<td>–OCH(_3)</td>
<td>–CH(_3)</td>
<td>–CH(_3)</td>
<td>–NHAc</td>
<td>34</td>
</tr>
</tbody>
</table>
The alkyl chain separating the terminal amine from the indole nucleus was shortened by a methylene group. Typically, such chain shortening in tryptamine analogs is not well tolerated by serotonin receptors\(^{62}\). However in this investigation, the chain-shortened analog 37a \((K_i = 3.1 \text{ nM})\) retained high affinity as compared to its tryptamine counterpart 31a.

![Chemical Structures](image.jpg)

**Figure 9.** Structures of \(N_1\)-benzenesulfonylgramines 37 and \(N_1\)-benzenesulfonylskatol- \es 38.\(^{96}\)

The obvious question at this point was whether or not the amine was required for binding. Removal of the amine of 37a would result in skatole analog 38a, a compound that lacks aqueous solubility. Hence, in order to examine the need for an aminooalkyl group at the indole 3-position, it was necessary to locate a position on the molecule that would tolerate a solubilizing group. An amino group was selected because a water-soluble salt could be formed. Several aryl amine derivatives of 14a, 31a, and 35 were examined to determine if the amine would be tolerated. Compound 39 \((K_i = 2.0 \text{ nM})\), the 4'-amino analog of 14a, was found to bind with the same affinity as its parent, 14a.
Likewise, the affinities of amino analogs 41, 31b, and 44 ($K_i = 0.6, 0.8,$ and 0.6 nM, respectively) are also quite high and indicate that an amino group is tolerated at this position.

In compound 45 ($K_i = 3.0$ nM), the presence of the amino group actually increased affinity by nearly 15-fold relative to its parent, 36. Furthermore, the affinity of 31b and 44 again indicate that the presence of a 5-methoxy group is not required for binding. The lower affinities of the corresponding acetamido derivatives 40, 42, 43, and 46 show that affinity is not directly related simply to the presence of an NH substituent on the aryl ring. As a further test of the tolerance of the $para$ amino substituent, compound 37b was examined. The affinity of 37b ($K_i = 6.9$ nM) was similar to that of 37a.\textsuperscript{96}

On the basis that a $para$ amino group is tolerated by 5-HT$_6$ receptors, compound 38b was examined. Compound 38b ($K_i = 12$ nM) retained affinity for 5-HT$_6$ receptors. Even though its affinity is about 3-fold lower than tryptamine derivative 31a, it, like 37, represented a novel type of 5-HT$_6$ receptors ligand.

Various tryptamines lacking an N$_1$ substituent generally bind with reduced affinity and, like 5-hydroxytryptamine (i.e., serotonin; $K_i$ ca. 100 nM), can display agonist character.\textsuperscript{62} On the basis of comparative structure-affinity and receptor modeling studies,\textsuperscript{93,98} Abate et al.\textsuperscript{99} proposed that tryptamine analogs, although likely utilizing a common aspartate moiety, bind at 5-HT$_6$ receptors differently depending upon whether or not they possess an N$_1$ (e.g., a benzenesulfonyl or benzyl) substituent. If the presence or absence of an N$_1$ substituent dictates how tryptamine analogs interact with 5-HT$_6$ receptors, it was reasoned that chiral analogs of N$_1$-unsubstituted tryptamines might bind differently than N$_1$-substituted tryptamines and that isomeric comparisons could prove to be informative.
In an extreme case, optical isomers of $N_1$-substituted tryptamines might even display opposite enantioselectivity for binding, as compared to their corresponding $N_1$-unsubstituted counterparts. Introduction of substituents $\alpha$ to the terminal amine of tryptamine analogs (e.g. an $\alpha$-methyl or $\alpha$-ethyl group) to create a chiral center has been shown to result in somewhat reduced $5-HT_6$ receptor affinity. Nevertheless, it was thought that reduced affinity might be an acceptable trade-off if it allowed enantiomeric potency comparisons to be made. Rather than examining isomers of $\alpha$-methyl- or $\alpha$-ethyltryptamines, however, Abate et al. opted to examine their cognate pyrrolidinylmethylindole counterparts. That is, because necessary optically active configurationally-defined starting materials were readily available, and because pyrrolidinylmethylindoles had been previously shown to bind at other (i.e., $5-HT_1$) serotonin receptor populations, compounds 47-50 were targeted for examination. Isomers of 47 (where $R = H$ or Me) were prepared for binding comparisons with their $N_1$-unsubstituted analogs 48. To evaluate this hypothesis further, isomers of isotryptamine 49 were compared with their ($C_3$) unsubstituted counterparts 50. The binding data are shown in Table 5.
**Table 5.** Binding affinities for h5-HT$_6$ receptor ligands.$^{99}$

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R'</th>
<th>Isomer</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48a</td>
<td>H</td>
<td>H</td>
<td>S(+)</td>
<td>2400</td>
</tr>
<tr>
<td>48b</td>
<td>H</td>
<td>H</td>
<td>R(–)</td>
<td>60</td>
</tr>
<tr>
<td>47a</td>
<td>H</td>
<td>SO$_2$Ph</td>
<td>S(+)</td>
<td>46</td>
</tr>
<tr>
<td>47b</td>
<td>H</td>
<td>SO$_2$Ph</td>
<td>R(–)</td>
<td>7.8</td>
</tr>
<tr>
<td>48c</td>
<td>Me</td>
<td>H</td>
<td>S(–)</td>
<td>640</td>
</tr>
<tr>
<td>48d</td>
<td>Me</td>
<td>H</td>
<td>R(+)</td>
<td>9.1</td>
</tr>
<tr>
<td>47c</td>
<td>Me</td>
<td>SO$_2$Ph</td>
<td>S(–)</td>
<td>1.7</td>
</tr>
<tr>
<td>47d</td>
<td>Me</td>
<td>SO$_2$Ph</td>
<td>R(+)</td>
<td>0.3</td>
</tr>
<tr>
<td>50a</td>
<td>–</td>
<td>H</td>
<td>S(+)</td>
<td>8750</td>
</tr>
<tr>
<td>50b</td>
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<td>H</td>
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<td>49a</td>
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<tr>
<td>49b</td>
<td>–</td>
<td>CH$_2$Ph</td>
<td>R(–)</td>
<td>9.9</td>
</tr>
<tr>
<td>51</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>32</td>
</tr>
</tbody>
</table>
As with structurally simpler \(N,N\)-dialkyltryptamines,\(^\text{62}\) introduction of an \(N_1\)-benzenesulfonfyl moiety led to enhanced affinity (comparing pyrrolinylmethylnindoles \(47a\) with \(48a\), \(47b\) with \(48b\), \(47c\) with \(48c\), and \(47d\) with \(48d\)) (Table 5). Likewise, in the isotryptamine series, benzyl analogs \(49a\) and \(49b\) displayed affinity higher than those of their parents, \(50a\) and \(50b\), respectively. In fact, the pyrrolidinylmethylnindoles bind with affinities higher than those of their simpler \(N,N\)-dimethyltryptamine counterparts; for example, compound \(47d\) binds with more than 10 times the affinity of \(31a\) and \(49b\) binds with 3 times the affinity of \(51\). Furthermore, it was shown that \(R\)-pyrrolidinylmethylnindoles bind with higher affinity than their \(S\)-enantiomers and that \(47b\) (\(K_i = 0.3\) nM) was found to bind with nearly 10-fold higher affinity than MS-245 (\(14a\)). Compound \(47d\) was described to be an antagonist, and found to bind at 5-HT\(_6\) receptors with a \(K_i <10\) nM.\(^\text{101}\) Although these results could not be used as evidence for different modes of binding, they were not inconsistent with the concept that \(N_1\)-substituted tryptamine-related analogs might bind differently than their \(N_1\)-unsubstituted counterparts. These results further identified \(R\)-pyrrolidinylmethylnindole analogs of MS-245 (\(14a\)) as binding with higher affinity than their \(S\)-enantiomers.\(^\text{99}\)

In continuing efforts by Kolanos et al.\(^\text{102}\) to identify a binding pharmacophore for MS-245-type compounds at h5-HT\(_6\) receptors, a question of interest was whether an indolic \(N_1\) nitrogen atom is required for high-affinity binding of tryptamine-related compounds. Two strategies were employed to address this issue. First, because isotryptamines have been shown to mimic tryptamines at certain 5-HT receptors,\(^\text{103,104}\) an isotryptamine analog of \(21\) (i.e., \(51\)) was examined. Second, the indole nitrogen atom of \(21\) was replaced by an \(sp^2\)-hybridized carbon atom to afford indene analog \(52\). It was reasoned
that the sp$^2$-hybridized carbon atom to which the ‘benzyl’ substituent would be attached, both in the isotryptamine and indene series, might mimic the electronic character of the indole nitrogen atom of 21. Because 38b retains affinity for 5-HT$_6$ receptors, abbreviated structures such as 53 were also evaluated.

![Chemical structures](image)

**Figure 10.** Some representative atypical 5-HT$_6$ receptor ligands.
Isotryptamines 51 ($K_i = 32$ nM) and 54 ($K_i = 50$ nM) were found to bind with high affinity, but with lower affinity than N1-benzyltryptamine 21 ($K_i = 6.5$ nM). Four explanations are possible for this somewhat lower affinity: (a) compounds 51 and 54 lack a 5-methoxy group, (b) the double bond is in the ‘wrong’ position, (c) relocation of the ring nitrogen atom is not well tolerated, and/or (d) the tryptamine N1-nitrogen atom is optimal for binding. The des-methoxy analog of 21 (i.e., 33a, $K_i = 6$ nM) retained affinity arguing that the methoxy group is not a major contributor to binding. Benzylindene 52 ($K_i = 57$ nM), which lacks the indole nitrogen atom, was found to bind with an affinity similar to that of 51 suggesting that an N1 indolic nitrogen atom might not be required. However, with 52 there is an additional complicating factor. Although the presence of the sp$^2$-hybridized ring carbon atom of 52 might electronically mimic the tryptamine nitrogen atom, it also imposes conformational constraint that could ‘lock’ the substituent into a conformation that is not particularly favored for binding. Consequently, benzylindene 55 was examined. The enhanced affinity of 55 ($K_i = 3.0$ nM) indicated that sp$^2$-hybridized ring carbon atom is not required for binding. Indeed racemic 55 (Figure 10) binds with an affinity between that of MS-245 (14a) and N1-benzyltryptamine 21. These results suggest that neither the tryptamine N1-nitrogen atom, nor an sp$^2$-hybridized carbon atom, is essential at this position for binding to 5-HT$_6$ receptors.

Apparently, an intact tryptamine moiety is not required for the binding of 39; that is, the abbreviated tryptamine 38b ($K_i = 12$ nM) binds with only 6-fold reduced affinity. The abbreviated isotryptamine 56 ($K_i = 2530$ nM), however, was found to bind with 50-fold lower affinity than isotryptamine 54 ($K_i = 50$ nM). Indene 53 ($K_i = 640$ nM) also binds with low affinity; again, this might be partly attributable to conformational constraint.
imposed by the exocyclic double bond. But, its reduced counterpart 1-(4-aminobenzyl)indene (57, \( K_i = 4470 \) nM) binds with even lower affinity. These results indicate that in the abbreviated series the indolic nitrogen atom or benzenesulfonyl group of 38b might contribute to binding. The low affinity of 3-(4-aminobenzyl)indene (58, \( K_i = 11,800 \) nM) provides evidence that the location of ring unsaturation can also influence affinity. Furthermore, the nearly 10-fold higher affinity of 59 (\( K_i = 740 \) nM) (the indane counterpart of 61) relative to 60 (\( K_i = 6100 \) nM) argues that in the abbreviated series, the benzenesulfonyl moiety might contribute to binding.\(^{102}\)

In summary then, arylsulfonyltryptamines, such as the benzenesulfonyltryptamine MS-245 (14a) and compound 31a, were developed in our laboratories as some of the first examples of 5-HT\(_6\) receptor antagonists. Structure-activity and structure-affinity studies have been conducted to identify what structural features of such compounds influence functional activity and receptor affinity. Today, the arylsulfonyltryptamines are considered a prototypical example of 5-HT\(_6\) receptor antagonists.
III. Specific aims and rationale

As discussed earlier in the historical narrative, simple tryptamine derivatives bind at 5-HT$_6$ receptors with modest affinity and ordinarily do so with little to no selectivity.\textsuperscript{91} We have also discussed that introduction of an N$_1$-arylsulfonyl substituent to the tryptamine nucleus can result in substantially (10- to > 100-fold) enhanced affinity. For example, the affinity of 5-OMe DMT (19; $K_i = 16$ nM) is enhanced upon introduction of an N$_1$-benzenesulfonyl group (MS-245, 14a, $K_i = 2.1$ nM), as is that of carbazole 28 ($K_i = 168$ nM) to 29 ($K_i = 1.5$ nM).\textsuperscript{93,95,103} Furthermore, these benzenesulfonyl-substituted tryptamine analogs (e.g., 14a and 29) behaved as 5-HT$_6$ receptor antagonists.\textsuperscript{93,95} On the basis of radioligand binding and modeling studies of the receptor, it has been suggested that simple N$_1$-unsubstituted tryptamines might bind differently at 5-HT$_6$ receptors.\textsuperscript{93,98} Kolanos et al.\textsuperscript{105} empirically addressed this issue.

It is commonly thought, when two series of agents are binding in a similar manner, that parallel structural changes between the two series will typically result in parallel affinity shifts. In this investigation Kolanos et al.\textsuperscript{105} compared the h5-HT$_6$ receptor affinities of 28 (i.e., 14 pairs of) N$_1$-unsubstituted tryptamines and their N$_1$-benzenesulfonyl-substituted counterparts to determine if such is the case. If the affinities of the two series parallel one another, this might be taken as evidence that two series are binding somewhat similarly at the 5-HT$_6$ receptors. A summary of the binding data is shown in Table 6.\textsuperscript{105}
Table 6. Affinities of some ligands at h5-HT₆ receptors.¹⁰⁵

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>h5-HT₆</th>
<th>Kᵢ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b: Z = H</td>
<td>a: Z = SO₂Ph</td>
</tr>
<tr>
<td>31</td>
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<td>Me</td>
<td>Me</td>
<td>H</td>
<td></td>
<td>4.1</td>
</tr>
<tr>
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<td>Et</td>
<td>Et</td>
<td>H</td>
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<td>-</td>
</tr>
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<td>Et</td>
<td>H</td>
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<td>14</td>
</tr>
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<td>-</td>
</tr>
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<td>Bn</td>
<td>H</td>
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<td>Et</td>
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<td>H</td>
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<td>14</td>
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</tr>
<tr>
<td>75</td>
<td>6-OMe</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>76</td>
<td>7-OMe</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
<td>19600</td>
<td>-</td>
</tr>
<tr>
<td>77</td>
<td>7-OMe</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
<td></td>
<td>138</td>
</tr>
<tr>
<td>82</td>
<td>H</td>
<td>Me</td>
<td>Me</td>
<td>H</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>

* This is compound 19 (Kᵢ = 13 nM).
In the comparative analysis utilizing 14 pairs of tryptamines, it was found that there was little correspondence ($r^2 = 0.201$; Figure 11) between the 5-HT$_6$ receptor affinities of the examined pairs. The results of this investigation (on compounds with $K_i$ values spanning a $>10,000$-fold range) support the prior suggestion$^{93}$ that $N_1$-unsubstituted and $N_1$-benzenesulfonyl-substituted tryptamines are probably binding (i.e., orienting) in a dissimilar fashion upon interaction with 5-HT$_6$ receptors.$^{105}$

![Figure 11](image.png)

**Figure 11.** Relationship between the 5-HT$_6$ receptor affinities ($pK_i$ values) of 14 $N_1$-unsubstituted tryptamines and their corresponding $N_1$-benzenesulfonyl counterparts ($r^2 = 0.201$).$^{105}$

As already discussed is that the $N_1$-arylsulfonyltryptamine MS-245 (14a; $K_i = 2.1$ nM) binds with a similar affinity as its des-methoxy counterpart 31a ($K_i = 4.1$ nM). Despite structure-affinity studies by our group,$^78$ and others,$^{98,106}$ a number of questions remain unanswered. For example, a structural feature common to the arylsulfonyltryptamines is an “aryl” moiety. Yet it has not been established that an aryl group is essential for binding. That is, if the aryl group binds at the receptor via a hydrophobic type of
interaction, its replacement by an alkyl group of similar or greater hydrophobicity could result in retention of affinity. Another feature that has not been fully explored is the necessity of the sulfonyl group. It is generally thought that the sulfonamide portion of MS-245 (14a) and 31a is important for binding; yet, replacement of the sulfonyl group of MS-245 (14a) by a methylene group (i.e., 21) has little impact on 5-HT<sub>6</sub> receptor affinity (see Table 2).

![Chemical structures](image)

14a, R = OCH<sub>3</sub>  
31a, R = H

21

33a, R = H

This would argue that the SO<sub>2</sub> moiety of MS-245 (14a) plays little, if any, role in binding. However, very few examples of N<sub>1</sub>-benzyltryptamines, such as 21 (K<sub>i</sub> = 6.5 nM), have been examined. This has prompted us to pose the following questions: (1) Is an aryl moiety required for high affinity binding at 5-HT<sub>6</sub> receptors? (2) Do benzyltryptamines, as a class, bind at 5-HT<sub>6</sub> receptors (i.e., is the sulfonyl moiety important for binding)?
A. Specific aims

1. To determine if the sulfonyl (–SO$_2$–) and aryl moieties of arylsulfonyltryptamines are required for high affinity binding at h5-HT$_6$ receptors.

2. To determine whether the N$_1$-benzenesulfonyl- and their corresponding N$_1$-benzyltryptamine counterparts bind in a similar fashion.

B. Hypotheses

1. If the aryl moiety interacts with receptor features via a hydrophobic interaction, it should be possible to replace this moiety with other hydrophobic groups.

2. If the sulfonyl moiety of arylsulfonyltryptamines is not required for binding, it should be possible to replace this group with a methylene group with retention of affinity.

3. If benzenesulfonyl derivatives (i.e., derivatives of 31) and benzyl derivatives (i.e., derivatives of 33) bind in a similar manner, then parallel structural changes should result in parallel affinity shifts.

C. Approach

To determine if the aryl moiety of 31 is required for binding, it is proposed to prepare a series of N$_1$-alkylsulfonyltryptamine analogs 78 for comparison with 31. The affinities of various 78 analogs will be examined where the $\pi$ values of the substituents range from 1.3 to 4.0. (see Table 7).
Table 7. Lipophilicities ($\pi$ values) of substituents to be examined.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Lipophilicity $(\pi$ value)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$i$Propyl</td>
<td>1.30</td>
</tr>
<tr>
<td>$n$Propyl</td>
<td>1.50</td>
</tr>
<tr>
<td>$n$Butyl</td>
<td>2.00</td>
</tr>
<tr>
<td>$n$Amyl</td>
<td>2.50</td>
</tr>
<tr>
<td>$n$Octyl</td>
<td>4.00</td>
</tr>
<tr>
<td>Cyclohexyl</td>
<td>2.51</td>
</tr>
</tbody>
</table>

$^a$ $\pi$ values are from Topliss$^{107}$ relative to $\pi = 2.13$ for phenyl.
Very few N₁-benzyltryptamines have been examined for their affinity at 5-HT₆ receptors. Those few that have been investigated (i.e., 21 and 33a, $K_i = 6.5$ nM and 6.0 nM, respectively) display an affinity comparable to their N₁-benzenesulfonyl counterparts (i.e., 14a and 31a, $K_i = 2.1$ nM and 4.1 nM, respectively) and suggest that a sulfonyl group might not be important for binding. We propose to examine an extended series of N₁-benzyltryptamine analogs 33 where R is varied. If the sulfonyl moiety is not required for binding, analogs 33 should display affinity comparable to their benzenesulfonyl counterparts 31. Several 33 analogs were selected for synthesis and evaluation. In some cases, their corresponding benzenesulfonyl counterparts had already been prepared and examined in our laboratory. In those instances where this was not the case, the required benzenesulfonyl analogs 31 also will be prepared and evaluated.

One of the goals of this work is to prepare a series of N₁-benzyl- and N₁-benzenesulfonyltryptamine analogs for comparison so that the necessity of the sulfonyl function can be determined. If the sulfonyl moiety can be replaced by a methylene group, this will indicate that the sulfonyl group is not essential for binding. Furthermore, if the sulfonyl group is not required, the benzyltryptamines 33 should bind with an affinity similar to their benzenesulfonyl counterparts 31, and there should be a significant correlation between the affinities of the two series. Indeed, if such a correlation exists, this would indicate that the two series are binding in a similar manner. That is, parallel substituent changes in two series of compounds that result in parallel affinity shifts will suggest a common mode of binding.⁹⁵
Finally, by examining the effect of substituted benzyl substituents on 5-HT₆ receptor affinity, it should be possible to determine how (e.g., electronic, lipophilic, steric) substituents contribute to 5-HT₆ receptor affinity.

**D. QSAR (Hansch relationships)**

The concept of QSAR was pioneered by Hansch and co-workers in the 1960s and, hence, is referred to as a Hansch relationship. The QSAR approach attempts to identify and quantify the physicochemical properties of a drug and to see whether one or more physicochemical properties of an agent has an effect on the drug’s biological activity. In a broader perspective, QSAR is defined by physicochemical properties (steric, electronic, lipophilic, and H-bonding), intrinsic properties (MWt, volume, and surface area), and biological properties (IC₅₀, EC₅₀, Kᵢ, and efficacy). Thus one of the applications of QSAR is lead compound optimization. What are these physicochemical features which we have mentioned? Essentially they refer to any structural, physical, or chemical property of a drug; as mentioned, most commonly studied are lipophilic, electronic, and steric properties. This is because it is possible to quantify these effects relatively easily. Thus, should the benzyltryptamines bind at 5-HT₆ receptors, QSAR studies will be performed to identify how various substituents influence affinity.

**E. Selection of benzyl substituents**

A very common problem in drug design is to find the optimum substitution on a benzene ring or on the benzenoid portion of a fused ring system in an active lead compound for maximization of drug potency. Since there are many possible substituents
and several different ring positions, the number of possible compounds to consider containing up to say two substituents is very large. Thus it would be highly advantageous to determine at an early stage which of these compounds might really be worth synthesizing. Historically, approaches to this problem have been rather haphazard, depending for the most part on the particular experience and intuition of the medicinal chemist involved and the relative availability of the starting materials required for synthesis. However, in the 1960s and 1970s the development of Hansch’s methods for structure-activity correlations brought about a more rational approach and the ability to generate quantitative structure-activity relationships (QSAR) within analog series. Commonly used property descriptors are a substituent hydrophobicity constant, $\pi$, a measure of a substituent’s hydrophobicity relative to hydrogen, the Hammett substituent constant, $\sigma$, a measure of the overall electron withdrawing or donating characteristics of a substituent, and Taft’s steric factor, $E_s$. Thus, a limited group of substituents which will give a good discrimination between $\sigma$, $\pi$, and $E_s$ can be selected (Figure 12) and an initial group of compounds synthesized. This procedure is suitable when the compounds are relatively easy to synthesize and a considerable lag time is encountered in obtaining activity data. However, it is less satisfactory under circumstances where synthesis is more difficult and test results are more readily forthcoming.
Figure 12. The Craig plot.\textsuperscript{112}

Figure 13. Topliss scheme for aromatic substituents.\textsuperscript{112}
In the context of the foregoing discussion the Topliss operational scheme for aromatic substitution may be considered (Figure 13). The assumption is made that the starting compound is the unsubstituted phenyl compound and that its biological activity has been measured. Since many systems are \( +\pi \) dependent, i.e., activity increases with increasing \( \pi \) values, the \( p \)-chloro analog is a good first choice, particularly since the ease of synthesis, relative to other substituted phenyl compounds, is generally favorable. For the purposes of this analysis the potency of the 4-chloro compound can be classified as greater than, equal to, or less than the activity of the parent compound. If the potency is increased, this can be attributed most probably to a \( +\pi \) effect, or a \( +\sigma \) effect (activity increases with increasing \( \sigma \) values), or to the combination of \( +\pi \) and \( +\sigma \). In this event the 3,4-dichloro compound would be selected for synthesis next since this would result in both larger \( +\pi \) and \( +\sigma \) values when summed for the two substituents. Moving to the point where the compound has been synthesized and tested, the compound can be classified as more potent, equipotent, or less potent than the 4-chloro analog. If potency did increase, then the 3-CF\(_3\), 4-Cl analog would be the next choice for synthesis since both \( \Sigma\pi \) and \( \Sigma\sigma \) would be larger.\(^{107}\)

If the 4-chloro compound was found to be equipotent with the parent compound this could result from a favorable \( +\pi \) effect offset by an unfavorable \( -\sigma \) dependency. If this is the case then the 4-methyl analog should show enhanced potency since this substituent is lipophilic and electron releasing, i.e., \( +\pi -\sigma \) type substituent. Assuming a favorable result the next selection would be the 4-C(CH\(_3\))\(_3\) compound with increased \( +\pi \) and \( -\sigma \) values. In the situation where the 4-CH\(_3\) compound is equipotent or less potent than the 4-Cl compound, it would seem reasonable to assume either an unfavorable steric effect from
the para substitution or a $-\pi$ effect. Since $-\pi$ effects, i.e., decreasing activity with increasing $\pi$ values are uncommon, the steric explanation seems more probable, hence the next move is to 3-Cl in the operational scheme.$^{107}$

The remaining segment of the scheme is concerned with the synthetic sequence to be followed where the 4-chloro analog was found to be significantly less potent than the parent compound. One may conclude that either there is an unfavorable effect from any kind of 4-substitution for steric reasons, or activity is $-\sigma$ or $-\pi$ controlled. Assuming that the $-\sigma$ effect is the most probable explanation, the 4-methoxy compound ought to be favorable selection for synthesis based on the electron releasing property of this substituent and its marginally hydrophilic character. Confirmation of this in the form of increased activity for the 4-OCH$_3$ compound would lead to the next choice of the 4-N(CH$_3$)$_2$ analog where there would be an even greater $-\sigma$ effect. A further trend in the right direction would prompt the synthesis of the 3-CH$_3$, 4-N(CH$_3$)$_2$ compound where the $-\sigma$ effect would be further reinforced. Further details on the continuation of the tree are contained in the original publication.$^{107}$

Ordinarily, the Topliss scheme is employed in an attempt to optimize a particular pharmacological activity. That was not the intent here. Rather, the scheme and the Craig Plot were use as guides for substituent selection so that representative substituents (i.e., substituents with different electronic and hydrophobic character) could be incorporated into the target benzyltryptamines. Selection of diverse substituents would also aid subsequent QSAR studies by insuring they didn’t have similar physicochemical properties. Hence, specific compounds targeted for initial synthesis and evaluation
included 33b-f for comparison with 33a. Also targeted were compounds 33g and 33h (i.e., 33 where R = 3-chloro and 3,4-dichloro, respectively).
IV. Results and Discussion

A. Synthesis

1. Synthesis of \(N, N\)-dimethyltryptamine (DMT)

The synthesis of all proposed compounds from the previous section was carried out starting from the compound DMT (82). DMT was prepared by the Speeter-Anthony reaction (Scheme 1).\(^{113}\) According to the reported procedure, a solution of indole (79) (in Et\(_2\)O/THF) was allowed to react with oxalyl chloride at 0 \(^\circ\)C and then at room temperature to yield compound 80, followed by addition of dimethylamine (aqueous) to 80, yielding the intermediate 3-indolylglyoxylamide 81. Intermediate 81 was reduced with lithium aluminium hydride (LiAlH\(_4\)) to afford DMT (82)

\[
\text{Scheme 1. a) Oxalyl chloride/anhydrous Et}_2\text{O/THF, 0 }^\circ\text{C; b) HN(CH}_3\text{)}_2, \text{rt; c) i. LiAlH}_4/\text{anhydrous THF, reflux; ii. NaOH, THF (hot).}
\]
2. Synthesis of N\textsubscript{1}-benzyltryptamines

Starting from \textit{N},\textit{N}-dimethyltryptamine (DMT) (82), two methods were used to generate the indolyl anion of compound 82. In one of the procedures, the anion of compound 82 was generated by reaction with NaH at 100 °C under anhydrous conditions (Scheme 2). In this reaction a mixture of DMT (82) and NaH was heated until evolution of H\textsubscript{2} gas ceased. The resultant product was then dissolved in anhydrous DMF resulting in a solution at 0 °C. This was treated with the appropriately substituted benzyl halide to afford the target compounds: analogs of 33 (33c-f, and 33h). Compound 33a had been previously reported and was available in our laboratory. Compound 33g was synthesized adopting the procedure\textsuperscript{114} where the DMT (82) anion was generated using \textit{t}-BuOK in anhydrous THF at room temperature and then allowed to react with the corresponding benzyl halide (Scheme 2). This latter procedure for generation of the indolyl anion of compound 82 was adopted in an effort to improve yields of target compounds; low yields of target compounds were obtained when the former procedure\textsuperscript{95} was used. Attempts to prepare amine analog 33b using a similar approach (i.e., utilizing a 4-nitro- or 4-acetamido-substituted benzyl halide) were unsuccessful. Subsequently, compound 33b was prepared via adopting the procedure\textsuperscript{116} shown in Scheme 3. In this synthetic procedure, the first step was to reduce the 4-nitro group of 83 to an amino group. Tin (II) chloride was added to a solution of 83 and then the reaction mixture was heated at reflux for 1h. After cooling, the solution was made basic (pH 8-9) and then extracted with EtOAc. The organic portion was used in the next reaction without further purification. The next step was to protect the 4-amino group of 84. Benzyl bromide was added to a suspension of the resultant compound in CH\textsubscript{2}Cl\textsubscript{2} and triethylamine, and the
mixture was allowed to stir for 2h followed by purification. The resultant compound 85 was allowed to react with phosphorus oxychloride in dry DMF in a Vilsmeier type reaction to afford 86. Ammonium acetate and MeCN were added to 86 yielding nitro olefin derivative 87. The final steps involved reduction of the nitro olefin side chain followed by dimethylation and finally deprotection of the 4-amino group to yield the target compound 33b. Compounds 33c-h were isolated as their oxalate salts whereas compound 33b was isolated as an HCl salt.

Scheme 2. a) i. NaH, DMF, 100 °C, ii. ArCH$_2$Cl, rt; b) i. t-BuOK, 18-crown-6, THF, rt; ii. 3-chlorobenzyl chloride.
3. Synthesis of $N_1$-benzenesulfonyltryptamines

Conditions used to synthesize the $N_1$-benzyltryptamines were adopted to synthesize this series of compounds. With the exception of compounds 31a$^{95}$ and 31b$^{96}$ which had been previously reported in our laboratory, compounds 31c-f were synthesized according to the procedure where the anion of DMT (82) was generated using $t$-BuOK in anhydrous THF at room temperature and then allowed to react with the appropriate benzenesulfonyl chloride (Scheme 4). The reaction mixture was allowed to stir overnight, then extracted with EtOAc. The extracts were washed (brine) and dried (Na$_2$SO$_4$), followed by evaporating the solvent and then the residue was purified to yield the target compounds (31c-f); these compounds were isolated as their oxalate salts.
Scheme 4. a) \( t \)-BuOK, 18-crown-6, THF; ii. \( \text{ArSO}_2\text{Cl} \), rt.

4. Synthesis of alkylsulfonyltryptamines

The two procedures used to synthesize the previous compounds were adopted to synthesize the alkylsulfonyltryptamines (Scheme 5). Two of the compounds (i.e., \( 78\text{d} \) and \( 78\text{f} \)) employed the procedure where the DMT (82) anion was generated using NaH at 100 °C in anhydrous conditions. This was followed by allowing the resultant anion to react with their appropriate alkylsulfonyl halides at room temperature. The reaction mixture was then extracted with EtOAc and the extracts were washed (brine) and dried (\( \text{Na}_2\text{SO}_4 \)). The solvent was evaporated and the residue purified to yield the target compound. On the other hand, compounds \( 78\text{a-c} \), and \( 78\text{e} \) were obtained by alkylation with the appropriate alkylsulfonyl halides following the generation of the DMT anion using \( t \)-BuOK in anhydrous THF at room temperature. In separate reactions, for each compound, the reaction mixture was extracted with EtOAc and the extracts were washed.
(brine) and dried (Na$_2$SO$_4$). The solvent was removed and the residue purified to yield the target compounds. The target compounds 78a-f were isolated as their oxalate salts.

![Diagram of Scheme 5]

**Scheme 5.** a) i. NaH, DMF, 100 °C, ii. ArCH$_2$Cl, rt; b) i. $t$-BuOK, 18-crown-6, THF, rt; ii. ArCH$_2$Cl, rt.

Suitable commercial alkylsulfonyl halides for compounds 78d and 78f were not available and, hence, required synthesis in our laboratory (Scheme 6$^{117}$ and Scheme 7$^{118}$ for n-amylsulfonyl chloride (90) and cyclohexylsulfonyl chloride (93), respectively). Synthesis of amylsulfonyl chloride was achieved by heating under reflux, amyl sodium sulfate (88) in acetone with cyanuric chloride (89) for 20h. The reaction mixture was cooled and filtered through a celite pad. The solvent was evaporated from the filtrate then
followed by distillation yielding 90. On the other hand, synthesis of cyclohexylsulfonyl chloride (93) was achieved by bubbling chlorine gas through a solution of cyclohexylthiol (92) in slightly diluted acetic acid maintained at 10-15 °C for 3h. Excess chlorine was removed and the resultant mixture was extracted with methylene chloride. The methylene chloride extract was washed (NaOH) and then dried (Na₂SO₄). The solvent was removed and then the residue was purified by distillation to yield 93.

Scheme 6. a) 18-crown-6, acetone, reflux.

Scheme 7. a) CH₃COOH, H₂O, Cl₂, rt.
B. Radioligand Binding Studies

Binding studies were conducted by the laboratory of Dr. Bryan Roth (Case Western University). In brief, h5-HT$_6$ receptor cDNA was transiently expressed in HEK-293 cells. All obtained target compounds were assayed for binding at h5-HT$_6$ receptors with $[^3]$H]LSD used as the radioligand, and $K_i$ values were determined. The $K_i$ values are the result of triplicate determinations. The binding data for compounds are shown in Tables 8 and Table 9.

Binding affinities for the alkylsulfonyl compounds 78a-78e ranged from $K_i = 135$ nM for 78c to $K_i = 590$ nM for 78a ($n$-butyl and $i$-propyl, respectively) (Table 8). This is a 30- to 150-fold lower affinity than that displayed by simple benzenesulfonyltryptamine 31a ($K_i = 4.1$ nM). The results show that an arylsulfonyl group at the tryptamine N$_1$-position is preferred, relative to an alkylsulfonyl group, for 5-HT$_6$ receptor affinity. We had proposed that if the aryl moiety interacts with receptor features via a hydrophobic interaction, it should be possible to replace this moiety with other hydrophobic groups. The alkyl moieties were all hydrophobic; on the contrary, we obtained lower binding affinities than expected. Also examined was cyclohexyl analog 78f, which is simply a reduced version of 31a, and with even higher hydrophobic character ($\pi = 2.51$) relative to phenyl ($\pi = 2.13$).$^{121}$ Compound 78f was found to bind with 50-fold lower affinity than 31a itself. The results suggest that the aryl moiety is optimal for binding, and that the interaction of this substituent with the receptor cannot be accounted for simply on the basis of hydrophobicity of the sulfonyl substituent.
Table 8. 5-HT\textsubscript{6} receptor binding affinities of alkylsulfonyltryptamines 78.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>(K_i), nM (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>78a</td>
<td>-SO\textsubscript{2}-\textit{i}-Propyl</td>
<td>590 (110)</td>
</tr>
<tr>
<td>78b</td>
<td>-SO\textsubscript{2}-\textit{n}-Propyl</td>
<td>280 (40)</td>
</tr>
<tr>
<td>78c</td>
<td>-SO\textsubscript{2}-\textit{n}-Butyl</td>
<td>135 (20)</td>
</tr>
<tr>
<td>78d</td>
<td>-SO\textsubscript{2}-\textit{n}-Amyl</td>
<td>220 (30)</td>
</tr>
<tr>
<td>78e</td>
<td>-SO\textsubscript{2}-\textit{n}-Octyl</td>
<td>440 (65)</td>
</tr>
<tr>
<td>78f</td>
<td>-SO\textsubscript{2}-Cyclohexyl</td>
<td>210 (30)</td>
</tr>
</tbody>
</table>

The hydrophobicity of a compound can be quantified by using the partition coefficient (\(P\)). Partition coefficients can be estimated by knowing the contribution that various substituents make to hydrophobicity. This contribution is known as the substituent hydrophobicity constant (\(\pi\)). The substituent hydrophobicity constant is a measure of how hydrophobic a substituent is, relative to hydrogen. The value can be obtained as follows. Partition coefficients are measured experimentally for a standard compound with and without a substituent (\(X\)). The hydrophobicity constant (\(\pi_X\)) for the substituent (\(X\)) is then obtained using the following equation.

\[
\pi = \log P_X - \log P_H
\]

Where \(P_H\) is the partition coefficient for the standard compound, and \(P_X\) is the partition coefficient for the standard compound with the substituent. A positive value of \(\pi\)
indicates that the substituent is more hydrophobic than hydrogen. A negative value indicates that the substituent is less hydrophobic.\textsuperscript{119}

Apart from the fact that the constants would be different, the two factors have different emphases. The partition coefficient P is a measure of the drug’s overall hydrophobicity and therefore an important measure of how efficiently a drug is transported to its target site and bound to its receptor. The $\pi$ factor is a measure of the hydrophobicity of a specific region on the drug’s skeleton. Thus, any hydrophobic bonding to a receptor involving that region will be more significant to the equation than the overall transport process. If the substituent is involved in hydrophobic binding to a receptor, then the QSAR equation using the $\pi$ factor will emphasize that contribution to biological activity more dramatically than the equation using P.\textsuperscript{119} A phenyl group, such as that found in \textbf{31a} has a $\pi$ value of 2.13.\textsuperscript{121} The alkyl substituents used in the present investigation (i.e., those found in \textbf{78a}-\textbf{78f}) possess $\pi$ values of 1.3 to 4.0 (see \textbf{Table 7}). If the N$_1$ substituent of the N$_1$-substituted tryptamines interacts with some receptor feature via a hydrophobic-type interaction, it should have been possible to replace the phenyl group with a similarly hydrophobic group with retention of affinity. The n-butyl and amyl analogs \textbf{78c} and \textbf{78d}, respectively possess N$_1$-substituents that are nearly as lipophilic as a phenyl group, yet their 5-HT$_6$ receptor affinities are substantially reduced relative to \textbf{31a}. The lipophilicity of the cyclohexyl analog (i.e, \textbf{78f}) is also comparable to that of a phenyl group and it, too, binds with reduced affinity. The n-octyl compound bears a group that is even more lipophilic than a phenyl group, and it might have been expected that the affinity of \textbf{78e} would have been higher than that of \textbf{31a} had the receptor interaction involved a simple
hydrophobic interaction. Yet, a plot of pKᵢ versus Pi (π) for the seven compounds 78a-78f and 31a reveals a poor correlation (r = 0.084) (Figure 14).

![Graph showing poor correlation between pKᵢ and Pi.]

**Figure 14.** Relationship between the affinities of 78a-78f and 31a versus π.

It might be noted that the more hydrophobic n-octyl group is longer than a phenyl group and reduced affinity could be rationalized by a less than optimal shape/length of the octyl substituent. In an attempt to relate binding affinities of the alkyl substituents to their steric effects we plotted pKᵢ versus Eₕ. N-Methylsulfonyltryptamine has been previously reported to bind with Kᵢ = 620 nM. We observed that increasing the size of the carbon-chain in a branched manner (i.e., i-propyl analog 78a) had little effect on affinity. However, the slightly longer n-propyl analog 78b improved binding affinity 2-fold (78b; Kᵢ = 280 nM). Increased chain length might result in further enhancement of affinity. However, the n-butyl and amyl analogs demonstrated comparable binding affinities (i.e., Kᵢ = 135 nM and Kᵢ = 220 nM respectively). A plot of pKᵢ versus Eₕ for the seven compounds 78a-78f and 31a shows this relationship (Figure 15). The results
suggest a high correlation between the binding affinity and bulkiness (size and shape) of the substituents \((r = 0.947)\). Since the alkyl side chains are conformationally flexible with rotation possible about the C-C bonds, it is not possible to determine the exact importance of chain length. Nevertheless, it seems likely that this is an important factor to the binding affinity at 5-HT\(_6\) receptors. However, we observe (Figure 15) that the data points of alphatic substituents are clustered around one area away from the data point of the phenyl substituent. The points typically appear like “two” data points. It is generally obvious that a line of best fit between two points would give a perfect correlation. Hence, this begs for further investigation and verification of this possible relationship. A feasible approach would be to select substituents with \(E_s\) values between the highest \(E_s\) value of the alphatic substituents already tested \((E_s = -0.79)\), and phenyl \((E_s = -2.58)\). Such substituents are for example \(t\)-butyl \((E_s = -0.93)\), \(t\)-butyl \((E_s = -1.54)\), and \(\text{Et}_2\text{CH} (-1.98)\). If the data points of affinity versus \(E_s\) of these substituents lie within an acceptable range on the line of best fit without greatly disrupting the significant relationship \((r = 0.947)\), then it would strongly suggest that there might be a correlation between the steric effect and affinity (i.e. increasing steric effect increases affinity).
To further describe the SAR of alkyl substituents, we considered the influence of electronic effects. The Hammett substituent constant (σ) is perhaps the most widely used electronic index in QSAR studies of drugs. Hammett correlations express quantitatively the relationship between chemical reactivity and the electron-donating or -accepting nature of a substituent. The Hammett substituent constant (σ) was originally defined for the purpose of quantifying the effect of a substituent on the dissociation constant of benzoic acid:

$$\sigma_X = \log \frac{K_X}{K_H} = \log K_X - \log K_H$$

Where $K_X$ is the dissociation constant of benzoic acid carrying substituent $X$; $K_H$ is the dissociation constant of unsubstituted benzoic acid.

Electron attracting substituents (such as $-\text{CO}_2\text{H}$, $-\text{NO}_2$, $\text{NR}_3^+$) have a positive σ value, while electron donating substituents ($-\text{OH}$, $-\text{OCH}_3$, $-\text{NH}_2$, $-\text{CH}_3$) have a negative σ. The
value of $\sigma$ also varies according to whether the substituent is in the meta- or para-position of an aromatic ring. Ortho substituents are subject to too many interferences and are not used in calculating $\sigma^{122}$.

In terms of electronic effect, alkyl substituents ranging from $-i$-propyl to $-octyl$ do not differ much and have similar $\sigma$ values (-0.13 to -0.16) (Table 9). It is observed from Table 9 that the phenyl substituent ($\sigma = -0.01$) is electron deficient with little electron donating effect compared to moderate electron donating effect demonstrated by alkyl substituents. If increasing electronegativity of $N_1$-substituted tryptamines would be expected to enhance binding affinity at h5-HT6 receptors, then $N_1$-alkylsulfonyltryptamines 78 should demonstrate a higher binding affinity than 31a. However, we do not observe this relationship. Thus, the electron donating ability of the $N_1$-alkyl substituents is probably not involved in the binding of alkylsulfonyltryptamines. The higher affinity of 31a over that of the alkyl derivatives suggests that the slightly greater electron deficient nature of the phenyl substituent might account for this effect.

Table 9. Electronegativities ($\sigma$ values) of alkyl substituents.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Electronegativity ($\sigma$ value)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$i$-Propyl</td>
<td>-0.15</td>
</tr>
<tr>
<td>$n$-Propyl</td>
<td>-0.13</td>
</tr>
<tr>
<td>$n$-Butyl</td>
<td>-0.16</td>
</tr>
<tr>
<td>$n$-Amyl</td>
<td>-0.15</td>
</tr>
<tr>
<td>$n$-Octyl</td>
<td>-0.16</td>
</tr>
<tr>
<td>Cyclohexyl</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

$^b$ $\sigma$ values are from Hansch et al.$^{121}$ relative to $\sigma = -0.01$ for phenyl.
Overall, then, the various N₁-alkylsulfonyltryptamines bind at 5-HT₆ receptors with modest affinity and with affinities substantially lower than that of 31a. Furthermore, their affinities which vary over only a very small range, are seemingly unaccounted for by the lipophilic, steric, or electronic character of their N₁ substituents.

Following the aforementioned conclusion, we went a step further to determine if the sulfonyl moiety of the arylsulfonyltryptamines is required for binding at h5-HT₆ receptors. López-Rodríguez et al.¹²⁰ have proposed a pharmacophore model for 5-HT₆ receptors suggesting that a sulfonyl or a sulfonamide moiety is a common pharmacophoric element of most ligands that interact with amino acids in the transmembrane domain of the 5-HT₆ receptors. However, as has already been mentioned, our laboratory had previously shown that the unsubstituted benzenesulfonyl analog 31a and its unsubstituted benzyl counterpart 33a bind with comparable affinities at h5-HT₆ receptors (Kᵢ = 4.1 nM and Kᵢ = 6.0 nM) respectively.⁹³,⁹⁵ Thus, while a sulfonyl moiety might be important for binding, its presence certainly is not required. However, the affinities of a series of benzenesulfonyl analogs, and their corresponding benzyl analogs, have never been compared.

To further investigate this concept, we proposed that if the sulfonyl moiety of arylsulfonyltryptamines is not required for binding, it should be possible to replace this group with a methylene group with retention of affinity. Furthermore, if the two series (i.e., benzenesulfonyl and benzyl) bind in the same manner, the two series should show comparable binding affinities. In light of this proposal, a small series of N₁-benzyl analogs 33 was prepared and examined (Table 10). Substituents selected for evaluation
included several electron donating and electron withdrawing groups; as mentioned earlier we employed the Topliss scheme\textsuperscript{107} as a guide for substituent selection.

**Table 10.** \(5\)-HT\(_6\) receptor binding affinities of benzenesulfonfyltryptamines 31 relative to their benzyl counterparts 33.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>R</th>
<th>X = SO\textsubscript{2} (31)</th>
<th>X = CH\textsubscript{2} (33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>H</td>
<td>4.1\textsuperscript{b}</td>
</tr>
<tr>
<td>b</td>
<td>4-NH\textsubscript{2}</td>
<td>0.8 (0.4)\textsuperscript{c}</td>
</tr>
<tr>
<td>c</td>
<td>4-OMe</td>
<td>13.0 (3)</td>
</tr>
<tr>
<td>d</td>
<td>4-Me</td>
<td>2.5 (0.6)</td>
</tr>
<tr>
<td>e</td>
<td>4-CF\textsubscript{3}</td>
<td>1.9 (0.4)</td>
</tr>
<tr>
<td>f</td>
<td>4-Cl</td>
<td>94 (25)</td>
</tr>
<tr>
<td>g</td>
<td>3-Cl</td>
<td>–</td>
</tr>
<tr>
<td>h</td>
<td>3,4 diCl</td>
<td>–</td>
</tr>
</tbody>
</table>

\textsuperscript{a} S.E.M are not shown for previously reported binding data.

\textsuperscript{b} Binding data previously reported from our laboratories\textsuperscript{78,96}

\textsuperscript{c} See Table 4.
Initially we prepared \(33f\) and \(33c\). Compound \(33f\) is the 4-chloro substituted benzyl analog; a chloro substituent is considered hydrophobic (+\(\pi\)) and electron withdrawing (+\(\sigma\)); details of the Hammett constant (\(\sigma\)) will be discussed later in this section. In contrast \(33c\) bears an electron donating methoxy group (-\(\sigma\), +\(\pi\)). As proposed by the Topliss scheme, a high affinity for the chloro analog might be attributed most probably to a +\(\pi\) effect, or +\(\sigma\) effect, or the combination of +\(\pi\) and +\(\sigma\). In this event the 3,4-dichloro compound would be selected for synthesis. Because chloro analog \(33f\) (\(K_i = 20\) nM) possessed higher affinity than \(33c\) (\(K_i = 132\) nM), we prepared the 3,4-dichloro benzyl analog \(33h\). Compound \(33h\) was found to bind with high affinity (\(K_i = 50\) nM); but, this was a slight drop in affinity relative to \(33f\) and contrary to what might be expected. This observation prompted us to prepare and examine the 3-chloro benzyl analog \(33g\). Interestingly, compound \(33g\) (\(K_i = 34\) nM) was found to bind with similar binding affinity as \(33f\) and \(33h\); a significant change in affinity was not demonstrated. However, since substituents at the 3-position might demonstrate rotameric binding it is confounding to explain what influence the 3-chloro substituent has on the binding of \(N_1\)-benzyl analogs at \(h5-HT_6\) receptors. Therefore, on examination of the aforementioned series of \(N_1\)-benzyl analogs, only 4-substituted benzyl analogs were examined to reduce any potential complications in data interpretation that might arise from possible rotameric binding or untoward effects associated with 3-position substituents. \(5-HT_6\) receptor affinities of the target compounds ranged from 20 nM to >400 nM (Table 10). But, none of the benzyl analogs retained the affinity of the unsubstituted benzyl analog \(33a\) (\(K_i = 6.0\) nM).
One explanation for the reduced affinity of 33b-f, compared with 33a, is that the receptor does not tolerate substituents on the benzylic nucleus. This seems unlikely because certain analogs of 14a bearing benzenesulfonyl substituents have been previously shown to bind with affinities comparable to that of 14a itself. However, with regard to these latter compounds, each possesses a methoxy group on the indolic ring. In order to make a more strict comparison, the benzenesulfonyl counterparts of 33c-f (i.e., 31c-f) were prepared and examined. The results (Table 10) show that 5-HT₆ receptors tolerate aryl substituents, and that the 4-amino, 4-methyl, and 4-trifluoromethyl analogs 31b, 31d, 31e (Kᵢ = 0.8 nM, 2.5 nM, and 1.9 nM, respectively) bind at least as well as their unsubstituted parent 31a. The results indicate that even though N₁-benzyl-substituted analogs bind at 5-HT₆ receptors, they bind with reduced affinity relative to their corresponding N₁-benzenesulfonyl counterparts.

If benzenesulfonyl derivatives (i.e., of 31) and benzyl derivatives (i.e., derivatives of 33) bind in a similar manner, then parallel structural changes should result in parallel affinity shifts. To test this hypothesis, we conducted a correlation analysis between the two series of compounds (i.e., derivatives of 31 and derivatives of 33) to determine whether their binding affinity data might show a linear relationship. Statistical analysis was performed using the GraphPad Prism program. A comparison of the affinities of the series 33 compounds with the series 31 compounds (r = -0.224; n = 6) shows little correspondence between the two (Figure 16). The calculated coefficient of determination, r² = 0.050 (i.e., variance), describes further that one of the 2 variables explains only 5% of the variation in the other variable. Consequently, these results
suggest that the two series of compounds bind in a dissimilar fashion or at least that parallel substituent changes result in non-parallel shifts in 5-HT$_6$ receptor affinity.

![Graph](image)

**Figure 16.** Relationship between the affinities of N$_1$-benzyl- and N$_1$-benzenesulfonyltryptamine analogs 33 and 31, respectively.

On the examination of the effect of substituted benzyl and benzenesulfonyl substituents on 5-HT$_6$ affinity, we have demonstrated dissimilarity in binding affinities. That is, substituent alteration results in different binding affinities for the two series. Since the substituents exhibit varying physicochemical properties (electronic, lipophilic, and steric), this begs the question of what influence these properties might have on the binding of 31 and 33 at 5-HT$_6$ receptors. In an effort to answer this question, the inherent relationship (if any) between these physicochemical properties and the binding at 5-HT$_6$ receptors was investigated in a further QSAR study. For this purpose, the influence of the parameters in Table 11 were examined.
Table 11. Summary of the physicochemical parameters employed.\textsuperscript{121,123}

<table>
<thead>
<tr>
<th>Substituent</th>
<th>$\sigma$</th>
<th>$\pi$</th>
<th>$E_s$</th>
<th>L (Å)</th>
<th>W(Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.06</td>
<td>1.00</td>
</tr>
<tr>
<td>NH$_2$</td>
<td>-0.66</td>
<td>-1.23</td>
<td>-0.61</td>
<td>2.93</td>
<td>1.50</td>
</tr>
<tr>
<td>OMe</td>
<td>-0.27</td>
<td>-0.02</td>
<td>-0.55</td>
<td>3.98</td>
<td>1.35</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>-0.17</td>
<td>0.56</td>
<td>-1.24</td>
<td>3.00</td>
<td>1.52</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>0.54</td>
<td>0.88</td>
<td>-2.4</td>
<td>3.30</td>
<td>1.98</td>
</tr>
<tr>
<td>Cl</td>
<td>0.23</td>
<td>0.71</td>
<td>-0.97</td>
<td>3.52</td>
<td>1.80</td>
</tr>
</tbody>
</table>

First considered was molecular “bulk” as an important factor in structure-activity relationships.\textsuperscript{122} Steric substituent constants ($E_s$) derived from Taft’s model, and STERIMOL parameters were considered in the present analyses. In order for a drug to interact with an enzyme or a receptor, it has to approach, and then interact with a binding site. The bulk, size, and shape of the drug may influence this process. For example, a bulky substituent may act like a shield and hinder the ideal interaction between drug and receptor. Alternatively, a bulky substituent may help to orientate a drug properly for maximum receptor binding and increase activity. Quantifying steric properties is more difficult than quantifying hydrophobic or electronic properties.\textsuperscript{109} Several methods have been tried and two are described here:

Taft’s steric ($E_s$) constants were the first widely used parameters for examining steric substituent effects in drug design.\textsuperscript{123} They were developed in studies of organic reaction mechanisms of hydrolysis of aliphatic esters as correlation parameters for intramolecular effects of substituents of nearby reaction centers (Figure 17). However, already indicated
by Taft and further substantiated by Charton,\textsuperscript{124} there is a linear correlation between $E_s$ constants and (minimum) radii of spherical and symmetrical substituents, so that evidently $E_s$ is a steric constant independent of electronic effects. This was further developed by Kutter and Hansch,\textsuperscript{125} who derived a linear regression equation linking $E_s$ and the (average) radii of several substituents that had not been determined experimentally. For nonsymmetrical substituents, these calculations were based either on minimum radii ($\text{NH}_2$, $\text{OCH}_3$) or on two different radii that resulted in two $E_s$ values ($\text{NO}_2$).

\[
\begin{align*}
E_s &= \log k_{\text{XCH}_2\text{COO}Me} - \log k_{\text{CH}_3\text{COO}Me} = \log \frac{k_x}{k_y}
\end{align*}
\]

**Figure 17.** Ester hydrolysis reaction and equation used to define the Taft steric parameter, $E_s$.\textsuperscript{123}

Another approach to measuring the steric factor involves a computer program called STERIMOL which calculates steric substituent values (Verloop steric parameters) from standard bond angles, van der Waals radii, bond lengths, and possible conformations for the substituent.\textsuperscript{123} This computer program has been devised to simulate the building of molecules or molecular groups from the well-known Corey-Pauling-Koltun (C.P.K.) atomic models. The partial bond lengths (covalent radii) and the bond angles used for defining the bond vectors of the various atom types in the program have been taken from the C.P.K. catalog as issued by The Ealing Corporation in the United States. The STERIMOL program is capable of giving different types of output with respect to the
shape of molecules and substituents. It is concluded that a length parameter $L$ for the substituents should be used. The minimum width was chosen as the initial width parameter $B_1$, and from this parameter, three additional width parameters $B_2$, $B_3$, and $B_4$ were derived in such a way that the four $B$ parameters would measure the width in four rectangular directions. Unlike $E_s$, the Verloop steric parameter can be measured for any substituent. For example, the Verloop steric parameter for carboxylic acid group are demonstrated in Figure 18. $L$ is the length of the substituent while $B_1$-$B_4$ are the radii of the group.

![Figure 18. Schematic representation of dimensions of the benzoic acid substituent.](image)

Considering the influence of steric factors on the binding of 31 and 33 derivatives at 5-HT$_6$ receptors, correlation analysis plots relating N$_1$-benzyl analogs (Figure 19) and N$_1$-benzenesulfonyl analogs (Figure 20) to $E_s$ are shown below.
Figure 19. Relationship between the affinities of N1-benzyltryptamine analogs 33 versus the $E_s$.

![Graph](image)

Figure 20. Relationship between the affinities of N1-benzenesulfonyltryptamine analogs 31 versus $E_s$.

![Graph](image)

A modest correlation ($r = 0.756$) is demonstrated between the benzyltryptamines and influence of steric effect on the binding at 5-HT$_6$ receptors (Figure 19). Compounds with large substituents show low affinity, whereas the compound with the smallest substituent...
has the highest affinity. These results suggest that the steric effect has an unfavorable influence on the binding affinities of benzyltryptamines at serotonin h5-HT₆ receptors. However, these observations could be coincidental and other possible parameters such hydrophobic and/or electronic interactions could be at play. On the other hand, the same substituents demonstrate a weaker correlation \( r = -0.158 \) with the binding of benzenesulfonyl analogs (Figure 20). These results suggest that the steric effects of the 4-position substituents on the benzenesulfonyltryptamine analogs demonstrate no significant relationship to the binding affinities.

In a relationship between the affinity of N₁-benzyltryptamine analogs and \( \sigma \) (electronic substituent constant), there is a weak correlation \( r = -0.270 \); Figure 21). Similarly, that of benzenesulfonyltryptamine analogs versus \( \sigma \) also showed a weak correlation \( r = -0.352 \); Figure 22). The results suggest that the binding affinities of the two series of compounds cannot be accounted for solely by the electronic effect of their 4-position substituents.

![Figure 21](image_url). Relationship between the affinities of N₁-benzyltryptamine analogs 33 versus \( \sigma \).
Figure 22. Relationship between the affinities of N$_1$-benzenesulfonyltryptamine analogs versus $\sigma$.

Comparison of the two series of compounds with $\pi$ is shown in Figure 23 and 24. There was no significant relationship between pK$_i$ (N$_1$-benzyltryptamine analogs) and $\pi$ ($r = -0.200$; Figure 23), whereas the relationship between pK$_i$ (N$_1$-benzenesulfonyltryptamine analogs) and $\pi$ showed a weak correlation ($r = -0.470$; Figure 24). Evidently, these results do not reveal a clear correlation of substituent lipophilicities with binding affinities. That is, increasing lipophilicity is not accompanied by increased binding affinities in either series.
Finally, we considered relationships between binding affinities and the Verloop parameters (i.e., length and width). A similar, moderate correlation ($r = -0.655$) (Figures 25, and 26 respectively) was obtained for the binding affinity of N$_1$-benzyltryptamine
analogs and length (L) and width (W). These results suggest that the binding affinities of the N1-benzyltryptamine analogs might be partially attributable to the length and breadth of the substituents at the 4-position of the phenyl ring.

**Figure 25.** Relationship between the affinities of N1-benzyltryptamine analogs 33 versus length.

**Figure 26.** Relationship between the affinities of N1-benzyltryptamine analogs 33 versus width.
On the other hand, a correlation between binding affinities of the N<sub>1</sub>-benzenesulfonyltryptamine analogs versus length, and width yielded weak correlations ($r = -0.447$; **Figure 27**), and ($r = -0.122$; **Figure 28**), respectively. No clear relationship is demonstrated between length or width and binding the affinities of the N<sub>1</sub>-benzenesulfonyltryptamine analogs. Thus, the directional parameters of the substituents might not contribute significantly to the changes in the binding affinities of N<sub>1</sub>-benzenesulfonyltryptamine analogs at 5-HT<sub>6</sub> receptors.

**Figure 27.** Relationship between the affinities of N<sub>1</sub>-benzenesulfonyltryptamine analogs versus Length.
Overall, none of the individual parameters examined (i.e., steric, electronic, lipophilic or Verloop) seemed to account for the binding of N$_1$-benzyl- or N$_1$-benzenesulfonyltryptamines at 5-HT$_6$ receptors. As already mentioned, sample size was small. Furthermore, the range of affinities is limited. There are some indications that inclusion of more than one parameter in attempted relating equations might afford more meaningful results. However, the small number of compounds available at this time does not allow examination of multiple parameters. Additional analogs will need to be prepared and examined in order to search for statistically significant relationships using multiple parameters.
V. Conclusions

N$_1$-Arylsulfonyltryptamines represent a class of 5-HT$_6$ receptor antagonists developed in our laboratories. One of the goals of the present investigation was to determine if the sulfonyl and aryl moieties of arylsulfonyltryptamines are required for high affinity binding at h5-HT$_6$ receptors. A series of N$_1$-alkylsulfonyl-, N$_1$-benzyl-, and N$_1$-benzenesulfonyltryptamine analogs was prepared and examined.

A general finding of the present investigation is that replacement of the benzenesulfonyl group of MS-245-like (i.e., 14a-like or 31a-like) benzenesulfonyltryptamines with an alkylsulfonyl group results in diminished affinity for h5-HT$_6$ receptors. The alkylsulfonyl derivatives 78 differed with respect to chain length, shape, and hydrophobicity, but none retained the affinity of the simplest MS-245-like compound 31a. No clear correlation between the binding affinities of these compounds and their physicochemical properties was identified, but, it would seem that π-π interactions better account for the binding of N$_1$-substituted tryptamines than do simple hydrophobic interactions because of the higher affinity of the arylsulfonyl derivatives.

Other goals were to determine if N$_1$-benzyltryptamines bind with high affinity and to determine whether the N$_1$-benzenesulfonyl- and their corresponding N$_1$-benzyltryptamine counterparts bind in a similar fashion. Upon comparison of their binding affinities, the N$_1$-benzyl-substituted compounds 33 were found to bind at 5-HT$_6$ receptors but typically
did so with affinities somewhat lower than their benzenesulfonyl counterparts 31. Evidence suggests that the two series (i.e., 31 and 33) are probably binding differently and, because the only structural difference between the two series is a sulfonfonyl versus methylene group, it would seem that the sulfonfonyl group determines the manner of binding. That is, the presence of the sulfonfonyl group results in a somewhat higher affinity. The phenyl-SO$_2$-N bond angle of $N$-benzenesulfonylpyrrole (ca. 105-106°)\(^{126}\) is only slightly less than the bond angle of a tetrahedral carbon atom. Furthermore, the N$_1$-S bond length (ca. 1.6-1.7 Å) found in such compounds is only slightly longer than the N$_1$-C bond length (ca. 1.5 Å) of N$_1$-benzylindoles.\(^{126-128}\) So, it is unlikely that geometry plays a substantial role in the affinity differences observed between the benzenesulfonyltryptamines and their benzyltryptamine counterparts. Thus, although it cannot be concluded that the sulfonfonyl group is essential for binding, it would appear that its presence is optimal when similarly-substituted pairs of compounds are examined, and that the oxygen atoms might form an anchoring interaction with some receptor-associated feature. The results also suggest, if the two series are binding differently, that structure-affinity findings from the benzenesulfonyl series cannot be extrapolated to the benzyl series. Nevertheless, because N$_1$-benzyltryptamines bind at 5-HT$_6$ receptors, additional studies will be required to optimize their affinity.

The overall findings of the present investigation are as follows: a) N$_1$-alkylsulfonyltryptamines bind at h5-HT$_6$ receptors with modest affinity lower than that of N$_1$-arylsulfonyltryptamines, b) the affinities of the N$_1$-alkylsulfonyltryptamines cannot be explained simply on the basis of their hydrophobicity or shape, c) N$_1$-benzyltryptamines bind at human 5-HT$_6$ receptors, but do so with somewhat lower affinity than their
corresponding N₁-benzenesulfonyltryptamines, d) there is no correlation between the affinities of the N₁-benzyl- and N₁-benzenesulfonyltryptamines suggesting that they might bind in a different manner, e) the affinities of the N₁-benzyl- and N₁-benzesulfonyltryptamines could not be readily explained on the basis of the hydrophobic, size, shape, or electronic character of their N₁-substituents. It is concluded that an aryl moiety is required for high affinity binding, but that a sulfonyl group, while seemingly making a contribution, is not essential. As such, we have substantially extended the structure-affinity relationships for the binding of the N₁-arylsulfonyltryptamines at h5-HT₆ receptors.
VI. Experimentals

A. Chemistry:

Melting points were determined in a glass capillary on a Thomas Hoover melting point apparatus and are uncorrected. Proton nuclear magnetic resonance ($^1$H NMR) spectra were obtained on a Varian Gemini 300 MHz spectrometer and the chemical shifts are reported in parts per million ($\delta$), using tetramethylsilane (TMS) as an internal standard. Elemental analysis was performed by Atlantic Microlab Inc. (Norcross, GA), and determined values are within 0.4% of theory (except where indicated). Column chromatography was performed on silica gel, grade 62-200 mesh, 150 Å (Sigma-Aldrich, MO). Routine thin-layer chromatography (TLC) was performed on silica gel GHLF (250 Microns, 2.5 x 10 cm; Analtech Inc., Newark, DE).

$N,N$-Dimethyl-2-[1-(4-methoxybenzenesulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (31c)

$N,N$-Dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of 4-methoxybenzenesulfonyl chloride (0.52 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried (Na$_2$SO$_4$). The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH$_2$Cl$_2$/MeOH (9.5:0.5) as eluent to give 0.48 g of a brown yellow oil. The product was isolated as an oxalate salt to give 31c (0.24 g, 31%) as white crystals
after recrystallization from Me$_2$CO: mp 164-164°C; $^1$H NMR (DMSO-d$_6$) δ 2.78 (s, 6H, CH$_3$), 3.03 (t, 2H, CH$_2$), 3.30 (t, 2H, CH$_2$), 3.78 (s, 3H, CH$_3$), 7.07 (d, 2H, ArH), 7.25-7.39 (m, 2H, ArH), 7.66 (d, 1H, ArH), 7.71 (s, 1H, ArH), 7.85 (m, 3H, ArH). Anal. Calcd for C$_{19}$H$_{22}$N$_2$O$_3$ · C$_2$H$_2$O$_4$: C, 56.23; H, 5.39; N, 6.24 Found: C, 56.05; H, 5.39; N, 6.28.

$\text{N,N-Dimethyl-2-[1-(p-toluenesulfonyl)-1H-indol-3-yl]-1-amoethane Oxalate (31d)}$

$\text{N,N-Dimethyltryptamine (82)}$ (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0°C for 15 min, and then a solution of $p$-toluenesulfonyl chloride (0.48 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried (Na$_2$SO$_4$). The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH$_2$Cl$_2$/MeOH (9.5:0.5) as eluent to give 0.57 g of a brown yellow oil. The product was isolated as an oxalate salt to give 31d (0.19 g, 27%) as white crystals after recrystallization from Me$_2$CO: mp 191-193°C; $^1$H NMR (DMSO-d$_6$) δ 2.31 (s, 3H, CH$_3$), 2.82 (d, 6H, CH$_3$), 3.05 (t, 2H, CH$_2$), 3.35 (d, 2H, CH$_2$), 7.29-7.40 (m, 4H, ArH), 7.66 (d, 1H, ArH), 7.73 (s, 1H, ArH), 7.83 (d, 2H, ArH), 7.91 (d, 1H, ArH). Anal. Calcd for C$_{19}$H$_{22}$N$_2$O$_2$S · C$_2$H$_2$O$_4$ · 0.25H$_2$O: C, 58.84; H, 5.49; N, 6.23 Found: C, 57.73; H, 5.60; N, 6.29.
$N,N$-Dimethyl-2-[1-(4-trifluoromethylbenzenesulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (31e)

$N,N$-Dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of 4-(trifluoromethyl)benzenesulfonyl chloride (0.62 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried (Na$_2$SO$_4$). The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH$_2$Cl$_2$/MeOH (9.5:0.5) as eluent to give 0.58 g of a brown yellow oil. The product was isolated as an oxalate salt to give 31e (0.32 g, 39%) as white crystals after recrystallization from Me$_2$CO: mp 176-179 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 2.78 (s, 6H, CH$_3$), 3.05 (t, 2H, CH$_2$), 3.31 (t, 2H, CH$_2$), 7.31-7.44 (m, 4H, ArH), 7.69 (d, 1H, ArH), 7.80 (s, 1H, ArH), 7.98 (t, 2H, ArH), 8.18 (d, 1H, ArH). Anal. Calcd for C$_{19}$H$_{19}$F$_3$N$_2$O$_2$S·C$_2$H$_2$O$_4$: C, 51.84; H, 4.35; N, 5.75 Found: C, 51.81; H, 4.35; N, 5.72.

$N,N$-Dimethyl-2-[1-(4-chlorobenzenesulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (31f)

$N,N$-Dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of 4-chlorobenzenesulfonyl chloride (0.53 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was
allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried (Na₂SO₄). The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH₂Cl₂/MeOH (9.5:0.5) as eluent to give 0.36 g of a brown yellow oil. The product was isolated as an oxalate salt to give 31f (0.24 g, 31%) as white powder after recrystallization from Me₂CO: mp 180-182 °C; ¹H NMR (DMSO-d₆) δ 2.78 (s, 6H, CH₃), 3.06 (t, 2H, CH₂), 3.29 (t, 2H, CH₂), 7.28-7.41 (m, 3H, ArH), 7.66-7.68 (m, 2H, ArH), 7.74 (s, 1H, ArH), 7.92-7.99 (m, 3H, ArH). Anal. Calcd for C₁₈H₁₉ClN₂O₂S·C₂H₂O₄: C, 53.03; H, 4.67; N, 6.18 Found: C, 53.15; H, 4.64; N, 6.21.

*N,N*-Dimethyl-2-[1-(4-aminobenzyl)-1H-indol-3-yl]-1-aminoethane Hydrochloride (33b)

A solution of 87 (0.47 g, 1 mmol) in dry THF (10 mL) was added in a dropwise manner to a stirred suspension of LiAlH₄ (0.21 g, 5.5 mmol) in dry THF (10 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was heated at reflux for 1.5 h. The excess LiAlH₄ was destroyed by addition of H₂O (0.2 mL), NaOH (15%, 0.2 mL) and H₂O (0.6 mL) at 0 °C. The precipitate was removed by filtration and washed with hot THF (25 mL). The combined washings and filtrate was dried (Na₂SO₄) and the solvent was evaporated under reduced pressure to give an oily residue. Chromatography on a silica gel column (Aldrich silica gel 60) using CH₂Cl₂/MeOH (9:1) as eluent gave the crude product, 2-[1-(4-*N,N*-dibenzylaminobenzyl)-1H-indol-3-yl]-1-aminoethane (0.28 g, 63%) as an oil: ¹H NMR (CDCl₃) δ 3.03 (t, 2H, CH₂), 3.09 (t, 2H, CH₂), 4.63 (s, 4H, CH₂),
5.14 (s, 2H, CH₂), 6.65 (d, 2H, ArH), 6.97 (d, 2H, ArH), 6.98 (s, 1H, ArH), 7.09 (t, 1H, ArH), 7.17 (t, 1H, ArH), 7.21-7.35 (m, 11H, ArH), 7.60 (d, 1H, ArH).

A solution of aqueous formaldehyde (38%, 190 µL) was added in a dropwise manner to a cooled (ice bath) and stirred mixture of 2-[1-(4-N,N-dibenzylaminobenzyl)-1H-indol-3-yl]-1-aminoethane (0.27 g, 0.6 mmol), sodium cyanoborohydride (0.08 g, 1.3 mmol), and glacial acetic acid (172 µL, 0.3 mmol) in MeOH/MeCN (10/3 mL). The reaction mixture was then allowed to warm to room temperature and allowed to stir for 2 h before saturated aqueous K₂CO₃ (5 mL) was added. The solvent was removed under reduced pressure. The remaining residue was diluted with H₂O (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic portions were washed with brine (1 x 15 mL) and dried (Na₂SO₄), and the solvent was evaporated under reduced pressure. The oily residue was chromatographed on a silica gel column (Aldrich silica gel 60) using CH₂Cl₂/MeOH (95:5) as eluent to give the crude product N,N-dimethyl-2-[1-(4-N,N-dibenzylaminobenzyl)-1H-indol-3-yl]-1-aminoethane (0.18 g, 61%) as a colorless oil: ¹H NMR (CDCl₃) δ 2.53 (s, 6H, CH₃), 2.86 (t, 2H, CH₂), 3.10 (t, 2H, CH₂), 4.65 (s, 4H, CH₂), 5.15 (s, 2H, CH₂), 6.66 (d, 2H, ArH), 6.98 (d, 2H, ArH), 6.98 (s, 1H, ArH), 7.12 (t, 1H, ArH), 7.20 (t, 1H, ArH), 7.22-7.37 (m, 11H, ArH), 7.61 (d, 1H, ArH).

Anhydrous ammonium formate (0.11 g, 1.8 mmol) was added in a single portion to a stirred suspension of N,N-dimethyl-2-[1-(4-N,N-dibenzylaminobenzyl)-1H-indol-3-yl]-1-aminoethane (0.17 g, 0.4 mmol) and an equal weight of Pd/C (10%, 0.17 g) in anhydrous MeOH (5 mL). The reaction mixture was heated at reflux for 1.5 h, filtered (Celite) and the solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column (Aldrich silica gel 60) using CH₂Cl₂/MeOH (95:5) as eluent to give a
crude product (0.09 g, 81%) as a semisolid: $^1$H NMR (CDCl$_3$) δ 2.58 (s, 6H, CH$_3$), 2.93 (t, 2H, CH$_2$), 3.10 (t, 2H, CH$_2$), 5.16 (s, 2H, CH$_2$), 6.63 (d, 2H, ArH), 6.97 (d, 2H, ArH), 6.97 (s, 1H, ArH), 7.13 (t, 1H, ArH), 7.20 (t, 1H, ArH), 7.33 (d, 1H, ArH), 7.62 (d, 1H, ArH). The product was converted to its hydrochloride salt in anhydrous Et$_2$O by addition of HCl/Et$_2$O. The orange precipitate was collected by filtration and recrystallized from MeOH/Et$_2$O to give 33b (0.07 g, 61%) as a yellow powder: mp 157-159 °C; $^1$H NMR (DMSO-d$_6$) δ 2.83 (s, 6H, CH$_3$), 3.13 (t, 2H, CH$_2$), 3.31 (t, 2H, CH$_2$), 5.39 (s, 2H, CH$_2$), 7.05 (t, 1H, ArH), 7.13 (t, 1H, ArH), 7.20-7.27 (m, 4H, ArH), 7.38 (s, 1H, ArH), 7.44 (d, 1H, ArH), 7.66 (d, 1H, ArH). Anal. calcd for C$_{19}$H$_{23}$N$_3$ x 2HCl x 0.5 H$_2$O: C, 60.81; H, 6.98; N, 11.20 Found: C, 60.69; H, 6.76; N, 10.74.

$N,N$-Dimethyl-2-[1-(4-methoxybenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33c)

A mixture of $N,N$-dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 4.17 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and 4-methoxybenzyl chloride (0.33 mL, 2.43 mmol) was added resulting in a solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH$_2$Cl$_2$ (2 x 50 mL). The organic portion was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CHCl$_3$ and MeOH (12:1) as eluent to give 0.35 g of a brown yellow oil. The product was isolated as an oxalate salt to give 33c (0.25 g, 51%) as a white powder after recrystallization from anhydrous MeOH/Et$_2$O: mp 172-173 °C; $^1$H NMR (DMSO-d$_6$) δ 2.79 (s, 6H, CH$_3$), 3.08 (t, 2H, CH$_2$), 3.25 (t, 2H, CH$_2$), 3.68 (s, 3H,
CH₃), 5.26 (s, 2H, CH₂), 6.84 (d, 2H, ArH), 7.01-7.17 (m, 4H, ArH), 7.33 (s, 1H, ArH), 7.45 (d, 1H, ArH), 7.60 (d, 1H, ArH). Anal. Calcd for C₂₀H₂₄N₂O · C₂H₂O₄ : C, 62.32; H, 6.58; N, 7.03 Found: C, 62.23; H, 6.66; N, 7.07.

**N,N-Dimethyl-2-[1-(4-methylbenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33d)**

N,N-Dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of 4-methylbenzyl chloride (0.35 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL). The combined organic portions were washed with brine (20 mL) and dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH₂Cl₂/MeOH (9.5:0.5) as eluent to give 0.13 g, of a brown yellow oil. The product was isolated as an oxalate salt to give 33d (0.13 g, 20%) as white powder after recrystallization from Me₂CO: mp 179-182 °C; ¹H NMR (DMSO-d₆) δ 2.23 (s, 3H, CH₃), 2.82 (s, 6H, CH₃), 3.07 (t, 2H, CH₂), 3.28 (t, 2H, CH₂), 5.30 (s, 2H, CH₂), 7.00-7.15 (m, 6H, ArH), 7.34 (s, 1H, ArH), 7.42 (d, 1H, ArH), 7.61 (d, 1H, ArH). Anal. Calcd for C₂₀H₂₄N₂ · C₂H₂O₄ : C, 69.09; H, 6.85; N, 7.32 Found: C, 69.17; H, 6.98; N, 7.29.
**N,N-Dimethyl-2-[1-(4-trifluorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33e)**

*N,N*-Dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of 4-(trifluoromethyl)benzyl chloride (0.49 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH₂Cl₂/MeOH (9.5:0.5) as eluent to give 0.25 g, of a brown yellow oil. The product was isolated as an oxalate salt to give 33e (0.18 g, 25%) as white powder after recrystallization from Me₂CO: mp 162-163 °C; ¹H NMR (DMSO-d₆) δ 2.82 (s, 6H, CH₃), 3.07 (d, 2H, CH₂), 3.27 (d, 2H, CH₂), 5.48 (s, 2H, CH₂), 7.04-7.13 (m, 2H, ArH), 7.34-7.44 (m, 4H, ArH), 7.64-7.66 (m, 3H, ArH). Anal. Calcd for C₂₀H₂₁F₃N₂·C₂H₂O₄: C, 60.54; H, 5.31; N, 6.41 Found: C, 60.60; H, 5.36; N, 6.43.

**N,N-Dimethyl-2-[1-(4-chlorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33f)**

A mixture of *N,N*-dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 4.17 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and 4-chlorobenzyl chloride (0.54 g, 3.34 mmol) was added resulting in a solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH₂Cl₂ (2 x 50 mL). The organic portion was dried over MgSO₄ and
the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH₂Cl₂ and MeOH (12:1) as eluent to give 0.40 g of a brown yellow oil. The product was isolated as an oxalate salt to give 33f (0.30 g, 61%) as a white powder after recrystallization from anhydrous MeOH/Et₂O: mp 170-172 °C; ¹H NMR (DMSO-d₆) δ 2.80 (s, 6H, CH₃), 3.09 (t, 2H, CH₂), 3.27 (t, 2H, CH₂), 5.36 (s, 2H, CH₂), 7.00-7.19 (m, 4H, ArH), 7.33-7.42 (m, 4H, ArH), 7.63 (d, 1H, ArH). Anal. Calcd for C₁₉H₂₁ClN₂·C₂H₂O₄: C, 62.60; H, 5.75; N, 6.95 Found: C, 62.35; H, 5.74; N, 6.93.

**N,N-Dimethyl-2-[1-(3-chlorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33g)**

N,N-Dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of 3-chlorobenzyl chloride (0.32 mL, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried (Na₂SO₄). The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH₂Cl₂/MeOH (9.5:0.5) as eluent to give 0.24 g of a brown yellow oil. The product was isolated as an oxalate salt to give 33g (0.18 g, 27%) as white crystals after recrystallization from Me₂CO: mp 140-141 °C; ¹H NMR (DMSO-d₆) δ 2.51 (s, 6H, CH₃), 3.10 (t, 2H, CH₂), 3.27 (d, 2H, CH₂), 5.40 (s, 2H, CH₂), 7.06-7.14 (m, 3H, ArH),
N,N-Dimethyl-2-[1-(3,4-dichlorobenzyl)-1H-indol-3-yl]-1-aminoethane Oxalate (33h)

A mixture of N,N-dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 4.17 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and 3,4-dichlorobenzyl chloride (0.46 mL, 3.32 mmol) was added resulting in a solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH₂Cl₂ (2 x 50 mL). The organic portion was dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CHCl₃ and MeOH (12:1) as eluent to give 0.30 g of a brown yellow oil. The product was isolated as an oxalate salt to give 33h (0.11 g, 10%) as yellowish crystals after recrystallization from anhydrous MeOH/Et₂O: mp 172-173 °C; $^1$H NMR (DMSO-d₆) δ 2.79 (s, 6H, CH₃), 3.09 (t, 2H, CH₂), 3.27 (t, 2H, CH₂), 5.38 (s, 2H, CH₂), 7.05 (t, 1H, ArH), 7.10-7.14 (m, 2H, ArH), 7.38-7.47 (m, 3H, ArH), 7.53-7.63 (m, 2H, ArH). Anal. Calcd for C₁₉H₂₁ClN₂ · C₂H₂O₄ : C, 62.61; H, 5.75; N, 6.95 Found: C, 62.50; H, 5.69; N, 6.88.

N,N-Dimethyl-2-[1-(Isopropylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78a)

A mixture of N,N-dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 2.52 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and isopropylsulfonyl
chloride (0.32 mL, 2.87 mmol) was added resulting in a solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH₂Cl₂ (2 x 50 mL). The organic portion was dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH₂Cl₂ and MeOH (12:1) as eluent to give 0.39 g of a brown yellow oil. The product was isolated as an oxalate salt to give 78a (0.24 g, 24%) as white powder after recrystallization from anhydrous MeOH/Et₂O: mp 175-178 °C; ¹H NMR (DMSO-d₆) δ 1.19 (d, 6H, CH₃), 2.82 (s, 6H, CH₃), 3.16 (t, 2H, CH₂), 3.37 (t, 2H, CH₂), 3.72-3.79 (m, 1H, CH), 7.32-7.42 (m, 2H, ArH), 7.55 (s, 1H, ArH), 7.78-7.85 (m, 2H, ArH). Anal. Calcd for (C₁₅H₂₂N₂O₂S)₂·C₂H₂O₄ x 1.5 H₂O : C, 54.44; H, 6.99; N, 7.93 Found: C, 54.00; H, 7.08; N, 8.31. C, a difference of 0.44.

**N,N-Dimethyl-2-[1-(n-propylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78b)**

A mixture of N,N-dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 2.52 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and n-propylsulfonyl chloride (0.32 mL, 2.87 mmol) was added resulting in a solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH₂Cl₂ (2 x 50 mL). The organic portion was dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH₂Cl₂ and MeOH (12:1) as eluent to give 0.24 g of a brown yellow oil. The product was isolated as an oxalate salt to give 78b (0.13 g, 12%) as a white powder after recrystallization from anhydrous MeOH/Et₂O: mp 144-145 °C;
$^1$H NMR (DMSO-d$_6$) $\delta$ 0.86 (t, 6H, CH$_3$), 1.51 (q, 4H, CH$_2$), 2.82 (s, 12H, CH$_3$), 3.15 (t, 4H, CH$_2$), 3.37 (t, 4H, CH$_2$), 3.55 (t, 4H, CH$_2$), 7.33-7.40 (m, 4H, ArH), 7.53 (s, 2H, ArH), 7.76-7.78 (m, 4H, ArH). Anal. Calcd for C$_{30}$H$_{44}$N$_4$O$_4$S$_2$ $\cdot$ C$_2$H$_2$O$_4$ : C, 54.44; H, 6.99; N, 7.93 Found: C, 54.17; H, 6.99; N, 8.21.

**N,N-Dimethyl-2-[1-(n-butylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78c)**

A mixture of N,N-Dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 2.52 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and n-butylsulfonyl chloride (0.37 mL, 2.87 mmol) was added resulting in a solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH$_2$Cl$_2$ (2 x 50 mL). The organic portion was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH$_2$Cl$_2$ and MeOH (12:1) as eluent to give 0.17 g of a brown yellow oil. The product was isolated as an oxalate salt to give 78c (0.03 g, 3%) as a white powder after recrystallization from anhydrous MeOH/Et$_2$O: mp 162-163 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 0.75 (s, 3H, CH$_3$), 1.22-1.29 (m, 2H, CH$_2$), 1.42-1.49 (m, 2H, CH$_2$), 2.79 (s, 6H, CH$_3$), 3.18 (d, 2H, CH$_2$), 3.31 (d, 2H, CH$_2$), 3.55 (t, 2H, CH$_2$), 7.32-7.43 (m, 2H, ArH), 7.53 (s, 1H, ArH), 7.75 (t, 1H, ArH), 7.83 (t, 1H, ArH). Anal. Calcd for C$_{16}$H$_{24}$N$_2$O$_2$S $\cdot$ C$_2$H$_2$O$_4$ : C, 54.25; H, 6.57; N, 7.03 Found: C, 54.06; H, 6.64; N, 6.97.
**N,N-Dimethyl-2-[1-(n-amylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78d)**

A mixture of N,N-dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.24 g, 1.68 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). After cooling at 0 °C for 15 min, a solution of n-amylsulfonyl chloride (90) (0.29 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to the mixture of N,N-dimethyltryptamine, potassium tert-butoxide and 18-crown-6. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL), washed with brine (20 mL) and dried with Na₂SO₄. The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH₂Cl₂/MeOH (9.5:0.5) as eluent to give 0.12 g of a brown yellow oil. The product was isolated as an oxalate salt to give 78d (0.05 g, 7%) as a white powder after recrystallization from anhydrous MeOH/Et₂O: mp 167-169 °C; ¹H NMR (DMSO-d₆) δ 0.75 (t, 3H, CH₃), 1.14-1.20 (m, 4H, CH₂), 1.48 (t, 2H, CH₂), 2.80 (s, 6H, CH₃), 3.10 (t, 2H, CH₂), 3.29 (t, 2H, CH₂), 3.55 (t, 2H, CH₂), 7.35-7.43 (m, 2H, ArH), 7.55 (s, 1H, ArH), 7.76 (d, 1H, ArH), 7.84 (d, 1H, ArH). Anal. Calcd for C₁₇H₂₆N₂O₂S·C₂H₄O₄: C, 55.32; H, 6.84; N, 6.79 Found: C, 55.22; H, 6.93; N, 6.75.

**N,N-Dimethyl-2-[1-(n-octylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78e)**

A mixture of N,N-dimethyltryptamine (82) (0.50 g, 2.66 mmol) and 60% NaH (0.10 g, 2.52 mmol) was heated at 100 °C under nitrogen until evolution of hydrogen gas ceased. The resultant product was dissolved in anhydrous DMF (5 mL), and n-octylsulfonyl chloride (0.56 mL, 2.87 mmol) was added resulting in a solution at 0 °C. The reaction
mixture was allowed to stir at room temperature for 3 h. Brine was added and the mixture was extracted with CH$_2$Cl$_2$ (2 x 50 mL). The organic portion was dried over MgSO$_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using CH$_2$Cl$_2$ and MeOH (12:1) as eluent to give 0.18 g of a brown yellow oil. The product was isolated as an oxalate salt to give 78e (0.06 g, 5%) as a white powder after recrystallization from anhydrous MeOH/Et$_2$O: mp 138-139 °C; $^1$H NMR (DMSO-d$_6$) $\delta$ 0.80 (t, 3H, CH$_3$), 1.10-1.19 (m, 10H, CH$_2$), 1.46 (t, 2H, CH$_2$), 2.78 (s, 6H, CH$_3$), 3.09 (t, 2H, CH$_2$), 3.30 (t, 2H, CH$_2$), 3.53 (t, 2H, CH$_2$), 7.33-7.41 (m, 2H, ArH), 7.52 (s, 1H, ArH), 7.74 (d, 1H, ArH), 7.81 (d, 1H, ArH). Anal. Calcd for C$_{20}$H$_{32}$N$_2$O$_2$S · C$_2$H$_2$O$_4$: C, 58.12; H, 7.53; N, 6.16 Found: C, 57.67; H, 7.47; N, 6.14. C, a difference of 0.45.

$N,N$-Dimethyl-2-[1-(cyclohexylsulfonyl)-1H-indol-3-yl]-1-aminoethane Oxalate (78f)

A mixture of $N,N$-dimethyltryptamine (82) (0.32 g, 1.68 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred mixture of potassium tert-butoxide (0.25 g, 2.18 mmol) and 18-crown-6 (0.04 g, 1.68 mmol) in dry THF (10 mL). The reaction mixture was cooled at 0 °C for 15 min, and then a solution of cyclohexylsulfonyl chloride (93) (0.46 g, 2.52 mmol) in dry THF (5 mL) was added in a dropwise manner. The reaction mixture was allowed to stir at room temperature overnight, then concentrated and extracted with EtOAc (3 x 15 mL). The combined organic portions were washed with brine (20 mL) and dried with Na$_2$SO$_4$. The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column using CH$_2$Cl$_2$/MeOH (9.5:0.5) as eluent to give 0.18 g of a brown yellow oil. The product was isolated as an
N,N-Dimethyltryptamine (82)

Compound 82 was prepared following a literature\textsuperscript{113} procedure. Oxalyl chloride (2.97 mL, 34.05 mmol) in N\textsubscript{2}-purged anhydrous Et\textsubscript{2}O (14 mL) was added dropwise to a solution of indole (4g, 34.04 mmol) in N\textsubscript{2}-purged mixture of anhydrous Et\textsubscript{2}O (27 mL), and THF (1.4 mL) at 0 °C under N\textsubscript{2}. The mixture was allowed to stir at 0 °C for 20 min and then at room temperature for 30 min. The bright yellow precipitate was collected by filtration and washed with anhydrous Et\textsubscript{2}O (40 mL) obtaining 3-indolylglyoxyl chloride (80) (6.73g).

3-Indolyglyoxyl chloride (80) (6.73 g, 32.41 mmol) was added with rigorous stirring to 30 mL of 40% dimethylamine in H\textsubscript{2}O. The mixture after sometime changed color from yellow to green. The mixture was allowed to stir overnight. The resulting solution was concentrated and the yellow precipitate was collected by filtration to give (4.69 g, 84 %) of a crude product (N,N-dimethyl-3-indolylglyoxylamide, 81); mp 152-155 °C (lit.\textsuperscript{129} mp 159-160 °C).

N,N-Dimethyl-3-indolylglyoxylamide (81) (5.69g, 26.32 mmol) as a solution in dry THF (32.8 mL) was added to a suspension of LiAlH\textsubscript{4} in dry THF (100 mL) in a dropwise manner at 0 °C. The mixture was heated under reflux for 3 h. The mixture was cooled at 0...
°C and the excess LiAlH₄ was destroyed by addition of H₂O (8.1 mL), NaOH (15%, 8.1 mL) and H₂O (24.3 mL) at 0 °C. The precipitate was removed by filtration and washed with 114 mL of hot THF. The filtrate was dried (MgSO₄) and the solvent was evaporated under reduced pressure to give a crude product as an oil. Distillation at 180 °C (0.2 mbar) yielded 82 (3.16 g) (56%) as a colorless oil that solidified on standing; mp 49-50 °C (lit.¹¹³ mp 44-46 °C); ¹H NMR (DMSO-d₆) δ 2.35 (s, 6H, CH₃), 2.65 (t, 2H, CH₂), 2.96 (t, 2H, CH₂), 7.01 (d, 1H, ArH), 7.14 (t, 1H, ArH), 7.19 (t, 1H, ArH), 7.34 (d, 1H, ArH), 7.62 (d, 1H, ArH), 8.08 (s br, 1H, ArH).

N₁-(4-Nitrobenzyl)indole (83)¹¹⁶
Indoline-2-carboxylic acid (2.45 g, 15 mmol) was heated with 4-nitrobenzaldehyde (2.27 g, 15 mmol) in MeCN (45 mL) overnight. The solvent was evaporated under reduced pressure, the residue was dissolved in H₂O (50 mL) and extracted with EtOAc (3 x 35 mL). The combined organic portions were dried (Na₂SO₄) and the solvent was evaporated under reduced pressure to give a dark, brown oil (4.05 g). Chromatography on a silica gel column (Aldrich silica gel 60) using CH₂Cl₂/hexane (1:1) as eluent gave crude product (2.27 g, 60 %) as a yellow oil which solidified on standing: mp 94-97 °C; ¹H NMR (DMSO-d₆) δ 5.54 (d, 1H, ArH), 7.03 (t, 1H, ArH), 7.10 (t, 1H, ArH), 7.36-7.42 (m, 3H, ArH), 7.55 (d, 1H, ArH), 7.59 (d, 1H, ArH), 8.19 (d,2H, ArH). The title compound has been cited in the literature¹¹⁵ but was not characterized.
**N$_1$-(4-Aminobenzyl)indole (84)**

A solution of 83 (1.09 g, 4.3 mmol) and tin (II) chloride dihydrate (9.75 g, 43 mmol) in absolute EtOH (100 mL) was heated at reflux for 1 h. The reaction mixture was allowed to cool to room temperature and then a small amount of ice was added. The solution was made slightly basic (pH 8-9) by addition of a saturated solution of NaHCO$_3$. Sodium chloride (ca. 4 g) was added to the solution and the mixture was extracted with EtOAc (2 x 300 mL). The combined organic portions were thoroughly washed with brine and dried (Na$_2$SO$_4$). The solvent was evaporated under reduced pressure to give a product as a yellow oil (0.81 g, 84%). The product was used in the next step without further purification.

**N$_1$-[4-(Dibenzylamino)benzyl]indole (85)**

Benzyl bromide (2.14 mL, 18 mmol) was added in a dropwise manner over 30 min to a well-stirred suspension of 84 (0.80 g, 3.6 mmol) in CH$_2$Cl$_2$ (10 mL) and triethylamine (1.52 mL, 10.8 mmol); this was allowed to stir for an additional 2 h. The solution was concentrated and the residue was chromatographed on a silica gel column (Aldrich silica gel 60) using hexane/CH$_2$Cl$_2$ (7:3) as eluent to give a crude product (1.31 g, 90 %) as a yellow oil. The product was used in the next step without further characterization.
(N\textsubscript{1}-[4-Dibenzylaminobenzyl]-3-[carboxaldehyde]) indole (86)

Phosphorus oxotrichloride (0.32 mL, 3.55 mmol) was added via a syringe to ice-cooled dry DMF (10 mL). The solution protected from moisture with a drying tube, was allowed to stir at room temperature for 15 min and then cooled to 0 °C. A solution of 85 (1.31 g, 3.25 mmol) in dry DMF (5 mL) was added over 5 min, and the solution was allowed to stir at room temperature for 2 h, poured into a mixture of ice and aqueous NaOH (1 N) and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 30 mL). The combined organic portions were washed with H\textsubscript{2}O (50 mL) and brine (50 mL), and dried (Na\textsubscript{2}SO\textsubscript{4}). The solvent was evaporated under reduced pressure to give the crude product (1.18 g, 84%) as a yellow solid: mp 137-138 °C. The homogenous product was used in the following reaction step without further purification. This compound has not been cited in the literature.

(N\textsubscript{1}-[4-Dibenzylaminobenzyl]-3-[2-nitrovinyl])indole (87)

A mixture of 86 (0.99 g, 2.3 mmol), ammonium acetate (0.09 g, 015 mmol) and MeNO\textsubscript{2} (8 mL) was heated at reflux for 3 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2}, washed with H\textsubscript{2}O (30 mL) and the solution dried (Na\textsubscript{2}SO\textsubscript{4}). The solvent was removed under reduced pressure to give the crude product (1.05 g, 96%) as a yellow oil: \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \( \delta \) 4.68 (s, 4H, CH\textsubscript{2}), 5.21 (s, 2H, CH\textsubscript{2}), 6.77 (d, 2H, ArH), 7.04 (d, 2H, CH\textsubscript{2}), 7.26-7.37 (m, 14H, ArH), 7.50 (s, 1H, ArH), 7.77 (d, 1H, ArH), 7.25 (d, 1H, ArH).
**Amylsulfonyl chloride (90)**

Cyanuric chloride (0.922 g, 5 mmol) (89) was added to a mixture of sodium n-amylsulfonate (88) (0.871 g, 5 mmol) and 0.25 mmol of 18-crown-6 ether in 10 mL of Me₂CO and the mixture was heated under reflux for 20 h under anhydrous conditions. After cooling the reaction mixture to room temperature the solution was filtered (celite pad). The solvent was removed under reduced pressure and the crude product was purified by Kugelrohr distillation yielding n-amylsulfonyl chloride (90) (0.51, 56%); \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 0.97 (t, 3H, CH\(_3\)), 1.41-1.45 (m, 4H, CH\(_2\)), 2.05 (t, 2H, CH\(_2\)), 3.67 (t, 2H, CH\(_2\)). This compound was used in the next step without further characterization.

**Cyclohexylsulfonyl chloride (93)**

Chlorine was bubbled through a solution of cyclohexylthiol (10.0 g) in 48 mL of acetic acid and 4.5 mL of water, maintained at 10-15 °C, until the yellow color persisted. Nitrogen was then bubbled through the resulting mixture to remove excess chlorine. The resulting mixture was poured into ice and then extracted with CH\(_2\)Cl\(_2\) (3 x 30 mL). The combined CH\(_2\)Cl\(_2\) extract was washed with H\(_2\)O (10 mL), 5% NaOH (10 mL), again with H\(_2\)O (10 mL), and then dried (Na\(_2\)SO\(_4\)). The solvent was removed under reduced pressure followed by Kugelrohr distillation of the residue to yield 3.1 g (23%) of cyclohexylsulfonyl chloride (93) as a yellowish liquid; \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 1.33-1.39 (m, 3H, CH\(_2\)), 1.74-1.75 (m, 3H, CH\(_2\)), 1.98-2.17 (m, 2H, CH\(_2\)), 2.41-2.44 (m, 2H, CH\(_2\)), 3.46-3.55 (m, 1H, CH). The bp of this compound was not determined.
B. Binding Assay
The h5-HT\textsubscript{6} radioligand binding assay was performed as previously described.\textsuperscript{71} In brief, h5-HT\textsubscript{6} cDNA was transiently expressed in HEK-293 cells using Fugene6 according to the manufacturer’s recommendations; 24 h after transfection the medium was replaced, and 24 h later, medium containing dialyzed serum (to remove 5-HT) was added. At 72 h after transfection, cells were harvested by scraping and centrifugation. Cells were then washed by centrifugation and resuspension in phosphate-buffered saline (pH = 7.40; PBS) and frozen as tight pellets at -80 °C until use. Binding assays were performed at room temperature for 90 min in binding buffer (50 mM Tris-Cl, 10 mM MgCl\textsubscript{2}, 0.1 mM EDTA, pH = 7.40) with [\textsuperscript{3}H]LSD (1 nM final concentration) using 10 µM clozapine for non specific binding. Concentrations of unlabeled test agent were used for K\textsubscript{i} determinations with K\textsubscript{i} values calculated using the program GraphPad Prism (V4.0). Specific binding represented 80-90% of total binding. K\textsubscript{i} values are the result of triplicate determinations.
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Vita

Abner Nyamwaro Nyandege was born on September 21, 1976 to the parents of Gideon and Agnes Nyandege. Abner graduated from Moi University in December 2000 with a Bachelor of Science degree in Chemistry. While working on his Master of Science degree, Abner received a publication titled “Further studies on the binding of N1-substituted tryptamines at serotonin 5-HT6 receptors” which was published in the Bioorganic and Medicinal Chemistry Letters in 2007.