Synthesis of Fused Triazolo-Pyridobenzodiazepine Analogs for Use as Potential Atypical Antipsychotic Treatments in Schizophrenia

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Thesis directed by
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Dedication

To my parents, without whom I would not be where I am in life today. Thank you for everything that you have done, and continue to do to support me.
Acknowledgements

I want to thank my family and close friends for all their support and love throughout this process. I want to thank them for listening to my chemistry related rants, sitting through practice presentations, and just generally being there to offer advice and listen.

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Abstract

Schizophrenia is a chronic mental illness that displays a wide variety of psychological symptoms and affects millions of people worldwide. It has a very complicated etiology, some of which is still not understood, but the altered activity of both dopamine and serotonin receptors have been implicated in the cause of this disease. Antipsychotics (APDs) developed over the years have split into two distinct categories: classical and atypical. Classical APDs target the dopamine receptors and are known to produce serious extrapyramidal side effects. There is a large percent of patients who are treatment-resistant to these drugs, which has aided in the development of atypical APDs. These compounds mainly target serotonin receptors, and tend to have very high affinity for the 5-HT$_{2A}$ receptor. Though atypical APDs currently on the market show reduced levels of extrapyramidal side effects, they are not without their own problems.

JL 13 is a benzoxazepine derivative with an atypical profile that has been shown to reduce extrapyramidal side effects consistent with atypical APDs. Based on its core structure, previous efforts were made to design and synthesize a large library of fused triazole derivatives of both benzoxazepine and benzodiazepine molecules. None of these analogs showed any significant binding affinity to the 5-HT$_{2A}$ serotonin receptor, thus reevaluation of compound structure was crucial. The relationship between the direction of the substituent attached to the triazole ring and its binding ability then came into question. Presented herein is a new synthetic route that was designed to create fused triazolo-analogs in which the direction of the substituent was reversed in an attempt to improve binding affinity.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>DISC1</td>
<td>disrupted in schizophrenia</td>
</tr>
<tr>
<td>MIA</td>
<td>maternal immune activation</td>
</tr>
<tr>
<td>ACE</td>
<td>adolescent cannabinoid exposure</td>
</tr>
<tr>
<td>D₂R</td>
<td>dopamine 2 receptor</td>
</tr>
<tr>
<td>EPS</td>
<td>extrapyramidal side effects</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>DRN</td>
<td>dorsal raphe nucleus</td>
</tr>
<tr>
<td>APD</td>
<td>antipsychotic drug</td>
</tr>
<tr>
<td>k_{off}</td>
<td>drug dissociation rate</td>
</tr>
<tr>
<td>JL 13</td>
<td>5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-\textit{b}][1,5]benzoxazepine fumarate</td>
</tr>
<tr>
<td>DOI</td>
<td>2,5-dimethoxy-4-iodoamphetamine</td>
</tr>
<tr>
<td>K_{i}</td>
<td>inhibitory constant</td>
</tr>
<tr>
<td>LCMS</td>
<td>liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
</tbody>
</table>
DMF  
N,N-dimethylformamide

TLC  
thin layer chromatography

BINAP  
2,2'-bis(diphenylphosphino)-1,1'-binaphthalene

HPLC  
high performance liquid chromatography

POCl₃  
phosphorus oxychloride

DMSO  
dimethyl sulfoxide

NMR  
nuclear magnetic resonance

DCM  
dichloromethane

EtOH  
ethanol

MeOH  
methanol

¹H NMR  
proton nuclear magnetic resonance

¹³C NMR  
carbon nuclear magnetic resonance
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General Experimental Information

All reactions were performed in glassware or 10 mL microwave vials. Glassware for dry reactions was dried for at least 12 hours at 60 °C or flame dried under argon atmosphere prior to use. Dry reactions were performed under an inert argon atmosphere. Reagents and solvents were purchased from Fisher Scientific, Sigma Aldrich or Acros. Microwave reactions were performed in a CEM Discover-SP Microwave operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W. Microwave reactions were performed in dry 10 mL pressurized vessels with rubber caps. NMR spectra were obtained from a Varian (400 MHz) NMR spectrometer and reported in ppm (δ) downfield relative to a trimethylsilyl (TMS) peak. Thin layer chromatography was performed using silica gel 60 F524 pre-coated plates (SiliCycle Inc.). Preparative thin layer chromatography was carried out using Silica Gel GF plates (Analtech, Inc). Flash chromatography was performed using SiliaFlash P60 (230-40 mesh) silica gel (Silicycle).
CHAPTER ONE: INTRODUCTION

1.1 Schizophrenia

Schizophrenia is a chronic mental illness with a constantly developing etiology that affects millions of people worldwide.¹ Schizophrenia exhibits three major categories of symptoms: positive symptoms (psychotic symptoms such as hallucinations, delusions, and catatonic behavior), negative symptoms (social withdrawal, apathy, etc), and cognitive impairment² (affecting language and executive function, episodic memory, processing speed, attention inhibition, etc).³ These symptoms have a profound social impact, as an estimated one-third of people living with schizophrenia are homeless and it is one of the most prevalent mental disorders found in inmates.⁴

Family studies conducted over the years have shown that schizophrenia is predominately a genetic disorder, with approximately an 80% risk of heritability. These studies suggest that the pathophysiology of the disease may be highly influenced by inherited genetic variants, with the strongest evidence coming from the DISC1 (disrupted in schizophrenia) translocation study. This translocation study showed that patients with mutated DISC1 genes had a higher likelihood of having and expressing the symptoms of schizophrenia. This study suggests that these disrupted genes may have a significant role in the development of this illness.⁵

However, the development of schizophrenia cannot be solely attributed to a person’s genetic makeup. Exposure to certain environmental factors during critical developmental stages can also be a contributing factor to the occurrence of the disease. There are many factors that could increase the likelihood of developing schizophrenia,
but one of the most studied and understood factors is prenatal infection. There have been a multitude of pre-clinical animal studies done that show that maternal immune activation (MIA) can disrupt gene expression in the brains of fetuses. The blocking of gene expression can lead to abnormal neurodevelopment, which can produce many of the behavioral symptoms that are associated with schizophrenia. Since the brain continues developing well into adolescence, this is another critical time period that can be largely influenced by outside factors. During this time, many young adults tend to experiment with psychotropic drugs, including cannabis. Hollins et al. conducted a study where the offspring of infected pregnant rats were exposed to cannabis during a time that corresponds to human adolescence. They found that the combination of these MIA and adolescent cannabinoid exposure (ACE) factors causes a two-to-five fold increase in the risk of developing schizophrenia. While these factors alone do not determine whether or not a person will develop schizophrenia, environmental influences cannot be discounted when analyzing the etiology of this disease.

1.2 Dopamine Receptors

While many of the underlying causes of this complex disease are still being determined, dopamine receptors and their pathways (Figure 1) have been implicated in the pathophysiology and symptoms of schizophrenia. There are 5 different subtypes of dopamine receptors that control almost all of the physiological functions of dopamine neurotransmitters: D₁, D₂, D₃, D₄, and D₅. These receptors are then split into two categories: D₁-class, which is receptors D₁ and D₅, and D₂-class, which is receptors D₂, D₃, and D₄. Research into these receptors and their various pathways resulted in the “original dopamine hypothesis,” which states that schizophrenic symptoms are induced
via excessive dopamine transmission throughout all regions in the brain. Over time, the dopamine expression-symptom relationship of this hypothesis was questioned. While the positive symptoms of schizophrenia are related to an increased release of dopamine and D2 receptor activation, it was determined that the negative symptoms are, at least in part, related to decreased D1 receptor stimulation. Due to this discovery, the “revised dopamine hypothesis” was formed, stating that increased dopamine transmission in regions involved with the mesolimbic pathway and decreased dopamine transmission in the prefrontal cortex (mesocortical pathway) contribute to both the positive and negative symptoms of schizophrenia.3,9

**Figure 1**: A diagram of the various dopamine pathways in the brain. Altered levels of dopamine transmission in many of these areas contribute to the development of schizophrenic symptoms6.

All effective antipsychotics currently on the market exhibit some form of D2 receptor (D2R) antagonism,8,10 suggesting that the D2 receptor plays a major part in the etiology of schizophrenia. These D2R antagonists do show an improvement in psychotic
symptoms in approximately 70% of schizophrenic patients, but they are not very effective when it comes to treating the other components of this disease, such as the negative symptoms or cognitive impairment.\textsuperscript{11} In fact, many D\textsubscript{2} antagonists have been found to produce negative symptoms in healthy individuals.\textsuperscript{12} This may suggest that many symptoms could be either exaggerated more or show no real improvement in some schizophrenic people using these D\textsubscript{2} receptor drugs. Though these D\textsubscript{2}R antagonists alleviate many positive symptoms in patients, they also induce unwanted extrapyramidal side effects (EPS). These side effects are most commonly observed when the D\textsubscript{2} receptors are more than 80% occupied.\textsuperscript{13} Due to these complications and negative side effects, many researchers are trying to determine new ways to obtain overall improvement in each symptom category while reducing side effects such as EPS.

More recently, there has been research done on the D\textsubscript{4} receptor (part of the D\textsubscript{2} receptor classification) that suggests that it plays an important role in schizophrenia and other psychotic diseases. mRNA distribution studies have shown that D\textsubscript{4} receptors were preferentially located in the cortical areas of the brain, which are associated with antipsychotic activity, and had a very low density in the striatum.\textsuperscript{14} There have been many reports in the past that state D\textsubscript{2} receptors must play a significant role in the etiology of schizophrenia due to the fact that elevations in this receptor have been found in schizophrenic brain tissue. However, this theory is controversial as many think that the elevation may be due to chronic drug treatment rather than pathological influences. While studies on the amount of D\textsubscript{4} receptors in schizophrenic brain tissue are sparse, three different independent studies have found a two- to six-fold increase in D\textsubscript{4} receptors in both treated and untreated schizophrenic patients. The elevation in D\textsubscript{4} receptor density
was found to be much higher and more significant than any elevation changes in D₂ or D₃ receptors. One could claim that the increase in receptors may be due to long term drug treatment, but the elevated presence in untreated patients supports the idea that it is not a completely drug mediated effect.¹⁵ Based on this data, the D₄ receptors could be an interested drug target, since they share many of the same qualities as D₂ receptors, and therefore could be affected by antipsychotic drugs meant to target D₂ receptors.

1.3 Serotonin Receptors

In an attempt to develop new drugs that improve more than just the positive symptoms of schizophrenia, researchers began looking into serotonin receptors as potential therapeutic targets. These receptors and their pathways (Figure 2)¹⁶ play an important role in the regulation of various physiological functions in the nervous system, such as cognition, sensori-motor functions, and autonomic and psycho-emotional responses. There are seven different families of serotonin neurons (5-hydroxytryptamine [5-HT] neurons) classified as 5-HT₁₋₇, and there are also at least 14 subtypes throughout these families. These neurons are highly expressed in the brain and send signals to many different regions, including the cerebral cortex, limbic regions, and basal ganglia, among others.¹⁷ These receptors have been associated with many aspects of the pathophysiology of schizophrenia, such as psychotic symptoms, cognitive impairment, negative symptoms, mood changes, and suicidal feelings.¹¹
Figure 2: A representative diagram of the serotonergic pathways in the brain. Upregulation of serotonin in the dorsal raphe nucleus (DRN) is thought to cause many of the symptoms of schizophrenia\textsuperscript{16}.

The development and research into serotonin receptors as major underlying factors in the etiology of schizophrenia lead to the development of a “serotonin hypothesis”. In a similar fashion to the original dopamine hypothesis, this theory states that any excessive serotonergic drive initiated in the dorsal raphe nucleus (DRN) in response to stress can interrupt the function of cortical neurons. The stimulation of the 5-HT\textsubscript{2A} receptors, the main cortical serotonergic receptors, via DRN upregulation causes the removal of phospholipids from the cell membrane. This process could lead to total phospholipid removal via an increase in phosphodiesters in the brain. This removal then decreases glutamate signaling, resulting in decreased action potential of the neurons, as well as hypo-metabolism of the affected neurons. Neurons that are not utilized properly
and frequently can cause synaptic volume loss, which can then induce both negative and cognitive symptoms.\textsuperscript{9}

These important 5-HT\textsubscript{2A} receptors are primarily located in the prefrontal cortex, the limbic system, and the basal ganglia. These structures and pathways are thought to be involved in the pathophysiology of schizophrenia, and the localization of many key neurons in those areas helps to confirm this hypothesis.\textsuperscript{18} Many of the 5-HT neurons send signals through the basal ganglia-thalamus-cerebral cortex network in the brain. Extrapyramidal motor functions are primarily mediated by the basal ganglia, where many of the neurons are located, so the blocking of the signals from 5-HT neurons may cause a reduction in observed EPS in schizophrenic patients\textsuperscript{17}.

1.4 Antipsychotic Drug Classifications

The antipsychotic drugs (APDs) used to treat schizophrenia are split into two main types: classical antipsychotic drugs and atypical antipsychotic drugs.\textsuperscript{19} One of the defining characteristics that separate the two classes is their tendency to generate extrapyramidal side effects. The atypical APDs currently on the market show a reduction in EPS compared to classical drugs, but can be associated with other serious side effects such as excessive weight gain and dangerous metabolic conditions.\textsuperscript{20} Acute treatment with classical APDs such as haloperidol or chlorpromazine may cause catalepsy, akathisia, tremors, body rigidity and bradykinesia, while chronic treatment may induce the development of tardive dyskinesia or tardive dystonia.\textsuperscript{20-22} Finally, classical antipsychotics target the dopamine receptors, while atypical antipsychotics target
dopamine receptors as well as others, such as the previously discussed serotonin receptors.\textsuperscript{11}

\textbf{1.5 Atypical Antipsychotic Drugs}

There is currently a high demand for the development of new and effective atypical antipsychotic drugs. As previously mentioned, there are a large percentage of patients who do not benefit from the classical antipsychotics, resulting in major health problems as a consequence. Schizophrenic individuals who are treatment-resistant tend to require more intensive care, and the persevering psychotic symptoms make these patients less receptive to psychosocial and vocational rehabilitation. In addition, around 20-30\% of patients that were initially receptive to classical antipsychotics may relapse during their first few years of treatment.\textsuperscript{22} These factors helped to contribute to the research and development of atypical antipsychotic drugs, as there were many patients who still could not receive the care they needed.

Though atypical antipsychotics are mainly focused on targeting the 5-HT receptors in the brain, they tend to have some reduced selectivity towards D\textsubscript{2} receptors as well. This selectivity is analyzed via a 5-HT\textsubscript{2A}/D\textsubscript{2} binding ratio, where a higher ratio would indicate a higher selectivity for 5-HT\textsubscript{2A} receptors over D\textsubscript{2} receptors. Since EPS tend to occur in relation to high D\textsubscript{2} receptor occupation, designing a ligand that has a high affinity for the 5-HT\textsubscript{2A} receptors and a lower affinity to D\textsubscript{2} receptors would likely result in an atypical classification as well as reduced observed extrapyramidal side effects.\textsuperscript{13}

The length of time that a drug interacts with its designated receptor may have a serious effect on the development of extrapyramidal side effects as well as its atypical
profile. It is thought that antagonists with a fast dissociation rate ($k_{\text{off}}$) from the dopamine receptors allow for the reduced display of these side effects. A fast $k_{\text{off}}$ would generate a prompt and reversible antagonistic effect, allowing the receptors to retain their signaling dynamics. However, studies showed that the difference in dissociation rates between classical APDs, such as chlorpromazine or haloperidol, and atypical APDs, such as clozapine, displayed only a two- to six-fold increase for atypical drugs. Compounds that have a high calculated lipophilicity or low solubility in water were also shown to have an slower $k_{\text{off}}$ and act in part as a long-lasting antagonist\textsuperscript{23}. There is controversy when it comes to this theory, because the atypical drug sertindole has a lower dissociation rate than the classical drug haloperidol.\textsuperscript{24} This suggests that while the dissociation rate could be a factor in determining if a drug has an atypical profile, not all compounds may fit this model.

1.5.1 Clozapine

![Figure 3: the structure of clozapine](image)

Clozapine (Figure 3), a dibenzodiazepine derivative, was the first antipsychotic drug that was shown to be more clinically effective than classical antipsychotics while also reducing extrapyramidal side effects.\textsuperscript{14} It induces little to no tardive dyskinesia, and has no reported plasma prolactin increases in patients, which could lead to infertility.
Clozapine has been shown to be effective in approximately 30% of treatment resistant schizophrenic patients, while classical APDs such as chlorpromazine have only been effective in around 4% of these patients.\textsuperscript{21}

Despite its beneficial EPS profile, treatment with clozapine has been shown to produce other serious side effects such as seizures, sialorrhea, orthostatic hypotension, and most significantly, agranulocytosis.\textsuperscript{21} In 1975, 16 patients in Finland developed granulocytopenia, and of those patients, 13 developed agranulocytosis (eight of whom died due to secondary infection). Since then, the use of clozapine has been significantly decreased. This tragic incident prompted an increase in restrictions on who can use clozapine, limiting its use to treatment-resistant patients, patients that are very sensitive to EPS, and dyskinetic patients. The government also made it so that patients were required to undergo intensive white blood cell monitoring during use to ensure that this blood disorder was not developing. While these new restrictions caused the overall incidence of agranulocytosis to decrease, the risk of developing this condition is still much greater than the risk associated with many other antipsychotics.\textsuperscript{22}

Clozapine is classified as an atypical antipsychotic drug due to its high affinity for the 5-HT receptors, as well as being a weak D\textsubscript{2} receptor antagonist. It interacts predominately with the 5-HT\textsubscript{2A} and D\textsubscript{2} receptors, though it has been shown to interact with muscarinic (M), 5-HT\textsubscript{3}, 5-HT\textsubscript{1C}, and D\textsubscript{4} receptors as well.\textsuperscript{21} The combination of these additional receptor interactions may have some role in the antipsychotic effect of clozapine. Another interesting aspect of clozapine is that it has a 10-fold higher affinity for D\textsubscript{4} receptors over D\textsubscript{2} and D\textsubscript{3} receptors. At therapeutic concentrations, clozapine occupies over 90% of D\textsubscript{4} receptors compared to only 30-60% of D\textsubscript{2} receptors. The lower
occupancy of D₂ receptors contributes to the lack of EPS observed, while the high occupancy of D₄ receptor may play a bigger role in the therapeutic action.¹⁵

The importance of serotonin receptors in the treatment of schizophrenia with clozapine and other atypical antipsychotics has been verified through various studies. In one test, the agonism of both 5-HT₂A and 5-HT₂C receptors resulted in hallucinations, which suggested that these receptors are likely highly connected to psychotic symptoms. Clozapine displays affinity for both of the 5-HT₂A and 5-HT₂C receptors to a much higher degree than it does for dopamine receptors, which can help with the treatment of many of the psychotic symptoms of this illness. Other effective atypical antipsychotics do not display 5-HT₂C affinity, which suggests this receptor subtype may not be a significant contributing factor to reducing both EPS and schizophrenic symptoms.²⁴

There are a few different mechanisms of action for clozapine that contribute to its superior side effect profile. One hypothesis is that clozapine decreases the dopaminergic activity in the mesolimbic system in a relatively selective manner over the dopamine neurons in the nigrostriatal regions of the brain, which is supported by electrophysiological studies. However, this hypothesis has mixed and controversial results when it comes to the chemical and biological evidence behind it. There have been many studies done that found that clozapine had a greater effect on dopamine turnover in the limbic systems, but there have been an even larger group of studies that found the opposite. Though there are varying experimental results, some studies have produced evidence that clozapine has some mesolimbic specificity, which may contribute to the lack of EPS. However, this cannot account for the greater efficacy in decreasing positive symptoms,¹⁰ so it must not be the only mechanism at play. While the other mechanisms
involved are not further discussed in this paper, it is important to note that there are many factors that contribute to a drug's clinical efficacy and success, and that cannot be overlooked when designing potential new therapeutics.

1.5.2 JL 13

The medications currently on the market are limited in their effectiveness, so there is a large unmet medical need for the 20-60% of patients that are unresponsive to the available antipsychotic drugs. A lengthy study conducted confirmed that clozapine had a greater efficacy both against negative symptoms as well as in patients that were treatment-resistant. This prompted researchers to develop a series of new antipsychotics that mimicked the therapeutic benefits of clozapine without the unwanted side effects. Eventually, 5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-b][1,5]benzoxazepine fumarate, more commonly known as JL 13 (Figure 4), was created. JL 13 is structurally similar to clozapine in many ways, such as the position of the chlorine and the methyl piperazine on the tricyclic moiety. Replacing the secondary amine in the central seven-membered ring with an oxygen and the introduction of a pyridine ring instead of a benzene ring helped JL 13 keep many of clozapine’s physicochemical properties while potentially decreasing unwanted side effects. One benefit derived from the change to an

Figure 4: The structure of JL 13
oxazepine is that JL 13 is much less sensitive to peroxidase-induced oxidation than clozapine is, reducing its possible toxicity to humans. In dog tests, JL 13 did not induce any of the significant side effects that clozapine does, such as sialorrhea or palpebral ptosis, or any motor side effects, such as Parkinsonian symptoms. All of these qualities make JL 13 a good atypical antipsychotic drug candidate.

It has been shown that most pyridine analogs of clozapine are weaker dopamine blockers than clozapine itself. Neurochemical studies with JL 13 confirmed that it had lower affinity for both D1 and D2 receptors compared to clozapine and the classical antipsychotic drug haloperidol (Table 1). This suggests that JL 13 will likely produce fewer extrapyramidal side effects than its predecessor clozapine as well as classical APDs.

**Table 1:** A comparison of various dopamine and 5-HT2 receptor binding affinity values for JL 13, clozapine, and haloperidol.

<table>
<thead>
<tr>
<th>Drug</th>
<th>D1</th>
<th>D2</th>
<th>D4</th>
<th>5-HT2A</th>
<th>5-HT2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>JL 13</td>
<td>302</td>
<td>1190</td>
<td>164</td>
<td>65</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Clozapine</td>
<td>115</td>
<td>45</td>
<td>21</td>
<td>3.8</td>
<td>8</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>25</td>
<td>1.0</td>
<td>0.88</td>
<td>78</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

JL 13 also displayed a higher selectivity ratio (7.25) than clozapine for the D4 receptor (Kᵢ =164 nM) over D2 receptors (Kᵢ = 1190 nM). Since an increased concentration of D4 receptors has been implicated in schizophrenic patients in several studies, the D4 antagonistic properties of JL 13 could be worth investigating. It is important to note that studies done with both a selective D4 antagonist as well as a mixed 5-HT2A/D4 antagonist showed no antipsychotic efficacy, so therefore the clinical relevance of D4 receptors in treatment is still controversial. However, the compounds
used in these studies had not demonstrated any in vivo blockage of D₄ receptors at clinical doses\textsuperscript{14}, so that may have contributed to the lack of antipsychotic action seen.

As a mixed 5-HT\textsubscript{2A}/D₂ antagonist, JL 13 also interacts with both the dopamine and serotonin systems. In a fashion similar to clozapine, JL 13 selectively acts on dopamine receptors in the mesolimbic system, while only interacting marginally with receptors in the neostriatum. This supports the hypothesis that JL 13 should display reduced EPS compared to classical antipsychotics. In vivo studies previously done showed that decreases in extracellular dopamine levels in the prefrontal cortex may be partially related to the development of both negative and cognitive symptoms. These studies found a strong correlation between the difference in affinities for 5-HT\textsubscript{2A} and D₂ receptors and a better ability to increase dopamine release in the prefrontal cortex. Both JL 13 and clozapine are weak D₂ antagonists and strong 5-HT\textsubscript{2A} antagonists, so the results found for these molecules should align with this hypothesis. As predicted, studies showed that clinical doses of clozapine, JL 13, as well as other atypical antipsychotics raised the levels of dopamine release in the medial prefrontal cortex more than in the striatum or nucleus accumbens while not modify the levels of dopamine in either of those areas.\textsuperscript{14} These results agree with JL 13’s atypical antipsychotic profile and again suggest that this compound will induce less EPS that many classical and atypical drugs.

In the serotonin system, JL 13 was shown to block (±)-DOI-induced head twitches (2,5-dimethoxy-4-iodoamphetamine), which are responses related to both 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptor agonism. Since JL 13 shows no significant 5-HT\textsubscript{2C} affinity, this effect can be attributed to JL 13 acting on 5-HT\textsubscript{2A} receptors, which is consistent with an atypical antipsychotic drug profile. The lack of 5-HT\textsubscript{2C} antagonism could also give JL
13 a more beneficial side effect profile. Many believe that the antagonism of 5-HT$_{2C}$ receptors causes patients to increase their intake in food, resulting in gain weight or obesity. Since JL 13 does not interact with this receptor subtype, it may aid in helping to reduce the amount of weight gained by patients on atypical antipsychotics. However, this is only one of many side effects of the drug profile that needs to be analyzed to ensure that JL 13 is not only effective, but safe as well.

With the safety and health issues presented in clozapine, JL 13 was analyzed to determine its propensity to generate many of the possible side effects and toxicities associated with many antipsychotic drugs. As a drug with an atypical profile, it is expected that treatment with JL 13 would decrease the amount of extrapyramidal side effects observed. When haloperidol-sensitized monkeys were dosed with JL 13, dystonia or bradykinetic Parkinsonian symptoms only occurred at the high doses of 50 mg/kg. The normal doses had some sedative effects, but reactivity levels in the animals were not affected, suggesting that the sedation is only mild and should not impair normal response ability. Another serious health problem that is associated with many classical antipsychotic treatments is increases in the concentration of prolactin in the endocrine system. Prolactin concentrations are closely related to the level of occupancy of antagonists in D$_2$ receptors - the more receptors that are occupied, the more prolactin concentrations increase. Increases in levels of prolactin are associated with many different health conditions in multiple physiological systems. Since JL 13 is a weak D$_2$ blocker, it does show a slight increase in circulating prolactin levels after 30 minutes post administration at a dose of 30 mg/kg. This increase is not observed at lower doses, but even at this high dose, prolactin levels return to normal after 150 minutes.
In addition, antipsychotics like clozapine and JL 13 have a potential to cause hematotoxicity, such as agranulocytosis. Studies show that an increased risk of developing disorders such as agranulocytosis is strongly related to a compound’s oxidation potential. Jean-François Liégeois et al. performed a series of experiments to determine how readily JL 13 undergoes oxidation via peroxidase enzymes compared to clozapine and another benzoxazepine derivative loxapine. They found that clozapine was very easily oxidized compared to both JL 13 and loxapine, enhancing thyl radical presence with very low concentration of peroxidase enzymes. With clozapine, many believe that this could be related to the ability to form a free radical when it comes in contact with peroxidase enzymes. The lone pair on the secondary amine in the central ring is highly available and can easily form the free radical, which can then also form the nitrenium cation via activation by neutrophils or acids. These cation structures can then form complexes with peroxidase enzymes as well as glutathione. However, oxazepines such as JL 13 have a lower propensity to undergo oxidation, as the two lone pairs on the oxygen in the central ring are much less available and the compound has no radical scavenger activity. There are no observed cation formations with benzoxazepines, as the delocalization observed in benzodiazepines such as clozapine is not possible within the structure of benzoxazepines. Therefore, benzoxazepine derivatives like that of JL 13 could be of significant interest not only in terms of efficacy, but overall drug safety as well.
1.6 Previously Synthesized Fused Triazole Benzoazepine and Benzodiazepine Derivatives

Based on the core structure and promising preclinical data of JL 13, Mongeau et al. designed various fused triazole derivatives of both benzodiazepine and benzoazepine molecules. The fused-ring molecules are of clinical interest in medicinal chemistry because they have been shown to possess beneficial pharmacologic and metabolic profiles. Benzodiazepine and benzoazepine molecules also produce therapeutic effects in different disease areas, including anxiety disorders, insomnia, and mood and movement disorders, in addition to treating many psychotic illnesses.

Ten fused triazolo-benzoazepine compounds (Figure 5) were designed for an initial screening in 5-HT$_{2A}$ affinity assays. These compounds were synthesized via conventional heating, microwave, and flow cytometry chemistry. Both microwave and flow conditions proved to produce higher yields (54-99%) than the conventional heating methods (17-66%). The substituents on the triazole ring for these analogs are described in Table 2.

![Figure 5: The structure of the first series of benzoazepine analogs](image-url)
Table 2: The various groups (R) of the triazolo-benzoazepine analog in figure 4.

<table>
<thead>
<tr>
<th>Substituent (R)</th>
<th>(2\text{-Cl-C}_6\text{H}_4)</th>
<th>(2\text{-OH-C}_6\text{H}_4)</th>
<th>(3\text{-OMe-C}_6\text{H}_4)</th>
<th>(2\text{-F-C}_6\text{H}_4)</th>
<th>(3\text{-NO}_2\text{-C}_6\text{H}_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>2-Cl-C(_6)H(_4)</td>
<td>2-OH-C(_6)H(_4)</td>
<td>3-OMe-C(_6)H(_4)</td>
<td>2-F-C(_6)H(_4)</td>
<td>3-NO(_2)-C(_6)H(_4)</td>
</tr>
</tbody>
</table>

However, none of the aryl-substituted triazole analogs showed significant affinity to 5-HT\(_2A\), which may have been caused by solubility issues with these compounds during screening. The assay used for this was performed in an aqueous matrix, requiring the compounds tested to be soluble in an aqueous mixture. These triazolo-compounds are fairly lipophilic which resulted in poor solubility for this assay. This problem was addressed when designing new analogs, with the newer compounds made into HCl salts to eliminate many of the solubility issues. However, there were still some problems using these new HCl salts, as they were fairly hygroscopic and did not give true binding values. In the literature, JL 13 is often synthesized as the fumarate salt form, so the next strategy was synthesizing these analogs as the fumarate salt. This may not only allow for more accurate binding results, but could also give values that would be comparable to those in the literature\(^{29,31}\).

After overcoming the solubility issues of these molecules, a new strategy for potentially increasing binding was developed by adding a basic aminoalkyl group off of the triazole. The formation of a compound that included a 1-(4-chlorophenyl)-N,N-dimethylymethanamine substituent off of the triazole showed moderate binding to the receptor \((K_i = 35 \text{ } \mu\text{M})\). This prompted a production of a large library of analogs, many of
which included an aminoalkyl group (Figure 6). A representative table (Table 3) of various R groups synthesized with these analogs is included below. However, even with these new additions, no significant 5-HT$_{2A}$ binding was observed.$^{29}$

![Figure 6: The structures of the second series of triazolo-benzoxazepine analogs](image)

Table 3: A small, representative sample of the various substitutions on the triazole ring of the structure in figure 5.$^{29}$

<table>
<thead>
<tr>
<th>Num.</th>
<th>R</th>
<th>Num.</th>
<th>R</th>
<th>Num.</th>
<th>R</th>
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<td><img src="image" alt="N" /></td>
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<td>2</td>
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<td>6</td>
<td><img src="image" alt="N" /></td>
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</tr>
<tr>
<td>4</td>
<td><img src="image" alt="N" /></td>
<td>8</td>
<td><img src="image" alt="NO$_2$" /></td>
<td>12</td>
<td><img src="image" alt="N" /></td>
</tr>
</tbody>
</table>
Molecular modeling of JL 13 and clozapine suggested that benzodiazepine molecules have better binding interactions with many of the key residues of the 5-HT\textsubscript{2A} receptor via hydrogen bonding and pi stacking. Piperazinyl benzodiazepine derivatives were then synthesized with a hydrogen, chlorine, or fluorine in the 8 position (Figure 7) to more closely mimic the structure of JL 13 and clozapine.\textsuperscript{29}

![Figure 7](image)

**Figure 7:** The structure of the piperazinyl benzodiazepine derivatives

While the hydrogen and fluorine derivatives displayed no apparent binding, the chlorine derivative showed a K\textsubscript{i} of 35 nM. Seeing as JL 13 also has a chlorine in the 8 position on the benzene ring, this suggested that the chlorine molecule may play an important role in the compounds ability to bind to the receptor. This then prompted the design and synthesis of 1,2,4-triazolo-benzodiazepine derivatives (Figure 8) with the chlorine atom on the benzene ring. Many of the same analogs described above in table 3 were synthesized with the new benzodiazepine structure. These analogs still displayed some solubility issues, and there was again no significant binding to the 5-HT\textsubscript{2A} receptor.\textsuperscript{29}
1.7 Synthesis of Novel Fused Triazole Benzodiazepine Derivatives

Due to the lack of binding affinity of the previously synthesized analogs, the structures of the compounds needed to be reevaluated. In comparing the structures of the triazole analogs to those of clozapine and JL 13, the orientation of the R group on the triazole became a topic of interest. In both clozapine and JL 13, the methyl piperazine is on the same carbon, labeled either C11 or C5, and facing away from the chlorine on the benzene ring. In the 1,2,4-triazole diazepine analogs that were previously synthesized, the R group, acting in a similar fashion to the methyl piperazine, is in the C3 position. More importantly, this leaves the R group facing in the same direction as the chlorine, which is the opposite orientation that the methyl piperazine is in for both clozapine and JL 13. This lead to a hypothesis that, because these groups are facing the opposite direction, it could affect the molecule’s ability to bind to the pocket in the 5-HT$_{2A}$ receptor. Based on this idea, a new scaffold for novel 1,2,4-triazolo-analogs was formed. Designing and utilizing a new synthetic route would generate the reverse amide benzodiazepine structure, and therefore a reverse chloroimine structure than previously used. This new structure should allow for triazole formation with the R group oriented towards the pyridine ring and away from the chlorine (Figure 9). Four triazolo-analogs were
synthesized containing the following functional groups: methyl, 2-fluorophenyl, 4-hydroxyphenyl, and 3-methoxyphenyl.

![Figure 9](image)

**Figure 9**: The structure of the novel triazolo-benzodiazepine analogs

The first attempt at preparing these new compounds was based on a one-pot synthesis procedure by Wolfhard W. Engel et al. The starting materials used to form 8-chloro-5,11-dihydro-6H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one were 2-chloro-3-aminopyridine and methyl 2-amino-5-chlorobenzoate (Scheme 1). The two reagents were coupled using potassium tert-butoxide, and the internal ring was closed with the addition of sulfuric acid then heating overnight. The first attempt on a 0.5 g scale resulted in a poor yield of 10%, and in the second attempt the compound mass was not observed via Liquid Chromatography Mass Spectrometry (LCMS). Due to the cost of the methyl 2-amino-5-chlorobenzoate, a new synthetic route seemed to be the best option moving forward.
A new synthetic route was then developed to form these triazolo-analogs. A variety of procedures were utilized for the synthesis of the desired intermediates, though some of them were unsuccessful. Described herein are the procedures that did not result in product formation.

The new route for the synthesis of these benzodiazepines began with the preparation of the acid chloride 1 from 5-chloro-2-nitrobenzoic acid (Scheme 2). The procedure called for dissolution of the benzoic acid and oxalyl chloride in tetrahydrofuran (THF) and N,N-dimethylformamide (DMF). The mixture was heated at reflux until the solution turned pale yellow in color after approximately one hour. The excess oxalyl chloride and solvent were then removed. However, an hour was insufficient time as there was little evidence of product formation so this reaction procedure was discarded.
Scheme 2:

![Scheme 2](image)

a) oxalyl chloride, THF, DMF, 80 °C, 1 hour

There were some issues with the coupling step involving the nitrobenzoyl chloride and 3-amino-2-chloropyridine as well (Scheme 3). The first attempt at the coupling was done by dissolving the nitrobenzoyl chloride in THF and pyridine, followed by the addition of 3-amino-2-chloropyridine. The reaction was then stirred for two hours at room temperature. LCMS and TLC suggested conversion to the desired product. An acid-base workup was then performed before recrystallization of the product. This step was attempted in both chloroform\textsuperscript{33} and 80% aqueous ethanol,\textsuperscript{29} but neither system worked. Additionally, another solvent system was tried for the coupling, where the two starting materials were dissolved in a mixture of 1,4-dioxane, cyclohexane, and pyridine then stirred overnight at room temperature,\textsuperscript{34} but that reaction was also unsuccessful.

Scheme 3:

![Scheme 3](image)

a) THF, room temperature, 2 hours; b) 1,4-dioxane, cyclohexane, pyridine, room temperature, overnight
To convert the nitro group to an amine on the coupled nitrobenzamide material (Scheme 4), the coupled material was dissolved in 2 M ammonium hydroxide. This nitrobenzamide did not dissolve well in this solution, but the reaction was warmed in an oil bath nonetheless. Sodium dithionite was added slowly, although the mixture still remained a suspension. After one hour of heating, TLC showed no product formation at all. Therefore, it was necessary to find a procedure for this step that had a solvent system that allowed for dissolution of the nitrobenzamide starting material.

Scheme 4:

To close the central seven-membered ring forming a secondary amine (Scheme 5), a Buchwald-Hartwig amination reaction was performed using the benzamide material, palladium(II) acetate, potassium tert-butoxide, and BINAP dissolved in toluene. The mixture was heated at 110 °C for a total of two days while being monitored via TLC and High Performance Liquid Chromatography (HPLC). Samples after one and two days showed no evidence of product formation. Research into literature suggested that the temperature of this reaction needed to be much higher in order to close the internal ring. When this reaction was done in a microwave at 200 °C for 15 minutes, there was full conversion to product which could be isolated in moderate yields through a simple ether workup. The downside of this procedure was that the microwave vials could only hold so much material, so this reaction had to be done many times in a row before getting enough
product to move through to the next two steps. Therefore, this microwave procedure, while successful, was not efficient for this overall synthetic route.

**Scheme 5:**

The chlorination of the closed-ring product (Scheme 6) originally met little success. The starting material was dissolved in toluene, then N,N-dimethylaniline and phosphorus(V) oxychloride (POCl₃) were added. The reaction was heated to 100 °C and stirred overnight.¹⁶ TLC suggested formation of chloroimine product. However, the column purification of the reaction mixture did not yield any product. The second attempt at this reaction used the same starting materials, but the reaction was warmed to reflux for 6 hours. The reaction mixture was monitored via TLC every hour, and LCMS every three hours. The LCMS at 6 hours showed consumption of both the starting material and intermediate. While the mass of the product was detected, another larger mass with m/z of 380 was detected in high quantities. This was determined to likely be the desired product with a POCl₂ adduct on either the nitrogen in the amide bond or the nitrogen in the pyridine ring (Scheme 7). The reaction mixture was too impure to move forward so another column purification was run. However, the product and byproduct co-eluted in a gradient of 0-30% ethyl acetate:hexanes and could not be successfully separated. This procedure gave a very low yield while sometimes not converting any of the starting
material to product. Therefore, it was best to find a new procedure that could form the chloroimine with better yield and less byproduct formation.

**Scheme 6:**

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{N} & \quad \text{N} \\
\text{O} & \\
\text{Cl} & \\
\end{align*}
\]

\[\overset{\text{a}}{\rightleftharpoons}\]

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{N} & \quad \text{N} \\
\text{O} & \\
\text{Cl} & \\
\end{align*}
\]

a) \(\text{POCl}_3, \text{N,N-dimethylaniline, toluene, 100 °C, overnight}\)

**Scheme 7:**

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{N} & \quad \text{N} \\
\text{O} & \\
\text{Cl} & \\
\end{align*}
\]

\[\overset{\text{a}}{\rightleftharpoons}\]

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{N} & \quad \text{N} \\
\text{O} & \\
\text{Cl} & \\
\end{align*}
\]

\[\overset{+}{\text{Cl}} \quad \overset{+}{\text{PO}} \quad \overset{+}{\text{Cl}}
\]

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{N} & \quad \text{N} \\
\text{O} & \\
\text{Cl} & \\
\end{align*}
\]

a) \(\text{POCl}_3, \text{N,N-dimethylaniline, toluene, 100 °C, 7 hours}\)
While experiencing problems with the chloroimine formation, a microwave reaction was attempted with the closed-ring diazepin-6-one product (Scheme 8) to see if it would form the triazole compound without needing to form the chloroimine intermediate. This reaction was performed with the same reaction conditions put forth by Mongeau et al. for the formation of the triazolo-analogs. The diazepin-6-one product, acetohydrazide, and 1-butanol were mixed in a microwave tube and the solution was irradiated at 200 °C for 15 minutes. The reaction was unsuccessful and saw no conversion to the desired product via LCMS. Therefore, it was determined that the chloroimine material was necessary to form the triazole.

Scheme 8:

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{HN} & \quad \text{O} \\
\text{HN} & \quad \text{N}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{Cl} \\
\text{HN} & \quad \text{N}
\end{align*}
\]

a) acetohydrazide, 1-butanol, 200 °C, 15 minutes
 CHAPTER TWO: Experimental Design

There were many synthetic route options that were unsuccessful in forming the desired intermediates of this project. However, after much trial and error a successful synthetic scheme was designed that achieved formation of each intermediate in moderate to good yields (Scheme 9).

Scheme 9:

a) oxalyl chloride, DCM, DMF, room temperature 3 hours; b) pyridine, 0 °C 1 hour, room temperature overnight; c) tin (II) chloride, ethanol, 80 °C 30 minutes; d) Sulfolane, conc. sulfuric acid, 140 °C, 1-4 hours e) phosphorus (V) oxychloride, toluene, 100 °C overnight; f) R-CONHNH₂, butanol, 200 °C, 15 mins
The acid chloride 1 needed to perform the coupling reaction with 3-amino-2-chloropyridine was synthesized using 5-chloro-2-nitrobenzoic acid as the starting material. The carboxylic acid was dissolved in dry dichloromethane (DCM) and dry DMF before being cooled to approximately 5 °C. Oxalyl chloride was added drop wise and the reaction was warmed back to room temperature. Product formation was seen after one hour, but the reaction took three hours to completely consume the starting material. This product conversion was monitored via TLC, as neither the starting material nor product have good ionizable groups and therefore were not observed using LCMS. The excess oxalyl chloride and solvent were removed in vacuo and product 1 was isolated in high yield.

To successfully couple together product 1 and the 3-amino-2-chloropyridine, the acid chloride was first dissolved in pyridine. The starting material tended to get somewhat sticky, and usually took extra stirring or sonication to completely dissolve. Once the acid chloride was completely in solution, 3-amino-2-chloropyridine was added and the solution was stirred at 0 °C for one hour, then at room temperature overnight. The conversion from starting material to product was observed by TLC as well as LCMS. The coupled product 2 was precipitated by the addition of water, and was then filtered, dried, and collected in moderate yields.

The next step in this synthetic route was the conversion of the nitro group on the benzene ring to an amine, which would then allow for the closing of the seven-membered central ring. Product 2 was dissolved in ethanol (EtOH), and in a separate flask tin(II) chloride was also dissolved in EtOH. The nitrobenzamide product did not completely go into solution, so once EtOH was added the mixture was sonicated until it was mostly
homogenous. This mixture was then slowly added to the tin(II) chloride solution and warmed to 70 °C. The undissolved nitrobenzamide almost immediately went into solution upon reaching temperature, giving a clear yellow solution. The reaction was monitored by TLC, and showed complete product conversion after 30 minutes, producing an orange solution. The solution was cooled to room temperature before being poured over ice water. It was then adjusted to pH 7-8 using a 5% sodium bicarbonate solution, and stirred for an hour to hydrolyze the tin salts. The benzamide product 3 was extracted with chloroform and the solvent removed to afford the desired product in moderate yields.

To close the internal seven-membered ring, product 3 was dissolved in sulfolane and concentrated sulfuric acid. The reaction was warmed to 140 °C and was completed within one to four hours, depending on how much starting material was used. The reaction was monitored by both TLC and LCMS and confirmed conversion to the desired product. The solution was then poured over ice water and the product precipitated, then ethyl acetate was added to remove any impurities. The final product 4 was filtered, dried, and collected in good yields. Something important to note is that there were a few instances where the hot plates did not appear to get the reaction mixture to 140 °C, and so the reaction was run at around 120 °C. However, the product was still formed in the same amount of time at these temperatures, so heating the reaction to 140 °C may not be necessary to form the final product. This would be something worth investigating at a later time when attempting to optimize all procedures.

The reaction to form the chloroimine intermediate 5 was done by heating product 4 with excess phosphorus(V) oxychloride (POCl₃) in toluene. Extra equivalents of
POCl$_3$ were added throughout the reaction process in 4-hour increments, but the reaction had still not completed after 8 hours. The reaction then was stirred overnight to allow for complete conversion to product 5. The solvent was evaporated and the crude product dried. Many attempts were made at isolating the chlorinated product via column chromatography, but seeing as the chloroimine was very susceptible to hydrolysis, they were met with little to no success. Therefore, the crude product was generally taken forward to the last step.

Finally, the triazolo-analogs were formed with the use of CEM microwave technology. The chloroimine product 5 was added to a microwave tube with a substituted hydrazide and 1-butanol. Microwave irradiation at 200 °C for 15 minutes resulted in moderate conversion to the substituted triazole compounds, 6a-d. The dried products were purified via preparative TLC to give moderate to low yields. Some of 5 was seen via LCMS when using 100% converted chloroimine material, so it is possible that butanol as a solvent may hydrolyze some of the starting material and therefore affect the yield. Another possibility is that the butanol used was not completely dry and contained water, which would easily hydrolyze the starting material. A test reaction was done with toluene as the solvent instead of 1-butanol. Complete conversion to the desired product was observed with a better crude yield than reactions done in butanol. Although toluene is not a conventional microwave solvent, the previous reaction done to form the chloroimine was run in toluene, so it was already clear that the starting material would have good solubility in this solvent. The Buchwald-Hartwig ring closing microwave reaction done earlier in this synthetic route was also done in toluene, so it seemed like a
logical solvent to test out first. Future work on selecting the most efficient solvent for this reaction would be necessary for optimization of this procedure.

**CHAPTER THREE: Results and Discussion**

Through this newly formed synthetic route, 4 novel fused triazolo-analogs were designed and synthesized. Unfortunately, these new compounds were not screened in the 5-HT$_{2A}$ binding assay due to time constraints. At this point, it is currently unknown whether or not the orientation of the R group on these triazolo-analogs has any effect on its ability to bind to the receptor. While the structures of both clozapine and JL 13 compared to the previously synthesized triazolo-analogs suggest that it may be important, it cannot be determined with reasonable certainty until the compounds are screened through binding assays.

While none of the analogs that were previously synthesized by Mongeau et al. showed any binding activity, the compounds synthesized with this general scaffold still hold promise. The molecular modeling done comparing clozapine and JL 13 demonstrated that the benzodiazepine structure likely conferred better binding interactions with the receptor through hydrogen bonding and pi stacking. The addition of the fused triazole ring may already help to increase the aromatic pi stacking interactions of these analogs with the receptors, but it may not be enough to result in any clinically relevant affinities. Synthesizing analogs that contain this triazole ring as well as a group that could increase hydrogen bonding may allow for this altered structure to display more significant binding.
Analogs with nitrogen-containing substituents off of the triazole ring could likely show decent binding affinity to the 5-HT\textsubscript{2A} receptors despite their relatively poor performance in the previously synthesized compounds. Studies done on many G protein-coupled receptors, such as the 5-HT receptors, show that all ligands that interact with these receptors contain at least one basic amine moiety. While all of the endogenous neurotransmitters contain primary amines, exogenous ligands that have secondary or tertiary amine groups bind to these receptors as well. Homology modeling studies done on the 5-HT\textsubscript{2C} receptor suggest that primary amines bind more tightly to this receptor than secondary or tertiary amines,\textsuperscript{42} so synthesizing molecules with tertiary amine substituents could confer receptor selectivity for 5-HT\textsubscript{2A}.

JL 13, which the structure of these compounds was partially based off of, showed no binding affinity to the 5-HT\textsubscript{2C} receptors while having a high affinity for 5-HT\textsubscript{2A}.\textsuperscript{29} Based off of the molecular modeling studies, this selectivity for 5-HT\textsubscript{2A} over 5-HT\textsubscript{2C} may be due in part to the tertiary amine in the methyl piperazine. These amine groups can form hydrogen bonds with some of the amino acids in the binding pocket, which could then increase binding affinity. This effect was not seen with the benzodiazepine and benzoazepine compounds synthesized previously, and that may in part be due to the orientation of the substituents. As previously stated, in JL 13 as well as clozapine, the methyl piperazine faces away from the chlorine on carbon 8.\textsuperscript{14} In all the triazolo-analogs, the chlorine is located on the same carbon, now labeled either carbon 6 or carbon 12. In the older analogs, the substituents off of the triazole carbon 3 face towards the chlorine while the substituents in the newly synthesized compounds described in this work face away from it. Since both clozapine and JL 13 show 5-HT\textsubscript{2A} binding affinity and the older
analogs do not, the difference in substituent directionality is one possible reason why this is observed.

Synthesizing analogs with various groups capable of hydrogen bonding off of the triazole ring would be of the upmost importance in continuing this project. Since tertiary amine groups have been significantly implicated in connection with increased 5-HT$_{2A}$ binding affinity, making compounds with groups such as dimethylamines, both free and as a part of a benzene ring system, would be some of the most likely candidates to show any binding affinity. Other groups of interest that could form hydrogen bonds within the receptor would be methoxy or hydroxyl moieties. Again, these could be tested as free groups or attached to a benzene ring. In all of these cases, the addition of another ring could help to promote hydrogen bonds, as it would likely shorten the distance between the substituent and the group to which it is binding. A benzene ring could also help to promote pi stacking between aromatic regions and therefore potentially increase binding affinity. Mongeau et al. previously synthesized many of these structures with the old triazole scaffold (as seen in figure 3), so being able to compare the observed binding constant of these very similar structures would help to validate or negate the hypothesis regarding the relationship between receptor affinity and orientation of the triazole substituent.

Although the previous molecular modeling done on this project suggested that diazepine analogs confer better binding to the receptor than oxazepine analogs, it would be beneficial to synthesize oxazepine analogs as well. Oxazepines are less sensitive to oxidation, which can decrease the probability of developing hematological diseases. Therefore, the recreation of any new diazepine analogs synthesized as oxazepines would
be of interest. This would require developing a new synthetic route to form the oxazepine version of the analogs created within this work. Once the new route is determined, a large library of analogs could be created and tested for binding affinity.
Experimental Procedures

**Synthesis of 5-chloro-2-nitrobenzoyl chloride (1)**

A flame-dried, 3-neck round bottom flask was fitted with a reflux condenser, thermocouple, and stir bar. The flask was cooled to room temperature under argon atmosphere. 5-chloro-2-nitrobenzoic acid (3.5 g, 0.017 mol, 1 eq), anhydrous dichloromethane (22 mL), and anhydrous dimethylformamide (1 drop) were added. The solution was stirred in an ice bath until it reached 5 °C. Oxalyl chloride (2.06 mL, 0.024 mol, 1.4 eq) was added dropwise to the solution. The solution was then warmed to room temperature and stirred for 3 hours. TLC in 4:1 DCM:MeOH shows conversion to product. Excess oxalyl chloride was removed in vacuo and the product was dried to give 5-chloro-2-nitrobenzoyl chloride as a yellow oil (3.7 g, 99% yield). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.09 (dd, $J$ = 8.8, 1.7 Hz, 1H), 7.69 (dt, $J$ = 8.7, 2.0 Hz, 1H), 7.65 (q, $J$ = 2.2 Hz, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 164.8, 143.2, 141.2, 134.0, 132.7, 128.2, 126.3.
Synthesis of 5-chloro-N-(2-chloropyridin-3-yl)-2-nitrobenzamide (2)

5-chloro-2-nitrobenzoyl chloride (3.7 g, 0.017 mol, 1 eq) was dissolved in pyridine (50 mL) in a 1-neck round bottom flask. The solution was cooled to 0 °C in an ice bath and 3-amino-2-chloropyridine (2.18 g, 0.017 mol, 1 eq) was added. The solution was stirred at 0 °C for one hour, then warmed to room temperature and stirred overnight. The product precipitated out upon addition of approximately 200 mL of water. The precipitate was filtered off and dried to give 5-chloro-N-(2-chloropyridin-3-yl)-2-nitrobenzamide as a white solid (3.63 g, 69% yield). $^1$H-NMR (400 MHz, CDCl$_3$): δ 8.79 (dd, $J = 8.0$, 1.8 Hz, 1H), 8.22 (dd, $J = 4.6$, 1.7 Hz, 1H), 8.16 (d, $J = 8.5$ Hz, 1H), 7.89 (s, 1H), 7.70-7.62 (m, 2H), 7.40-7.33 (m, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ 163.4, 145.3, 141.3, 133.6, 131.6, 131.4, 130.0, 128.8, 126.7, 123.7. LCMS (M+H), C$_{12}$H$_7$Cl$_2$N$_3$O$_3$ m/z calc: 311.99, found: 311.93.
Synthesis of 2-amino-5-chloro-N-(2-chloropyridin-3-yl)benzamide (3)

In a round bottom flask, 5-chloro-N-(2-chloropyridin-3-yl)−2-nitrobenzamide (3.63 g, 0.012 mol, 1 eq) was mixed with ethanol (60 mL) until it became a mostly homogenous mixture. In a separate round bottom flask, SnCl₂ (11.1 g, 0.058 mol, 5 eq) was dissolved in ethanol (10 mL). The 5-chloro-N-(2-chloropyridin-3-yl)-2-nitrobenzamide solution was then slowly added to the SnCl₂ solution. The reaction mixture was warmed to 70 °C, and the precipitate dissolved almost immediately to give a yellow solution. The reaction was stirred for 30 minutes to ensure conversion to product. TLC in 4:1 ethyl acetate:hexanes showed product formation and the solution turned orange in color. The mixture was cooled to room temperature and then poured over ice into a 1 L beaker. The pH of the solution was adjusted to 7-8 using a 5% sodium bicarbonate solution, giving the mixture a creamy yellow color. The solution was stirred for one hour to hydrolyze the tin salts. The mixture was then extracted with chloroform (5 x 300 mL), dried over MgSO₄ and filtered. The solvent was removed in vacuo to give 2-amino-5-chloro-N-(2-chloropyridin-3-yl)benzamide as yellow solid (2.5 g, 76% yield).

¹H-NMR (400 MHz, CDCl₃): δ 6.70 (d, 1H), 5.59 (s, 2H). δ 8.76 (dd, J = 8.2, 1.8 Hz, 1H), 8.26 (s, 1H), 8.16 (dd, J = 4.7, 1.7 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.31 (dd, J = 8.2, 4.7 Hz, 1H), 7.24 (d, J = 2.3 Hz, 1H), 6.70 (d, J = 8.8 Hz, 1H), 5.59 (s, 2H).¹³C-NMR
NMR (100 MHz, CDCl₃): δ 166.6, 148.1, 144.3, 133.7, 132.0, 126.9, 123.5, 121.7, 115.9.

LCMS (M+H), C₁₂H₉Cl₂N₃O m/z calc: 282.01, found: 281.96.

**Synthesis of 8-chloro-5,11-dihydro-6H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one (4)**³

A flame-dried, 3-neck round bottom flask was equipped with a reflux condenser and stir bar, then cooled to room temperature under argon atmosphere. 2-amino-5-chloro-N-(2-chloropyridin-3-yl)benzamide (2.5 g, 0.009 mol, 1 eq), Sulfolane (10.9 mL, 0.114 mol, 12.8 eq), and concentrated sulfuric acid (0.47 mL, 0.009 mol, 1 eq) were added. The solution was then warmed to 140 °C, and progress was monitored via LCMS. After 2 hours, the starting material was completely consumed. The solution was cooled to room temperature, and then poured over 50 mL of ice water. The product precipitated out, and the solution was washed with ethyl acetate to extract impurities. The precipitate was filtered and dried to give 8-chloro-5,11-dihydro-6H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one as a yellow solid (1.67 g, 76% yield).¹H-NMR (400 MHz, CDCl₃): δ 10.03 (s, 1H), 8.76 (s, 1H), 7.88 (dd, J = 4.9, 1.6 Hz, 1H), 7.63 (d, J = 2.7 Hz, 1H), 7.41 (dd, J = 8.6, 2.6 Hz, 1H), 7.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.14 (d, J = 8.7 Hz, 1H), 6.96 (dd, J = 7.8, 4.7 Hz, 1H).¹³C-NMR (100 MHz, DMSO): δ 166.6, 151.1, 146.8, 143.3, 133.9, 131.8, 129.6, 125.4, 124.7, 123.6, 122.2, 119.5. LCMS (M+H), C₁₂H₉ClN₃O m/z calc: 246.04, found: 245.90.
Synthesis of 6,8-dichloro-11H-benzo[e]pyrido[3,2-b][1,4]diazepine

A flame-dried, 3-neck round bottom flask was equipped with a reflux condenser and stir bar, then cooled to room temperature under argon atmosphere. 8-chloro-5,11-dihydro-6H-benzo[e]pyrido[3,2-b][1,4]diazepin-6-one (0.5 g, 0.002 mol, 1 eq) and anhydrous toluene (3 mL) were added and the solution was stirred. Phosphorus (V) oxychloride (2.09 mL, 0.022 mol, 11 eq) was added via syringe. The solution was warmed to 100 °C and stirred for 4 hours. LCMS suggested 50% conversion to product after 4 hours, and the phosphorus (V) oxychloride was mostly reacted or evaporated. Added more phosphorus (V) oxychloride (2.09 mL, 0.022 mol, 11 eq) and stirred for another 4 hours. LCMS after 8 hours suggested 75% conversion to product with no remaining phosphorus (V) oxychloride. Added more phosphorus (V) oxychloride (2.09 mL, 0.022 mol, 11 eq) and stirred the solution overnight. LCMS showed full conversion to product. The solution was transferred to a 1-neck round bottom and the solvent was evaporated in vacuo. The crude product was dried on the high vac to give 6,8-dichloro-11H-benzo[e]pyrido[3,2-b][1,4]diazepine as a dark brown solid (99% crude yield). 1H-NMR (400 MHz, CDCl3): δ 9.62 (s, 1H), 7.74 (q, J = 5.6 Hz, 2H), 7.59 (d, J = 2.5 Hz, 1H), 7.37 (dd, J = 8.4, 2.4 Hz, 1H), 7.14 (dd, J = 7.8, 6.0 Hz, 1H), 7.09 (d, J = 8.6 Hz,
Synthesis of 12-chloro-3-methyl-9H-benzo[\textit{f}]pyrido[2,3-\textit{b}][1,2,4]triazolo[4,3-\textit{d}][1,4]diazepine (6a)

6,8-dichloro-11\textit{H}-benzo[\textit{e}]pyrido[3,2-\textit{b}][1,4]diazepine (0.100 g, 0.3786 mmol, 1 eq), acetohydrazide (0.028 g, 0.3786 mmol, 1 eq), and 1-butanol (3 mL) were added to a dry 10 mL microwave tube. Microwave irradiation at 200 °C, 300 W, 300 PSI for 15 minutes and resulted in a clear yellow solution. Conversion to the desired product was observed via LCMS. The solvent was evaporated \textit{in vacuo} and the product dried on the high vac. Purification with preparative TLC in 5% DCM:MeOH afforded 12-chloro-3-methyl-9\textit{H}-benzo[\textit{f}]pyrido[2,3-\textit{b}][1,2,4]triazolo[4,3-\textit{d}][1,4]diazepine as a light yellow solid (41 mg, 38% yield). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.24 (dt, $J = 5.0, 2.5$ Hz, 1H), 8.01 (dd, $J = 4.8, 2.6$ Hz, 1H), 7.62-7.54 (m, 1H), 7.34-7.22 (m, 1H), 7.14 (dt, $J = 8.8, 4.9$ Hz, 1H), 6.89 (dd, $J = 8.6, 4.8$ Hz, 1H), 6.43 (d, $J = 3.9$ Hz, 1H), 2.58 (d, $J = 4.6$ Hz, 3H). $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 153.6, 152.1, 151.4, 147.6, 143.0, 132.6, 131.8, 129.5, 121.5, 121.5, 120.8, 119.5, 119.4, 12.8. LCMS (M+H), C$_{14}$H$_{10}$ClN$_5$ m/z calc: 284.06, found: 283.99.
Synthesis of 12-chloro-3-(2-fluorophenyl)-9H-benzo[f]pyrido[2,3-b][1,2,4]triazolo[4,3-d][1,4]diazepine (6b)

The desired product 12-chloro-3-(2-fluorophenyl)-9H-benzo[f]pyrido[2,3-b][1,2,4]triazolo[4,3-d][1,4]diazepine was produced following the same procedure for product 6a. Isolated as a yellow solid (37 mg, 27% yield). $^1$H-NMR (400 MHz, CDCl$_3$): δ 8.20-8.14 (m, 1H), 7.82 (t, J = 7.2 Hz, 1H), 7.51 (q, J = 7.2 Hz, 1H), 7.35 (t, J = 7.5 Hz, 2H), 7.03 (dd, J = 9.8, 8.3 Hz, 2H), 6.92 (d, J = 9.0 Hz, 1H), 6.79 (dd, J = 8.0, 4.7 Hz, 1H), 6.34 (s, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ 160.5, 153.2, 153.1, 147.8, 143.3, 133.2, 133.1, 132.2, 132.1, 131.9, 129.5, 129.4, 125.4, 121.6, 121.4, 119.3, 118.7, 116.7, 116.4, 115.4. LCMS (M+H), C$_{19}$H$_{11}$ClFN$_{5}$ m/z calc: 364.07, found: 363.99.
Synthesis of 4-(12-chloro-9H-benzo[f]pyrido[2,3-b][1,2,4]triazolo[4,3-d][1,4]diazepin-3-yl)phenol (6c)

The desired product 4-(12-chloro-9H-benzo[f]pyrido[2,3-b][1,2,4]triazolo[4,3-d][1,4]diazepin-3-yl)phenol was produced following the same procedure for product 6a. Isolated as an orange solid (12 mg, 9% yield). ¹H-NMR (400 MHz, DMSO): δ 10.00 (s, 1H), 8.90 (s, 1H), 8.19 (d, J = 4.7 Hz, 1H), 7.88 (d, J = 2.6 Hz, 1H), 7.45 (dd, J = 8.5, 2.7 Hz, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.20 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 7.7 Hz, 1H), 6.93 (dd, J = 8.0, 4.7 Hz, 1H), 6.80 (d, J = 7.9 Hz, 2H). LCMS (M+H), C_{19}H_{12}ClN_{5}O m/z calc: 362.07, found: 361.96.
Synthesis of 12-chloro-3-(3-methoxyphenyl)-9H-benzo[f]pyrido[2,3-b][1,2,4]triazolo[4,3-d][1,4]diazepine (6d)

![Chemical structure](image)

6,8-dichloro-11H-benzo[e]pyrido[3,2-b][1,4]diazepine (0.050 g, 0.379 mmol, 1 eq), m-Anisic hydrazide (0.0315 g, 0.379 mmol, 1 eq), and toluene (2 mL) were added to a dry 10 mL microwave tube. Microwave irradiation at 200°C, 300 W, 300 PSI for 15 minutes and resulted in an orange solution. LCMS suggested full conversion to product. The solvent was evaporated in vacuo and the product dried to afford crude 12-chloro-3-(3-methoxyphenyl)-9H-benzo[f]pyrido[2,3-b][1,2,4]triazolo[4,3-d][1,4]diazepine as an orange solid (42 mg, 64% yield). A small amount of product was purified via preparative TLC in 5% MeOH:DCM, which gave a mixture of both the desired product and some remaining m-anisic hydrazide, which complicated the NMR spectrum. ¹H-NMR (400 MHz, DMSO): δ 8.96 (s, 1H), 8.21 (d, J = 5.0 Hz, 1H), 7.52-7.40 (m, 2H), 7.35 (dt, J = 8.1, 3.7 Hz, 2H), 7.15 (dd, J = 9.6, 5.1 Hz, 1H), 7.06 (dd, J = 8.2, 2.7 Hz, 1H), 6.99 (s, 1H), 6.97-6.86 (m, 1H), 3.71 (s, 3H). LCMS (M+H), C₂₀H₁₄ClN₅O m/z calcd: 376.09, found: 375.99.
References


