Investigation of the Structure-Activity Relationship of a Dopamine Receptor 4 Antagonist to Treat Cocaine Addiction

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BRIEF. This study demonstrates the successful synthesis of drug candidates for potential use as PET tracers and *in vivo* tool compounds to study and potentially treat cocaine addiction.

ABSTRACT. Cocaine addiction is a serious and currently untreatable problem, with over 4.8 million Americans having abused the substance in 2009 [1]. Studies have shown that antagonism of dopamine receptor 4 is a possible treatment for substance addiction [1]. The lead compound for this project is a D4 antagonist that is being investigated as a PET tracer, a compound that is made radioactive in order show up on a PET scan to image D4 receptors in the brain as well as an *in vivo* tool to study cocaine addiction. Derivatives of the benzimidazole lead compound were previously investigated for these purposes [2]. This study identified a para-chlorobenzyl analog, termed ML398 [2], with excellent potency, though it lacked the ability to be a PET tracer [2]. The para-chlorobenzyl analog also displayed superior biological qualities, so the western portion of the molecule (western phenyl ring) was further investigated for PET imaging. In order to synthesize these analogs, a six step synthesis was employed. Three of the analogs were created with the aim of being PET tracers, while the fourth was created to maintain potency. Biological data has yet to be attained, but if a suitable candidate is found, it will be tested in animal models for treatment of cocaine addiction.

INTRODUCTION.

Cocaine is a dangerous and highly prevalent drug, with approximately two million users in the U.S. [1]. This drug has highly addictive properties, and long-term use can prove detrimental to the body. Cocaine increases synaptic levels of dopamine, which is perceived as a positive feeling, reinforcing its effects, potentially resulting in addiction [1, 2]. There are currently no medications approved to treat cocaine addiction [1].

Many processes within the central nervous system (CNS) are controlled by dopamine receptors. These receptors have been found to be involved in the pathogenesis of a variety of diseases including Attention Deficit Hyperactivity Disorder (ADHD), schizophrenia, Alzheimer's, Parkinson's, and substance addiction [2, 3, 4]. There are five different types of dopamine receptors: D1, D2, D3, D4, and D5, grouped into two families. The D1-like family contains the D1 and D5 receptors, and the D2-like family contains the D2, D3, and D4 receptors. While there has been extensive research on D1 and D2 receptors, there is still much to be discovered about the involvement of D3 and D4 in various diseases [2]. Using indirect methods, it has been shown that the D4 receptor is expressed in the frontal cortex, hippocampus, hypothalamus, retina, and amygdala regions of the brain [1, 3]. However, a successful Positron Emission Tomography (PET) tracer for the D4 receptor has yet to be discovered [5]. A PET tracer is a radiolabeled compound (compound that is made radioactive) that will show up on a PET scan that can be used to image receptors in the brain. In regards to the function of D4, a D4 knockout study suggested that D4 plays a role in drug sensitivity and locomotion [1, 4].

A receptor antagonist is a compound that inhibits the function of an agonist, which would normally create a biological response when bound to a receptor. Research has shown that D1 and D2 receptor antagonists were not successful at treating side effects of cocaine addiction such as withdrawal [1]. Buspirone has been researched as a possible treatment for the side effects of cocaine addiction [1]. Buspirone binds to D3 and D4 receptors with a high affinity [1]. Known D4 antagonists have properties that can be significantly improved upon, such as binding affinity, potency, metabolic rate, and selectivity [2]. Potency describes the amount of a drug necessary for an adequate biological effect to occur. Binding affinity is the ability of a drug to bind with the desired target. Poor selectivity, or promiscuity, is problematic because if a drug interacts with other receptors there could be severe side effects [2]. One promising D4 antagonist is a benzimidazole compound, which displayed many sought after attributes, such as potency, but lacks the desired binding affinity to be effective *in vivo* [2]. A derivative of this benzimidazole compound, ML398, displays promising qualities such as high selectivity and a slightly improved binding affinity.

The goal of this project is to synthesize a highly selective D4 antagonist with high binding affinity for PET tracing and useful *in vivo* properties to test in animal models. The use of these molecules will be beneficial to study cocaine addiction more thoroughly. A new D4 antagonist was created using organic synthesis, or production of a complex chemical compound from more simple compounds. A second project goal was to create a library of compounds that will be tested for affinity, potency, and other aforementioned biological properties in the hopes that they will perform better than previous D4 antagonists. The future aim is to keep exploring compounds until one is found that meets the criteria and can be used *in vivo*.

Discovering a new, selective D4 antagonist will be highly beneficial to the scientific community because it furthers the current understanding of the D4 receptor in various CNS related diseases. The successful synthesis of a D4 antagonist and multiple analogs presented later demonstrate a step in the right direction towards a suitable PET tracer and *in vivo* tool. A drug that could effectively treat cocaine addiction would be monumental and can improve the lives of millions.

MATERIALS AND METHODS.

All reagents and solvents were commercial grade.

Instrumentation

Agilent 6130 Quadrupole LC/MS with electrospray ionization

Low resolution mass spectra were obtained on an Agilent 6130 Quadrupole LC/MS with electrospray ionization. The LCMS (liquid chromatography-mass spectrometry) is a highly sensitive machine that aids in identifying compounds in a sample by analyzing their retention times. To prepare for using the LCMS, 0.5 to 1.0 mgs of a sample is placed into a vial, which is then filled with methanol. The vial is sealed and placed in a tray inside the machine to be analyzed.

Teledyne ISCO - Silica Gel 60 (230-400 mesh)

Automated chromatography on silica gel was performed using a Teledyne ISCO. The ISCO performs column chromatography, a process that purifies chemical compounds by separating the mixtures. The ISCO is "normal phase" in the sense that it runs from less polar (i.e. hexane) to more polar (i.e. ethyl acetate) solvents.

Luna 5uC18(2) 100A AXIA

Preparative purification was performed on a Gilson chromatograph using a Luna 5u C18(2) 100A AXIA column (30x50 mm) and a water/acetonitrile gradient. The Gilson performs reverse phase chromatography, which separates a solution based on polarity. This particular type of chromatography is reverse phase due to the fact that it starts with a more polar solvent such as water, and transitions to a less polar solvent like acetonitrile.

Bruker DRX-400 NMR

1H and 13C NMR spectra were recorded on a Bruker DRX-400 NMR instrument. NMR helps identify the quantity and position of protons of a material. This is used to determine purity of a sample.

Sorbent Technologies HL 0.25 mm silica gel plates with UV indicator

Analytical thin layer chromatography (TLC) was performed on Sorbent Technologies HL 0.25 mm silica gel plates with UV indicator. Visualization was accomplished by irradiation under a 254 nm UV lamp and/or the use of an iodine chamber. TLC plates are used to track reaction progress as well as determine how many different compounds are in a mixture.

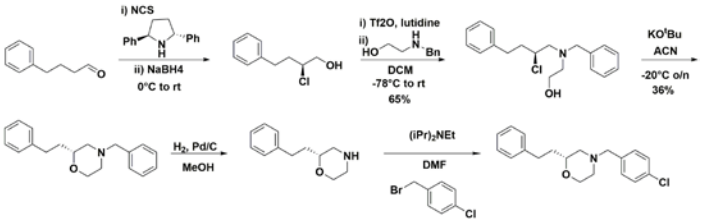
General Overview of Methods

Before any bench work can be done in the lab, the properties and quantities of chemicals needed must be known. In order to do this, stoichiometry is used to determine equivalents needed. Properties such as molecular weight and density are also needed. A large majority of the bench work done can be broken up into two categories: running step-by-step reactions and synthesizing libraries. The process of running a reaction begins with flame drying a flask containing a stir bar, and then placing the flask under argon. Flasks are placed under an argon atmosphere in case any of the reagents being worked with are air-sensitive. Next, reagents and solvents are added to the flask. The order of adding chemicals depends on the procedure and mechanism of the reaction. Reactions are able to be run at various temperatures, and the stir bar is used to mix reagents. While the reaction is taking place, its progress is monitored by analyzing the reaction using TLC plates or LCMS. Once a reaction is complete, it is quenched with water to stop the reaction. Then, the products are extracted from an aqueous solvent with an organic solvent in triplicate. Next, the organic layer is dried over sodium sulfate and filtered. The next step is to purify the reaction via column chromatography. Column chromatography is performed using the ISCO, which isolates the product based on polarity as described above. The desired product is located from the column using TLC plates and then collected. A purified sample is usually analyzed using NMR to ensure it is the correct product.

Synthesizing a library involves setting up multiple reactions that have the same reagents except one. The reactions' progress is followed by LCMS. A work up is not always necessary in library synthesis. Each reaction is then purified using reverse phase chromatography on the Gilson. Each product is then dried and its purity is checked via LCMS. It is then transferred to a barcoded vial, weighed, and registered.

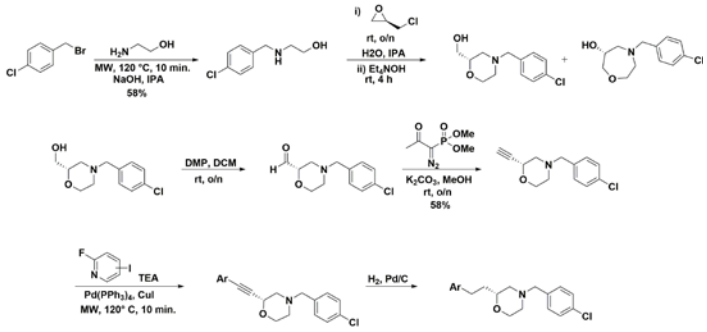
RESULTS.

The synthesis of the ML398 probe began with an Asymmetric alpha-chlorination, as shown in Scheme 1. This reaction enantioselectively placed a chlorine on the carbon backbone of the compound in 51% yield. The second step was a SN2 reaction in which ethanolamine displaced the triflate in situ (in its original place) to create a chloro-amino alcohol. This reaction proceeded in 65% yield. This intermediate compound underwent a second SN2 reaction, which formed the desired morpholine core in 36% yield. Once the morpholine core was formed, a hydrogenolysis (cleavage of a carbon-carbon bond) was performed to remove the benzyl protecting group, or eastern ring of the compound. The final step of the synthesis was an SN2 reaction to add the para-chloro benzyl substituent to the nitrogen of the morpholine core.



Scheme 1. Synthesis of ML398.

The synthesis of phenyl derivatives began with a nucleophilic substitution (SN2) reaction, in which ethanolamine displaces the bromine of para-chlorobenzyl bromide. This reaction proceeded in 58% yield. The second step was a cascade of three SN2 reactions in which a chiral epoxide is opened to form the morpholine core. The subsequent reaction was a Dess Martin Periodinane (DMP) oxidation, in which the primary alcohol was converted into the aldehyde in 31% yield. The aforementioned aldehyde was then converted into a terminal alkyne using a Seyfirth-Gilbert homologation with the Ohira-Bestmann modification in 53% yield. Four different analogs of the phenyl were created through a Sonogashira coupling reaction. 2- fluoropyridine analogs were synthesized in the hopes of becoming a PET, and the meta-cyanophenyl was synthesized to maintain potency. The alkynes were then reduced to the desired final products by a palladium catalyzed hydrogenation.



Scheme 2. Synthesis of ML398 analogs.

Library

Four analogs were successfully synthesized using the aforementioned synthesis. The three analogs in Figure 1A were created with the aim of becoming PET tracers. To achieve this, each analog had a variation of 2-fluoropyridine added in replacement of the phenyl of ML398. This substituent was added at three different positions in order to investigate the structure activity relationship of the analogs. Structure activity relationship describes how the structure of a chemical affects its activity. For example, the basic nitrogens on the morpholine cores of this D4 are essential because that is the necessary structure used to attach to the binding site of the D4 receptor. 2-Fluoropyridines were synthesized for the purpose of testing these compounds as PET tracers. While there are other atoms, such as carbon-11 and could be used for the same purpose, ^{18}F has a radioactive half-life of about 110 minutes that is desirable for practical implementation.

The fourth analog in Figure 1B was created in an attempt to maintain potency similar to ML398, but achieve more desirable biological properties.

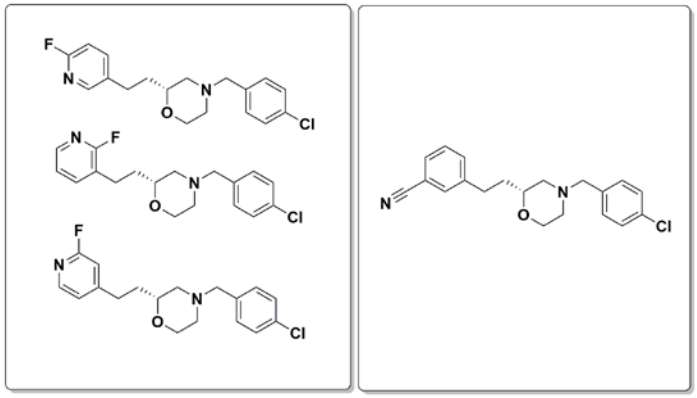


Figure 1. A: 2-Fluoropyridine Analogs (Potential PET tracers). B: (Benzonitrile Analog Potential *in vivo* tool).

DISCUSSION.

The methodology and resulting synthesized analogs are indeed progress toward evaluating D4 antagonists as a possible treatment for the side effects of cocaine addiction, especially since there has been minimal research on D4 antagonists as a viable treatment. The analogs that were made are significantly different from other D4 ligands. Most previous D4 ligands are 1, 4 disubstituted and have a piperidine or piperazine core (benzyl ring core with two nitrogens opposite of each other). The analogs made in this study are 2, 4 disubstituted morpholines (similar to piperazine or piperidine but with an oxygen atom in the benzyl ring). The synthesis of a successful D4 antagonist with a morpholine core would be a novel achievement. Furthermore, as a successful D4 PET tracer has never been created, a compound with desired properties would be a contribution to the continued neurological study of these receptors. Most literature has focused on D4 antagonists as a treatment for schizophrenia, which they failed to be [2]. Much of this is attributed to the lack of knowledge about D4 receptors in general. Prior studies have demonstrated that D4 antagonists may curtail addictive behavior in animal models [2]. D1 and D2 antagonists were shown to be largely ineffective for treating addiction, which reinforced the use of a D4 antagonist [2]. While the synthesis of these analogs was successful, testing will dictate whether they are viable during *in vivo* testing. The synthesis is original and nearly optimized due to its small number of steps as well as its decent yields.

CONCLUSIONS AND FUTURE DIRECTIONS.

In addition to the biological testing of the current analogs, further research of potential D4 antagonists could be advanced through a matrix library. A matrix library includes every combination of desired substituents selected to be tested. For example, if there were five different phenyl ring substituents and five different benzimidazole substituents, twenty-five analogs in total would be synthesized. This would also further the understanding of the structure activity relationships. More compounds mean that more data can be collected, and a better understanding of what substituents will be beneficial to use in a selective D4 antagonist can be gained. There are a few possible outcomes for the analogs. If an analog exhibits binding affinity of less than 10 nM, as well as complete selectivity, it will continue into PET studies. If a compound displays better potency and drug metabolism and pharmacokinetic (DMPK) properties, it will continue into *in vivo* testing. However, if none of the analogs display improved properties, more analogs will be made.

In addition to analyzing aryl substituents, core modifications are also worth exploring. Finding an optimal core, would prove beneficial, and cores can be tested much like other substituents. A novel core for a D4 antagonist could provide new information to be patented.

Selecting the best analog to use as a PET tracer is also imperative in order to fully understand the location of D4 receptors. DMPK is necessary to optimize before an analog can be used as a PET tracer or tested *in vivo*, so naturally a future endeavor would be to further improve DMPK.

Cocaine addiction is a disease that is detrimental to the overall wellbeing of the public; taking action to treat it is a critical scientific pursuit with many humane benefits. The goal of this project was to synthesize highly selective D4 antagonists based on previous literature, specifically ML398. ML398 was successfully resynthesized, and four analogs were successfully created using a six step synthesis that produced acceptable yields. The creation of analogs marks progress toward creating a drug that will treat the side effects of cocaine addiction.

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SUPPORTING INFORMATION.

Supplemental Methods.

Creating ML398 Probe:

Figure S1: 4-phenylbutanal: product of Swern Oxidation

Figure S2: (*S*)-2-chloro-4-phenylbutan-1-ol: product of Asymmetric alpha-chlorination

Figure S3: (*S*)-2-(benzyl(2-chloro-4-phenylbutyl)amino)ethanol: product of S_N2 Reaction

Figure S4: (*R*)-4-benzyl-2-phenethylmorpholine: product of S_N2 Reaction

Figure S5: (*R*)-2-phenethylmorpholine: product of reaction to complete ML398 synthesis

Figure S6: (*R*)-4-(4-chlorobenzyl)-2-phenethylmorpholine: ML398 Probe

Creating Analogs of ML398 Probe:

Figure S7: 2-((4-chlorobenzyl)amino)ethan-1-ol: product of S_N2 Reaction

Figure S8: (*S*)-(4-(4-chlorobenzyl)2-morpholin-2-yl)methanol: product of S_N2 Reaction

Figure S9: (*S*)-(4-(4-chlorobenzyl)2-morpholine-2-carbaldehyde: product of Dess Martin Periodinane Oxidation

Figure S10: (*R*)-4-(4-chlorobenzyl)-2-ethynylmorpholine: Product of Seyfirth Gilbert Homologation.

Figure S11: General Procedure for Sonogashira Coupling Reaction

Figure S12: General Procedure for Hydrogenation

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