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Synthetic Progress Towards Asymmetrical Pyridine-Based and Thiophene-Based CXCR4 Modulators

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Modulators

by

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ABSTRACT

Chemokine receptor type 4 (CXCR4) is overexpressed in cells associated with various disease pathways such as autoimmune disorders, inflammation, HIV-1 proliferation, and cancer. Healthy functions such as stem cell differentiation and migration are linked with the CXCR4/CXCL12 signaling pathway. Hence, the binding interaction of CXCR4 with its natural cognate CXCL12 ligand can be partially blocked with small molecules to hinder the mechanism of cancer metastasis or inflammation but still allow normal cell functions to occur. Various antagonists and modulators for CXCR4 have been synthesized and tested, but new compounds with better efficacy are necessary due to cardiotoxicity and/or poor oral bioavailability of these compounds. The overall goal for this research is to synthesize asymmetrical, pyridine-based and thiophene-based compounds as potential modulators of CXCR4-CXCL12 activity. A new research scheme is being investigated and tested using 2,5-thiophene as the central ring and modifications to the side chains with a different hit functional group on each side to yield an asymmetrical small molecule antagonist. After the synthesis process is completed, the analogues are analyzed through NMR and MS and proceed to be tested with further biological tests.

INTRODUCTION

1.1 Background

The chemokine receptor type 4 (CXCR4) is widely found in a variety of tissues and expressed in hematopoietic, cardiovascular, nervous, and immune systems.[1] CXCR4 interacts with mediators such as CXCL12, MIF, and gp120 and by heterodimerization with other chemokine receptors like ACKR3 or CCR5 to form a complex signaling network.[2] The CXCR4 signaling network and its related physiological processes in the hematopoietic and immune systems are associated with various diseases including HIV-1 infection and proliferation, autoimmune diseases, acute/chronic inflammation, and cancer.[2] The binding interaction of the CXCR4 with its only natural endogenous cognate chemokine ligand 12[3] is overexpressed in the pathological pathways of cancer metastasis and inflammation.[4] Various antagonists and modulators for CXCR4 have been synthesized and tested, but new compounds with better efficacy are necessary due to side effects like cardiotoxicity and poor oral bioavailability of certain compounds.[5,6] This project focuses on the design and synthesis of compounds that may show potential as CXCR4 antagonists based on aromatic heterocyclic rings as the core of the compound and stepwise modifications for asymmetrical functional group attachments.

1.2 Chemokines and CXCR4

Chemokines are a large family of secreted small proteins belonging to the cytokine superfamily with molecular weight of 8-12 kDA (usually ~70-80 amino acid residues) that mediate cellular activation, differentiation, and trafficking.[2,7] Chemokines are grouped into four families – CXC, CC, CX3C, and XC – based on how the spacing pattern of the two conserved cysteine motifs on the N-terminus involved in disulfide bond formation are arranged.[8] Cells must express a

complimentary chemokine receptor in order for the cell to recognize and respond to a chemokine. Similarly, chemokine receptors are divided into four families – CCR, CXCR, XCR, and CX3CR – classified according to the subfamily of chemokines to which most of its ligand binds.[9] Many promiscuous chemokines bind to multiple receptors, and each receptor can interact with more than one chemokine.[2] Chemokine receptors belong to the 7-transmembrane α-helices G-Protein Coupled Receptor (GPCR) family. When the ligand binds to the extracellular domains of its cognate receptor, conformational changes in the seven transmembrane domains facilitates interactions with intracellular heterotrimeric G protein to transmit the signal.[2]

CXCR4 is one in six out of eighteen known chemokine receptors to bind to a single ligand.[10] Resembling other GPCRs, CXCR4 is made up of seven transmembrane alpha helices connected by three extracellular (ECL) and three intracellular loops (ICL) and a C-terminus located in the cytoplasm.[3] With slight differences compared to other GPCR structures in the 34 amino acid chain extracellular N-terminus and the orientation of the alpha helices mostly on the extracellular side, CXCR4 forms specific disulfide bonds to shape the entrance of the ligand-binding pocket for CXCL12. The structure of CXCR4 is shown in Figure 1. Crystallization studies reveal that CXCR4 exists as a homodimer but can bind 1:2 ligand to receptor stoichiometrically though computational, functional, and biophysical methods support a 1:1 ratio of CXCL12 to CXCR4 instead.[3]
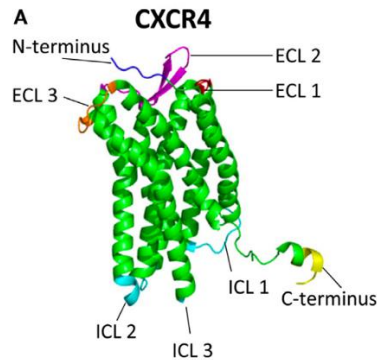
Figure 1. Three-dimensional structure of CXCR4.
Image taken without permission from Pawig, L.; Klasen, C.; Weber, C.; Bernhagen, J.; Noels, H. Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family: molecular perspectives. Frontiers in Immunology **2015,** 6 (429).

CXCL12, also known as the stromal cell-derived factor-1 (SDF-1), is the endogenous chemokine ligand specific to CXCR4; however, this ligand also binds to ACKR3, which is an atypical chemokine receptor that modulates CXCR4 functions and also overexpressed in a variety of diseases.[1] CXCL12 exists as six different splice variants in humans from CXCL12α to φ, and these different isoforms have different activities and functions and are expressed differently among tissues.[11] The CXCL12α is the major and most-studied variant. Mature, biologically active CXCL12 undergoes a cleavage of the 21 amino acids at the N-terminus and is characterized by three strands of the β-sheet packed against an alpha helix.[3] The RFFESH motif depicted in Figure 2 in red is the most important binding motif for CXCR4 and the CXCL12 core.
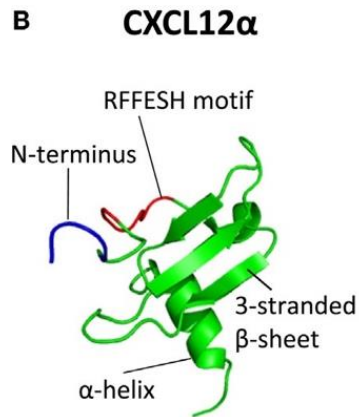
Figure 2. Three-dimensional structure of CXCL12.
Image taken without permission from Pawig, L.; Klasen, C.; Weber, C.; Bernhagen, J.; Noels, H. Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family: molecular perspectives. Frontiers in Immunology **2015,** 6 (429).

CXCR4 is one of the most studied chemokine receptors, and it along with its ligand is a major therapeutic target in the treatment of cancer as they support migration, proliferation, and survival of cancer cells.[12] CXCR4 is also intensively studied for its role in autoimmune diseases and disorders. Furthermore, CXCR4 acts as a coreceptor for human immunodeficiency virus-1 (HIV-1), allowing for its entry into host CD4+ T-cells and homing stem and progenitor cells in the bone marrow to control their mobilization into the peripheral blood stream and tissues for homeostasis as well as after tissue injury.[7] Knock out studies with mice reveal that CXCR4 is involved in cardiogenesis, cerebellum development, hemopoiesis, vascularization, and embryogenesis.[13] In healthy cells, the interactions of CXCR4 and CXCL12 trigger the mobilization of stem cells[2], immune cell survival and trafficking[14], gene transcription[15], intracellular calcium modulation[15], and cell adhesion[16]. This means CXCR4 and CXCL12 trigger stem cell differentiation to repair tissue damage, neural crest cells migration for normal brain development, and embryonic stem cells induction for cardiogenesis.[13,17]

1.3 CXCR4 Mechanism of Action

The missing N-terminus piece for the crystal structure of CXCR4 was discovered in 2010 through binding small molecule antagonist IT1t and cyclic peptide CVX15.[18] The NMR structure of CXCL12 bound to the N-terminus of CXCR4 gave insights into their critical interactions[19], and models like atomic-level dynamics[20] are currently being investigated to determine the activation mechanism and ultimately, a completed crystal structure of CXCR4 bound to CXCL12.

Following the generalized two-site model of chemokine ligand binding to receptor[21], the mechanism proposes binding between the CXCL12 N-loop (RFFESH) with the CXCR4 N-domain, or site one, to dock the chemokine to its receptor following the receptor's recognition of the chemokine core. In site two, the interactions of the CXCL12 N-terminal with the CXCR4 ECL residues activate CXCR4 signaling[22]. Related interaction mechanisms of chemokine to receptor have been suggested due to the lack of comprehensive and quantitative methods describing how the two-site model explains binding affinity and modes, ligand specificity, and activation. Recent research contests there may be intermediate-site interactions such as when CXCR4 assumes a bent conformation that directs the N-terminus towards the major pocket instead of the minor pocket.[23] To that end, the two-site model fails to address these overlooked and perhaps dependent interactions, and a completed complex model regulating how chemokines interact with their receptors will provide functional outcomes, such as in the drug development of chemokine antagonists used for cancer treatment.

Given the vital interactions of CXCR4 and CXCL12 in physiological functions like hemostasis and trafficking cells, completely blocking the active site of CXCR4 with an antagonist will have unwarranted damaging side effects. Instead, to modulate the

CXCL12/CXCR4 axis involved in the disease pathways of cancer metastasis and inflammation, our approach is to design and synthesize molecules intended to allow the first binding interaction step of CXCR4 and CXCL12 but would block the second the interaction of the CXCL12 N-terminal with CXCR4 ECL. Our molecules should also be reversible in that that they are able to dissociate after binding for a certain period of time.
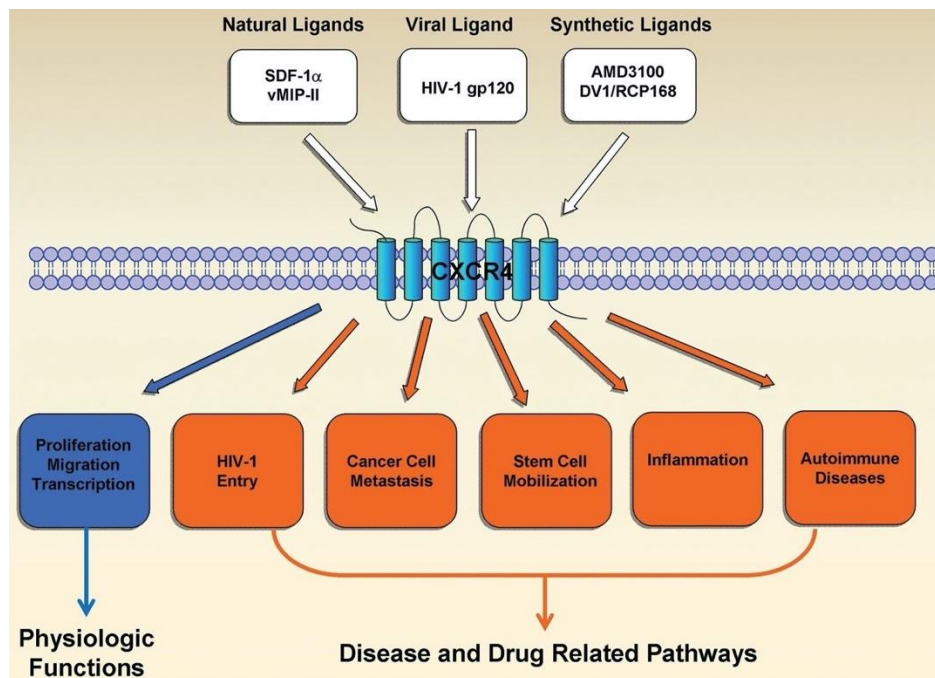
Figure 3. Pathways regulated by CXCR4.
Image taken without permission from: Choi, W.-T.; Duggineni, S.; Xu, Y.; Huang, Z.; An, J. Drug Discovery Research Targeting the CXC Chemokine Receptor 4 (CXCR4). J. Med. Chem. **2012**, 55. 977-994.

2.1 Disease Pathways Related to CXCR4

2.1.2   Cancer

For millions of people in the United States, the struggle with cancer both shapes and is shaped by many day-to-day life choices. These are the numerous avoidable risk factors such as tobacco use, alcohol consumption, physical inactivity, and obesity. Other cancers are caused by uncontrollable risk factors such as inherited genetic mutations and a family history of cancer.

More than 16.9 million Americans who battled cancer many years ago with no current evidence of relapse were alive on January 1, 2019, and more than 1.8 million Americans are expected to be diagnosed in 2020.[24] The top three leading cancers by death rates are lung and bronchus, female breast, and prostate in the United States.[25]

Cancer can develop almost anywhere in and on the human body in an unregulated and proliferated manner different from normal, healthy cells. These cells deviate from healthy cell behaviors because of damaged and unrepaired DNA either acquired genetically as a mutation or exposures to the environment such as ultraviolet rays and smoking. The development of cancer involves a multistep process, carcinogenesis, where changes in tissue structures and cell phenotypes induces local regions of hypoxia, promoting the formation of precancerous and cancerous tumors.[26] Cancer cells no longer follow the regular cell cycle which includes programmed cell death, or apoptosis. Instead, the defining fundamental trait of cancer cells is their ability to survive beyond the normal life span of the cell by resisting apoptosis and evading growth suppressors to eventually generate tumors and metastasize.[26]

The variability in type and unpredictable nature of cancer makes treatment rather difficult. However, modern technology and science methods have enabled the screening, detection, treatment and even prevention of many cancers. Several advancements in treatment of cancer include less invasive surgeries through the use of ultrasound, computed tomography (CT) scans, magnetic resonance imaging (MRI), and positron emission tomography (PET) scans, lasers, or cryosurgery. Chemotherapy is a popular application of chemotherapeutic drugs to kill cancer cells and can be combined with radiation therapy, and the effects are systemic.[27] These approaches were targeted to kill cancer cells but could also damage and kill some normal cells. Other methods of treatment include targeting cancer cells directly through growth signal

inhibitors, endogenous angioinhibitors, and drugs inducing apoptosis. Research is currently

being conducted on nanotechnology, RNA expression profiling and proteomics, and

antiangiogenic chemotherapy.

In the United States, the overall cancer death rate has declined since the 1990s. According to

the Annual Report to the Nation released in March of 2020, the overall cancer death rate among

men from 2001 to 2017 decreased by 1.8% per year and among women by 1.4%.[28] Among

children from the ages of 0 to 14, the overall cancer death rate decreased by 1.4% per year from

2013 to 2017.[28] This fact provides a hopeful outlook that cancer will one day be completely

cured.

2.1.2.2 Cancer Metastasis

Metastasis is the general term that describes cancer cells spreading from the primary source

tumor into other areas of tissues and distant organs.[29] Responsible for as much as 90% of cancer-

related deaths, cancerous cells follow a sequence of interrelated steps to disseminate: 1) entry

into the circulatory and lymphatic systems (intravasation), 2) survival or evasion of the immune

system's attacks to translocate to other tissues and organs, 3) exit the bloodstream

(extravasation), before finally, 4) survival, adaptation, and invasion of the foreign

microenvironment to proliferate and form a secondary tumor (colonization).[30] When cancer has

metastasized, the cancer is still known and named after the part of the body where it originated

(i.e. Breast cancer that has spread to the lungs is known as metastatic breast cancer not lung

cancer), and treatment is based on the original cancer cells' location.[31]

The interactions of cancer cells with the microenvironment drives cancer pathogenesis

and metastasis using communication pathways such as the CXCR4-CXCL12 signaling axis.

These malignant cells utilize similar migration pathways of healthy cells during tissue

regeneration and repair. In common tissues and organs of metastasis such as the brain, lung, liver and lymph nodes, CXCL12 is highly expressed, which facilitates and guides the migration of CXCR4-expressing cancer cells to infiltrate and provide support to a local tumor with growth and angiogenic factors.[32] Otherwise, the binding of CXCR4 and CXCL12 can promote metastasis through multiple signaling cascades such as the phosphoinositide 3-kinase/Protein kinase B (PI3K/AKT) and mitogen-activated protein kinase (MAPK) pathways.[32] The axis also contributes to therapeutic resistance with the recruitment of myeloid bone marrow-derived cells that indirectly encourages tumor recurrence and metastasis.[33] CXCR4 over-expression predicts a poor overall survival prognosis for many different types of cancers such as breast, colorectal, lung, liver, prostate, and renal cancer; irrespective of any cancer type, high expression levels of CXCR4 is associated with poorer progression-free survival.[34]

Stopping the progression of cancer's natural course to spread and invade other areas of the body will help improve cancer survival rates and keep tumors localized for treatments to more effectively work. Thus, the CXCR4-CXCL12 axis is a major target of therapeutic approaches. The development of drug candidates to inhibit the migration of cancer cells by disrupting these binding interactions is the overarching goal for this project.

2.1.3   Inflammation: Inflammatory bowel disease, allergic asthma, and pulmonary fibrosis

2.1.3.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is comprised of two major idiopathic forms, ulcerative colitis and Crohn's disease, which are chronic inflammatory disorders of the gastrointestinal tract (GI) empirically characterized by clinical, pathological, endoscopic, and radiological features such as intestinal inflammation and epithelial injury.[35, 36] Through examination of the CXCR4/CXCL12

axis, CXCR4 and CXCL12 were found to exert pro-inflammatory properties, and CXCR4 was a requisite for these pro-inflammatory effects. In experiments of CXCR4 knock-out mice and small molecule antagonists of CXCR4, the results demonstrated blocking CXCR4 reduced inflammation. CXCL12 and CXCR4 are upregulated in IBD intestinal epithelial cells, and by modulating the CXCR4/CXCL12 axis with CXCR4 antagonists, potential for therapeutic purposes to reduce inflammation and improve colonic pathology is shown.[37]

2.1.3.2 Allergic asthma

Allergic asthma is defined by airway hyperresponsiveness (AHR) to a variety of direct-acting and indirect-acting stimuli[38] and excessive airway mucus production. Mixed results on the factors of chronic pulmonary eosinophilia and elevated serum immunoglobulin E[39] affecting AHR show a mere association, but perhaps not a requirement for the development of an allergen-induced response[40]. CXCR4 is upregulated by the proinflammatory cytokine IL-4 in CD4+ T cells including Th2 cells, and the expression of CXCR4 is higher in BAL CD4+ T cells of asthmatic people compared to their peripheral blood CD4+ lymphocytes. Because of this, there will likely be a significant response to CXCL12 that occurs in the narrowing airway, leading to inflammation. When CXCR4 signaling is inhibited, invasion of the lungs by eosinophils is reduced by ~50%, highlighting the role CXCR4 with its ligand on either the allergic response onset or in its maintenance.[41]

2.1.3.3 Pulmonary fibrosis

Pulmonary fibrosis is a progressive disorder of tissue remodeling, fibroproliferation, and deposition of extracellular matrix in the lung parenchyma that causes respiratory failure resulting

in premature death and no effective treatment intervention to date[42]. In human interstitial lung disease, plasma and lung levels of CXCL12 associated with ~90% of CXCR4 expression in circulating fibrocytes were elevated.[43, 44] The expanded pool of circulating fibrocytes is an independent predictor of mortality, supporting the fact that these cells are relevant in the pathogenesis of pulmonary fibrosis[45]. The mechanism of CXCR4/CXCL12 expression mediates fibrocytes influx to the lung, and pharmacologic inhibition of this process as a therapeutic strategy for fibrotic lung disease results in attenuated disease severity.

2.1.4   HIV

Human Immunodeficiency Virus (HIV) is a bloodborne pathogen that attacks the body's immune system, and if left untreated, can lead to Acquired Immunodeficiency Syndrome (AIDS) and related complications. The origins of HIV are traced to the chimpanzee version of the virus, simian immunodeficiency virus (SIV), and perhaps was passed to humans through the consumption of their infected blood. The virus destroys CD4 (also known as T-cells) cells which makes it difficult for the immune system to fight off infections and other diseases. Globally, 75.7 million people have become infected with HIV since the start of the epidemic in the 1980s, and in 2019, there were 36.2 million adults and 1.8 million children ages from 0 to 14 living with HIV.[46] The rate of HIV infection has been reduced by 40% since the peak of the epidemic in 1998; around 1.7 million of people were newly infected in 2019 compared to the 2.8 million in 1998.[46]

The two main types of HIV are HIV-1, which is most common, and HIV-2, less common and infectious. HIV-1 accounts for around 95% of all infections worldwide; the most common strain of HIV-1 is Group M. The other groups – N, O, and P – are uncommon. Within Group M,

there are at least nine genetically different subtypes A, B, C, D, F, G, H, J and K. Subtypes can join to combine genetic material to form a hybrid mixture virus, known as a circulating recombinant form.[47]

HIV-1 infection begins when the virus enters into mucosal dendritic cells and CD4+ lymphocytes[48] and then multiplies, leading to acute HIV which is characterized by a temporary drop in CD4+ lymphocytes but also further entry into other cell populations such as T-lymphocytes, macrophages, and dendritic cells. From this, the HIV reservoir is started, and the virus can multiply and bud from these cells in the future. For HIV and the host cell to interact, CD4 surface proteins coordinate with the trimer of the HIV-1 envelope glycoprotein, gp120. A conformational change in gp120 exposes more binding sites[49], and these new binding sites allow for the second trimeric protein of HIV-1, gp41, to change conformation and insert the N-terminus of its fusion peptide into the host cell membrane.[50] The principle coreceptor for gp120 is CXCR4 and CCR5.[51]

Currently with no cure that completely eradicates or suppresses HIV and its reservoirs, prevention strategies for HIV are the most important and effective methods to stop the transmission of HIV which includes condoms, HIV testing, blood precautions and safety, male circumcision, informative programs, and harm reduction efforts for injecting drug users. Pre-exposure prophylaxis (PrEP) is also a form of prevention for high-risk individuals in combination with other prevention methods.[52] HIV treatment involves combination antiretroviral therapy (ART) along with medications to prevent and treat the opportunistic infections that take advantage when the immune system is compromised by HIV until the viral loads are undetectable and there is no risk of transmission to others.[53]

2.1.5 Autoimmune Diseases

Rheumatoid arthritis (RA) is a disease of inflammation and the autoimmune system whereby the immune system mistakenly attacks healthy cells, and this leads to inflammation of the affected parts of the body. Specifically, in RA, the joints of the hands, wrists, and knees are attacked, which causes the lining of the joint to become swollen and damaged chronically.[54] RA can also affect other tissues like the lungs, heart, and eyes. CXCR4 and CXCL12 is highly expressed in the serum and joint synovial fluid of patients with RA, and CXCL12 can be secreted and produced by joint synovial cells which further activates and supports the migration of inflammatory cells to these synovial tissues.[55] These expressions of CXCL12 enhance CXCR4 expression by immune cells, and the CXCR4-CXCL12 axis will keep the activated immune cells in the inflamed joint.[1] Treatment options for RA include medications such as steroids, nonsteroidal anti-inflammatory drugs (NSAIDS), and disease-modifying antirheumatic drugs (DMARDS).[56] However, due to various side effects, biologic agents are becoming a newer treatment option instead. Biologic agents target the specific part of the overactive immune system that triggers inflammation. Acting similarly like a biologic agent, a CXCR4 antagonist that disrupts the CXCR4-CXCL12 pathway shows potential as a drug to treat RA in patients.

3.1 CXCR4 Antagonists and Modulators

3.1.1 AMD3100

The CXCR4 bicyclam reversible antagonist plerixafor (AMD3100) was approved by the FDA in 2008 for autologous transplantation in patients with Non-Hodgkin's Lymphoma or multiple myeloma though it has been shown to benefit other malignant diseases involving CXCR4 as well.[57] The initial purpose was to prevent HIV entry but given CXCR4's connections

with stem cell mobilization, this became the purpose to administer the drug. AMD3100 directly antagonizes CXCR4 without affecting other receptors for either CXC- or C-C chemokines[58], and Ca^{++} flux measurements confirmed that the CXCR4 signal pathway was blocked.[59] Other aromatic derivatives of AMD3100 were synthesized and tested, but only the compounds retaining the central benzene ring structure were active.[58]

Due to various and adverse side effects, AMD3100 is currently the only FDA-approved CXCR4 antagonist and can be administered at a dosage of 240 µg/kg for a limited to single time use. A recent study in 2017 shows that a higher dose of plerixafor at 480 µg/kg can be administered in healthy stem cell donors, which mobilizes greater numbers of $CD34^+$ cells than the conventional dose.[60] AMD3100 can induce lung or liver fibrosis, and mobilized cells may act as potential cancer stem cells.[61] Other studies have noted the impractically of daily dosing requirements[62], lack of oral bioavailability[59], and cardiac arrythmia in two patients.[62]

3.1.2 WZ811

In search for a new class of CXCR4 antagonists due to the cyclam's metal ion chelating property contributing to cardiotoxicities and short-term usage, an extensive structure-activity profile indicated the central aromatic ring and a one-carbon separation between the central aromatic phenyl ring and the nitrogen of the acyclic linker is critical to the potency and affinity of the molecule to CXCR4.[63] A potent compound, WZ811, was synthesized however it has poor bioavailability and unable to exhibit any in vivo efficacy due to rapid oxidative metabolism.[64]

4.1 Precursor Molecules

The project of AMD3100 (Figure 4) started off as an impurity characterized with a bicyclam in which the two cyclam rings were tethered by a direct carbon-carbon linkage known as JM1657; when unable to re-synthesize JM1657, a new approach to synthesize a bicyclam derivative with cyclam rings tethered by an aliphatic, or propyl bridge, JM2763, was developed.[65] From there, the replacement of the aliphatic bridge with an aromatic bridge as in JM2987 showed an increase in potency by 100-fold.[65] AMD3100 is highly potent as a CXCR4 antagonist, but lacks oral bioavailability and must be administered by intraveneous infusion, which limits its clinical applicability for long-term anti-HIV therapy.[66] AMD3465 builds upon the basic structure of AMD3100 by modifying the two cyclam rings into a monocyclam macrocyclic N-pyridinylmethylene molecule to reduce the basic amine groups attached.[66] Also lacking oral bioavailability due to a high positive charge at physiological pH, WZ811 was synthesized in an effort to combat this problem with a molecule structure composed of two aromatic amine moieties connected by a para-xylylene group.[66] Ultimately, WZ811 also lacked oral bioavailability.[64] Other groups have based chosen amide-sulfamide side chains to add to the central phenyl ring of the bis-secondary amine compound with promising results as candidates for the intervention of breast cancer metastasis.[67]
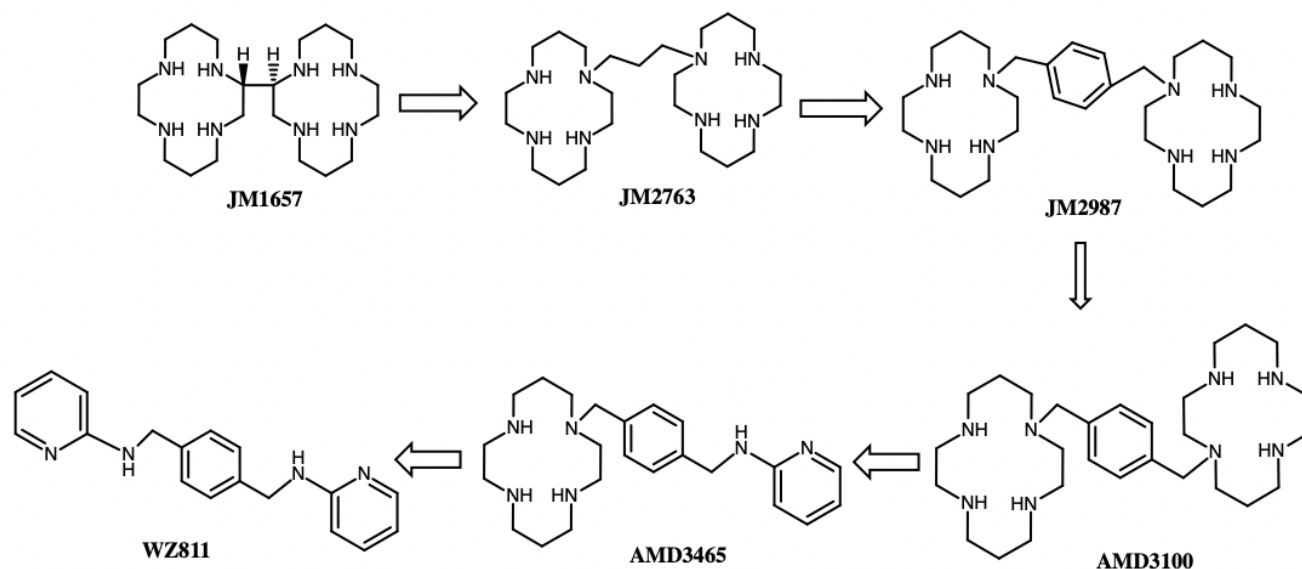
Figure 4. Progression of JM1657 to WZ811

To increase efficacy and oral bioavailability, the synthesis of better CXCR4 antagonists continues. Based on WZ811, analogues with modifications to the ring chain have been synthesized. The Lipinski rule of 5 guides the consideration of various properties and structural components that could potentially lead to a successful design of new analogs for oral delivery.[68] The rule prioritizes compounds to be more orally active if these requirements are met - molecular weight less than 500 daltons, calculated logarithm of the octanol-water partition coefficient (cLog P) of less than 5, 5 or fewer hydrogen bond donors, and 10 or fewer hydrogen bond acceptors.[69] However, this research was conducted two decades ago in 1997, and drug developers are urged to reconsider how the rule of 5 is used because new modalities of drug synthesis are challenging the Lipinski hypothesis.[69] Given exceptions to the rule[70] and perhaps an outdated rule compared to modern times, the Lipinski rule of 5 was used as a guideline to assist in the direction of our drug development.

WZ811 has a molecular weight less than 500 g/mol, 4 nitrogen atoms, and a calculated cLog P value of 2.65.[71] Current research within our group has focused on designing and

synthesizing analogues based on WZ811 by altering the phenyl ring with other heterocyclic

aromatic rings with a lower p value than benzene. The cLog P values for the aromatic rings of

interest, which details how much of a solute dissolve in the water portion versus in an organic

portion, have been listed in Table 1.

Table 1. Aromatic Ring Structure and cLog P values
Values taken from Exploring QSAR[72] and Human Metabolome Database[73]

Ring	Structure	cLog P
Benzene		2.13
Thiophene		1.81
Furan		1.34
Pyridine		0.65
Pyrazine		-0.26

Analogues of 2,5-furan and 2,5-pyrazine, 3,4-thiophene, and 2,6-pyridine have been

synthesized by Dr. Theresa Gaines (Figure 5), and 2,6-pyridine had the best results; especially

the symmetrical substituents, 3-methyl aniline, 4-ethyl aniline, and the morpholine, exhibiting

above 60% inhibition in the Matrigel invasion assay following passing the preliminary screening

in the binding assay[74]. Other symmetrical substituents, the 4-ethyl pyridine and the morpholine,

on the 2,6-pyridine reduced inflammation on par with WZ811 at 42% and 39% respectively.[74]

The 2,5-furan and 3,4-thiophene series had derivatives, specifically the 4-methyl aniline, 2-fluoro aniline, and 1-methylpiperazine substituents, with invasion assay results above 70%.[74] The 2,5-thiophene series was synthesized by Francisco Garcia (Figure 5) with the best invasion assay inhibition out of all the five core molecules synthesized at 80% though these were not tested in the paw edema test against inflammation.[74]

Analogues of pyridine with substituents at 2,5 positions was synthesized by Virani Saniya (Figure 5). Of the 2,5-diamino pyridine and 2,5-dicarbaldehyde pyridine analogues, the 2,5-dicarbaldehyde compounds had the best results.[75] Specifically, the 2,5-diamino pyridines with smaller substituents like methyl, chloro, and fluoro showed more activity in the assays than the larger substituents, and for the 2,5-dicarbaldehyde pyridine compounds, longer substituents like methyl, methoxy, and nitro yielded more activity compared to small substituent attachments.[75] In the Carrageenan paw edema test, only the 4-chloro substituent on the 2,5-dicarbealdhyde compound reduced inflammation by 8%, a number much less than the 40% reduction by WZ811.[75]
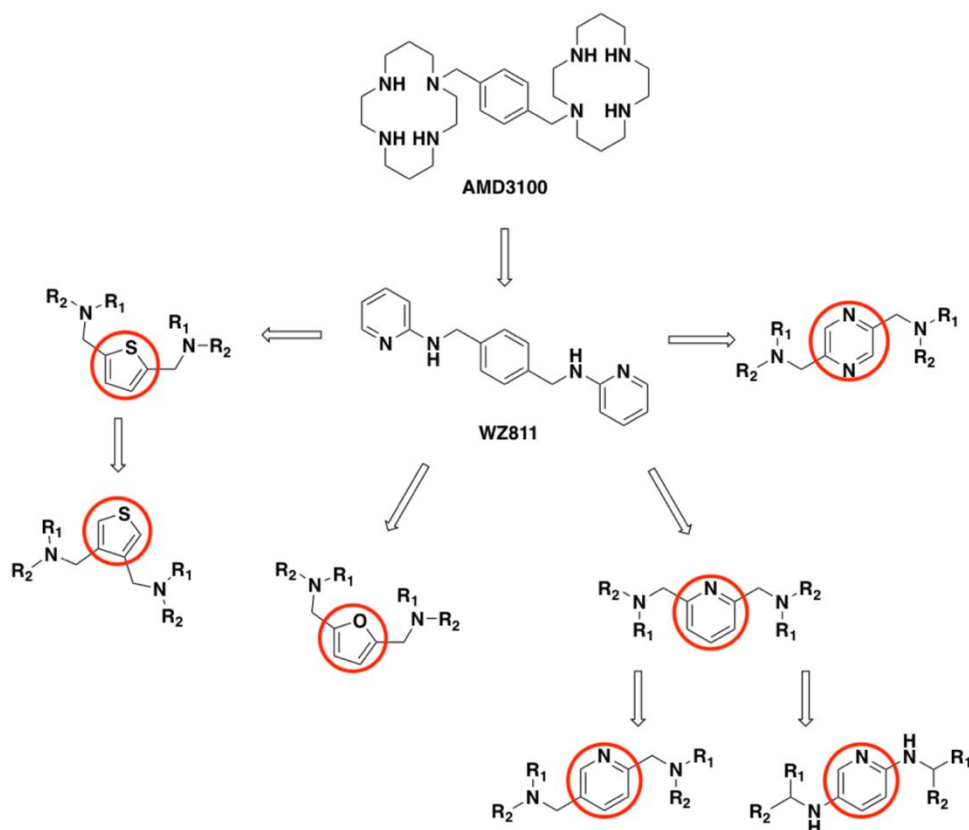
Figure 5. Progression of CXCR4 antagonists to synthesized symmetrical analogues with modified aromatic center rings and its substituents

5.1 Design Rationale

Our current project builds upon the group's previous work. Following the suggestions of Dr. Theresa Gaines, our project expands upon the library of compounds with asymmetrical 2,5-thiophene and 2,6-pyridine analogues with the hypothesis that this will help increase the activity of the compounds due to a better fit into the active site. Support for this hypothesis is also provided with dipyrimidine amine attachments to the dual pyrimidinyl class of molecules with different functional groups like methoxy and morpholine showing potent inhibition, and one compound displayed promising properties in three mouse models to block CXR4.[64]

To synthesize asymmetrical pyridine-based and thiophene-based compounds, the central phenyl ring of WZ811 is replaced with a pyridine or thiophene ring since it is a common motif

for therapeutic purposes and has a lower cLog P value than benzene. The central pyridine ring is modified through stepwise reactions leading to two different functional group attachments using reductive amination (Figure 6). Our first goal was to accomplish the synthesis of the precursor for the overall synthesis of our target compounds. The analogues will be verified with mass spectrometry (MS) and proton nuclear magnetic resonance (^{1}H NMR). Once successful, the synthesized analogues with different subsequent functional group attachments on each side will undergo binding affinity assay and Matrigel invasion assay. The compounds will proceed to the Matrigel invasion assay if the effective concentration is 100 nM or less. These biological assays are conducted to test how well the compounds bind to the CXCR4 receptor and the inhibiting effectiveness.[75] Analogues with the best results from the invasion assay will be tested using the Carrageenan paw edema test to assess the anti-inflammatory activity of the compounds. Dr. Hyunsuk Shim's laboratory at Emory University School of Medicine will conduct these biological assays.
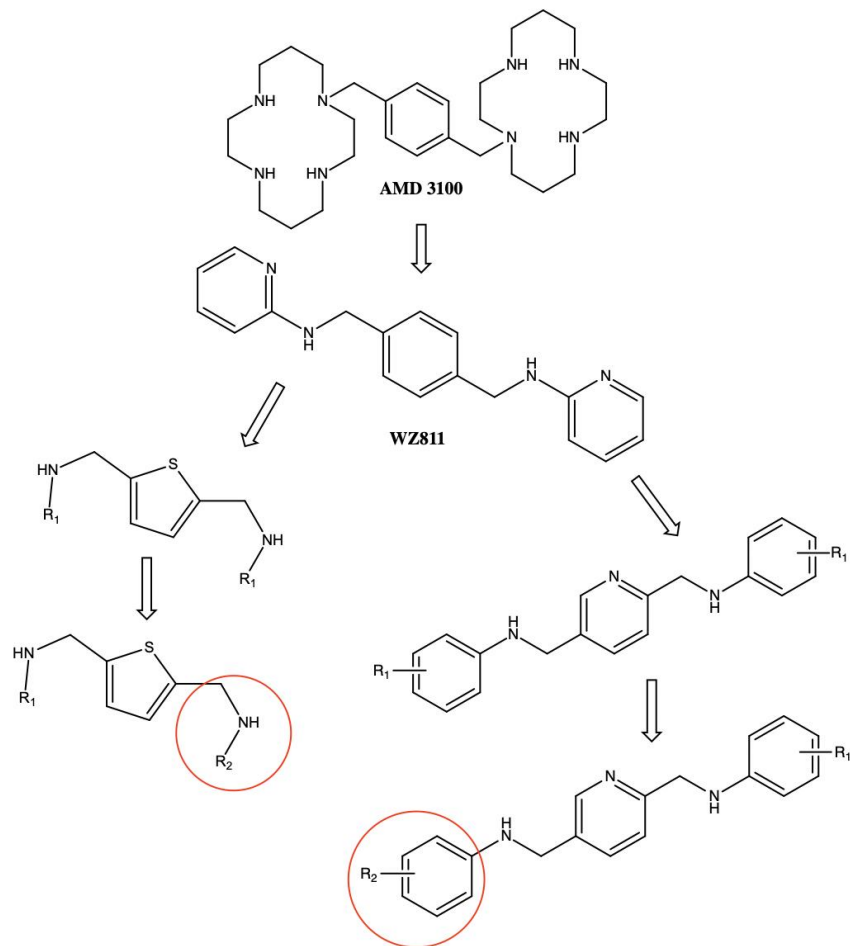
Figure 6. Progression of CXCR4 antagonists leading up to current work in our group

RESULTS and DISCUSSIONS

6.1 Chemistry

6.1.1 2,6-pyridine analogues

The design of these asymmetrical pyridine analogues involved conversion of one ester to an

alcohol to an aldehyde and protection of the aldehyde with ethylene glycol. The other ester was

then converted to an aldehyde followed by reductive amination for the first substituent

attachment. Finally, deprotection of the ethylene glycol and the final reductive amination

attachment to the last aldehyde would yield the asymmetrical pyridine-based modulator. The

reactions are shown in Scheme 1. One of the esters on dimethyl pyridine-2,5-dicarboxylate (**1**)

was reduced to an alcohol using sodium borohydride (**2**). This alcohol was oxidized with Dess-Martin Periodinane to form an aldehyde as seen on **3**. An ethylene glycol group was attached to protect this aldehyde through the Dean-Stark reaction (**4**). The other ester was treated with DIBAL-H to form another aldehyde (**5**). These steps were to synthesize the precursor molecule. Our design involved reductive amination as a point of attachment of a hit functional group in the last three steps from **6 to 8**. Care had to be taken with the reductive amination so that the ethylene glycol protected aldehyde would remain intact through the reaction. A strong acid would deprotect and return the original aldehyde (**7**) for the final amine attachment (**8**). The substituted amines were chosen based on the results from Dr. Theresa Gaines' and Francisco Garcia's synthesized analogues; substituents with high affinity in the binding assay and demonstrated reduction in inflammation during the paw edema test were to be used.

The first three steps to synthesize the precursor compound were successful. In the fourth and final step with DIBAL-H, the reduction of the ester reacted completely to an alcohol instead of the aldehyde desired. This alcohol can then be oxidized to the aldehyde. However, successful completion of this series was not finished, and a new approach was tested to reduce complexity in steps.
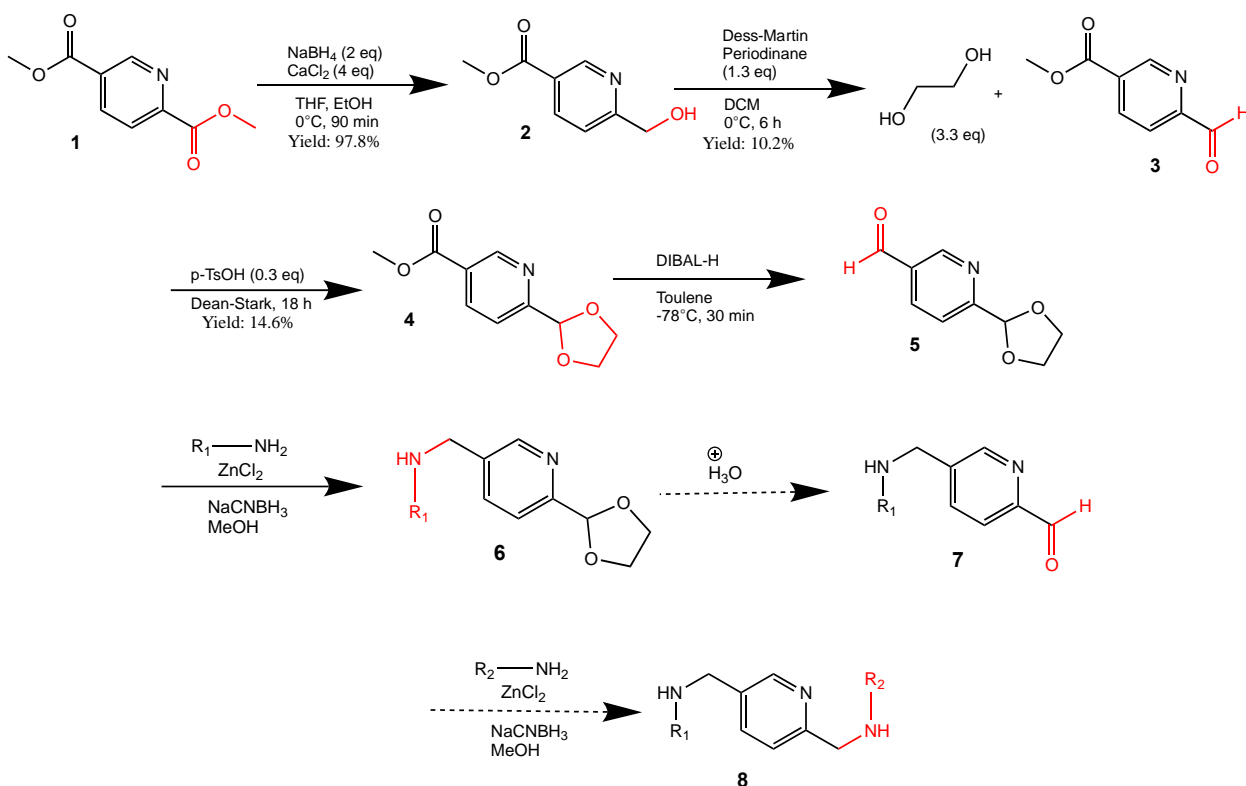
Scheme 1. Synthesis of 2,6-pyridine analogues from the 2,6-pyridine precursor molecules

6.1.2 2,5-thiophene analogues

In our next approach, we started over with 2,5-thiophenedicarboxaldehyde because of its

availability in the laboratory. If the thiophene synthesis is successful, this route will also be

extended to pyridine-based compounds as originally planned. We continue to use the protection

step with ethylene glycol but start off with an aldehyde already part of the compound. Using 0.3

equivalence of a catalyst and the starting compound (**1**), we were able to isolate 40% of **2** after

column chromatography. We hoped that by using a lower equivalence of ethylene glycol, less

would become bis-substituted and more would remain mono-substituted, leaving one side

available for a hit functional group. Protecting one side should have made the exposed aldehyde

more reactive for the amine attachment. The resulting mono-protected compound underwent

reductive amination with a hit functional group, 4-methoxy (**3**). The deprotection was completed

with citrate ammonium nitrate (**4**). After the deprotection, the final reductive amination would

take place with a different hit aniline (**5**). Following the completed synthesis and analytical

verification by ^{1}H NMR and MS of these analogues, they will be sent to Dr. Shim's lab for

further biological testing.
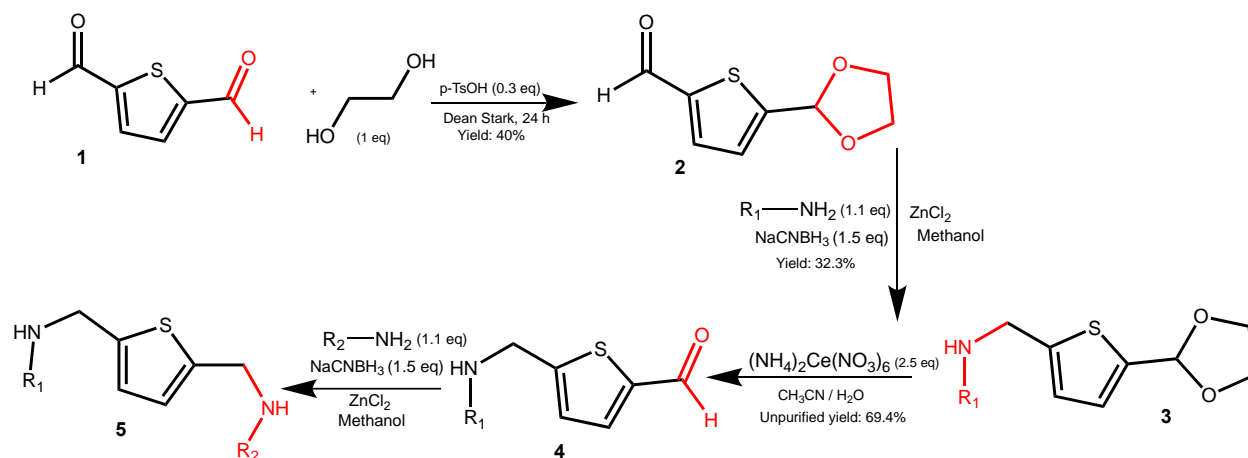
Scheme 2. Synthesis of 2,5-thiophene analogues from 2,5-thiophene precursor molecules

6.1.3. Challenges

Throughout the synthesis process, several challenges were encountered. The need for a more

simplistic synthetic route due to the stepwise nature of reactions that builds upon the last

molecule was recognized. An important lesson learned with the first synthetic route is the need

for analytical verification with both MS and NMR following the completion of a reaction before

moving on to the next step. With the switch to 2,5-thiophenedicarboxaldehyde, an equivalence

amount of ethylene glycol was considered thoroughly to produce enough of the mono-protected

compound. Thus, many of these procedures were continuously modified to maximize yield and

give the desired product.

In the first reductive amination with 4-methoxy (**3**), which is one of the hit functional

groups chosen to be attached, purification of the product was difficult. The excess amine and

product have similar retention factors (R_f values), and the column chromatography required at

least 6 hours of time to develop enough separation between the two compounds despite using a solvent system in which the compound of interest has a retention factor of 0.2. Therefore, there was not enough time to run the column while also managing other class times. To prevent excess amine, the 2.3 equivalence was reduced to 1.1 in the reductive amination reactions. However, to further reduce the excess amine obtained, it is suggested that the amine be added slowly and in smaller increments at a time while monitoring the progress of the reaction. It was also found that zinc chloride was a necessary reagent in the reductive amination reaction because it worked as a catalyst.

In the next step of deprotection, the original procedure that was used was 37% hydrochloric acid (HCl). However, surprisingly, the analytical analysis with H'NMR revealed that the compound had three aldehyde signals, and the 4-methoxy substituent was no longer attached to the thiophene ring. It appears that the compound may be acid sensitive. The mechanism of action is still unclear. Alternatively, citrate ammonium nitrate was used to deprotect the ethylene glycol[80], and this reaction worked better though there continues to be two aldehyde peak signals. It is unknown currently what could be causing two aldehyde signals. The rest of the H'NMR showed that the 4-methoxy substituent remains attached to the thiophene ring. However, since this deprotected compound was not completely purified before NMR analysis, impurities were noted. The final reductive amination attachment (**5**) is currently being worked on.

CONCLUSIONS

The CXCR4 small molecule antagonist synthesis is currently 75% completed with one last step to a final asymmetrical compound pending. The methods to synthesize the precursor were modified continuously through trial and error in order to synthesize our target compounds and

maximize yield of products; hence, an extended duration of time was devoted on the design of the asymmetrical 2,5-thiophene and 2,6-pyridine analogues. Work is currently being expanded to 2,6-pyridinedicarboxyaldehyde using the modeled synthetic route with 2,5-thiophene. With promising results in the current synthesis design, we hope to expand upon the library of CXCR4 antagonists with these asymmetrical small molecules and analyze their efficacy as CXCR4 antagonists.

EXPERIMENTAL DETAILS

7.1 Chemistry

The ^{1}H NMR (400 MHz) spectra were recorded on a Bruker Ac 400 FT NMR spectrometer in deuterated chloroform (CDCl3). Mass spectra were recorded on a JEOL spectrometer at Georgia State University Mass Spectrometry Center.

7.1.1 General Procedures[76] for the Synthesis of the methyl 6-(hydroxymethyl) nicotinate Analogues

To a stirring mixture of 500 mg of dimethyl pyridine-2,5-dicarboxylate, THF, ethanol, and 1.13 g of calcium chloride (CaCl$_2$), was added and cooled to 0°C. The mixture was maintained for 15 minutes, and sodium borohydride (NaBH$_4$) was added slowly. After being stirred for another 90 minutes while keeping the temperature constant, the bulk of ethanol was removed in vacuo. The resulting mixture was extracted with ethyl acetate, and the organic layer was dried and concentrated in vacuo. This procedure was used to synthesize **2**.

Methyl 6-(hydroxymethyl) nicotinate (2) MS ESI+: MW 167.17, found 168.0

7.1.1.2 General Procedures[77] for the Synthesis of the 3-pyridinecarboxylic acid, 6-formyl-, methyl ester Analogues

Crude methyl 6-(hydroxymethyl) nicotinate was dissolved in DCM, after which Dess-Martin periodinane was added portion-wise with stirring on ice. After 6 hours, the reaction was quenched by the dropwise addition of a solution of 5% w/w sodium thiosulfate in half saturated sodium bicarbonate. The aqueous layer was extracted with DCM, and the combined organic extracts were dried over sodium sulfate and concentrated under reduced pressure. This procedure was used to synthesize **3**.

3-Pyridinecarboxylic acid, 6-formyl-, methyl ester (3) MS ESI+: MW 165.15, found 166.0

7.1.1.3 General Procedures[78] for the Synthesis of the 3-pyridinecarboxylic acid, 6-(1,3-dioxolan-2-yl)-, methyl ester Analogues

In 10 milliliters of toluene, 3-pyridinecarboxylic acid, 6-formyl-, methyl ester (**3**) was added followed by ethylene glycol. p-Toluenesulfonic acid was added lastly. This reaction was refluxed using the Dean-Stark apparatus overnight. Sodium bicarbonate was added for the reaction work-up. Brine was added in the very last step of extraction, and the top layer was saved and concentrated under rotovap. This procedure was used to synthesize **4**.

3-pyridinecarboxylic acid, 6-(1,3-dioxolan-2-yl)- (4) MS ESI+: MW 209.20, found 210.0

7.1.1.4 General Procedures[79] for the Synthesis of the 3-pyridinecarboxaldehyde, 6-(1,3-dioxolan-2-yl)- Analogues

Toluene was added in a round bottom flask with 3-pyridinecarboxylic acid, 6-(1,3-dioxolan-2-yl)-, methyl ester. The round bottom flask was placed into a container with dry ice and acetone. The contents were stirred constantly with a stirring bar. DIBAL-H was added last. Once the reaction was finished in 30 minutes, the reaction was quenched with sodium bicarbonate, and brine was used for the work-up. This procedure was used to synthesize **5**.

7.1.1.5 General Procedures for the Synthesis of mono-aniline 2,6 Pyridine Analogues

To a solution of methanol, 100 mg of 3-pyridinecarboxaldehyde, 6-(1,3-dioxolan-2-yl)- (**5**) was combined in a dry vial with the aniline derivative, sodium cyanoborohydride, and zinc chloride. The solution was stirred for 2 hours and 5 minutes to overnight depending on the aniline. This procedure was used to synthesize **6** and **8** and used in Saniya Virani's thesis[75].

7.1.2. General Procedures for the Synthesis of the mono-protected 2,5-thiophene Analogues

Using a Dean-Stark Apparatus, 2-5-dicarboxyaldehyde (**1**) was dissolved in p-TsOH. To the stirring mixture, 1 equivalence of ethylene glycol was added dropwise. The completed reaction would yield compound **2** after column chromatography separation (15:1 hexane, ethyl acetate).

2-Thiophenecarboxaldehyde, 5-(1,3-dioxolan-2-yl)- (2) 40% as a yellow oil. ^{1}H NMR (400 MHz): 9.90 (s, 1H), 7.68 (d, 2H), 7.24 (d, 2H), 6.14 (s, 1H), 4.09 (m, 2H). MS EPI+: MW of compound 184.21, found, 185.0.

7.1.2.1 General Procedures for the Synthesis of the mono-aniline 2,5-thiophene Analogues

To a solution of methanol, 80 mg of monoprotected 2,5-thiophene (**2**) was combined with the aniline derivative of choice (1.1 eq) and zinc chloride (1 equivalence). The solution was stirred at room temperature for 5 min, and then treated with 1.5 equivalence of sodium cyanoborohydride (NaCNBH$_3$). The solution was then stirred for 5 h to overnight depending on the amine. The product was purified via flash column chromatography (5:1 DCM to hexane). This was used to synthesize **3**.

2-Thiophene-methoxyaniline, 5-(1,3-dioxolan-2-yl)- (3) Yellow oil. ^{1}H NMR (400 MHz): 7.26 (s, 1H), 7.01 (d, 2H), 6.87 (d, 2H), 6.78 (d, 2H), 6.63 (d, 2H), 6.04 (s, 1H), 4.11 (d, 2H), 4.01 (d, 2H), 3.98 (s, 1H). MS EPI+: MW of compound 291.37.21, found, 292.0.

7.1.2.2 General Procedures for the Synthesis of the deprotected 2,5-thiophene Analogues

17 mg of compound **3** (0.058 mmol) was dissolved in about half a milliliter of acetonitrile. Citrate ammonium nitrate (2.5 equivalence, 0.145 mmol, 79.5 mg) was dissolved in about half a milliliter of water and added to compound 3. The reaction was stirred for several hours. This was used to synthesize **4**.

2-Thiophene-methoxyaniline, 5-carboxyaldehyde (4) Not completely purified. Yellow oil. ^{1}H NMR (400 MHz): 9.51 (s, 1H), 8.619-6.628 (m, 2H), 4.58 (s, 1H), 3.82 (s, 1H). MS EPI+: MW of compound 247.07, found, 248.0.

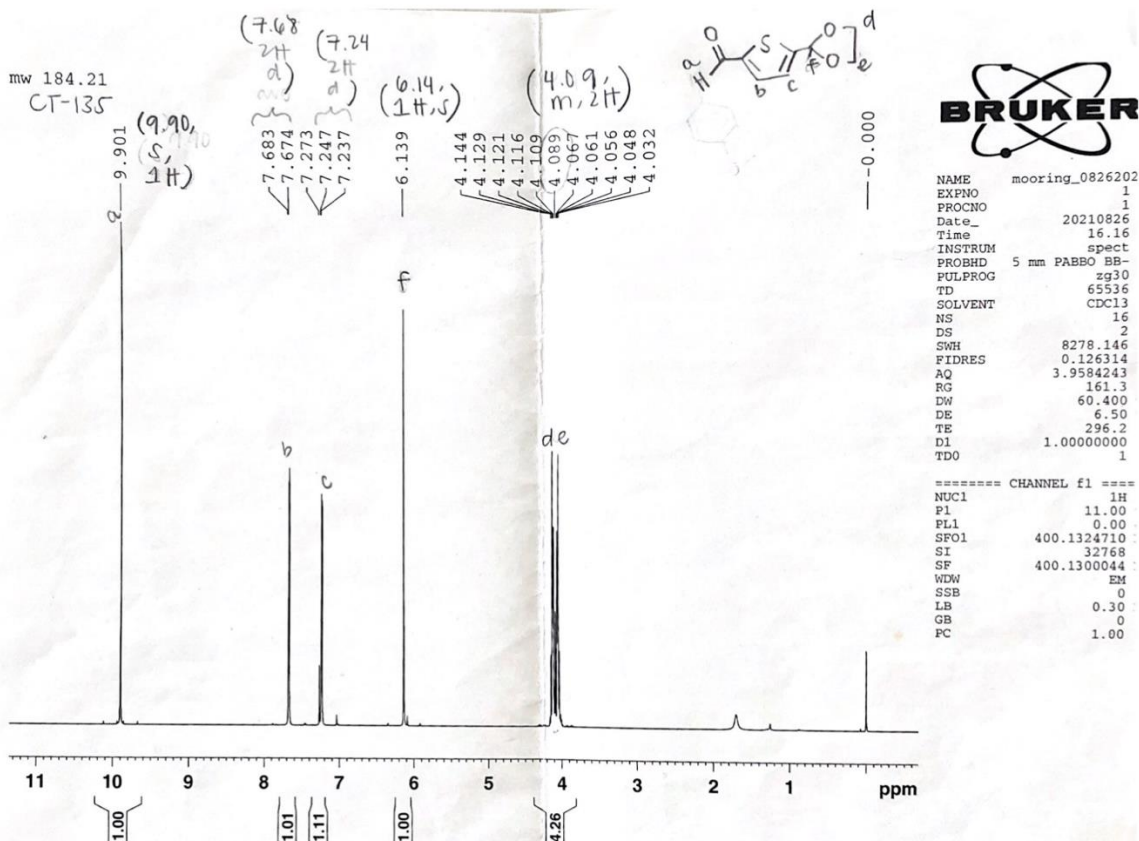**7.1.2.3 General Procedures for the Synthesis of the di-amino 2,5-thiophene Analogues**

To a solution of methanol, the unprotected 2,5-thiophene (**4**) was combined with the aniline derivative of choice (1.1 eq) and zinc chloride (1 equivalence). The solution was stirred at room temperature for 5 min, and then treated with 1.5 equivalence of sodium cyanoborohydride (NaCNBH₃). The solution was then stirred for 5 h to overnight depending on the amine. The product was purified via flash column chromatography (5:1 DCM to hexane or 15:1 hexane, ethyl acetate). This was used to synthesize **5**.

SUPPLEMENTAL

^{1}H NMR (400 MHz) of 2-Thiophenecarboxaldehyde, 5-(1,3-dioxolan-2-yl)-

CT-139

7.258
7.017
7.008
6.874
6.866
6.789
6.783
6.766
6.758
6.636
6.631
6.614
6.035

4.427
4.137
4.119
4.110
4.102
4.014
4.006
3.997
3.979
3.743

-0.000

e. (7.26, s, 1H)
c. (7.01, d, 2H)
d. (6.87, d, 2H)
a. (6.78, d, 2H)
b. (6.63, d, 2H)
i. (6.04, s, 1H)

f. (4.11, d, 2H)
g. (4.01, d, 2H)
h. (3.98, s, 1H)

0.56 1.00 1.01 2.19 2.12 0.97 2.05 2.18 2.05 3.51

¹H NMR (400 MHz) of 2-Thiophene-methoxyaniline, 5-(1,3-dioxolan-2-yl)-

CT-158

h
10.037
9.951
9.828

8.619
7.840
7.756
7.644
7.634
7.303
7.281
7.260
7.107
7.097
6.947
6.925
6.790
6.784
6.773
6.767
6.697
6.651
6.645
6.634
6.628

4.537

3.841
3.819
3.740

impurities

2.171
1.419
1.412
1.369
1.330
1.303
1.290
1.282
1.251
1.229
1.215
0.892
0.877
0.849
0.839
0.832
0.827
0.807

b
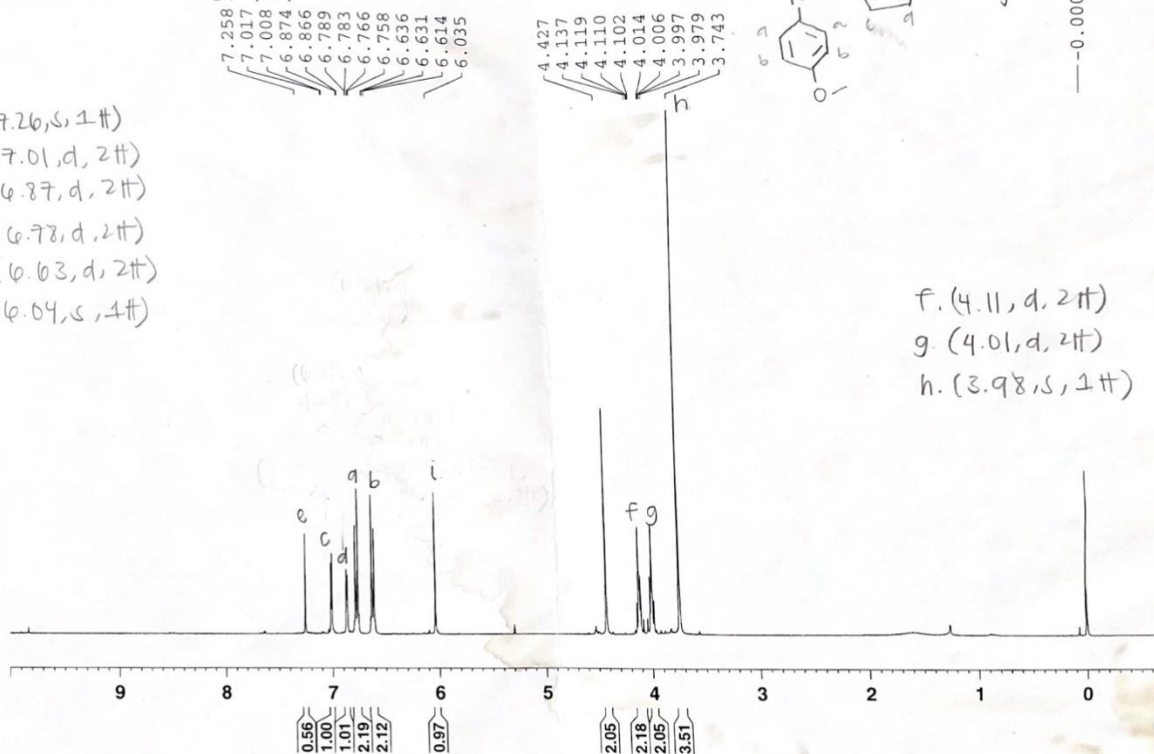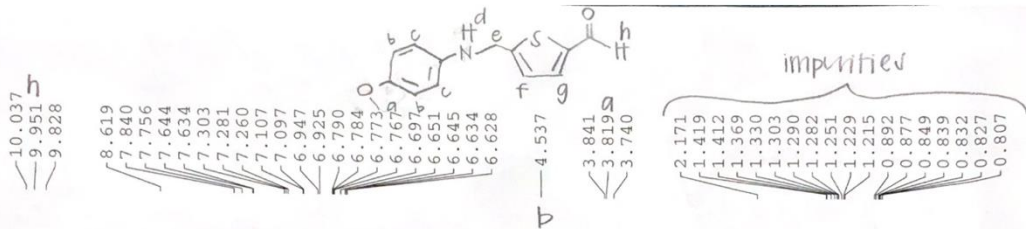
h. (9.51, s, 1 H)

g, h, e, d (8.619-6.628,
m, 2 H)

b. (4.58, s, 1 H)

a (3.82, s, 1 H)

g + h + e + d
and f
somewhere
all in this area

f

10 9 8 7 6 5 4 3 2 1 0 ppm
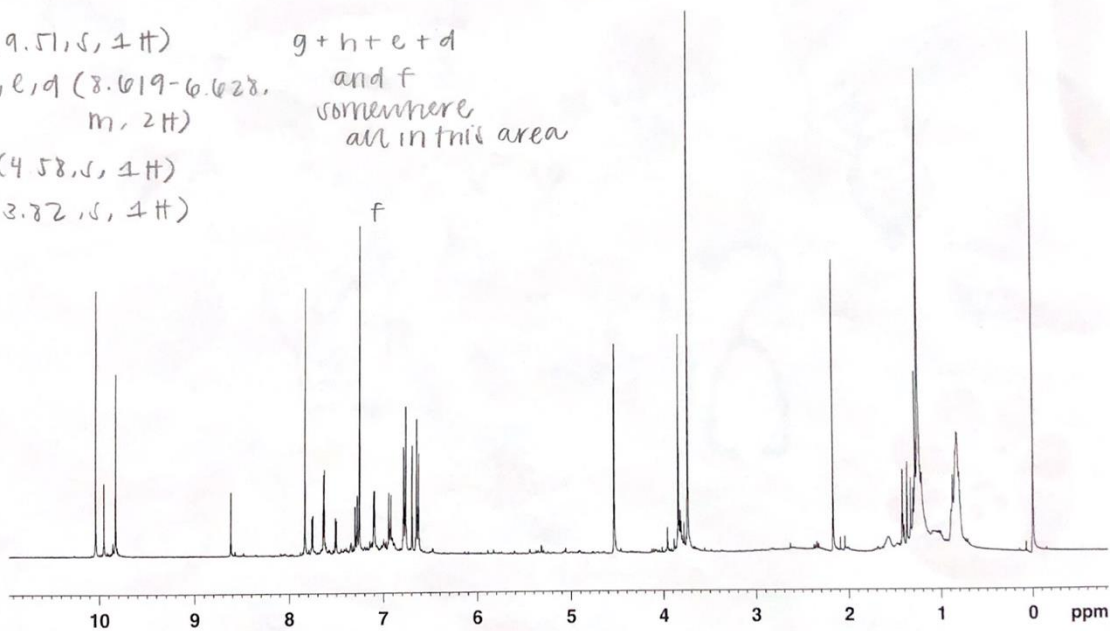
¹H NMR (400 MHz) of 2-Thiophene-methoxyaniline, 5-carboxyaldehyde

REFERENCES

1.   García-Cuesta, E.M.; Santiago, C.A.; Vallejo-Díaz, J.; Juarranz, Y.; Rodríguez-Frade, J.M.; Mellado M. The Role of the CXCL12/CXCR4/ACKR3 Axis in Autoimmune Diseases. Front, Endocrinol. (Lausanne) **2019**, 10 (585), 1-16.

2.   Tsou, L.K.; Huang, Y.H.; Song, J.S.; Ke, Y.Y.; Huang, J.K.; Shia, K.S. Harnessing CXCR4 antagonists in stem cell mobilization, HIV infection, ischemic diseases, and oncology. Med Res Rev. **2018**, 38 (4), 1188-1234.

3.   Pawig, L.; Klasen, C.; Weber, C.; Bernhagen, J.; Noels, H. Diversity and inter-connections in the CXCR4 chemokine receptor/ligand family: molecular perspectives. Front. Immunol. **2015**, 6 (429).

4.   Yu, X., Wang, D., Wang, X. et al. CXCL12/CXCR4 promotes inflammation-driven colorectal cancer progression through activation of RhoA signaling by sponging miR-133a-3p. J Exp Clin Cancer Res **2019**, 38 (32).

5.   Luo, Y.; Zhao, X.; Zhou, X.; Ji, W.; Zhang, L.; Luo, T.; Liu, H.; Huang, T.; Jiang, T.; Li, Y. Short-term intermittent administration of CXCR4 antagonist AMD3100 facilitates myocardial repair in experimental myocardial infarction. Acta Biochimica et Biophysica Sinica **2013**, 45 (7), 561–569.

6.   Liu, T.; Li, X.; You, S.; Bhuyan, S.S.; Dong, L. Effectiveness of AMD3100 in treatment of leukemia and solid tumors: from original discovery to use in current clinical practice. Exp Hematol Oncol **2015**, 5 (19).

7.   Yvonne, D.; Lukas, P.; Christian, W.; Heidi, N. The CXCL12/CXCR4 chemokine ligand/receptor axis in cardiovascular disease. Front. Physio. **2014**, 5 (212), 1-23.

8.	Miller, M.C. and Mayo, K.H. Chemokines from a Structural Perspective. Int J Mol Sci. **2017**, 18 (10), 2088-2104.

9.	Stone, M.J.; Hayward, J.A.; Huang, C.; Huma, Z. E.; Sanchez, J. Mechanisms of Regulation of the Chemokine-Receptor Network. Int J Mol Sci. **2017**, 18 (2), 342-375.

10.	Busillo, J.M. and Benovic, J.L. Regulation of CXCR4 Signaling. Biochimica et Biophysica Acta (BBA) – Biomembranes **2007**, 1768 (4), 952-963.

11.	Janssens, R.; Struyf, S.; Proost, P. The unique structural and functional features of CXCL12. Cellular and Molecular Immunol. **2018**, 15, 299-311.

12.	Bianchi, M.E. and Mezzapelle R. The Chemokine Receptor CXCR4 in Cell Proliferation and Tissue Regeneration. Front. Immunol. **2020**, 11 (2109), 1-8.

13.	Kawaguchi, N.; Zhang, T.-T.; Nakanishi, T. Involvement of CXCR4 in Normal and Abnormal Development. Cells **2019**, 8 (185), 1-14.

14.	Pozzobon, T.; Goldoni, G.; Viola, A.; Molon, B. CXCR4 Signaling in health and disease. Immunol. Letters **2016**, 177, 6-15.

15.	Lombardi, L.; Tayano, F.; Morelli, F.; Latiano, T.P.; Sebastiano, P.D.; Maiello, E. Chemokine receptor CXCR4: Role in gastrointestinal cancer. Crit Reviews in Oncology and Hematology **2013**, 88 (3), 696-705.

16.	Byrant, J.; Ahern, D.J,; Brennan, F.M. CXCR4 and vascular cell adhesion molecule 1 are key chemokine/adhesion receptors in the migration of cytokine-activated T cells. Arthritis and Rheumatism **2012**, 64 (7), 2137-2146.

17.	Ceholski, D.K.; Turnball, I.C.; Pothula, V.; Lecce, L.; Jarrah, A.A.; Kho, C.; Lee, A.; Hadri, L.; Costa, K.D.; Hajjar, R.J.; Tarzami, S.T. CXCR4 and CXCR7 play distinct roles in

cardiac lineage specification and pharmacologic β-adrenergic response. Stem Cell Res. **2017**, 23, 77-86.

18. Wu, B.; Chien, E.Y.; Mol, C.D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F.C.; Hamel, D.J.; Kuhn, P.; Handel, T.M.; Cherezov, V.; Stevens, R.C. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. Science **2010**, 330 (6007), 1066-1071.

19. Veldkamp, C.T.; Seibert, C.; Peterson, F.C.; De la Cruz, N.B.; Haughner, J.C. 3rd; Basnet, H.; Sakmar, T.P.; Volkman, B.F. Structural basis of CXCR4 sulfotyrosine recognition by the chemokine SDF-1/CXCL12. Sci Signal **2008**, 1 (37), 1-18.

20. Chang, C.C.; Liou, J.W.; Dass, K.T.P.; Li, Y.-T.; Jiang, S.-J.; Pan, S.-F.; Yeh, Y.-C.; Hsu, H.-J. Internal water channel formation in CXCR4 is crucial for Gi-protein coupling upon activation by CXCL12. Commun Chem **2020**, 3 (133), 1-12.

21. Crump, M.P.; Gong, J.H.; Loetscher, P.; Rajarathnam, K.; Amara, A.; Arenzana-Seisdedos, F.; Virelizier, J.-L.; Baggiolini, M.; Skyes, B.D.; Clark-Lewis, I. Solution structure and basis for functional activity of stromal cell-derived factor-1; dissociation of CXCR4 activation from binding and inhibition of HIV-1. Embo J. **1997**, 16, 6996-7007.

22. Rajagopalan, L. and Rajarathnam, K. Structural basis of chemokine receptor function- a model for binding affinity and ligand selectivity. Biosci Rep. **2006**, 26 (5), 325-339.

23. Kleist, A.B.; Getschman, A.E.; Ziarek, J.J.; Nevins, A.M.; Gauthier, P.-A.; Chevigné, A.; Szpakowska, M.; Volkman, B.F. New paradigms in chemokine receptor signal transduction: Moving beyond the two-site model. Biochem Pharm. **2016**, 114, 63-68.

24. American Cancer Society. Cancer Facts & Figures 2020. Atlanta: American Cancer Society; **2020**.

25.   U.S. Cancer Statistics Working Group. U.S. Cancer Statistics Data Visualizations Tool, based on 2019 submission data (1999-2017): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; www.cdc.gov/cancer/dataviz, released in June **2020**.

26.   Bidram, E.; Esmaeili, Y.; Ranji-Burachaloo, H.; Al-Zaubai, N.; Zarrabi, A.; Stewart, A.; Dunstan, D.E. A concise review on cancer treatment methods and delivery systems. Journal of Drug Delivery Science and Technology **2019**, 54, 101350-101378.

27.   Huang, C.Y.; Ju, D.T.; Chang, C.F.; Muralidhar Reddy, P.; Velmurugan, B.K. A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. Biomedicine **2017**, 7(4), 23.

28.   Cancer Statistics, **2020.** National Cancer Institute website. https://www.cancer.gov/about-cancer/understanding/statistics

29.   Seyfried, T.N. and Huysentruyt, L.C. On the origin of cancer metastasis. Critical reviews in oncogenesis **2013**, 18, 1-2 (2013): 43-73.

30.   Chaffer, C.L. and Weinberg, R.A. A perspective on cancer cell metastasis. Science **2011**, 331 (6024), 1559-1564.

31.   Understanding Advanced and Metastatic Cancer, **2020**. American Cancer Society website. https://www.cancer.org/treatment/understanding-your-diagnosis/advanced-cancer/what-is.html

32.   Tulotta, C.; Stefanescu, C.; Chen, Q.; Torraca, V.; Meijer, A.H.; Snaar-Jagalska, B.E. CXCR4 signaling regulates metastatic onset by controlling neutrophil motility and response to malignant cells. Scientific Reports **2019**, 9 (2399), 1-16.

33.   Chatterjee, S.; Behnam Azad, B.; Nimmagadda, S. The intricate role of CXCR4 in cancer. Adv Cancer Res. **2014**, 124, 31-82.

34.   Zhao, H.; Guo, L.; Zhao, H.; Zhao, J.; Weng, H.; Zhao, B. CXCR4 over-expression and survival in cancer: a system review and meta-analysis. Oncotarget **2015**, 6(7), 5022-5040.

35.   Poldolsky, D.K. Inflammatory bowel disease. N. Engl. J. Med. **2002**, 347, 417-429.

36.   Xavier, R. and Podolsky, D.Unravelling the pathogenesis of inflammatory bowel disease. Nature **2007**, 448, 427-434.

37.   Werner, L.; Guzner-Gur, H.; Dotan, I. Involvement of CXCR4/CXCR7/CXCL Interactions in Inflammatory bowel disease. Theranostics **2013**, 3(1), 40-46.

38.   O'Byrne, P.M. and Inman, M.D. Airway Hyperresponsiveness. Chest **2003**, 123 (3), 411S-416S.

39.   Wilder, J.A.; Collie, D.D.; Wilson, B.S.; Bice, D.E.; Lyons, C.R.; Lipscomb, M.F. Dissociation of airway hyperresponsiveness from immunoglobulin E and airway eosinophilia in a murine model of allergic asthma. Am J Respir Cell Mol Biol. **1999**, 20 (6), 1326-34

40.   Tournoy, K.G.; Kips, J.C.; Schou, C.; Pauwels, R.A. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. Clin Exp Allergy. **2000**, 30 (1), 79-85

41.   Daubeuf, F.; Hachet-Haas, M.; Gizzi, P.; Gasparik, V.; Bonnet, D.; Utard, V.; Hibert, M.; Frossard, N.; Galzi, JL. An antedrug of the CXCL12 neutraligand blocks experimental allergic asthma without systemic effect in mice. J Biol Chem. **2013**, 288 (17), 11865-76.

42.   Mehrad, B.; Burdick, M.D.; Strieter, R.M. Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. Int J Biochem Cell Biol. **2009**, 41(8-9), 1708-18.

43.   Mehrad, B.; Burdick, M.D.; Zisman, D.A.; Keane, M.P.; Belperio, J.A.; Strieter, R.M. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. Biochemical & Biophysical Research Communications **2007**, 353, 104–108.

44.   Phillips, R.J.; Burdick, M.D.; Hong, K.; Lutz, M.A.; Murray, L.A.; Xue, Y.Y.; Belperio, J.A.; Keane, M.P.; Strieter, R.M. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. Journal of Clinical Investigation **2004**, 114, 438–446.

45.   Moeller, A.; Gilpin, S.E.; Ask, K.; Cox, G.; Cook, D.; Gauldie, J.; Margetts, P.J.; Farkas, L.; Dobranowski, J.; Boylan, C.; O'Byrne, P.M.; Strieter, R.M.; Kolb, M. Circulating Fibrocytes Are an Indicator for Poor Prognosis in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med. **2009**, 179 (7), 588-94

46.   Global HIV Statistics, **2020**. UNAIDS Agency website. https://www.unaids.org/en/resources/fact-sheet

47.   Taylor, B.S.; Sobieszczyk, M.E.; McCutchan, F.E.; Hammer, S.M. The challenge of HIV-1 subtype diversity [published correction. N Engl J Med. **2008**, 358 (15), 1590-1602.

48.   Hladik, F.; Sakchalathorn, P.; Ballweber, L.; Lentz, G.; Fialkow, M.; Eschenbach, D.; McElrath, M. J. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity **2007**, 26(2), 257–270.

49.   Kwong, P,D,; Wyatt, R.; Robinson, J.; Sweet, R.W.; Sodroski, J.; Hendrickson, W.A. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature **1998**, 393 (6686), 648-59.

50.   Denizot, M.; Varbanov, M.; Espert, L.; Robert-Hebmann, V.; Sagnier, S.; Garcia, E.; Curriu, M.; Mamoun, R.; Blanco, J.; Biard-Piechaczyk, M. HIV-1 gp41 fusogenic function triggers autophagy in uninfected cells. Autophagy **2008**, 4 (8), 998-1008.

51.   Panos, G. and Watson, D.C. Effect of HIV-1 subtype and tropism on treatment with chemokine coreceptor entry inhibitors; overview of viral entry inhibition. Crit Rev Microbiol. **2015**, 41 (4), 473-87.

52.   McCormack, S.; Dunn, D.T.; Desai, M.; Dolling, D.I.; Gafos, M.; Gilson, R.; Sullivan, A.K.; Clarke, A.; Reeves, I.; Schembri, G.; Mackie, N.; Bowman, C.; Lacey, C.J.; Apea, V.; Brady, M.; Fox, J.; Taylor, S.; Antonucci, S.; Khoo, S.H,; Rooney, J.; Nardone, A.; Fisher, M.; McOwan, A.; Phillips, A.N.; Johnson, A.M.; Gazzard, B.; Gill, O.N. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. The Lancet **2016**, 387 (10013), 53-60.

53.   Günthard, H. F.; Saag, M. S.; Benson, C. A.; del Rio, C.; Eron, J. J.; Gallant, J. E.; Hoy, J. F.; Mugavero, M. J.; Sax, P. E.; Thompson, M. A.; Gandhi, R. T.; Landovitz, R. J.; Smith, D. M.; Jacobsen, D. M.; Volberding, P. A. Antiretroviral Drugs for Treatment and Prevention of HIV Infection in Adults: 2016 Recommendations of the International Antiviral Society-USA Panel. JAMA **2016**, 316 (2), 191–210.

54.   Rheumatoid Arthritis (RA), **2020**. Centers for Disease Control and Prevention Website. https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html

55.   Peng, L.; Zhu, N.; Mao, J.; Huang, L.; Yang, Y.; Zhou, Z.; Wang, L.; and Wu, B. Expression levels of CXCR4 and CXCL12 in patients with rheumatoid arthritis and its correlation with disease activity. Experimental and therapeutic medicine **2020**, 20 (3), 1925–1934.

56.   Bullock, J.; Rizvi, S.; Saleh, A. M.; Ahmed, S. S.; Do, D. P.; Ansari, R. A.; Ahmed, J. Rheumatoid Arthritis: A Brief Overview of the Treatment. Medical principles and practice: international journal of the Kuwait University, Health Science Centre, 2018, 27 (6), 501–507.

57.	De Clercq, E. Mozobil® (Plerixafor, AMD3100), 10 years after its approval by the US Food and Drug Administration. Antiviral chemistry & chemotherapy **2019**, 27, 2040206619829382.

58.	Hatse, S.; Princen, K.; Bridger, G.; De Clercq, E.; Schols, D. Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4, FEBS Letters **2002**, 527.

59.	De Clercq, E. AMD3100/CXCR4 Inhibitor. Front. Immunol. **2015**, 6, 276.

60.	Pantin, J.; Purev, E.; Tian, X.; Cook, L.; Donohue-Jerussi, T.; Cho, E.; Reger, R.; Hseieh, M.; Khuu, H.; Calandra, G.; Geller, N.L.; Childs, R.W. Effect of high-dose plerixafor on CD34+ cell mobilization in healthy stem cell donors: results of a randomized crossover trial. Haematologica **2017**, 102 (3), 600-609.

61.	Liang, Z.; Zhan, W.; Zhu, A.; Yoon, Y.; Lin, S.; Sasaki, M.; Klapproth, J.-M. A.; Yang, H.; Grossniklaus, H.E.; Xu, J.; Rojas, M.; Voll, R.J.; Goodman, M.M.; Arrendale, R.F.; Liu, J.; Yun, C.C.; Synder, J.P.; Liotta, D.C.; Hyunsik, S. Development of a Unique Small Molecule Modulator of CXCR4. PLOS ONE **2012**, 7 (4), e34038.

62.	Liu, T.; Li, X.; You, S.; Soumitra, S.B.; Dong, L. Effectiveness of AMD3100 in treatment of leukemia and solid tumors: from original discovery to use in current clinical practice. Exp Hematol Oncol **2015**, 5, 19.

63.	Zhu, A.; Zhan, W.; Liang, Z.; Yoon, Y.; Yang, H.; Grossniklaus, H. E.; Xu, J.; Rojas, M.; Lockwood, M.; Snyder, J. P.; Liotta, D. C.; Shim, H. Dipyrimidine amines: a novel class of chemokine receptor type 4 antagonists with high specificity. J Med Chem **2010**, 53 (24), 8556–8568.

64.	Debnath, B.; Xu, S.; Grande, F.; Garofalo, A.; Neamati N. Small molecule inhibitors of CXCR4. Theranostics **2013**, 3 (1), 47-75.

65.   De Clercq, E. The AMD3100 story: The path to the discovery of a stem cell mobilizer (Mozobil). Biochemical Pharmacology **2009**, 77 (11), 1655-1664.

66.   Hatse, S.; Princen, K.; De Clercq, E.; Rosenkilde, M.M.; Schwartz, T.W.; Hernandez-Abad, P.E.; Skerli, R.T. Bridger, G.J.; Schols, D. AMD3465, a monomacrocyclic CXCR4 antagonist and potent HIV entry inhibitor. Biochemical Pharmacology **2005**, 70 (5), 752-761.

67.   Wu, R.; Yu, W.; Yao, C.; Liang, Z.; Yoon, Y.; Xie, Y.; Shim, H.; Bai, R. Amide-sulfamide modulators as effective anti-tumor metastatic agents targeting CXCR4/CXCL12 axis. European Journal of Medicinal Chemistry **2020**, 185, 111823.

68.   Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews **1997**, 23 (1-3), 3-25.

69.   Mullard, A. Re-assessing the rule of 5, two decades on. Nat Rev Drug Discov **2018,** 17, 777.

70.   Ghose, A.K.; Viswanadhan, V.N.; Wendoloski, J.J. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. Journal of combinatorial chemistry **1999**, 1 (1), 55-68.

71.   De Clercq, E.; Yamamoto, N.; Pauwels, R.; Balzarini, J.; Witvrouw, M.; De Vreese, K.; Debyser, Z.; Rosenwirth, B.; Peichl, P.; Datema, R., Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. Antimicrobial agents and chemotherapy **1994**, 38 (4), 668-674.

72.   Hansch, C.; Leo, A.; Hoekman, D.; Livingstone, D., Exploring QSAR: hydrophobic, electronic, and steric constants. American Chemical Society Washington, DC: **1995**; Vol. 48.

73.   Wishart, D. S.; Tzur, D.; Knox, C.; Eisner, R.; Guo, A. C.; Young, N.; Cheng, D.; Jewell, K.; Arndt, D.; Sawhney, S., HMDB: the human metabolome database. Nucleic acids research **2007**, 35 (suppl_1), D521-D526.

74.   Gaines, T.D. Design, Synthesis and Analysis of Small Molecule Heterocyclic Aromatic-Based CXCR4 Modulators. Ph.D. Dissertation, Georgia State University, Atlanta, GA, **2017**.

75.   Virani, S. Synthesis and Analysis of Pyridine Derivatives as Small Molecule Antagonists of CXCR4. Masters Thesis, Georgia State University, Atlanta, GA, **2018**.

76.   Kozhevnikov, V.N., Shabunina, O.V., Kopchuk, D.S., Ustinova, M.M., Konig, B, Kozhevnikov, D.N., Facile synthesis of 6-aryl-3-pyridyl-1,2,4-triazines as a key step toward highly fluorescent 5-substituted bipyridines and their Zn(II) and Ru(II) complexes. Tetrahedron **2008**, 64 (37), 8963-8973.

77.   Vasta, J.D., Anderson, K.A., Deck, K.M., Nizzi, C.P, Eisenstein, R.S., Raines, R.T., Selective Inhibition of Collagen Prolyl 4-Hydroxylase in Human Cells. ACS Chem Bio **2016**, 11(1), 193-199.

78.   Hannon, M., Rodger, A., Mann, N. H. Supramolecular compounds and their use as antitumor and antiviral agents. PCT Int. Appl. **2005**, 115 pp. CODEN:PIXXD2; WO2005033119.

79.   French, J.B., Cen, Y., Vrablik, T.L., Xu, P., Allen, E. Hanna-Rose, W., Sauve, A.A., Characterization of Nicotinamidases: Steady State Kinetic Parameters, Classwide Inhibition by Nicotinaldehydes, and Catalytic Mechanism. Biochemistry **2010**, 49(49), 10421-10439.

80.   Ates, A., Gautier, A., Leroy, B., Plancer, J-M., Quesnel, Y., Markó, I.E. Remarkably Efficient Deprotection of Cyclic Acetals and Ketals. Tetrahedron Letters **1999**, 40, 1799-1802.