SULFONAMIDE DERIVATIVES AS TUBULIN INHIBITORS AND SELECTIVE
ANTI-TRYPANOSOME AGENTS – DESIGN, SYNTHESIS & BIOLOGICAL
EVALUATION

VIHARIKA BOBBA

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Andhra University
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We hereby approve this thesis for

(VIHARIKA BOBBA)

Candidate for the Master of Science in Chemistry degree for the

Department of Chemistry

and the CLEVELAND STATE UNIVERSITY

College of Graduate Studies

Thesis Chairperson, (Dr. Bin Su)

Department & Date

Thesis Committee Member, (Dr. Xue-Long Sun)

Department & Date

Thesis Committee Member, (Dr. Yana Sandlers)

Department & Date

Student’s Date of Defense: (6/29/2016)
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VIHARIKA BOBBA

ABSTRACT

Background

Human African Trypanosomiasis (HAT), is an endemic vector borne disease and a serious threat to the huge population living in sub-Saharan Africa where, the health systems are least effective, or even non-existent. Two major sub-species are responsible for HAT, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* and these are extracellular parasite that resides in blood and tissue fluids of humans or cattle. The parasites are transmitted between the hosts through the bite of tsetse fly. Current treatment strategies are relied upon few drugs which are least effective with high drug toxicity and low selectivity towards parasite cells.

Methods

We developed a synthetic scheme for the synthesis of sulfonamide derivatives and have generated a library of compounds, which are targeting tubulin protein of the *Trypanosoma* parasite. All the compounds are purified by column chromatography methods and analyzed using different analytical techniques like HPLC and mass spectrometry. Latter, the cell inhibitory effect of all these compounds were analyzed using cell viability assay (MTT assay and MTS assay). HEK293 and mouse macrophage
RAW267.4 cells are taken as normal mammalian cells and *T. b. brucei* cells are the parasite cells. The selective index for all the compounds were obtained to study the selectivity of the compounds.

**Results and Conclusions**

All the compounds were synthesized following the scheme and were obtained in >98% purity. From, the cell inhibitory studies all the compounds showed better inhibition of parasite cell growth compared to normal cells, of which one compound (compound 15) exhibited a very higher selective index. In comparison, with all the previously synthesized library of compounds, these compounds showed better selective inhibition. The compounds with higher selective index were considered for further research.
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ABBREVIATIONS

NTD’s – Neglected Tropical Diseases

HAT – Human African Trypanosomiasis

CNS – Central Nervous System

CSF – Cerebrospinal fluid

NECT – Nifurtimox-Eflornithine Combination Therapy

DFMO – Alpha-difluoro methyl ornithine

DMF – N’, N – Dimethylformamide

TLC – Thin layer chromatography

NMR – Nuclear Magnetic Resonance Spectroscopy

MS – Mass Spectrometry

HPLC – High Performance Liquid Chromatography

ACN – Acetonitrile

MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

MTS – 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

HEK293 – Human Embryonic Kidney cell lines 293

DMEM – Dulbecco’s modified Eagle’s medium
DMSO – Dimethylsulfoxide

$K_2CO_3$ – Potassium Carbonate

NaH – Sodium hydride

NaOH – Sodium hydroxide

$Na_2CO_3$ – Sodium carbonate

FeCl$_3$ – Iron Chloride

Zn – Zinc
CHAPTER I
INTRODUCTION

Neglected tropical diseases (NTD’s) are a medically diverse group of tropical infections. They are caused by a variety of pathogens (viruses, bacteria, protozoa and helminths) and are especially common in low-income populations in the regions of Africa, Asia, and the Americas effecting more than one billion people globally. Few studies reported that more than 500,000 deaths may result annually from NTD’s. [1] One such NTD is Human African Trypanosomiasis (HAT) which is ranked as second among NTD’s that are responsible for substantial global morbidity, mortality and economic losses. It is estimated that 9000 to 500,000 people die from this disease every year. [2,3]

1.1 Human African Trypanosomiasis

Human African Trypanosomiasis (HAT), also known as African sleeping sickness, is an endemic vector borne disease and a serious threat to the huge population living in sub-Saharan Africa. [2] It is caused by the protozoan parasite Trypanosoma brucei belonging to the kinetoplastid order. [4]

The Trypanosoma is an extracellular parasite that resides in blood and tissue fluids of humans or cattle. [3, 4] Once infected, the host’s immune system initiates humoral immune response against the parasite surface antigens. However, eradication is not
achieved as the parasite confronts the immune system by switching the variant surface glycoproteins (VSG’s) of each generation. [5, 6]

Several species of Trypanosoma exist in the African continent. Studies have shown that two major sub-species responsible for HAT, Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense. T. b. gambiense having Humans as their host are commonly seen in West and Central Africa [8], whereas T. b. rhodesiense that primarily infects cattle and domestic animals are found in sub-Saharan Africa [6]. Another species, T. b. brucei is known to be less infectious to humans, but is more harmful towards cattle and agricultural production is also found in African regions, therefore T. b. brucei is most commonly used for laboratory and animal studies as it shares many common features (such as antigenic variation) with the two parasites that infects humans. [10, 11, 17]

The HAT infection is transmitted through the bite of a specific species that belongs to the genus Glossina, also known as tsetse fly. HAT is a two-stage infectious disease; the first stage, the hemolymphatic stage where the patients show symptoms like anemia, thrombocytopenia, impaired liver and kidney function and raised inflammatory markers is acute. The second stage which is chronic cerebral stage, (meningoencephalitic stage) is a major threat where the parasite enters the cerebrospinal fluid (CSF). [14] Most common symptoms of this stage are fever, headache, lymphadenopathy, memory loss, hallucination, nocturnal sleeping pattern, personality changes, cognitive decline, and coma. Without effective initial treatment, the infection results in coma and ultimately leads to death. The damages caused by the parasite after entering the CNS are irreversible even after treatment.
1.2 Available treatment strategies

The diagnosis of HAT can be done through an inexpensive serological test. [5] Once diagnosed, the current chemotherapy strategies of HAT rely on only few drugs including Pentamidine, Suramin, Melarsoprol, Eflornithine and Nifurtimox-Eflornithine combination therapy (NECT), which results in high toxicity and limited efficacy against late stages of the disease. [7, 8, 9] Hence, there is an immediate need for effective and specific drugs with no or fewer adverse effects.

Pentamidine, a water soluble aromatic diamidine is used in the early stages of the disease against *T. b. gambiense*. [7] Its trypanocidal activity involves binding to the minor groove of mitochondrial kDNA and also acting as inhibitor of the S-adenosylmethionine decarboxylase. Another early stage drug most commonly used is Suramin, a sulfonated naphthylamine. It is taken up into the *T. b. rhodesiense* cells by receptor-mediated LDL endocytosis. It is known that Suramin inhibits various glycolytic enzymes and LDL receptors thereby limiting the parasite’s supply of cholesterol and phospholipids.

Melarsoprol, a melaminophenyl arsenical is the most universal drug introduced in the 1940s. It is the only approved drug for effectively treating advanced stages of infection in both *T. b. gambiense* and *T. b. rhodesiense*. [7] However, the adverse effects like encephalopathy in every 1 out of 20 patients and drug resistance in some of the *T. brucei* species are two major shortcomings. Another late stage drug, α-difluoro-methylornithine (DFMO, eflornithine) is safer than the other drugs, but its efficacy is limited to only *T. b. gambiense*. It acts as an irreversible inhibitor of ornithine decarboxylase (ODC), thereby
inhibiting polyamine synthesis. However, DFMO is known to be less active against *T. rhodesiense*.

As an alternative approach, combination therapies are given to patients with second stage disease, where the parasite enters the central nervous system (CNS). For example, Nifurtimox is taken orally for 1 to 2 months and α-difluoromethylornithine (α-DFMO) is administered by a specific scheme over 5 weeks which includes 14 days of intravenous injections. [10]

Recent developments such as derivatizing imidazoles, arsenicals and antibiotics have been successfully tested in experimental models. Combinations of drugs with additive or potentiating effects mainly based on inhibition of decarboxylase enzymes or exposure to oxidative stress appear to be promising. [13]

The major limitations associated with these drugs are poor selectivity and specificity of drugs towards parasite cells, high toxic effects, route of drug administration (intramuscular or intravenous injection), a very high cost of hospitalization for treatment, and drug resistance. [11, 12, 17] Overall, these drugs are not effective in successful treatment of the African sleeping sickness. Thus for, there is a lack of effective and inexpensive chemotherapeutic agents for the treatment of Human African Trypanosomiasis. [12, 13] Therefore, improved chemotherapeutic agents with better selectivity and specificity for HAT are required to combat this disease.

1.3 Tubulins as target

Tubulin is a highly conserved protein and an important structural unit of microtubules. Tubulin is a heterodimer, that consists of α-tubulin and β-tubulin; both subunits have a
molecular weight of 55 kDa. These tubulin structures are present in almost all eukaryotic cells. Recent studies have found tubulin like structures in prokaryotes as well. [16, 17] Microtubules are highly dynamic structures and switch stochastically between contraction and relaxation phases both in vivo and in vitro. This non-equilibrium behavior is known as dynamic instability. The intrinsic microtubule dynamics are further modified in the cell by interaction with cellular factors that stabilize or destabilize microtubules, which operate in specific ways to generate different microtubule assemblies during the cell cycle. Tubulin-containing structures play a significant role in many important cellular functions such as development and maintenance of cytoskeleton, cell signaling, intracellular transport, chromosome segregation during cell division, and cell locomotion. [16, 17]

![Figure 1: Tubulin protein](image)
Studies have shown that, tubulins play major role during Trypanosoma cell division and locomotion as well. The population doubling rate of trypanosomes depends mainly on the tubulin polymerization and depolymerization. The flagellum emerging from the basal body on the posterior end of the trypanosome possess canonical ‘9+2’ microtubule axoneme that drives flagellar movement. The flagellar pocket is known to be an important structure in the uptake and internalization of molecules that are essential for the trypanosomes survival. [17, 21]

As it is widely known, tubulins are a very attractive target in anticancer drug discovery field, and several successful tubulin binders are found to be first line chemotherapeutic agents.

Tubulin inhibitors also prove to be promising drug candidates against HAT because they can interrupt the *T. brucei* cell division and also affect the locomotion function of flagellum which can then lead to cell death. The specific binding of tubulin inhibitors to the parasite cells also reduces the toxic effects of the drugs on the host cells. [17, 21]

In addition, identification of binding pockets uniquely located on *T. brucei* tubulin would allow development of selective tubulin inhibitors, which could dramatically reduce the toxic effects of the anti-parasite drugs to the host cells. [21] These factors, therefore, indicate that there are potential advantages to using tubulin inhibitors as agents for treating trypanosomiasis.
CHAPTER II
EXPERIMENTAL SECTION

2.1 Materials and methods

All commercially available chemicals were used without further purification unless otherwise noted. All reactions are carried out at normal room temperature under a closed fume hood. Following, synthesis all the compounds are purified and analyzed using different analytical techniques, Column chromatography, Nuclear Magnetic Resonance spectroscopy, High performance liquid chromatography and Mass spectrometry.

2.2 Synthetic scheme for sulfonamide derivatives

2-amino, 5-nitro phenol (1mmol, 1eq), the starting material 1 was reacted with different benzyl chlorides and/or alkyl chlorides (1.2eq) in the presence of anhydrous potassium carbonate (K$_2$CO$_3$, 3eq) and DMF as solvent to give compound 2a-2e. This step was carried out at room temperature for overnight. The reaction was quenched by adding water and saturated aqueous Na$_2$CO$_3$ solution and then left it stirring. Later, solid product formed was dried and purified.

The 2-amino position of the compound 2a-2e (1mmol, 1eq) was reacted with various substituted sulfonyl chlorides (1.2eq) in the presence of sodium hydride (NaH, 1.5eq) and DMF to generate sulfonamide derivative, which followed by hydrolysis with 3M sodium hydroxide (NaOH) and methanol to generate compound 3a-3i. The other hydrogen on the
2-amino group of compound 3a-3i (1mmol, 1eq) was methylated by using methyl iodide (1.2eq) in the presence of sodium hydride (NaH, 3eq) and DMF to generate compound 4a-4i. Further, reduction of compound 4a-4i (1mmol, 1eq) was carried out in the presence of Zn (10eq)/FeCl₃ (4eq) and DMF/H₂O (in 3:1 ratio) at 5-nitro group to generate aniline derivatives, 5a-5i. Finally, coupling reaction was performed to compound 5a-5i (1mmol, 1eq) with substituted acyl chlorides (1.2eq) in the presence of K₂CO₃ (5 eq) and 1,4-Dioxane to generate compound 6a-6p. [17-20]

The reaction progress in each and every step is known by thin layer chromatography.
Scheme 1: Synthesis of sulfonamide derivatives

i) K$_2$CO$_3$, DMF; ii) NaH, DMF; iii) NaH, DMF; iv) FeCl$_3$-Zn, DMF-H$_2$O; v) K$_2$CO$_3$, 1,4-Dioxane

2a. R$_1$: 3-(trifluoromethyl)benzyl
2b. R$_1$: 4-(trifluoromethyl)benzyl
2c. R$_1$: 2-(trifluoromethyl)benzyl
2d. R$_1$: 2-methyl, 5-(trifluoromethyl)benzyl
2e. R$_1$: 1-hexyl
2f. R$_1$: 4-cyano benzyl

3a. R$_1$: 3-(trifluoromethyl)benzyl; R$_2$: Methanesulfonyl
3b. R$_1$: 2-(trifluoromethyl)benzyl; R$_2$: Methanesulfonyl
3c. R$_1$: 2-methyl, 5-(trifluoromethyl)benzyl R$_2$: Methanesulfonyl
3d. R$_1$: 1-hexyl; R$_2$: Methanesulfonyl
3e. R$_1$: 4-cyano benzyl; R$_2$: Ethanesulfonyl
3f. R$_1$: 3-(trifluoromethyl)benzyl; R$_2$: Alpha-Toluene sulfonyl
3g. R$_1$: 4-(trifluoromethyl)benzyl; R$_2$: Alpha-Toluene sulfonyl
3h. R$_1$: 2-(trifluoromethyl)benzyl; R$_2$: Alpha-Toluene sulfonyl
3i. R$_1$: 1-hexyl; R$_2$: Alpha-Toluene sulfonyl
Figure 2: Scaffold of sulfonamide derivatives

Table I: R1, R2 and R3 positions of sulfonamide derivatives

<table>
<thead>
<tr>
<th>Compound name</th>
<th>R1 group</th>
<th>R2 group</th>
<th>R3 group</th>
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| 6a            | \[
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\] | \[
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\] | \[
\begin{array}{c}
\text{O} \\
\text{CF}_3 \\
\text{CF}_3
\end{array}
\] |
| 6b            | \[
\begin{array}{c}
\text{CF}_3 \\
\text{CF}_3
\end{array}
\] | \[
\begin{array}{c}
\text{O} \\
\text{S} \\
\text{S}
\end{array}
\] | \[
\begin{array}{c}
\text{O} \\
\text{CF}_3 \\
\text{CF}_3
\end{array}
\] |
| 6c            | \[
\begin{array}{c}
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\text{CF}_3
\end{array}
\] | \[
\begin{array}{c}
\text{O} \\
\text{S} \\
\text{S}
\end{array}
\] | \[
\begin{array}{c}
\text{O} \\
\text{CF}_3 \\
\text{CF}_3
\end{array}
\] |
| 6d            | \[
\begin{array}{c}
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\text{CF}_3
\end{array}
\] | \[
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\text{S}
\end{array}
\] | \[
\begin{array}{c}
\text{O} \\
\text{CF}_3 \\
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<th>6e</th>
<th>6f</th>
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<td><img src="image6.png" alt="Chemical Structure" /></td>
<td><img src="image7.png" alt="Chemical Structure" /></td>
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</table>
Thin Layer Chromatography

Thin Layer Chromatography (TLC) is a separation technique and is most commonly used to determine the reaction stages in an organic chemistry lab. TLC is performed on a sheet of glass, plastic or aluminum foil coated with a layer of adsorbent material (silica gel, aluminium oxide or cellulose) which is known as the stationary phase. A solvent or
mixture of solvents, known as the mobile phase, is used to separate the analyte spotted on the stationary phase by capillary action.

Identification is performed by the calculation of the retention factor (Rf), which is the calculated from the distance travelled by the analyte divided by the distance travelled by the solvent (mobile phase). This Rf value is used as reference to further purify the compounds using column chromatography, which should be around 0.2 to achieve a good separation.

Precoated silica gel 60 F254 plates (Stationary phase) were taken and both the starting material and the reaction mixture were spotted on it. Hexane (non-polar) and Ethyl acetate (polar) solvents were used in different ratio specific to the compound as mobile phase. Initially, the starting material was seen in both samples, then eventually it faded away in the reaction sample leaving a single spot, the product, hence proving the completion of reaction. All these compounds were readily visible under ultraviolet light.

Figure 3: 3a: TLC of starting materials and the reaction mixture; 3b: TLC showing completion of reaction
2.3 Compound purification and analysis

2.3.1 Column Chromatography

Column chromatography is the most commonly used method to separate and purify individual chemical compounds from a mixture. The phenomenon of separation is due to the differences in the equilibrium distribution (K) of the components between two phases: the mobile phase and the stationary phase. The latter is typically a granular material made of solid particles for e.g. silica, alumina, polymers, etc. the particle size ranges between 2-50 mm. The mobile phase is either mixture of polar (ethyl acetate, methanol, etc.) and non-polar (hexane, toluene, etc.) solvents or a single solvent.

The rate of migration of a component is inversely proportional to its distribution coefficient, so the components with a high distribution in the stationary phase will move slowly through column and hence be separated from the components with a lower distribution in the stationary phase. This differential rate of migration also depends upon the composition of the mobile and the stationary phases and the temperature.
Figure 4: Images representing the separation of components from a mixture in column chromatography

In this study, all column chromatography experiments were carried out using silica gel ultrapore (40-60 µm) 60A as stationary phase and hexane and ethyl acetate solvents (in different ratio obtained from the Rf values calculated from TLC) as mobile phase.

2.3.2 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy (NMR spectroscopy), is the most commonly used research technique that exploits the magnetic properties of certain atomic nuclei in a given molecule. It identifies the physical and chemical properties of atoms or the molecules and provides the information regarding its molecular structure, reaction state,
reaction dynamics and the chemical environment. The principle governing the NMR technique relies on the induction of transitions between different Zeeman levels of a particular nucleus induced by a variable radiofrequency (RF) in a magnetic field. The resonance frequencies of an atom changes with the differences in the chemical environment it exists. This difference is known as “chemical shift” and it is given in units of parts per million (ppm). The intramolecular magnetic field around an atom in any given molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule.

Figure 5: NMR spectroscopy

NMR spectra are very unique, well-resolved and are highly predictable for small molecules. Therefore, in organic chemistry practice, NMR analysis is used to confirm the
identity of a substance. Different functional groups, and identical functional groups with differing neighboring substituents are definitely distinguishable with noticeable signals. A disadvantage of NMR is that, it requires a large amount (5-50 mg) of purified substance and needs to be dissolved in a solvent. Moreover, NMR analysis of solid substances may not give equally well-resolved spectra.

All the NMR spectra were recorded on a Varian 400 MHz spectrometer (\(^{13}\text{C} \) NMR at 100 MHz) using Chloroform-d (CDCl\(_3\)) and Methyl sulfoxide-d\(_{6}\) (DMSO) as solvent. Chemical shifts (\(\delta\)) for \(^1\text{H} \) NMR spectra were reported in parts per million to residual solvent protons.

2.3.3  **Mass Spectrometry**

Mass Spectrometry (MS) is an analytical technique that sorts ions based on their mass to charge ratio. It works by ionizing chemical compounds to generate charged molecules or molecular fragments and sort them by measuring their mass-to-charge ratios. The mass spectrum generated is a plot of the ion signal characterizing the mass-to-charge ratio of the sample. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and molecules, and to elucidate the chemical structures of molecules.

The MS instrument consists of three major components: an ion source, an analyzer (ion trap), and a detector. Many types of ion source devices are available to generate charged samples: electron ionization, chemical ionization, photoionization, gas discharge ionization and desorption ionization. For, liquid sample the ionization can be enhanced by adding additives such as formic acid, acetic acid, ammonium acetate, ammonium
formate, etc., to the solvent being used. The analyzer is a combination of electric or magnetic fields used resolve the projected beam into its characteristic mass components and the electron multiplier detector is used to record and determine the ion beam.

![Diagram of ion-trap mass spectrometry](image)

**Figure 6: Ion-trap mass spectrometry**

Mass spectra for all the compounds were obtained on the Bruker Ion-Trap mass spectrometer at Cleveland State University MS facility center. The stock solutions of all the compounds standard 1mg/ml with 90% Acetonitrile and 0.1 % formic acid (to enhance ionization). All the working standard solutions were prepared in the range 0.05-10 µg/ml from serial dilution of stock with 90% ACN.
2.3.4 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a widely used analytical techniques to separate, identify and quantify components from a mixture based on their physical and chemical properties. The main principle of the HPLC technique relies on the adsorption phenomenon, which is adhesion of analyte molecules to the solid surface (stationary phase). The HPLC technique is distinguished from traditional liquid chromatography (column chromatography) by having significantly higher operational pressures (50-350 bar). A very small amounts of sample can be separated through analytical HPLC. The typical column dimensions are 2.1-4.6 mm diameter, and 30-250 mm length. Hence, HPLC possess higher resolving power compared to other separation methods.

The typical HPLC instrument includes a sampler, pumps and a detector. The sampler is used to introduce the sample mixture into the column, the stationary phase, which contains a solid adsorbent material. The pumps are operated under different pressures and velocities to allow the desired flow rate and composition of the mobile phase which consists of the solvents used to separate the mixture. The sample mixture travelling through the column at different velocities, forms specific physical interactions (such as hydrophobic, dipole-dipole, ionic, etc.) with the adsorbent. The rate of migration of each component depends on its chemical composition, on the nature of the stationary phase (column), and on the composition of the mobile phase. The time at which a separated component elutes (emerges from the column) is called its retention time. This retention time is measured as one of the characteristic of the analyte separated.
The most common mobile phases are water, acetonitrile and/or methanol (used in different ratio), however, some methods may use water-free mobile phases. Acids (such as formic, phosphoric or trifluoroacetic acid) or salts may also be used to enhance the separation of the sample components in few methods. During the chromatographic analysis, the composition of the mobile phase can be kept constant (isocratic elution mode) or can be varied (gradient elution mode) to achieve the best separation. Afterwards a signal is generated proportional to the amount of sample component emerging from the column form the detector, hence providing quantitative analysis of the sample. A digital microprocessor and user software control the HPLC instrument and provide data analysis.
Reverse-phase HPLC analysis of compounds were conducted on Beckman HPLC system with AutoSampler. The chromatographic separation was performed on a C18 column (2.0 mm x 150 mm, 5 µm) from Phenomenex (Torrance, CA, USA). The mobile phase of 90% acetonitrile and 10% water was employed for isocratic elution with a flow rate of 0.2 mL/min. the injection volume was 20 µL and the UV detector was set up at 260 and 320 nm.

2.4 Biological studies

2.4.1 Reagents

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagents were from Promega life science (Madison, WI). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma-Aldrich (Milwaukie, WI).

2.4.2 Cell culture

HEK293 kidney cells, mouse macrophage RAW267.4 cells were obtained from ATCC (Rockville, MD) and maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS), 2% penicillin-streptomycin and 2ul/ml of Cipro at 37 °C in a Heraeus water-jacketed incubator with 5% CO₂. T. b. brucei Lister 427 cells were cultured in HMI-9 medium with 10% FBS at 37 °C in a Heraeus water-jacketed incubator with 7.5% CO₂.

2.4.3 Cell Viability assay
Cell viability assays are often used to determine the cell proliferation or to show the direct cytotoxic effects.

2.4.4 MTT assay

Following the synthesis of the sulfonamide derivatives, the MTT assay was used to examine the effect of these candidates on HEK293 and mice macrophage RAW267.4 cell growth in three replicates. Cells (approx. 9000 cells/well) were grown in DMEM medium in 96-well flat-bottomed plates for 24 hrs and were exposed to different concentrations of the compounds dissolved in DMSO (final concentration ≤0.1%) in medium for 48 hrs. Controls received DMSO at a concentration similar to that in compound-treated cells. Subsequently, the medium was replaced by 120 µl of 0.5 mg/ml of MTT reagent diluted in fresh media and incubated at 37 °C for 2 hrs. Supernatants were removed from the wells, and the reduced MTT dye was solubilized in 120 µl /well DMSO. Absorbance at 570 nm was determined using a SpectroMax Plus 384 spectrophotometer (Molecular Devices) and the data obtained with quadruplication were normalized and fitted to a dose-response curve using GraphPad Prism v.5 (GraphPad).
**DMEM media**: Dulbecco’s modified Eagle’s medium (DMEM) is a cell culture media, that is used to maintain cells in tissue culture. It is composed of vitamins (folic acid, nicotinamide, riboflavin, B12), amino acids, salts (calcium chloride, sodium chloride, potassium chloride, magnesium sulfate and monosodium phosphate), iron, and carbohydrates (sugars). This media is suitable for most cell types used in biochemistry and microbiology labs. In this study, this media is also supplemented with fetal bovine serum (FBS), Cipro, and penicillin and/or streptomycin for which provides additional support for cell growth.

### 2.4.5 MTS assay

(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) MTS dye. The MTS assay was used to examine the effect of sulfonamide derivatives on *T. b. brucei* cell growth in three replicates. Five thousand cells of *T. b. brucei* were seeded in a 96-well flat-bottomed plates and were treated with different concentrations of the compounds dissolved in DMSO in HMI-9 medium for 48 hrs at 37 °C. Controls received DMSO at a concentration similar to that in compound-treated cells. Later, 20 µl of MTS (5% PMS) from the CellTiter cell proliferation assay was added to the 200 µL of *T. b. brucei* cell culture in each well and incubated at 37 °C for 3 hrs. Soluble formazan, produced by viable cells due to reduction of MTS was measured at 490 nm on a SpectroMax Plus 384 spectrophotometer (Molecular Devices). Data obtained with quadruplication were normalized and fitted to a dose-response curve using GraphPad Prism v.5 (GraphPad).
**HMI-9 Media:** HMI-9 media is the specific cell culture media used for the *Trypanosoma* cell culturing. This media is composed of Iscove’s Modified Dulbecco’s Medium (IMDM), fetal bovine serum (FBS), serum, hypoxanthine, bathocuproine disulfonic acid, cysteine, pyruvic acid, uracil, cytosine, and 2-mercaptoethanol.
CHAPTER III
RESULTS AND DISCUSSIONS

3.1 Chemistry

General procedure for the synthesis of 6a-6p:

K$_2$CO$_3$ (5mmol, 5eq) and corresponding substituted acyl chloride (1.2mmol, 1.2eq) were successfully added to a solution of 5a-5i (1mmol, 1eq) in 3 ml of dry 1, 4- Dioxane and the mixture was stirred at room temperature for hours. 10 ml of H$_2$O and 3 ml of saturated Na$_2$CO$_3$ was added to the reaction mixture and it was left stirred for hours. The solid product precipitated was collected by filtration and purified by silica gel column chromatography.

Experimental data and results of all compounds

1) N-(4-(N-methylmethylsulfonamido)-3-((3-(trifluoromethyl)benzyl)oxy)phenyl)-3,5-bis(trifluoromethyl)benzamide(M1A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5a and stirred for 2 hrs. Yield 80.3%, Pale Yellow solid. $^1$H NMR (400MHz, CDCl$_3$) δH 8.36 (s, 3H), 8.09 (s, 1H), 7.93 (d, 1H), 7.74 (s, 1H), 7.66 (d, 2H), 7.59 (t, 1H), 7.32 (d, 1H), 6.97 (q, 1H), 5.19 (s, 2H), 3.26 (s, 3H), 2.88 (s, 3H); $^{13}$C NMR (100MHz, CDCl$_3$) δC 162.9,155.2, 138.7, 136.7, 136.4, 132.7, 130.9, 129.4, 127.5, 125.4, 124.3, 113.0, 105.5, 69.8, 38.3, 37.7,
29.7. ESI-MS calculated for \((\text{C}_{25}\text{H}_{19}\text{N}_2\text{SO}_4\text{F}_9) [\text{M-H}]^-\): 613.0; Molecular Weight (calculated from structure): 614.48

2) N-(4-(N-methylmethylsulfonamido)-3-((3-(trifluoromethyl)benzyl)oxy)phenyl)-2,4-bis(trifluoromethyl)benzamide (M1B). 2,4-bis(trifluoromethyl)benzoyl chloride was used with 5a and stirred for 2 hrs. Yield 92.6%, Pale Yellow solid. ¹H NMR (400MHz, DMSO) δH 10.84 (s, 1H), 8.23 (d, 2H), 7.97 (d, 1H), 7.92 (s, 1H), 7.84 (d, 1H), 7.73 (d, 1H), 7.67 (d, 1H), 7.63 (d, 1H), 7.32 (d, 2H), 7.23 (d, 1H), 7.21 (d, 1H), 5.27 (s, 2H), 3.14 (s, 3H), 2.92 (s, 3H); ¹³C NMR (100 MHz, DMSO) δC 164.83, 155.54, 139.94, 138.30, 132.29, 131.97, 130.50, 130.06, 125.98, 125.73, 125.22, 124.87, 112.46, 105.34, 69.33. ESI-MS calculated for \((\text{C}_{25}\text{H}_{19}\text{N}_2\text{SO}_4\text{F}_9) [\text{M-H}]^-\): 613.02; Molecular Weight (calculated from structure): 614.48

3) N-(4-(N-methylmethylsulfonamido)-3-((2-(trifluoromethyl)benzyl)oxy)phenyl)-3,5-bis(trifluoromethyl)benzamide (M3A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5b and stirred at room temperature for 3 hrs. Yield 78.1%, Pale Yellow solid. ¹H NMR (400MHz, DMSO) δH 10.78 (s, 1H), 8.60 (s, 2H), 8.40 (s, 1H), 7.88 (d, 1H), 7.83 (d, 1H), 7.69 (t, 1H), 7.62 (s, 1H), 7.43 (d, 1H), 7.35 (d, 1H), 5.32 (s, 2H), 3.12 (s, 3H), 2.87 (s, 3H); ¹³C NMR (100 MHz, DMSO) δC 163.24, 155.34, 140.03, 137.40, 134.71, 133.49, 131.54, 130.84, 129.40, 129.10, 126.59, 125.82, 124.93, 122.22, 113.69, 106.12, 66.85. ESI-MS calculated for \((\text{C}_{25}\text{H}_{19}\text{N}_2\text{SO}_4\text{F}_9) [\text{M-H}]^-\): 612.92; Molecular Weight (calculated from structure): 614.48
4) N-(4-(N-methylmethylsulfonamido)-3-((2-(trifluoromethyl)benzyl)oxy)phenyl)-
2,4-bis(trifluoromethyl)benzamide (M3B). 2,4-bis(trifluoromethyl)benzoyl
chloride was used with 5b and stirred for 2.5 hrs. Yield 67.1%, pale yellow solid.

$^1$H NMR (400 MHz, DMSO) $\delta_{H}$ 10.83 (s, 1H), 8.23 (d, 2H), 7.98 (d, 1H), 7.87 (q,
2H), 7.76 (t, 1H), 7.62 (t, 1H), 7.51 (s, 1H), 7.34 (s, 2H), 5.30 (s, 2H), 3.13 (s, 3H), 2.88 (s, 3H);
$^{13}$C NMR (100 MHz, DMSO) $\delta_{C}$ 164.81, 155.45, 140.01,
134.76, 133.46, 132.20, 130.63, 130.53, 129.30, 126.57, 125.70, 112.64, 105.18,
66.76, 38.15, 37.95. ESI-MS calculated for (C$_{25}$H$_{19}$N$_2$SO$_4$F$_9$) [M-H]-: 612.86;
Molecular Weight (calculated from structure): 614.48

5) N-(3-((2-methyl-5-(trifluoromethyl)benzyl)oxy)-4-(N-
 methylmethylsulfonamido)phenyl)-3,5-bis(trifluoromethyl)benzamide (M4A).
3,5-bis(trifluoromethyl)benzoyl chloride was used with 5c and stirred for 3 hrs.
Yield 71.3%, white solid. $^1$H NMR (400 MHz, DMSO) $\delta_{H}$ 10.80 (s, 1H), 8.60 (s,
2H), 8.39 (s, 1H), 7.90-7.36 (m, 6H), 5.25 (s, 2H), 3.13 (s, 3H), 2.91 (s, 3H), 2.45
(s, 3H), 2.08 (s, 1H); $^{13}$C NMR (100 MHz, DMSO) $\delta_{C}$ 163.21, 141.76, 139.89,
137.31, 136.39, 131.49, 131.18, 129.03, 126.08, 125.15, 124.92, 113.60, 106.46,
67.81, 66.81, 38.18, 38.05, 18.86. ESI-MS calculated for (C$_{26}$H$_{21}$N$_2$SO$_4$F$_9$) [M-H]-: 626.86; Molecular Weight (calculated from structure): 628.51

6) N-(3-((2-methyl-5-trifluoromethyl)benzyl)oxy)-4-(N-
methylmethylsulfonamido)phenyl)-2,4-bis(trifluoromethyl)benzamide (M4B).
2,4-bis(trifluoromethyl)benzoyl chloride was used with 5c and stirred for 3 hrs.
Yield 59.3%, white solid. $^1$H NMR (400 MHz, DMSO) $\delta_{H}$ 10.86 (s, 1H), 8.23 (d,
2H), 7.98-7.27 (m, 7H), 5.23 (s, 2H), 3.12 (s, 3H), 2.89 (s, 3H), 2.44 (s, 3H); $^{13}$C
NMR (100 MHz, DMSO) δC 164.85, 155.67, 141.82, 139.94, 136.35, 131.69, 131.47, 130.49, 125.88, 125.15, 112.49, 105.36, 67.75, 66.81, 38.13, 38.04, 18.85.

ESI-MS calculated for (C₂₆H₂₁N₂SO₄F₉) [M-H]-: 626.91; Molecular Weight (calculated from structure): 628.51

7) N-(3-(hexyloxy)-4-N-methylmethylsulfonamido)phenyl)-3,5-
   bis(trifluoromethyl)benzamide (M5A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5d and stirred for 3 hrs. Yield 80.2%, pale yellow solid. ¹H NMR (400 MHz, CDCl₃) δH 8.53 (s, 1H), 8.38 (s, 2H), 8.07 (s, 1H), 7.80 (d, 1H), 7.21 (d, 1H), 6.79 (q, 1H), 4.06 (t, 2H), 3.26 (s, 3H), 2.97 (s, 3H), 1.82 (m, 2H), 1.47 (m, 2H), 1.36 (m, 5H), 0.93 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δC 163.03, 155.76, 138.81, 136.57, 132.59, 127.69, 125.47, 125.07, 124.26, 121.55, 112.48, 105.27, 68.55, 38.11, 37.67, 31.45, 29.19, 25.77, 22.55, 13.96. ESI-MS calculated for (C₂₃H₂₆N₂SO₄F₆) [M-H]-: 539.00; Molecular Weight (calculated from structure): 540.52

8) N-(3-(hexyloxy)-4-N-methylmethylsulfonamido)phenyl)-2,4-
   bis(trifluoromethyl)benzamide (M5B). 2,4-bis(trifluoromethyl)benzoyl chloride was used with 5d and stirred for 3 hrs. Yield 74.0%, pale yellow solid. ¹H NMR (400 MHz, CDCl₃) δH 7.95 (s, 1H), 7.92 (t, 2H), 7.78 (d, 2H), 7.28 (t, 1H), 6.67 (q, 1H), 4.09 (t, 2H), 3.26 (s, 3H), 2.90 (s, 3H), 1.85 (q, 2H), 1.61-1.35 (m, 6H), 0.94 (t, 3H); ¹³C NMR (100 MHz, CDCl₃) δC 164.44, 155.92, 138.60, 132.86, 132.82, 132.53, 129.43, 129.25, 128.58, 125.23, 124.21, 124.16, 123.90, 121.54, 121.44, 119.91, 104.88, 68.55, 37.97, 37.59, 31.45, 29.19, 25.78, 22.55, 13.97.
ESI-MS calculated for (C_{23}H_{26}N_{2}SO_{4}F_{6}) [M-H]-: 539.01; Molecular Weight (calculated from structure): 540.52

9) N-(4-(N-methyl-1-phenylmethylsulfonamido)-3-((3-(trifluoromethyl)benzyl)oxy)phenyl)-3,5-bis(trifluoromethyl)benzamide (M6A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5e and stirred for 3 hrs. Yield 71.2%, white solid. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\)H 8.35 (d, 3H), 8.07 (s, 1H), 7.69-7.28 (m, 10H), 6.96 (q, 1H), 6.88 (d, 1H), 5.00 (s, 2H), 4.32 (s, 2H), 3.11 (s, 3H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\)C 155.49, 138.56, 136.90, 136.47, 131.55, 130.77, 130.68, 129.29, 129.02, 128.70, 127.61, 126.26, 125.53, 1225.10, 112.96, 105.63, 69.60, 58.12, 38.54, -0.007. ESI-MS calculated for (C_{31}H_{23}N_{2}SO_{4}F_{9}) [M-H]-: 689.01; Molecular Weight (calculated from structure): 690.58

10) N-(4-(N-methyl-1-phenylmethylsulfonamido)-3-((3-(trifluoromethyl)benzyl)oxy)phenyl)-2,4-bis(trifluoromethyl)benzamide (M6B). 2,4-bis(trifluoromethyl)benzoyl chloride was used with 5e and stirred for 3 hrs. Yield 67.5%, white solid. \textsuperscript{1}H NMR (400 MHz, DMSO) \(\delta\)H 7.88 (s, 1H), 7.80-7.63 (m, 6H), 7.56 (t, 2H), 7.35 (d, 5H), 7.08 (d, 1H), 6.76 (q, 1H), 5.28 (s, 2H), 4.26 (s, 2H), 3.16 (s, 3H); \textsuperscript{13}C NMR (100 MHz, DMSO) \(\delta\)C 155.70, 139.87, 138.41, 131.66, 131.36, 130.24, 130.00, 128.75, 112.40, 69.32, 57.08, 40.62, 40.41, 40.20, 39.99, 39.79. ESI-MS calculated for (C_{31}H_{23}N_{2}SO_{4}F_{9}) [M-H]-: 688.94; Molecular Weight (calculated from structure): 690.58
11) N-(4-(N-methyl-1-phenylmethylsulfonamido)-3-((4-(trifluoromethyl)benzyl)oxy)phenyl)-3,5-bis(trifluoromethyl)benzamide (M7A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5f and stirred for 3 hrs. Yield 98.3%, white solid. $^1$H NMR (400 MHz, DMSO) $\delta$H 10.74 (s, 1H), 8.59 (s, 2H), 8.40 (s, 1H), 7.69 (q, 5H), 7.37 (q, 6H), 7.08 (d, 1H), 5.32 (s, 2H), 4.46 (s, 2H), 3.12 (s, 3H); $^{13}$C NMR (100 MHz, DMSO) $\delta$C 141.80, 139.83, 137.32, 131.40, 131.17, 130.84, 130.24, 129.05, 128.76, 128.58, 128.54, 126.15, 125.87, 125.83, 113.45, 106.30, 69.42, 57.12, 38.54. ESI-MS calculated for (C$_{31}$H$_{23}$N$_2$SO$_4$F$_9$) [M-H]-: 688.87; Molecular Weight (calculated from structure): 690.58

12) N-(4-(N-methyl-1-phenylmethylsulfonamido)-3-((2-(trifluoromethyl)benzyl)oxy)phenyl)-3,5-bis(trifluoromethyl)benzamide (M8A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5g and stirred for 3 hrs. Yield 99%, white solid. $^1$H NMR (400 MHz, DMSO) $\delta$H 10.76 (s, 1H), 8.60 (s, 2H), 8.39 (s, 1H), 7.96-7.12 (m, 12H), 5.34 (s, 2H), 4.39 (s, 2H), 3.08 (s, 3H); $^{13}$C NMR (100 MHz, DMSO) $\delta$C 163.21, 155.48, 139.98, 137.40, 134.78, 133.44, 131.67, 131.34, 131.14, 130.81, 130.78, 130.20, 129.34, 129.08, 128.74, 128.55, 127.18, 126.88, 126.55, 126.49, 126.18, 126.05, 125.76, 124.93, 122.22, 113.62, 106.02, 66.82, 57.11, 38.50. ESI-MS calculated for (C$_{31}$H$_{23}$N$_2$SO$_4$F$_9$) [M-H]-: 688.96; Molecular Weight (calculated from structure): 690.58

13) N-(4-(N-methyl-1-phenylmethylsulfonamido)-3-((2-(trifluoromethyl)benzyl)oxy)phenyl)-2,4-bis(trifluoromethyl)benzamide (M8B). 2,4-bis(trifluoromethyl)benzoyl chloride was used with 5g and stirred for 3 hrs.

30
Yield 98%, white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 7.57-7.28 (m, 14H), 7.05 (d, 1H), 6.96 (q, 1H), 5.31 (s, 2H), 4.18 (s, 2H), 3.10 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 164.41, 155.53, 138.45, 134.10, 132.46, 132.05, 130.75, 130.05, 129.42, 129.00, 128.62, 128.58, 128.53, 127.88, 126.21, 112.90, 105.29, 57.87, 38.42. ESI-MS calculated for (C$_{31}$H$_{23}$N$_2$SO$_4$F$_9$) [M-H]: 688.92; Molecular Weight (calculated from structure): 690.58

14) N-(3-(hexyloxy)-4-(N-methyl-1-phenylmethylsulfonamido)phenyl)-3,5-bis(trifluoromethyl)benzamide (M9A). 3,5-bis(trifluoromethyl)benzoyl chloride was used with 5h and stirred for 3 hrs. Yield 99%, white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta_H$ 8.10 (s, 2H), 7.89 (s, 1H), 7.70 (s, 1H), 7.60-7.22 (m, 6H), 6.97 (q, 1H), 6.72 (s, 1H), 4.34 (s, 2H), 4.01 (t, 2H), 1.68 (q, 2H), 1.35 (quin, 8H), 0.92 (t, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta_C$ 130.68, 128.87, 128.75, 31.48, 25.60, 22.52, 13.98, -0.008. ESI-MS calculated for (C$_{29}$H$_{30}$N$_2$SO$_4$F$_6$) [M-H]: 615.56; Molecular Weight (calculated from structure): 616.61

15) 4-cyano-N-(4-(N-methyl-1-phenylmethylsulfonamido)-3-((4-(trifluoromethyl)benzyl)oxy)phenyl)benzamide (M2C). 4-cyano benzoyl chloride was used with 5f and stirred for 2.5 hrs. Yield 96%, Pale brown solid. $^1$H NMR (400 MHz, DMSO) $\delta_H$ 10.57 (S, 1H), 8.06 (t, 4H), 7.73 (d, 5H), 7.36 (d, 6H), 7.07 (d, 1H), 5.31 (s, 2H), 4.46 (s, 2H), 3.12 (s, 3H); $^{13}$C NMR (100 MHz, DMSO) $\delta_C$ 206.97, 164.75, 139.15, 132.99, 131.39, 130.27, 129.00, 128.75, 128.56, 125.86, 113.19, 106.07, 69.38, 57.11, 31.14. ESI-MS calculated for (C$_{30}$H$_{24}$N$_3$SO$_4$F$_3$) [M-H]: 578.12; Molecular Weight (calculated from structure): 579.59
16) N-(3-((3-cyanobenzyl)oxy)-4-(N-methylethylsulfonamido)phenyl)benzo[d][1,3]dioxole-5-carboxamide (MCN). benzo[d][1,3]dioxole-5-carbonyl chloride was used with 5i and stirred for 2 hrs. Yield 94%, white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$H 7.56-6.81 (m, 11H), 6.09 (d, 2H), 5.20 (s, 2H), 3.27 (s, 3H), 3.08 (q, 2H), 1.32 (t, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$C 148.38, 137.64, 132.14, 132.09, 131.15, 129.68, 128.55, 121.78, 112.34, 108.26, 107.65, 105.11, 101.98, 69.42, 46.38, 38.36, -8.12, -0.003. ESI-MS calculated for (C$_{30}$H$_{24}$N$_3$SO$_6$) [M-H]: 492.34; Molecular Weight (calculated from structure): 493.53

3.2 Cell growth inhibition of sulfonamide derivatives and selective inhibition of *T. b.*

brucei cell growth:

All the sulfonamide derivatives synthesized in our laboratory were screened for their inhibitory activity using cell proliferation assay. *T. b. brucei* Lister 427 cells were used as the parasite cells, and human embryonic kidney HEK293 cells and mouse macrophage RAW267.4 cells were used as the normal mammalian cells. Results of cell growth inhibition of all the compounds and the selective index are shown in Table 2. The selective index was calculated by dividing the IC$_{50}$’s of the mammalian cell growth inhibition by the IC$_{50}$’s of the *T. brucei* cell growth inhibition.
Table II: Comparison of IC$_{50}$’s of sulfonamide derivatives against normal cells (HEK293 and macrophages) and *T. b. brucei* cells.

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<th>Entry</th>
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<th>IC$_{50}$ against <em>T. brucei</em> cell growth (µM)</th>
<th>IC$_{50}$ of macrophage RAW 267.4 cell growth (µM)</th>
<th>IC$_{50}$ of <em>T. brucei</em> cell growth (µM)</th>
<th>IC$<em>{50}$ of HEK293 cells/IC$</em>{50}$ of <em>T. brucei</em></th>
<th>IC$<em>{50}$ of HEK293 cells/IC$</em>{50}$ of <em>T. brucei</em></th>
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<td>11</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>3.39±1.22</td>
<td>293.1±18.23</td>
<td>86.46</td>
<td>&gt;500</td>
<td>&gt;147.49</td>
</tr>
<tr>
<td>12</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
<td>7.13±3.27</td>
<td>&gt;500</td>
<td>&gt;70.12</td>
<td>&gt;500</td>
<td>&gt;70.12</td>
</tr>
<tr>
<td>13</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>10.46±5.23</td>
<td>&gt;500</td>
<td>&gt;47.8</td>
<td>158.1±39.91</td>
<td>15.11</td>
</tr>
<tr>
<td>14</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>7.81±3.83</td>
<td>69.85±0.9</td>
<td>8.94</td>
<td>13.2±2.88</td>
<td>1.69</td>
</tr>
<tr>
<td>15</td>
<td><img src="image5.png" alt="Chemical Structure" /></td>
<td>0.07±0.02</td>
<td>&gt;500</td>
<td>&gt;7142.85</td>
<td>&gt;500</td>
<td>&gt;7142.85</td>
</tr>
</tbody>
</table>
For the three moieties (R1-R3) of the compound scaffold (Figure 2), various functional groups specifically focusing on electron withdrawing substituents were introduced to enhance the anti-trypanosomal activity and decrease anti-mammalian cell activity. From the previous studies conducted in our lab we found that electron withdrawing group substitutions at R1 domain decrease the inhibition of mammalian cell growth. Ortho- or meta- or para-trifluoro methyl substitution on phenyl showed almost similar inhibition to that of alkyl (hexyl) group at R1 position. To further optimize, the R2 domain was derivatized with a bulky substitution group (α-toluene) which showed better inhibition of cell growth in parasite cells compared to simple alkyl groups (methyl, ethyl) substitutions and, also in comparison with mammalian cells. The R3 domain is confined to electron withdrawing substitutions on the aryl groups. Overall, electron withdrawing substitutions to the aryl groups at R1 and R3 domains along with bulky R2 domain showed increased inhibition of *T. brucei* cell growth and decreased inhibition of mammalian cell growth. Compounds 11, 12, and 15 showed higher selective index and better inhibition compared to the other compounds.
CHAPTER IV

CONCLUSIONS AND FUTURE DIRECTIONS

Human African Trypanosomiasis is a serious threat to people living in sub-Saharan Africa. Due to the lack of effective treatment, an inadequate number of medical facilities and expensive hospital administration process, there is an immediate need for low cost and easily administrable drugs. From, the previous research conducted in our lab we found a unique molecular scaffold that selectively targets *T. brucei* tubulin and these drugs showed notable effects through oral administration. Among the previously generated library of compounds, the main scaffold (figure 2) with electron withdrawing group substitutions showed a higher cell growth inhibition and also selective inhibition of *T. brucei* cells in comparison with mammalian cells. Hence, the current research was focused on further derivatization of those compounds to identify ideal drug candidates possessing higher potency and selectivity toward *T. brucei* cell growth inhibition. In this study, we synthesized 16 compounds with different electron withdrawing group substitutions on the phenyl rings of R1 and R3 domains, with also considering the position of the substituents, - ortho, meta, and para. From the cell inhibitory studies, we found that one compounds out of 16 showed better cell growth inhibition and a very higher selective index. This compound (15), possess a cyano group at para position on the benzyl ring of R3 domain, a bulky phenyl group at the R2 domain, and a trifluoromethyl
substitution at para position of benzyl ring at the R1 domain. From this study, we found that strong electron withdrawing groups at para position of R1 and R3 domains showed better selective index. Further research of this compound needs yet to be conducted, by performing western blotting studies, tubulin polymerization studies to know the potency.

From my work, it can be concluded that the library of compounds generated have shown better inhibition selectively towards the *T. brucei* (parasite) cells. These results are proving that the sulfonamide derivatives are promising drug candidates for effective and simpler way to eradicate HAT infection.
REFERENCES


5. S.M. Nzou et al. / Parasitology International 65 (2016) 121–127


17. R. Lama; R. Sandhu; B. Zhong; B. Li; B. Su; Identification of selective tubulin inhibitors as potential anti-trypanosomal agents, Bioorg. Med. Chem. Lett. 22 (2012) 5508–5516


