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Synthesis of Dipicolinic Acid Derivatives of Potential Anti-leishmanial Activity

A Thesis Submitted in Partial Fulfilment of the Requirements
for the M.Sc.Degree in Environmental Chemistry

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Abstract

Leishmaniasis is a parasitic disease caused by *Leishmania* species, transmitted through the bites of infected sandflies. It poses a significant public health challenge in many tropical and subtropical regions, leading to severe morbidity and mortality. Current treatments, such as miltefosine, are limited by toxicity, resistance, and accessibility issues. Therefore, developing new compounds with improved efficacy and safety profiles is essential to combat this disease effectively and provide better treatment options for affected populations.

This research focuses on synthesizing and characterizing dipicolinic acid derivatives, evaluating their anti-leishmanial activities, and elucidating their mechanisms of action. Molecular docking studies were conducted to predict the binding interactions of these compounds with key targets in the *Leishmania* metabolic pathways. Additionally, cytotoxicity assessments and predictions regarding the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles of the most active compounds were performed. The findings aim to provide better treatment options for affected populations and contribute to the ongoing efforts to combat leishmaniasis.

The current study successfully synthesized dipicolinic acid derivatives, achieving percentage yields between 33.1% and 85.46%, with relatively easy synthetic method making these compounds attractive candidates for further study. The structures of the synthesized compounds were confirmed through spectroscopic and elemental analyses. *In vitro* screening against two forms of *L. major* demonstrated that all compounds exhibited activity, with compound **IV** showing the highest efficacy with IC_{50} of 2.86 ± 0.14 μ M against promastigotes and 3.84 ± 0.16 μ M against amastigotes, outperforming the reference drug miltefosine by nearly three times. Mechanistic studies indicated that compound **4** inhibits the folic acid pathway, as evidenced by anti-folate

tests and molecular docking, which revealed a docking score of -13.73 kcal/mol for *Lm*-PTR1, surpassing that of trimethoprim (-11.91 kcal/mol).

Additionally, *in vitro* cytotoxicity assays indicated a superior safety profile for compound **IV**, with an IS value of 108.2, significantly better than miltefosine. *In silico* analyses further suggested favourable bioavailability and the potential for enhanced toxicity profiles, reinforcing the viability of these compounds as promising candidates for anti-leishmanial therapies.

Dedication

This work is dedicated to future generations of researchers, with the hope that it contributes to the advancement of knowledge and inspires further exploration.

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List of Abbreviations

Abbreviation	Name
Absorption, Distribution, Metabolism, Excretion and Toxicity	ADMET
Adenosine Monophosphate ion	AMP ²⁻
Amphotericin B deoxycholate	AmB-D
Blood-Brain Barrier	BBB
Cutaneous Leishmaniasis	CL
Cytochrome-P enzymes	CYP
Dichloromethane	DCM
Dihydrofolate Reductase	DHFR
Dimethyl Sulfoxide	DMSO
Gastrointestinal Absorption	GIA
IR	Infrared
Lipophosphoglycan	LPG
LogP	Lipophilicity
Mucocutaneous Leishmaniasis	MCL
Neglected Tropical Diseases	NTDs
Nuclear Magnetic Resonance	NMR
Pan-Assay Interference Compounds	PAINS
Permeability Glycoprotein	P-gp
post-kala-azar dermal leishmaniasis	PKDL
Pteridine Reductase 1	PTR1
Selectivity Index	SI
Standard Deviation	SD
Thymidylate Synthase	TS
Ultraviolet	UV
Vector-borne diseases	VBDs
Visceral Leishmaniasis	VL

Chapter (1)

1. Introduction

Neglected tropical diseases (NTDs) are infections caused by bacteria, parasites, viruses, and fungi that thrive in tropical and subtropical low to middle-income countries where access to health services is limited (Mitra & Mawson, 2017; Molyneux et al., 2017). Biological and transmission characteristics of NTDs are diverse yet a lot of them are categorized as vector-borne diseases (VBDs) (Mitra & Mawson, 2017; Wilson et al., 2020). A vector is an organism -usually an arthropod- transmits infectious pathogens from infected hosts to uninfected humans. The major vectors that spread VBDs are lice, mosquitoes, triatomine bugs, blackflies, tsetse flies ticks and sandflies (Rocklöv & Dubrow, 2020; Wilson et al., 2020).

Sandflies belong to suborder Nematocera of order Diptera. The further classification of them is not agreed upon universally, yet in general they are put in Psychodidae family and Phlebotomine subfamily. Among the six types of them present, two of them are of medical interest, namely: *Lutzomyia* that is divided into 25 subgenera and *Phlebotomus* which is furtherly divided into 12 subgenera. With body length not exceeding 3 mm and colour ranges from sharp white to dark black, Phlebotomine sandflies are easily recognised. Unlike mosquitos, Phlebotomine sandflies' attack is silent and before engorging they usually hop around the host and then settle to bite. Their hopping behaviour gave the indication that their site of breeding is not far away. Sandflies are of crepuscular or nocturnal activity, yet some species bite in the daylight. Cool and humid places during the daytime are resting places for such species that is including houses, dense vegetation areas, birds' nests and etc...(Killick-Kendrick, 1999)

Males and females of sandflies feed on natural sugar sources yet females during the period of egg gestation feed on blood as a source of nutrients for their eggs. This process of feeding is believed to play a vital role in the successful establishment of VBDs as females' bite includes the injection of their saliva into the host's skin and that the successful development of eggs requires multiple blood-feedings (Cecílio et al., 2022; Killick-Kendrick, 1999). On average 30 to 70 eggs are deposited by sandfly female that will be protected in a place that is humid and has organic matter. Once the latter process succeed, oviposition occurs after five to eight days. Life cycle of such species is divided into four main stages: eggs, larvae, pupae, and adults. Hatching of eggs happens between four to 20 days, 20 to 30 days for complete development of larval and adults emerge during dark hours, often just before dawn after six to 13 days. All stages of their life cycle depends on availability of food and temperature. Warm temperature and humidity are key in the rapid and complete growth of such species which explains their distribution in regions where temperature exceeds 15°C for three months a year yet they cannot withstand temperatures above 40°C for two hours. Per say, the optimum conditions for flourishing of sandflies-warm and humid-covers the largest portion of the world as it stretches from latitude 50°N to 40°S as seen in Figure 1, with exception of New Zealand and Pacific islands (Cecílio et al., 2022).

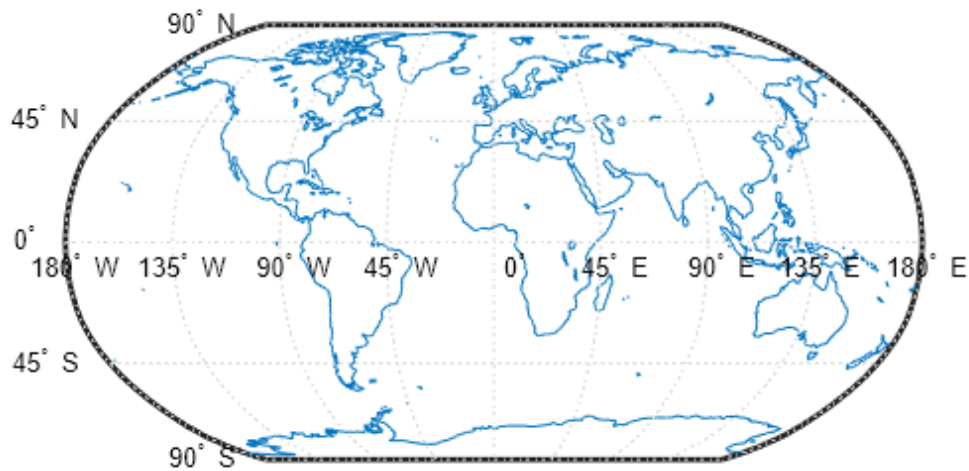


Figure 1: World Map Labelled with Latitude and Longitude.

Yet due to climate change, the distribution of sandflies around the globe as well as their spread in areas that they have not been before was suggested by different studies that aimed to understand the desperation capacity, as much more lands are being favourable for the survival and growth of such species (Cross & Hyams, 1996; Medlock et al., 2014; Trájer & Sebestyén, 2019).

Climate change is among the challenges imposed by industrial revolution (Dogaru, 2020). The continuous emission of greenhouse gases has raised mean global temperature by 1°C. Even though such increase could be perceived to be negligible, yet it has a profound impact on the overall climate of the world including increasing warm days and night, increasing extreme heat events, decreasing snow cover of lands, decreasing cold days and nights and catalysing sea level rise. The impact of global warming is found to be heterogeneous meaning that geosphere is more suffering compared to aquasphere. Global warming induced changes affected the hydrological cycle in a way that many regions became dryer, yet others became wit. According to Intergovernmental Panel on Climate Change, if such trend in greenhouse gases emission continued, it's more likely that further warming and irreversible changes will be brought into the scene and as a result this will impact the whole ecosystem (Intergovernmental Panel on Climate Change, 2014). Among the major hidden threats that are lurking are the transmission season duration as well as spread of VBDs. Dynamics of transmission, the geographical distribution, and re-emergence of VBDs all could be affected by climate change by indirect and direct routes. Directly, ecosystem habitants will be affected in a way that would promote or demote the growth and localisation of vectors (Rocklöv & Dubrow, 2020). Vectors of VBDs are ectotherms (cold-blooded) thus their survival, abundance and feeding activities will increase as temperature increases. Also, their development rate will be catalysed as temperature rises, yet they will not be able surviving beyond the previously mentioned conditions (Levesque & Marshall, 2021; Rocklöv & Dubrow, 2020). Once the external conditions are sat for the growth and development of such species, a lot of diseases could be transmitted including dengue, chikungunya, Zika virus, lymphatic

filariasis, onchocerciasis, Japanese encephalitis, Chagas disease, yellow fever, schistosomiasis, malaria, and leishmaniasis which are known to be the eight NTDs (Caminade et al., 2019; Rocklöv & Dubrow, 2020; Semenza & Suk, 2018).

1.1. Leishmaniasis

Leishmaniasis is a NTD that is caused by different species that carry an intracellular parasite namely *Leishmania*. As Figure 2 shows, the infection by *Leishmania* starts with a bite of an infected female of *Phlebotomus* and *Lutzomyia* sandflies that seeks for a blood meal as mentioned previously. When an infected female sandfly digests the blood meal, promastigotes of leishmania grow on the wall of its guts, yet it could lose it upon defecation. For their survival, the promastigotes get attached to the inner portion of their guts that is mediated by lipophosphoglycan (LPG), which also plays a huge role in their colonisation in the host's body (Killick-Kendrick, 1999). *Leishmania*'s LPG protects the parasites from complement-mediated lysis and simultaneously induces the activation of complement, therefore promotes the phagocytosis of parasites. The formation of intracellular amastigotes is mediated also by LPG as it delays the formation of phagolysosomes thus allowing the parasites taking this form (Rossi & Fasel, 2018). The cell surface glycoconjugate LPG varies from one to another *Leishmania* species yet it is believed that sufficient number of ligands must be present in the midgut of a female sandfly so that the promastigotes could attach. It was reported that after the blood meal, promastigotes will not just attach to LPG, but they will attach to the produced lectins, too (Killick-Kendrick, 1999). In the host's body, the parasites multiply within the phagolysosomal compartment in an amastigote form. Now that promastigotes converted into amastigotes, different diseases could be manifested clinically and each of which is correlated with a species of *Leishmania* (Roatt et al., 2020; Sangshetti et al., 2015).

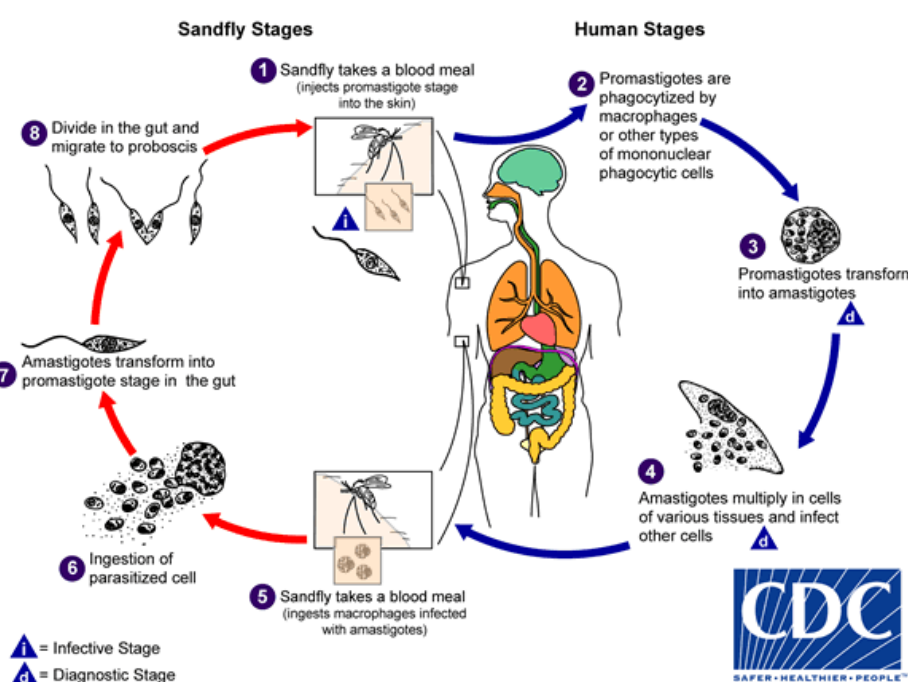


Figure 2: Life cycle of leishmania (DPDx, 2024) :

Leishmania species are more than 20 yet there are four main leishmaniasis, namely: visceral leishmaniasis (VL), mucocutaneous leishmaniasis (MCL), cutaneous leishmaniasis (CL) and post-kala-azar dermal leishmaniasis (PKDL). Each one of them requires a certain protocol of treatment (Arenas et al., 2017; Killick-Kendrick, 1999; Rossi & Fasel, 2018; Sangshetti et al., 2015). *L. peruviana*, *L. tropica*, *L. panamensis*, *L. braziliensis*, *L. mexicana*, *L. venezuelensis*, *L. amazonensis*, *L. major*, *L. guyanensis*, *L. infantum*, *L. aethiopica*, *L. donovani*, etc. all are causative organisms that infect a wide variety of the population. VL is caused mainly by *L. donovani* and that it is deadly if untreated. After treatment of VL, PKDL could evolve with clinical manifestations as maculopapular macular, and nodular rash. *L. major*, *L. mexicana*, *L. braziliensis*, and *L. panamensis* cause CL, which is associated with morbidity, however it could heal by itself after 3-18 months leaving scars on the healed skin depicted in Figure 3.



Figure 3: An example of a scar left by CL. (Gurel et al., 2020)

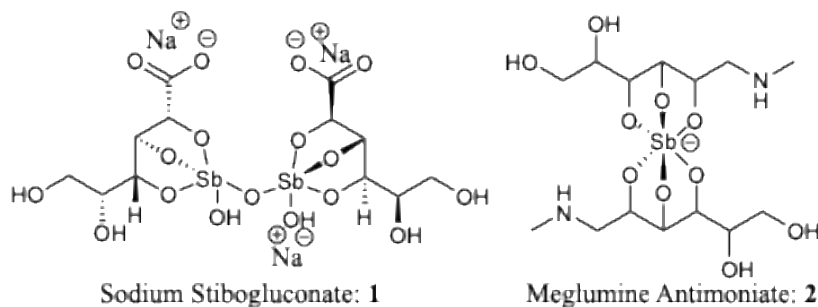
Such scars affect the psychosocial status due to the social isolation and stigma caused by the disease. Several species are able to cause MCL, but the most important among them all is *L. braziliensis*. MCL, unlike the latter destruct the cartilage and mucosa of the pharynx and mouth, which develops to involve more facial tissues (Arenas et al., 2017; Killick-Kendrick, 1999; Roatt et al., 2020; Rossi & Fasel, 2018; Sangshetti et al., 2015).

In Africa, the Mediterranean region, the Americas, and Asia, Leishmaniasis is considered to be an endemic. All over the globe, 12 to 15 million people are infected, and 350 million are under the risk of getting infected and each year between 1.5 to 2 million new cases are reported with 70,000 deaths (Arenas et al., 2017; Cecílio et al., 2022; Killick-Kendrick, 1999; Mitra & Mawson, 2017; Roatt et al., 2020; Rossi & Fasel, 2018; Sangshetti et al., 2015).

1.2. Treatments used for leishmaniasis

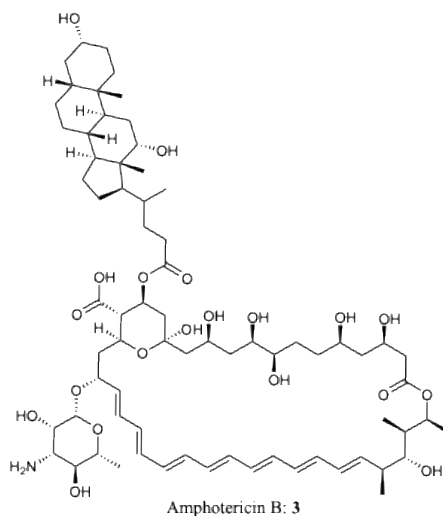
Up to this date, no vaccine has been developed for leishmaniasis and the available drugs are tools to treat and control VL and CL. Disease's severity depends on the host-immune response as well as the infecting leishmania species (Roatt et al., 2020; Sangshetti et al., 2015). Currently, there are many available drugs, each has its pros and cons. The beginning of treatment of CL and VL started with pentavalent

antimonials. Pentavalent antimonials (Sb^{5+}) are considered as prodrugs (drugs that become active upon entering the body) and have been available for seven decades as they are first line treatment for leishmaniasis. The active form Sb^{3+} is achieved as Sb^{5+} gets reduced in the body such ion has high affinity to thiol and nitrogenous biomolecules, such as protein, enzymes, thiols etc. sodium stibogluconate **1** Meglumine antimoniate **2** and are the main two formulations of pentavalent antimonials drugs (Roatt et al., 2020; Sangshetti et al., 2015).

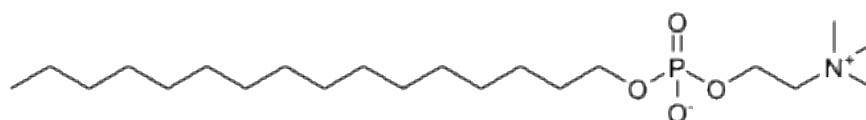


Despite the effectiveness these drugs, major drawbacks were reported such as high dosage required to give an acceptable anti-leishmanial activity that affects heart, kidney, and liver as well as the accompanied side effects such as local irritations, hepatic and cardiac alteration, vomiting, nausea, and anorexia. The latter issues forced experts to bring upon a new solution.

Amphotericin B deoxycholate (AmB-D) **3** is an antifungal and of activity against leishmania species that was developed thereafter. Such drug binds to sterols, and it is of high affinity towards ergosterol, thus increasing protozoan membrane permeability. Although it is an effective treatment, yet severe side effects were produced by it such as hypokalaemia, fever, myocarditis and especially nephrotoxicity. To circumvent the issue, lipid formulations were developed to compensate the side effects of AmB-D. Liposomal AmB-D is the only of all the lipid formulations of AmB-D to be approved by FDA. The main issue with this drug was its high cost -keeping in mind that poorest regions will be infected- and low efficiency in treating CL (Roatt et al., 2020; Sangshetti et al., 2015).

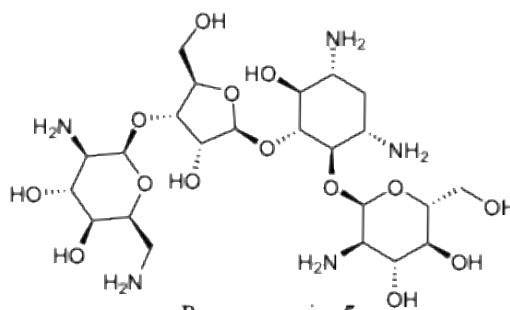


Miltefosine **4**, hexadecylphosphocholine, is an antineoplastic agent that was developed mainly for skin metastases of breast cancer, yet in the mid-1980 it was used as the first oral anti-leishmanial agent. Its activity as anti-leishmanial agent comes from its ability to change signaling pathways as well as cell membrane synthesis thus leading to apoptosis. Besides its apparent curing activity, its prolonged treatment period - VL, 50–100 mg/day for 28 days and CL 2.5 mg/kg per day for 28 days – as well as teratogenicity, and high potential of resistance led to decline in its application in treating leishmania (Roatt et al., 2020; Sangshetti et al., 2015).



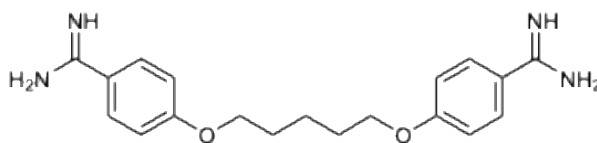
Miltefosine: **4**

Another drug that has been used in leishmania treatment is paromomycin **5**. Paromomycin is an aminoglycoside antibiotic that has shown an antileishmanial activity as it alters protein synthesis and inhibits respiration by changing mitochondrial membrane fluidity. VL and CL were both treated with paromomycin, yet it is more effective for CL. Among the cons of this drug is limited availability in endemic regions that restricts its usage. Also, many side effects were observed upon usage such as ototoxicity, pain at injection site, elevated hepatic transaminases, nausea, diarrhea and abdominal cramps. Promastigotes of *L. donovani* has developed resistance to paromomycin which is another drawback to be put on the list (Roatt et al., 2020; Sangshetti et al., 2015).



Paromomycin: **5**

As days go by, leishmanial species built resistance towards antimonial drugs and since 1940s when pentamidine **6** was first synthesised, its antileishmanial activity was investigated. The exact mechanism of action against leishmania organisms is unknown yet it is to be explored. Initial investigations show that pentamidine accumulates in the parasites and has many actions in there such as inhibition of mitochondrial topoisomerase II, and binding to kinetoplast DNA. Though it is effective, yet its usage was put into a halt due to its acute toxicity (Roatt et al., 2020; Sangshetti et al., 2015).



Pentamidine: **6**

1.3. Future control of leishmaniasis

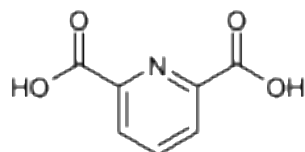
In the recent years, the range of available anti-leishmanial drugs has expanded, yet none of them matches the requirements for best management of the infection in different patient populations. Most infectious diseases spread across regions where high poverty, low surveillance and limited medical are predominant. The latter along with resistance and toxicity of drugs are leading causes for demanding new therapeutic agents and methods (Croft et al., 2006; Maarouf et al., 1998). With low investments for development and discovery of new drugs as well as the small arsenal of therapy, scientist and world health agencies are encountered with a gigantic challenge in controlling and compacting this crucial neglected disease (Roatt et al., 2020). An urgent need for new and efficient drug for leishmaniasis is atop of WHO's list along with other 19 diseases, according to their reports (WHO, 2020). With the enormous challenges imposed in development of new therapeutic agents, scientists are still on the road to determine a radical cure for all forms of leishmaniasis. Nowadays many approaches were used including thermotherapy and CO₂ laser administration (Valencia et al., 2013), cryotherapy and electrotherapy (Chakravarty & Sundar, 2019), topical drug therapy using nitric oxide derivatives (López-Jaramillo et al., 2010), intralesional drug administration (Arboleda et al., 2019), combination therapy (Mahajan et al., 2015), immunomodulators (Mayrink et al., 2005), nanotechnology (P. Kumar et al., 2019), and drug repurposing (Bustamante et al., 2019). Besides all of these approaches, characterisation, and identification of new targets within the cell of parasites is core approach as it deals with targets of the drugs that will interfere with essential biochemical and metabolic pathways for parasite survival. To achieve success in this approach, the target enzyme must be inhibited with finding significant differences between parasite and mammalian in terms of function and structure (Singh et al., 2012).

1.4. Pyridine-2,6-dicarboxylic acid as versatile biological heterocyclic ring

Heterocyclic compounds are widely spread in nature due to their necessity for the living beings as they are present in biochemicals such as DNA, RNA, hormones, vitamins amino acids and so on. Due to their availability, they have been used to serve as precursors for different number of synthetic products. Pyridine was discovered in the nineteenth century and its derivatives have attached enormous interest for the preparation of vast variety of compounds. The interest they have attracted was not in vain as the derivatives have shown that they could be applied in different causes such as anti-bacterial, anti-microbial, anti-diabetic, anti-oxidant, anti-cancer, anti-viral, anti-fungal and more. Among pyridine derivatives, 2,6-disubstituted ones are greatly important as they could be employed in various fields due to ability to produce further derivatives through synthesis (Stellenboom & Baykan, 2019).

Pyridine-2,6-dicarboxylic acid **7**, or commonly known Dipicolinic Acid (DPA) is an aromatic dicarboxylic acid that occurs naturally. In nature, DPA is found as a secondary metabolite in aerobic bacteria such as *Bacillus* and anaerobic ones like *Clostridium* which are considered as in endospores of grampositive bacteria. Under stressful conditions in the environment e.g., starvation for nutrients, high temperature, surrounding of toxic compounds and the emission of radiation, DPA becomes

necessary for the initiation of the sporulation. 10% of the weight of dry *Bacillus subtilis* is attributed to the presence of DPA chelated with Calcium ions. And by this, it helps it in resisting the heat as well as in the prevention of the denaturation of the DNA (Schwardmann et al., 2022; Takahashi et al., 2015).



Dipicolinic acid: 7

Biosynthetically, one of the intermediate of L-aspartate amino acid anabolism is converted to DPA in sporulating bacteria through a single step, Figure 4 (McClintock et al., 2018; Schwardmann et al., 2022; Takahashi et al., 2015).

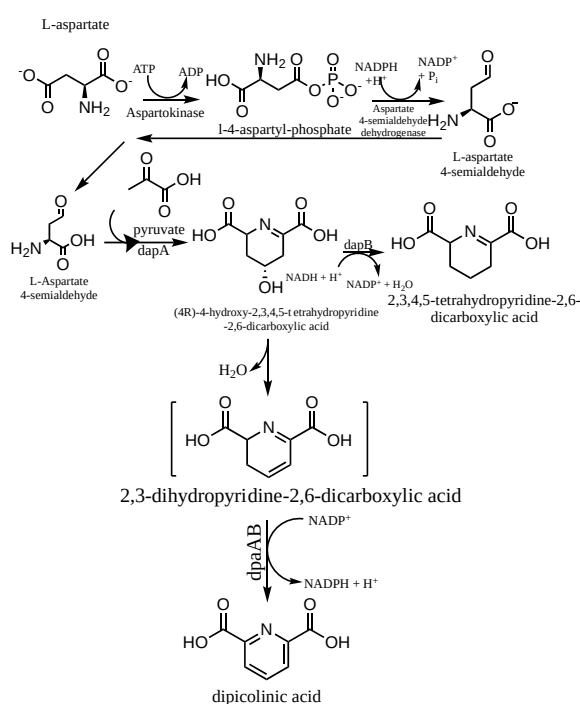
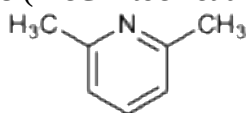


Figure 4: Scheme of the anabolic pathway of DPA.

Chemically, DPA could be synthesized through the oxidation of one of the extracts of natural coal, namely 2,6-lutidine **8** (McClintock et al., 2018).



2,6-lutidine: 8

Due to the non-toxicity, biodegradability, stability against heat, DPA has been employed in various fields including polymerization industrial, medical, cosmetics, and cleaning sectors (Schwardmann et al., 2022; Takahashi et al., 2015).

1.5. Problem statement:

There are a few available drugs that are widely used for the treatment of leishmaniasis. All of these drugs have many limitations such as long course of treatment, toxic side effects, and high cost. So, the need to develop new potent, cheaper, and less toxic drugs for the treatment of leishmaniasis is important.

1.6. Significance of the study:

The need for new anti-leishmanial agents is critical due to limitations in availability of effective drugs and the rise of drug resistant strains of leishmaniasis.

1.7. Research questions:

In the present perspective, emergence of resistant strains of leishmania and severe side effects resulting from the use of conventional anti-leishmanial therapies present an urgent need for developing novel agents against this parasite. This study questions what is the effectiveness of dipicolinic acid as alternative choices in the treatment for leishmaniasis? Also, what are factors determining the anti-leishmanial potential of the dipicolinic acid in terms of functional groups present?

1.8. Research objectives:

The objectives of this research are to synthesize and characterize the structures of the prepared dipicolinic acid derivatives. Then, to evaluate the anti-leishmanial activities of the synthesized derivatives using standard methods. And finally, to discuss the structure-activity relationship of the most active compounds.

1.9. Research method:

This research begins with a literature review on the recent synthetic pathways and the possible derivatives that could be synthesized out of Dipicolinic acid. After that, the synthesis of these derivatives will commence, followed by purification and chemical analysis, including Nuclear Magnetic Resonance (NMR) spectroscopy, Infrared (IR) spectroscopy, and elemental analysis (CHN). These analyses will confirm the structures of the derivatives and ensure their purity and quality. Anti-leishmanial activity will be determined using standard anti-leishmanial assays such as Alamar blue assay (Bekhit et al., 2015). The assays will provide an assessment of growth inhibition activity, the minimum inhibitory concentration (MIC) of each derivative, and an overall evaluation of their anti-leishmanial potential. A study of activity-structure relationship will be held through studying the effect of modifications on structure and the resulting activity. The latter will aid future researchers in designing more potent derivatives and improving anti-leishmanial activity.

1.10. Expected outcomes:

By achieving the objectives of this research, a contribution to the discovery and development of novel anti-leishmanial agents that might combat the overgrown challenges, and finally could lead to improved treatment options.

Chapter (2)

2. Literature review

2.1. Overview

In the recent 10 years, many cores and derivatives have been applied to determine their potential serving as anti-leishmanial agents such as those listed on Figure 5, that include quinolines, purines, benzodiazepines, quinazolines, chromenes and coumarins, chalcones, triazines, steroids, azoles, furans, indoles, acridines, quinones, thiophenes, pyrimidines, acridines, hydrazones and Schiff's bases and pyridines (Sangshetti et al., 2015).

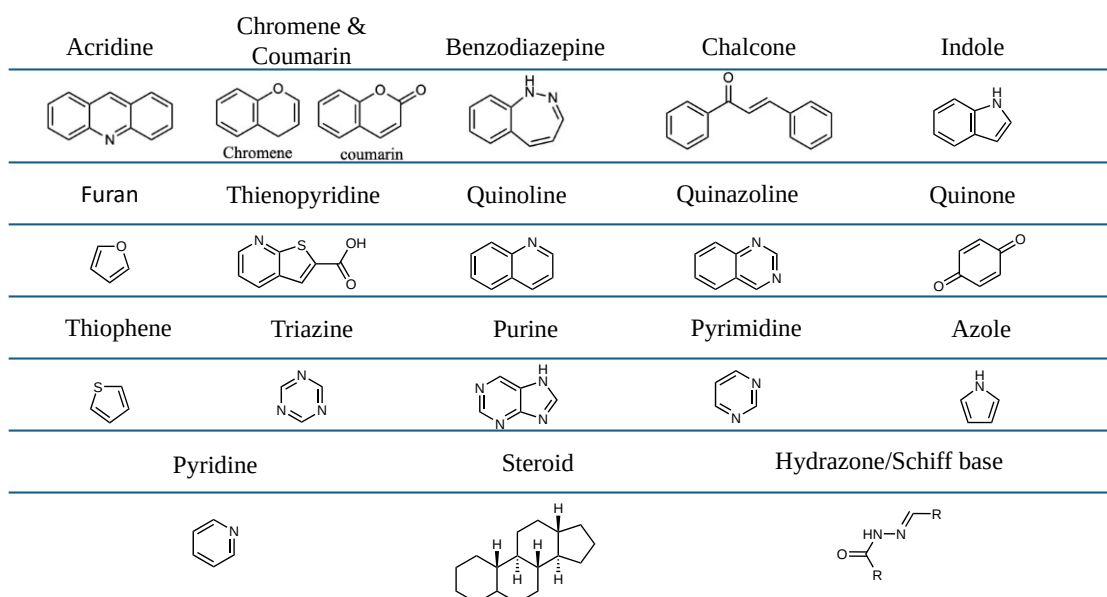
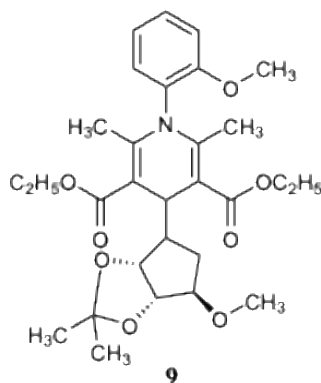


Figure 5: cores reported to have anti-leishmanial activity.

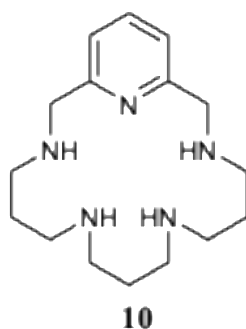
2.2. Pyridines

Pyridine core has been used several times by many researchers as it has great potential as anti-leishmanial entity. Pandey et al. (Pandey et al., 2010) have synthesized 1-phenyl-4-glycosyl-dihydropyridines series through multicomponent, one pot reaction in the presence of tetrabutylammonium hydrogensulfate as a catalyst. The synthesized compounds were screen against promastigote and amastigote *L. donovani*, *in vivo* and *vitro*. The compounds have shown good to moderate activity yet compound **9** was of potent activity with 0.04 and 1.16 mM as *in vitro* IC₅₀ values for promastigote and amastigote forms, respectively (Pandey et al., 2010). Selectivity index (SI) which is defined as CC₅₀ divided by IC₅₀ is used to indicate the safety margin between the concentration of a compound to kill a pathogen and the concentration that is toxic to mammalian counterparts. The higher the value of SI, the better it is as it is more likely that compound's activity is not due to a general

metabolic toxin (Dzoyem et al., 2016). SI value was reported in this study to be 8.04 which is higher than pentamidine and miltefosine as their SI values are 2.58 and 1.08. Further, it was reported that using intracellular Hamster model, *in vivo* results were $49.73 \pm 12.0\%$ inhibition range of *L. donovani*. These results were also compared with pentamidine and miltefosine, but these standard drugs have shown better inhibition activity by approximately two folds. All in all, these findings suggest that **9** compound could be furtherly optimized, and better profiles of drug could be obtained.



In another study, a series of xylene, pyridine and, pyrazole azamacrocyclic derivatives were synthesized and screened *in vitro* against promastigote and amastigote forms of three leishmania species namely: *L. donovani*, *L. braziliensis* and *L. infantum*. Instead of conducting *in vivo* experiment, J774.2 macrophage cell line were used as host cell models as they get infected by leishmania parasites naturally. SI value was calculated mathematically in this research by dividing J774.2 macrophage IC_{50} by Parasite IC_{50} . Compound **10** was the most promising one with best results obtained for all the leishmania species with IC_{50} for *L. infantum* in promastigote form of $30.4 \pm 1.4 \mu M$, macrophage IC_{50} of $309.9 \pm 43.8 \mu M$ and 10 SI value. For *L. braziliensis* SI value was reported to be 12 with IC_{50} its promastigote form of $26.2 \pm 1.1 \mu M$ and IC_{50} of macrophage of $309.9 \pm 43.8 \mu M$. Finally, for *L. donovani*, 1549 SI value was reported with IC_{50} of macrophage of $309.9 \pm 43.8 \mu M$ and $0.2 \pm 0.0 \mu M$ as IC_{50} of promastigotes of *L. donovani*. Meglumine antimoniate was used as a reference drug and it has higher IC_{50} for promastigotes and lower SI values of all leishmania species compared with **10**. With these results, it was suggested by the authors that such drug will be most active in reducing the infectivity and dividability of parasites (Martín-Montes et al., 2023).



Unlike **9**, compound **10** was studied solely *in vitro*, thus comparison of results will not be reliable, but both cores could be optimised and studied to obtain better results.

2.3. Hydrazone/Schiff's bases

Hydrazones or Schiff's bases are important class of compounds that have wide range of activity and thus they have been applied in different studies such as theoretical studies (Ali et al., 2020), metal ion complexations (Liu et al., 2022) studies and biological studies (Pahlavani et al., 2015). Hydrazones are of wide range of biological activities, and they have shown antioxidant, anti-inflammatory, antihypertensive, anti-infective, antimicrobial, anticancer, antimalarial and antileishmanial activities (Kapusterynska et al., 2023).

Synthetically, hydrazones are obtained by reaction of ketone (**A**) or aldehyde (**B**) with a hydrazide or hydrazine in the presence of a catalyst, most commonly an acid as Figure 6 shows (Kapusterynska et al., 2023).

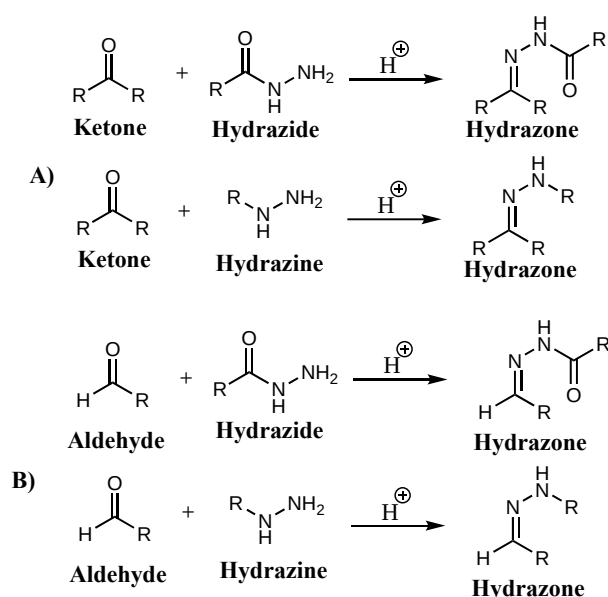
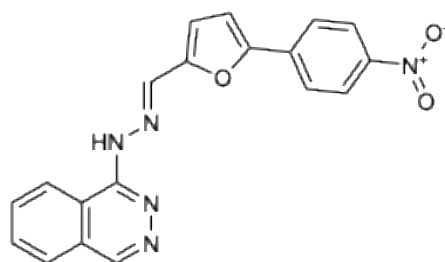


Figure 6: General reaction for formation of hydrazone starting with **A**) Ketone with hydrazide or hydrazine, and **B**) Aldehyde hydrazide or hydrazine in the presence of an acid as a catalyst.

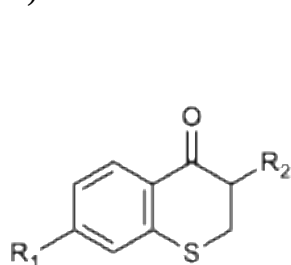
Kapusterynska et al. (Kapusterynska et al., 2023) have synthesized 17 hydrazone compounds by mechanochemical synthetic approach by operating the reaction on a planetary ball mill while keeping reaction on going for 180 min with 6 cycles per 30 minutes. In mechanochemical reactions, mechanical energy is used to bring up the chemical change, that includes using compression, friction, or sheer energy (Leonardi et al., 2018). They could be defined too as mechanical energy-induced reactions as they absorb energy coming from milling or grinding processes (Horie et al., 2004). The chosen scaffolds were furanyl or phenolic aldehydes with different heterocyclic hydrazinamides or hydrazines. The synthesized products were screened for their anti-bacterial and anti-leishmanial activities. In vitro screening for anti-leishmanial activity was done against *L. donovani* intramacrophage and axenic amastigotes. Between all the synthesized compounds, compound **11** was of the best results with IC_{50} of 0.3 ± 0.1 μ M for intramacrophage and 0.2 ± 0.1 μ M for axenic amastigotes and SI more than 333. Miltefosine was used a reference drug, and its IC_{50} for both amastigotes was very close to **11** yet its SI was 9 which is lower than **9** by far (Kapusterynska et al., 2023).

The previous results are promising, and *in vivo* experiments are needed for further investigations.

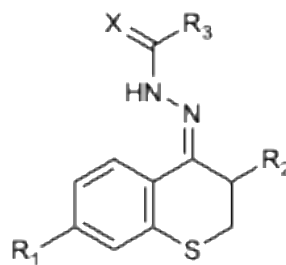


11

The impact of addition of hydrazone on anti-leishmanial activity of a compound was illustrated by Vargas et al. (Vargas et al., 2018) as thiochroman-4-ones **12** and acyl hydrazone derivatives of thiochroman-4-ones **13** were tested against intracellular amastigote of *L.panamensis*, *in vitro*. For cytotoxicity, human monocytes (U-937 ATCC CRL-1593.2) were used.



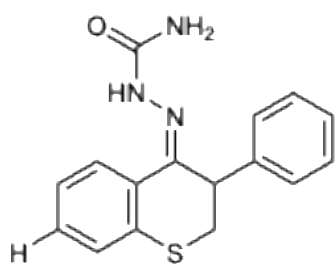
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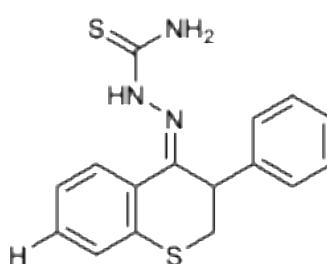
13

X = O or S

The enhancement was translated upon the changes in SI values of the core and its hydrazone counterparts as it increased by 1 up to 36 fold/s. from 17 compounds synthesized, compounds **14** and **15** were most active.



14



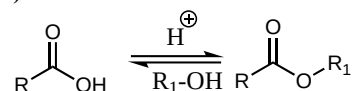
15

EC₅₀ values were 5.4 μ M for **14** and 5.1 μ M for **15**, and LC₅₀ of 100.2 and 50.1 μ M, respectively. The overall SI values were 18.6 and 9.8. Even though the results are shown good activity, yet they are not better than the reference drug which is Amphotericin B in any profile. But the point is, hydrazone formation could enhance the overall anti-leishmanial profile of a given core.

2.4. Hydrazones/Schiff's bases of pyridine

Based on reaction in Figure 6, for pyridine core to be able to form hydrazone bond, it must be in hydrazine or hydrazide form. In this review hydrazide will be considered. Pyridine hydrazide could be taking many different forms based on the form of pyridine carboxylic acid as the standard method for synthesis of hydrazides is a two steps reaction as Figure 7 illustrates. The first is esterification of the carboxylic acid, avoiding any acid base reaction between hydrazine and carboxylic acid and the second is hydrazinolysis of the ester in alcohol (X. Zhang et al., 2002).

1) Esterification



2) Hydrazinolysis

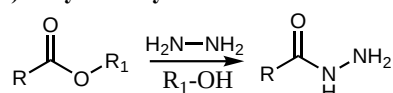
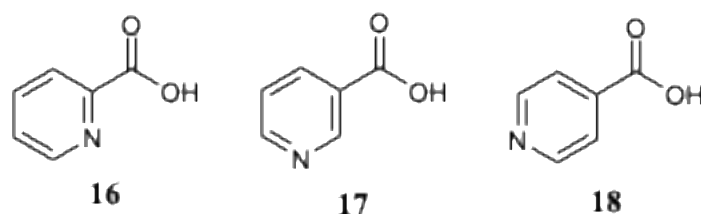
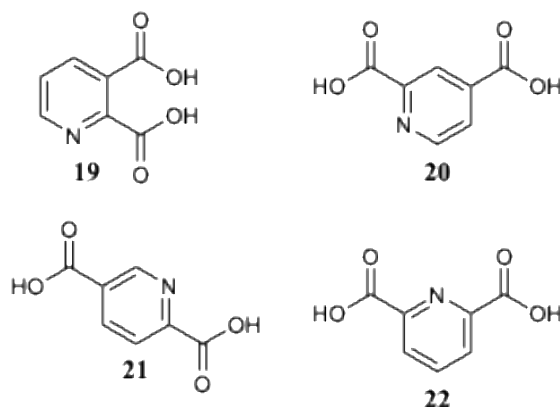


Figure 7: Standard method for hydrazide formation step 1) Esterification and 2) Hydrazinolysis.

Now, pyridine could be substituted by mono-, di-, or tri carboxylic acid/s. There are three pyridine mono-carboxylic acid isomers, namely: pyridine-2-carboxylic acid **16**, pyridine-3-carboxylic acid, **17**, and pyridine-4-carboxylic acid **18** (Seo et al., 2024).



Disubstituted pyridine with carboxylic acids are found in four isomers, pyridine 2,3-dicarboxylic acid **19**, pyridine 2,4-dicarboxylic acid **20**, pyridine 2,5-dicarboxylic acid **21**, and pyridine 2,6-dicarboxylic acid **22** (Hassan et al., 2020).



Pyridine 2,6-dicarboxylic acid is of interesting properties as mentioned in a previous section, so this review focuses on its hydrazone derivative namely: Bis(aldehyde/ketone)-2,6-dipicolinoylhydrazone.

2.5. Bis(aldehyde/ketone) dipicolinoyl hydrazones, synthesis and applications

2.5.1. Synthesis

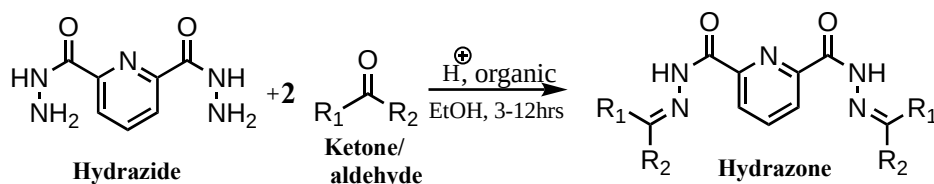
In 1985, Paolucci et al. (Paolucci et al., 1985) were first to report a method for synthesizing hydrazone of Dipicolinic hydrazide. They reported preparation of bis(salicylaldehyde) dipicolinoyl hydrazone **23** by dissolving two equivalents of salicylaldehyde in absolute ethanol at 60 °C and then addition of one equivalent of 2,6- dipicolinoylhydrazine dissolved in ethanol to the solution that was kept refluxing for 3 hours. The obtained product was recrystallised from ethanol.

Starting with ketones, Chen et al. (Chen et al., 1996) used the same previous method to obtain four different bis(4-acyl-1-phenyl-3-methyl-pyrazolones-5) dipicolinoyl hydrazone derivatives **24**, **25**, **26**, and **26**. The products were recrystallised from methanol. Gudasi et al. (Gudasi et al., 2006) was first to synthesize bis-(3-methoxysalicylaldehyde)-2,6-dipicolinoyl hydrazone **27** based on the previous method, and it was recrystallised from 2-ethoxy ethanol.

Modifications on the previous method were reported by Li et al. (Li et al., 2007), as *p*-toluenesulfonic acid was used as a catalyst in synthesizing **27**. Also, instead of 2-ethoxy ethanol, methanol was used for recrystallisation. Acid catalyst could be generalised into an organic acid, as acetic acid was used in other studies (Amini et al., 2020; R. Kumar et al., 2018; Zamani et al., 2023).

Structures conformation was developing with the subsequent development in analytical tools. In the old days, Infrared (IR) and ¹H-Nuclear Magnetic Resonance (NMR) were the only things to be used (Chen et al., 1996; Paolucci et al., 1985), but then, melting point was to be reported (Arora et al., 1986). Nowadays, along with the others, ¹³C-NMR and MS are necessary (Amini et al., 2020; Yadav & Singh, 2018; Yuan et al., 2021; Zamani et al., 2023).

After having a brief sight over the development of synthetic methods, a general conclusion could be obtained to optimize the reaction conditions as Scheme 1 shows. In general, to synthesize bis(aldehyde/ketone) dipicolinoyl hydrazone, a molar ratio of 2:1 between aldehyde/ketone and dipicolinic hydrazide is needed. Separately, each one is dissolved in ethanol, then aldehyde/ketones solution is brought into 60°C. To the heated solution, dipicolinic hydrazide solution is added. Next, drops of an organic acid as catalyst are added. The reaction time is ranging from 3 (Paolucci et al., 1985)-12 (Zamani et al., 2023) hours. After that, the solid product is collected through filtration and washed with water and ethanol. Finally, recrystallisation is done by a solvent that dissolves the crude product at high temperatures and not when it cools down.



Scheme 1: A general synthetic method of hydrazone deduced from previous studies in which a hydrazide is reacted with 2 moles of ketones or aldehyde in the presence of an organic acid and refluxed for 3 to 12 hours.

2.6. Applications of bis(aldehyde/ketone) dipicolinoyl hydrazones

2.6.1. Metal complexation

Ligands of hydrazones are widely used in coordination chemistry due to the presence of azomethine group. Azomethine' nitrogen is π -electrons acceptor as well as basic in nature that can be seen in Figure 8 (Joshi, 2022).

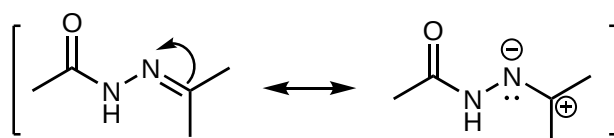


Figure 8: Hydrazone's imine resonance structure that shows its ability forming π^* -d coordination bond.

The presence of other hetero atoms along with the azomethine group, makes the compound flexi-dentate complex (Joshi, 2022). Chelation of flexidentate complexes involves different attachment points of the ligand to metal ion (E. Sloan & H. Busch, 1978). This variation in attachment is controlled by various factors including solvent, pH, counter anion, metal ion and ratio between reactants (El-Tabl et al., 2007). With the relative easiness of synthesis of this class of compounds along with the wide diverse potential, hydrazones have been studied and applied by many researchers (Shah et al., 2022).

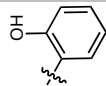
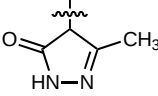
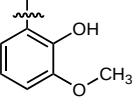
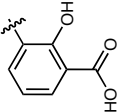
The demand over raw materials and energy has grown exponentially with the rapid growth in population and industrialisation. with such growth, countries started seeking for new sources of energy such as nuclear energy. Uranium is among the most preferred raw materials in nuclear energy. Yet, with the usage of this element, many aqueous resources have been contaminated with its soluble form that is uranyl ion (UO_2^+). Such seepage has horrific impact on environment and human health. Thus, it is of huge necessity seeking ways removing uranyl ion from aqueous medias (Şimşek et al., 2022). Paolucci et al. (Paolucci et al., 1985) investigated compound **23** with variant amounts of uranyl salts and it was found that mono- bi- and trinuclear stable complexes were formed. In another study, compounds **24**, **25**, **26** and **27** were synthesized and reacted with excess amount of uranyl to find that stable trinuclear complexes formed (Chen et al., 1996). Since these studies did not mention an application of these complexes, it is to be suggested that their selectivity towards uranyl gets investigated.

Lanthanide complexes are of high significance as they are applicable in many advanced technologies such as nuclear, energy-efficient lights, batteries, and sensors technologies (Abu Elgoud et al., 2022). Pr^{3+} , La^{3+} , Sm^{3+} , Eu^{3+} , Tb^{3+} , Gd^{3+} , Dy^{3+} and Yb^{3+} complexes of **29** were synthesized by Gudasi et al. (Gudasi et al., 2006) and it was reported that formed complexes were binuclear with attachment of nitrate ions and water to the metal centre due to the high coordination requirements of lanthanide ions. Further, the biological activity of these complexes were evaluated against *Pseudomonas aeruginosa* and *Bacillus cirroflagellosus* bacteria and *Aspergillus niger* and *Penicillium notatum* fungi using cup-plate method. The results were ranging from non-active to moderately active with reference to norfloxacin and grisofulvin. Although their activity was mild in this application, yet it could serve as a starting point for applications in different fields. **29** coordination polymers with Eu^{3+} , Er^{3+} , Dy^{3+} and Yb^{3+} were synthesized by vapour diffusion method with chloride ion as a templet. Among the synthesized compounds, Dy^{3+} complex showed single magnet molecular behaviour (L. Zhang et al., 2018).

Ligand-metal complexes chemical features differ from the individual properties of free metal ion or organic ligand (Gil-Moles & Concepción Gimeno, 2024). This difference gives raise to unusual features such as electrical properties, biological activity, adsorption capacity, magnetism, and nonlinear optical properties 1-D pentanuclear, W-like, Zn^{2+} coordination polymer of **29** was synthesized and studied. It was reported that it possesses fluorescence property with emission at 522 nm and excitation at 450 nm. Comparing the complex with the ligand, free ligand's excitation wavelength was at 365 nm and emission at 581 nm (S. Qin et al., 2011). These results show the capacity of **29** coordination polymer to be applied as sensor for Zn^{2+} , still other ions must be infused in a solution to investigate its selectivity and different fluorescence responses that would be obtained upon complexation with other ions. Yet it could be applied in different fields as well.

Crystal engineering is chemical field that focuses on the design, properties, and applications of crystals. This field was the initial spark that led to emergence of hybrid coordination networks and metal-organic frameworks that are part of porous coordination networks (Mukherjee et al., 2020). This field is highly significant as such materials are capable of being applied across different fields such as nonlinear optics, microelectronics, and others. Copper supramolecular complexes of **28** and **29** were synthesized with variation in solvent, anion, and ligands. Such variation led to obtaining diversly constructed supramolecular through hydrogen bonding (S. N. Qin et al., 2011). **29** square-grid neutral complex of Cu^{2+} was constructed by Adhikary et al. (Adhikary et al., 2014) and its magnetic properties were investigated. Results showed that such complex has ferro and anti-ferromagnetic spin exchanges, even though anti-ferromagnetic exchange was found to be dominant at regions of low temperatures. Macrocyclic of **29** with transition metal ions, namely: Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+} were synthesized and studied. It was reported that such ligand is nona-dentate. Further these complexes are of potential being applied in catalysis field (Vadavi et al., 2011). Table 1 summarizes the significant data of the previous studies.

Table 1: A summary of metal complexation applications of bis(aldehyde/ketone) dipicolinoyl hydrazones, where R₁ and R₂ are based on Scheme.1:

Number	R ₁	R ₂	% Yield (%)	Recrystallisation Solvent	Type of Study	Reference
23			90.5	Ethanol	Uranyl Complexation	(Paolucci et al., 1985)
24			95	Methanol	Uranyl Complexation	(Chen et al., 1996)
25			90	Methanol	Uranyl Complexation	
26			93	Methanol	Uranyl Complexation	
27			91	Methanol	Uranyl Complexation	
28			90	2-ethoxy ethanol	Lanthanide Complexation and Biological Screening	(Gudasi et al., 2006)
			85	Methanol	Crystal Structure Study	(Li et al., 2007)
			NR		Complexation with Zinc.	(S. Qin et al., 2011)
			93	NR	Supramolecular Complexation	(S. N. Qin et al., 2011)
			70	2-ethoxy ethanol	Crystal Structure Study, and Complexation	(Vadavi et al., 2011)
			NR	NR	Magnetic Properties investigation	(Adhikary et al., 2014)
			82	2-ethoxy ethanol	Coordination Polymers synthesis	(L. Zhang et al., 2018)
29			92	NR	Supramolecular complexation	(S. N. Qin et al., 2011)

*NR = Not reported.

2.6.2. Chemosensory

Research in potential applications of bis(aldehyde/ketone) dipicolinoyl hydrazones has taken a new leap after investigating their coordination chemistry. With the shown chelating abilities, researches' attention is turned into their application as chemosensors. Chemosensors are defined as a receptor that transduce chemical signal into changes in emission, colour, or redox potential (Wang & Anslyn, 2011).

Novel compound **30** was reported by Bhattacharyya et al. (Bhattacharyya et al., 2016) and was applied as naked eye sensor for Cu^{2+} ions in aqueous medium. Upon complexing with Cu^{2+} ions, change from yellow to brown was observed. **30**'s affinity towards Cu^{2+} was further studied as other metals were used and slight change in colour was observed as Figure 9 shows. Detection limit was calculated to be almost $0.004 \mu\text{M}$, which is lower than other naked eye chemosensors for Cu^{2+} .



Figure 9: Compound **30** affinity towards Cu^{2+} as **1** is **30** and the others are complexes with it. minor changes are observed with other ions compared to Cu^{2+} . reproduced with permission of the author.

DPA-based chemosensors are not just capable of detecting cations but also, they are capable of detecting anions. In another study, compound **31** was synthesized to be another naked eye chemosensor for Cu^{2+} , F^- , Acetate and adenosine monophosphate (AMP^{2-}) ions all of which if taken in excess cause severe physiological problems such as inhibition of enzymes function, urolithiasis, fluorosis, high blood pressure, Menke's, Alzheimer's, Wilson's, and Parkinson's diseases and more. Thus, having a chemosensor that detects all of these ions at once is crucial. The study reports that lower detection limit for Cu^{2+} was $0.12 \mu\text{M}$, which is higher than **30**. Further, **32** showed good affinity towards F^- and acetate with $1.4 \mu\text{M}$ detection limit yet the highest was obtained for AMP^{2-} ions with lower detection limit of $0.08 \mu\text{M}$. The selectivity towards these ions was represented by observing changes in colours of solutions upon using different ions and anions. Figure 10 shows change in colour of solution from colourless to yellow upon detection of F^- , acetate, AMP^{2-} and Cu^{2+} whereas no other ions gave such change (R. Kumar et al., 2018).



Figure 10: selectivity representation of compound **31**(L1) towards acetate (AcO^-), F^- , AMP^{2-} and Cu^{2+} . Reproduced with permission of the authors.

Cyanide ion is among the most poisonous anions to living organisms as it affects many physiological processes such as central nervous system and metabolic actions. As industries grow, more cyanide gets excreted to environment thus it is crucial thinking of detecting cyanide. Loads of methods were developed in the recent years, yet colorimetric detection stands in a different edge due to simplicity and rapidity. The usual mechanism of cyanide sensing involves photo-induced electron transfer (Chakraborty et al., 2021). Yadav and Singh (Yadav & Singh, 2018) synthesized compounds **30**, **32**, **33** and **34** and applied as chemosensors to CN^- and Cu^{2+} . detection limit for Cu^{2+} ranged from 0.098 to 0.12 μM . the obtained complexes with Cu^{2+} were used to detect CN^- and detection limits ranged from 0.31 up to 0.53. Unlike the previous studies, the selectivity was studied through spectroscopic and electrochemical methods.

Compound **32** potential acting as a chemosensor for Cu^{2+} was investigated by Amini et al. (Amini et al., 2020). the compound is not new as it was mentioned by the author as it was synthesized previously, see table.1. Anyhow, the selectivity of compound towards Cu^{2+} was studied and the results revealed that no interferences were observed from other ions. Still, with detection limit of 0.311 μM , such compound seems to be out of the race as the value is relatively high. Thus, structural modifications might enhance the detection limit.

Recently, indole containing derivative, **34**, was synthesized and used as a colorimetric chemosensor for Cu^{2+} . Like the previous studies, naked eye observation of colour change upon complexation with Cu^{2+} was represented by noticing the difference in colour between solutions of free cations. The sharp change from colourless solution to dark brown was reported by the study as it is represented in Figure 11. limit of detection was among the lowest which is close enough to that of **35** (Zamani et al., 2023). Table summarizes the previous studies.

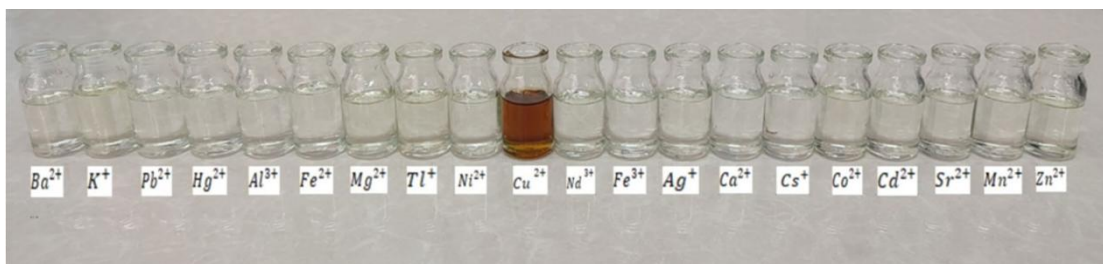


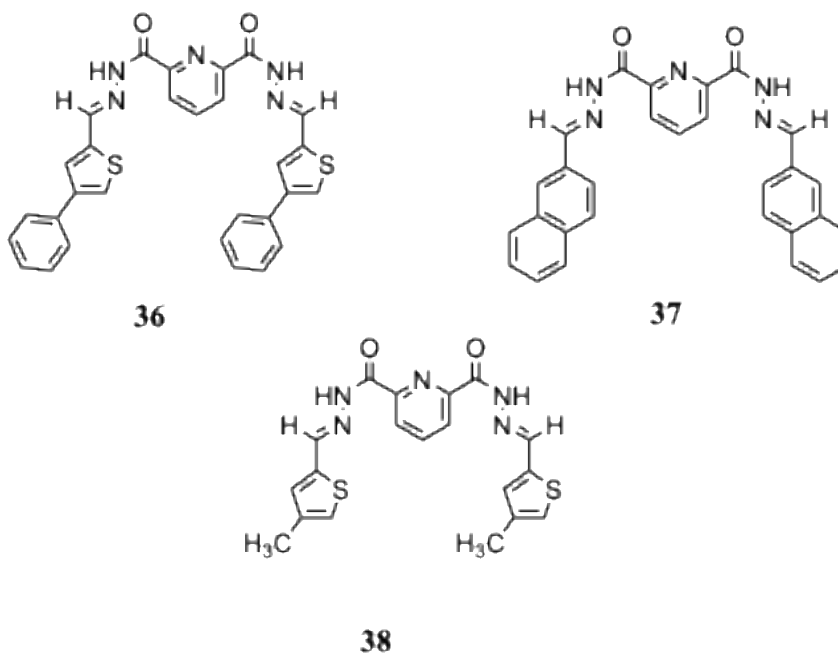
Figure 11: a representation of naked eye sensing of Cu^{2+} by compound 35. Reproduced with permission of the authors.

Table 2: Summary of chemosensing section with radicals attached to core based on Scheme 1. along with reported % yield, detected ions and detection limit.

Number	R ₁	R ₂	% Yield	Ions Detected	Detection Limit (μM)	Reference
23			68.45	Cu^{2+}	0.31	(Amini et al., 2020b)
30			79.6	Cu^{2+}	0.004	(Bhattacharyya et al., 2016)
			50	CN^-	0.10 N.R..	(Yadav & Singh, 2018b)
31			90	Cu^{2+}	0.12	(R. Kumar et al., 2018b)
				F^-	1.4	
				Acetate	1.4	
				AMP^{2-}	0.08	
32			74	Cu^{2+}	0.12	(Yadav & Singh, 2018b)
				CN^-	0.31	
33			60	Cu^{2+}	0.097	
				CN^-	N.R.	
34			50	Cu^{2+}	0.098	
				CN^-	0.53	
35			95	Cu^{2+}	0.0042	(Zamani et al., 2023b)

2.6.3. Biological activity

The attention towards bis(aldehyde/ketone)-2,6-dipicolinoylhydrazones is growing with the development of science in many fields due to their great potential. Biologically, anti-cancer activity of such derivatives was studied through synthesizing 12 derivatives and screen them against human colon cancer cell line (HT-29) and Ishikawa human endometrial cancer cell line (ISH). Compounds **36** and **37** were of significant activity against (HT-29) cell line with IC_{50} of 8.88 μ M and 6.78 μ M, respectively. For (ISH) cell line, compound **38** was of best IC_{50} value with 8.26 μ M (Şenkardeş et al., 2021).



A computational study held by Desai et al. (Desai et al., 2004) 241,000 compounds were screened in three stages virtually for their potential anti-parasitic against cysteine proteases enzyme. First stage involved filtration of the molecular database by ADME protocol to eliminate non-drugs candidates. The result was obtaining 60,000 molecules out of 241,000. Then, docking was done by GOLD software against malarial and leishmanial cysteine protease. Long story short, after the docking, 100 compounds were atop of the list and they passed to next stage in which they were biologically screened in vitro against falcipain-2, falcipain-3, *P. falciparum*, and *L. donovani* promastigotes. 24 compounds were reported to be active and among them is compound **23**, see Table 1 and 2. Yet, the compound's anti-leishmanial activity was reported to be null. Still, that compound has passed through all of these stages in between 241,000 indicates that structural modifications and choosing another target might open the way for new drug candidate that is a derivative of **23**. Away from this study, up to our knowledge, these derivatives have not been applied previously to such application, and that further investigation might be a steppingstone towards paving the way for new drug candidates.

2.7. Targets of anti-leishmanial drugs

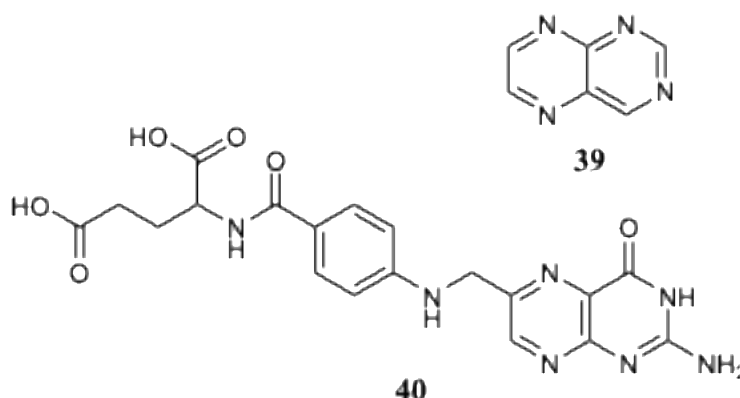
2.7.1. Overview

Leishmania parasites metabolic pathways are crucial in its proliferation and growth. Therefore, it would be rational to encounter leishmania parasites infections by targeting these metabolic pathways. Since these pathways are crucial, their impediment must cause death or growth suppression of parasites. Also, targets must be completely absent or different from the host homolog (Jain et al., 2022).

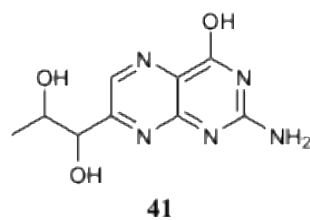
In the recent years, many drugs targets in leishmania species have been identified including sterol biosynthesis pathway (de Macedo-Silva et al., 2022), thiol metabolism pathway (Battista et al., 2020), hypusine pathway (Chawla et al., 2012), glycosylphosphatidylinositol pathway (Mohammed et al., 2020), glycolytic pathway (Ohms et al., 2021), purine salvage pathway (Barazorda-Ccahuana et al., 2024), nucleoside transporters (Aldfer et al., 2022), cyclin dependent kinase (Efsthathiou & Smirlis, 2021), mitogen activated protein kinase (Baker et al., 2021), enzymes of polyamine biosynthesis pathway (Carter et al., 2022), peptidase (Bhat & Qureshi, 2021), DNA topoisomerase (Rosa-Teijeiro et al., 2021), metacaspase (Eyssen & Coetzer, 2020), glyoxalase system (Ezema et al., 2023), and enzymes of folate biosynthesis pathway (Bigot et al., 2023).

2.7.2. Folate biosynthesis pathway

Pteridine **39** is a crucial cofactor in all living organisms, and it plays a significant role in metabolic functions as it is known to be transferring one-carbon units. Folic acid **40** is a pteridine unconjugated derivative that has *p*-aminobenzoic acid moiety that is glutamylated (Vickers & Beverley, 2011).



Studies on protozoan showed that such species are pteridine auxotrophs, meaning that they require an exo-sources of conjugated and unconjugated pteridines to complete their growth process. Along with folic acid, parasites salvage an important unconjugated pteridine from their hosts that is known as biopterin **41**. The extent in which leishmania species are capable of converting biopterin into folate in a matter of dispute yet it was revealed that they could live on biopterin alone (Vickers & Beverley, 2011).



Folates cofactors play a crucial role in various metabolic functions such as nucleic acid synthesis and amino acid metabolism. Thus, the growth and development of these protozoan are highly associated with this pathway (Jain et al., 2022). Dihydrofolate reductase (DHFR) and thymidylate synthase (TS) are key enzymes in this metabolic pathway. Homology studies showed that DHFR is 58% identical to humans and TS is 31%. Folic acid is inactive as it is and thus it needs to get activated by conversion into tetrahydrofolate that is essential in the process of DNA synthesis. In the process, folic acid is converted by two steps mediated by DHFR-TS into tetrahydrofolate as represented in Figure 12 (Raj et al., 2020).

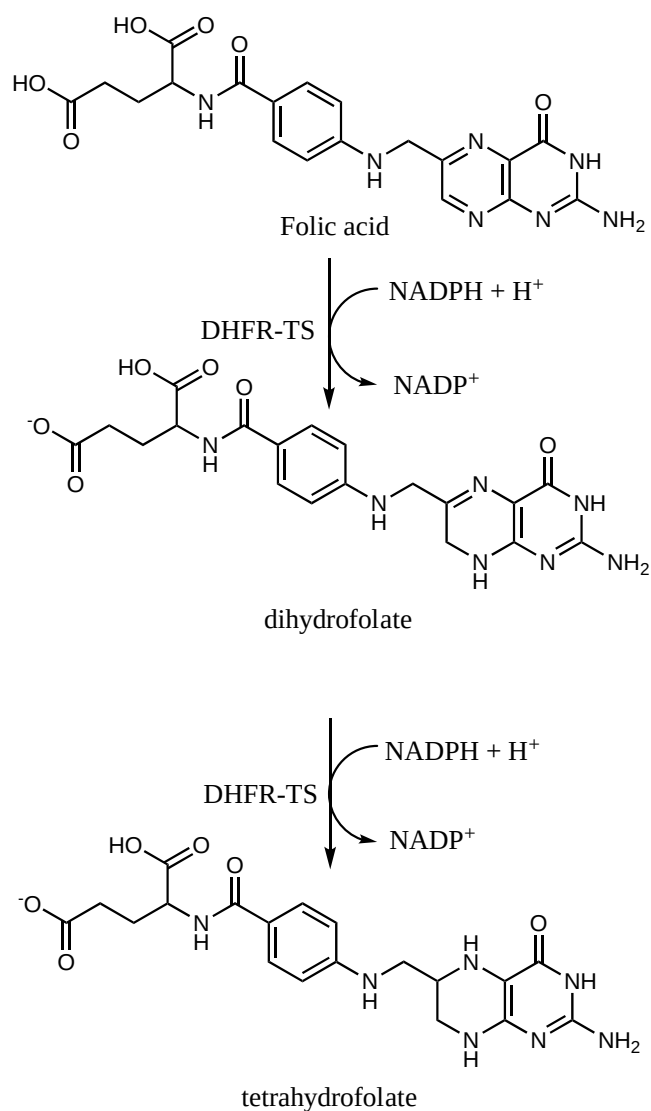


Figure 12: Folic acid conversion into tetrahydrofolate mediated by DHFR-TS enzymes.

Targeting DHFR-TS is a beneficial way to stop the growth of leishmania parasites, yet it was observed that inhibition of these enzymes led to activation of another pathway in which the salvaged biopterin is used to produce an analog of tetrahydrofolate named tetrahydrobiopterin **42** (Jain et al., 2022; Vickers & Beverley, 2011). After investigations, pteridine reductase 1 (PTR1, PDB ID: 7PXX) enzyme was identified and it was reported that it mediates this process, as illustrated in Figure 11. It was reported that participates too in the first process of conversion of folic acid into tetrahydrofolate (Jain et al., 2022; Raj et al., 2020; Vickers & Beverley, 2011).

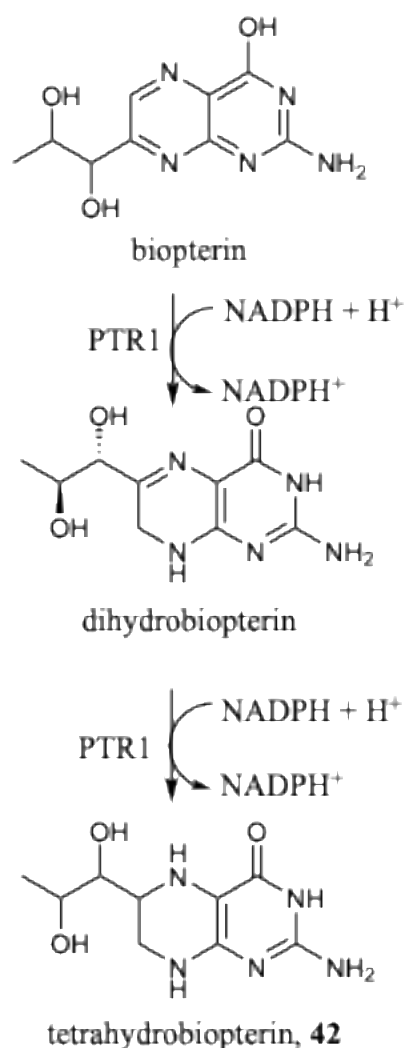
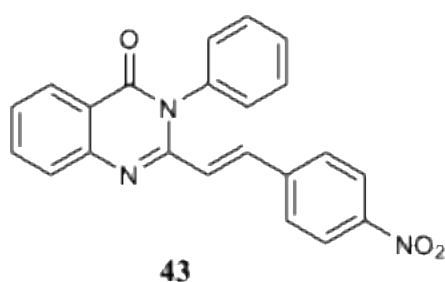


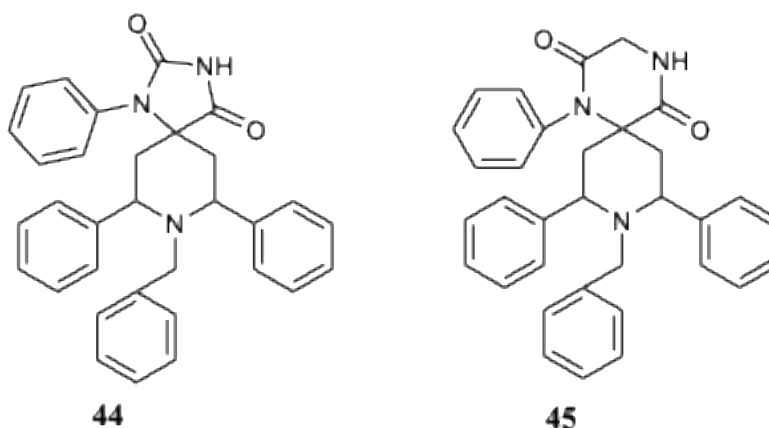
Figure 13: conversion of biopterin into tetrahydrobiopterin by PTR1 enzyme.

Unlike DHFR and TS, PTR1 is not found in humans (Jain et al., 2022). Also, DHFR-TS inhibitors are no longer working due to resistance of leishmania species (Vickers & Beverley, 2011) and no resistance of PTR1 inhibitors was reported yet (Jain et al., 2022). Therefore, targeting DHFR-TS and PTR1 simultaneously is a rational strategy at this stage.

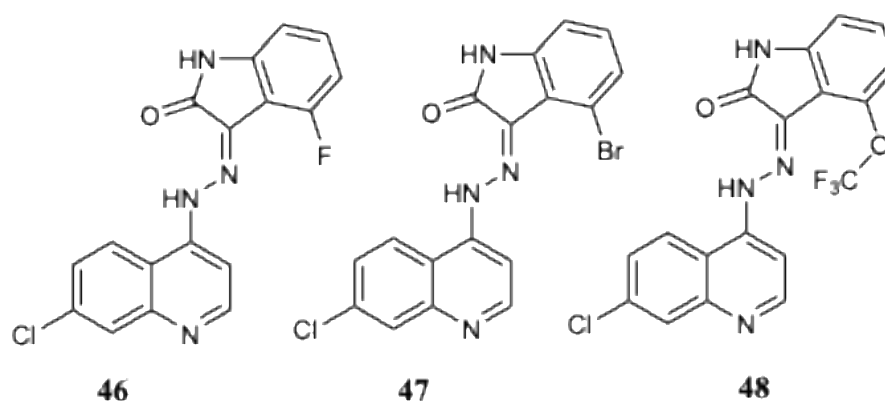
In a study anti-malarial and anti-leishmanial activities of quinolones derivatives were investigated after synthesizing the compounds through cyclisation, condensation, and hydrolysis. For anti-leishmanial activity, *L. donovani* strains were screened in vitro against the synthesized compounds and compound **43** showed promising activity with IC_{50} less than standard drugs AmB-D and miltefosine by 2 and 150 times, respectively. These results made **43** candidate to be screened via molecular docking against Lm-PTR1 to further understanding its observed potential. Docking study revealed that the compound has fit the binding site perfectly with binding energy of -9.2 kcal/mol. Further, hydrogen bondings were detected with His241, and phenyl group moiety was packed deeply between Phe113, Leu188, Leu226, Leu229, and Val230. Since PTR1 is NADPH-dependent, thus inhibiting its function is useful to block the action of the enzyme and that was demonstrated as the quinolone moiety exhibited hydrogen bonding and π - π stacking with NADPH. All of this justifies the obtained results of *in vitro* of compound **43** (Seifu et al., 2022).



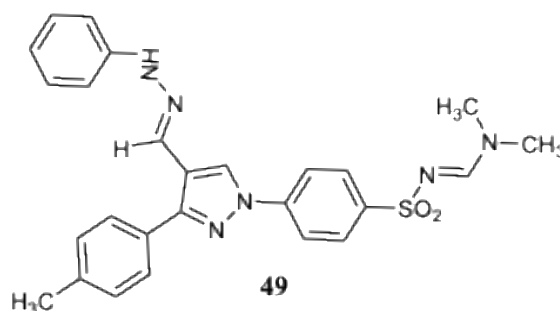
Mohamed et al. (Mohamed et al., 2023) synthesized spiro-piperidine derivatives using ionic liquids and the products were tested for their anti-leishmanial in vitro activity against *L. major* amastigotes and promastigotes. All compounds were of good activity yet compounds **44** and **45** were of superior activity compared to the standard drug miltefosine ($IC_{50} = 8.08 \mu M$) with IC_{50} values of $0.89 \mu M$ and $0.50 \mu M$. The observed *in vitro* results of these compounds were further investigated by molecular docking against Lm-PTR1. The docking score of compounds was -7.8 and -8.0 kcal/mol, respectively. In both compounds, the piperidinyl group formed ionic interactions with through its nitrogen with the phosphate of NADPH and thus blocking its action. Unlike the pervious study, the inhibition of this pathway was validated by reversal anti-leishmanial activity by folic acid and folinic acid test. The results showed that compounds **44** and **45** both were both acting as anti-folate agents with survival percentage after the addition of folic and folinic acids in the range of 72-87%, compared to 20-26% before the addition.



In another study held by Sabt et al. (Sabt et al., 2023) 20 quinolone-isatin derivatives were synthesized and tested *in vitro* against *L. major* species. All compounds were better than miltefosine reference drug against promastigotes of this parasite with IC₅₀ values ranging from 0.5084–5.9486 μ M compared to 7.8976 μ M of reference drug. In amastigote form, compounds' activity was ranging from 0.60442–8.2948 μ M vs. 8.08 μ M of miltefosine as IC₅₀ values. Furthermore, three compounds (**46**, **47**, and **48**) were the most active in this series against both forms of leishmania species. Molecular docking study on these compounds showed that they do inhibit the catalytic activity of *Lm*-PTR1 as they pack through the NADPH pocket with docking scores ranging from -11.71 and -12.43. Validation of docking results was done by folate test in which survival percentage before the addition of a competitor was 21–25% and after the addition of the competitors survival percentage increased to 74–98%, thus such compounds are believed to act as anti-folate agents in parasites. Compared to the previous study, the growth inhibition expressed by folate test is better in this study as the values are higher which suggests that hydrazone bond and aromatic nitrogens might enhance the inhibition.



In a recent study, pyrazole hydrazone derivatives were synthesized and their anti-malarial and anti-leishmanial activities were studied *in vitro*. For Compound **49** was of the best anti-promastigote activity against *L. aethiopica* with IC₅₀ of 0.018 μ M that is better than reference drugs miltefosine and AmB-D by 174 and 2.6 folds. The active compound's activity was further studied by molecular docking and the results showed that compound **49** was successfully fitting the active site of the enzyme *Lm*-PTR1 with binding energy of -9.8 kcal/mol. Also, most importantly π - π stacking with NADPH was observed and thus the enzyme got deactivated (Berhe et al., 2024). These results, suggest too that such activity could be attributed to the presence of hydrazone, aromatic nitrogens and aryl groups.



Chapter (3)

3. Experimental

3.1. Materials

Melting point was determined by open capillaries via Stuart Scientific (SMP-1). IR spectra were obtained using SHIMADZU FT-IR spectrometer (IRPrestige-21) for all samples except sample **II** that was dissolved in Dichloromethane (DCM) and analysed by Perkin Elmer FT-IR spectrometer (Spectrum-1). ^{13}C and ^1H NMR were recorded by JNM-ECZ500R, Jeol FT-NMR 500MHz, spectrometer at University of Alexandria, department of chemistry, Egypt and MNova software was used to analyse the data at University of Bahrain. Elemental analysis was performed at University of Ain Shams, Cairo, Egypt. Reaction was monitored by Silica gel TLC plates of 0.25 mm thickness purchased from Merck (25 HPTLC Aluminium Sheets 20 x 20 cm Silica gel 60 F₂₅₄) and spots were visualised by ultraviolet (UV) light (UVitec).

Anti-leishmanial evaluation, the following materials were used: polystyrene tissue culture flasks obtained from Corning, 96-well chambered plates and micropipettes from Pipetman, ultra, multichannel pipettes bought from Hamilton, haemocytometer, eppendroff tube,), pipette tips by Oxford syringes (20 and 25 gauges) and aluminium foil. Instrumentations used for latter purpose are: light microscope, inverted and normal types by Motic & Olympus, class II biosafety cabinet from Labconco, Pristage's autoclave, analytical balance by Mettler Toledo (AB204-S), microwell plate reader by PerkinElmer, Thermo Electro's carbon dioxide (CO₂) incubator, and centrifuge from Hettich/ Thermo Electro, vortex mixer (whirl VIB2).

3.2. Chemicals

Aldehyde, ketones and DPA were purchased from Fluka and Aldrich. Solvents used dimethyl sulfoxide (DMSO), DCM, absolute ethanol, pyridine, and methanol were purchased from Fluka. Solids including MgSO₄, Na₂CO₃ and CaCl₂ were obtained too from Fluka.

Miltefosine was obtained from AG Scientific, San Diego, CA, USA, and AmB-D was purchased from Fungizone®, ER Squibb, UK.

3.3. Culture medium for anti-leishmanial activity

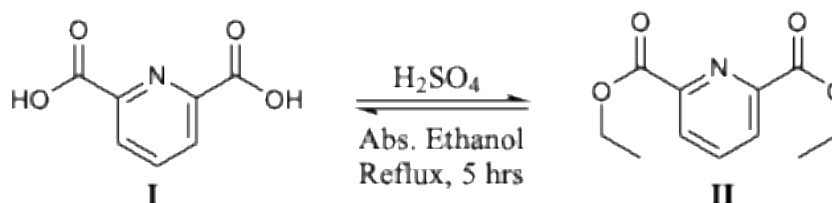
Axenic *L.major* culture mediums were supplied with RPMI-1640 from Gibco, Invitrogen Co., UK, 10% heat- inactivated fetal calf serum (HIFCS), 1% penicilline-streptomycin and 1% L-glutamine all from Sigma Chem. Co., St. Louis, USA.

3.4. Chemistry

3.4.1. Method

3.4.1.1. *Synthesis of diethyl dipicolinate, II*

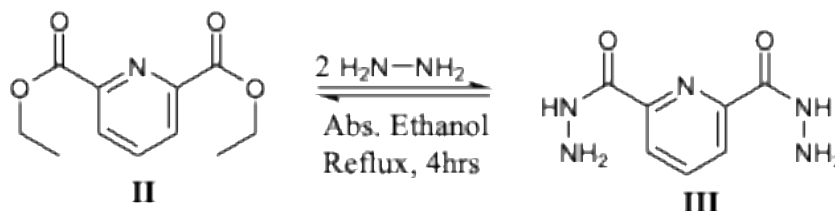
Diethyl dipicolinate, was synthesized from dipicolinic acid and absolute ethanol according to Scheme 2. Dipicolinic acid, (15g, 0.090mol) was dissolved in absolute ethanol and kept refluxing while stirring. TLC was checked to determine the end of reaction. After 5 hours, ethanol was evaporated by rotary evaporator and crude product was dissolved in DCM and washed with 5% NaCO₃ solution several times. The solution was then dried by MgSO₄, and excess DCM was evaporated by the rotatory evaporator. Obtained product was dried over CaCl₂ and was used for the next step.



Scheme 2: Synthesis of diethyl dipicolinate by reacting DPA with ethanol in the presence of drops of H₂SO₄:

3.4.1.2. *Synthesis of dipicolinic hydrazide, III*

5g (0.022mol) of the obtained product of compound **II** were dissolved in absolute ethanol and kept on ice bath and the 2 equivalences of hydrazine hydrate (1.4mL, 0.045mol) were added dropwise using a syringe. Then, reaction was brought into room temperature and solution turned from colourless into greenish-white. Next, reaction pot was heated on oil bath for 4 hours and white precipitate was obtained according to Scheme.3. The precipitate was then collected by filtration. Crude product was recrystallised from distilled water and white needles-like crystals were obtained of 2,6- dipicolinoylhydrazine, **III**. The product was dried over CaCl₂ and was used in further synthetic stages.

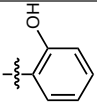
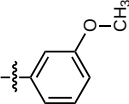
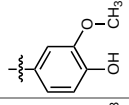
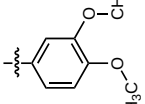
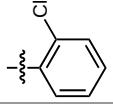
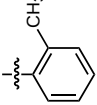
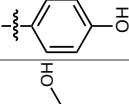
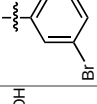
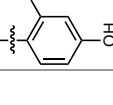


Scheme 3: reaction of **II** with hydrazine to obtain **III**.

3.4.1.3. Synthesis of bis(aldehyde/ketone) dipicolinoyl hydrazones, IV-XII:

Bis(aldehyde/ketone)-2,6-dipicolinoylhydrazones, **IV-XII**, were synthesized according to Scheme 1. A mixture of aldehyde or ketone (0.0021mol) and 10 drops of glacial acetic acid were heated under reflux in absolute ethanol on an oil bath for 15 minutes. Then, 0.2g (0.00101mol) of corresponding the hydrazide (3) was dissolved in the same solvent and added to the mixture portion-wisely for 30 minutes. Then, reaction continued for 6 hours while monitoring the progress over TLC plates. The crude product was collected by filtration and recrystallised from suitable solvents as shown in Table 3. Finally, products were kept in desiccator over CaCl₂ to dry up and used in further stages of the research.

Table 3: Bis(aldehyde/ketone) dipicolinoyl hydrazones products along with recrystallisation solvents. where R₁ and R₂ are based on Scheme 1:

Number	R ₁	R ₂	Recrystallisation Solvent
IV			Methanol
V			DMSO
VI			Pyridine
VII			Ethanol
VIII			DMSO
IX			DMSO
X			Ethanol
XI			DMSO
XII			Water

3.5. Biological assay

3.5.1. *In vitro* antileishmanial activity

The synthesized compounds were evaluated against both life cycle stages of *L. major* parasite namely intracellular amastigotes and extracellular promastigotes at university of Alexandria, Egypt. Throughout the assay, highest concentration of DMSO was 1% as it has no impact upon the growth of parasites. *L. major* isolates were studied and treated also with standard drugs (miltefosine and AmB-D) to compare their activity with the synthesized compounds in this research. The method of assay was done with reference to reported methods by Sabt et al. (Sabt et al., 2023), Mohamed et al. (Mohamed et al., 2023) and Bekhit et al. (Bekhit et al., 2015). For the anti-promastigotes assay, a 96-well flat-bottom plate was used, where 100 μ L of culture media containing 3×10^6 *L. major* promastigotes were seeded into each well. Various dilutions of the newly synthesized compounds (10, 3.33, 1.11, 0.37, 0.12, and 0.04 μ M) were then added to the promastigote cultures. Positive control wells contained only the promastigotes, while negative control wells contained 1% DMSO and media alone. The tests were performed in duplicate, and the plates were incubated at room temperature ($21 \pm 1^\circ\text{C}$). After 24 hours, 10 μ L of Alamar Blue reagent (prepared by dissolving 12.5 mg of resazurin in 100 mL of distilled water) was added to each well. The fluorescence intensity of the resulting mixture was measured after 48 hours using an ELISA plate reader at wavelengths of 540 and 630 nm. The Alamar Blue assay works by the conversion of the active ingredient resazurin (blue and non-fluorescent) to resorufin (pink and highly fluorescent) by reduction reactions in metabolically active cells.

With reference to the latter authors, anti-amastigotes assay was done by diluting test compounds serially in a 96-well plate to achieve a final test concentration range of 0.04-10 μ M in 50 μ L of culture medium. Subsequently, 50 μ L of a suspension containing 2×10^7 axenic amastigotes per mL was added to each well. The plate contents were then incubated in a humidified atmosphere at 31°C under 5% CO_2 for 72 hours. After 68 hours of incubation, 10 μ L of a fluorochrome resazurin solution (consisting of 12.5 mg of resazurin dissolved in 100 mL of phosphate-buffered saline (PBS) at pH 7.2) was added to each well. The absorbance of the resulting mixture was measured after a total incubation time of 72 hours using a Victor 3 Multi-label Counter, with an excitation wavelength of 530 nm and an emission wavelength of 590 nm.

The IC_{50} value for each compound was then evaluated from sigmoidal dose-response curves using the Graphpad Prism 3.0 software, and the results were expressed as the mean $\pm$ standard deviation (SD) of triplicate experiments, with each test concentration measured in duplicate. Assays were also performed using standard antileishmanial drugs and negative controls (medium alone and 1% DMSO) to obtain reference values. Additionally, the background fluorescence intensity of each compound and reference drug was measured.

3.5.2. Reversal of antileishmanial activity of most active compound by folic acid and folinic acid

Leishmania promastigotes were cultured in the logarithmic phase of growth and used for the *in vitro* growth inhibition assay. The most active compound was tested at a concentration above its IC₅₀ value, while trimethoprim, a known antifolate drug, was used as a positive control at a concentration of 100 µM. To investigate the reversal of antileishmanial activity by folinic acid and folic acid, *L.major* promastigotes (1 × 10⁶ cells/mL) were incubated with either 20 µM or 100 µM of folinic acid or folic acid for an hour at room temperature. After the incubation, the parasites were washed and resuspended in fresh culture media. The test compound or trimethoprim were then added to the parasite suspensions at the previously specified concentrations, and the 24-well plates containing the treated parasites were incubated for 48 hours. Finally, the percentage survival of parasites was calculated using the formula:

$$\% \text{ Survival} = 100 - \% \text{ AP};$$

where % AP is the percentage growth inhibition, and it is calculated by:

$$\% \text{ AP} = (T_c - T_p) / T_c \times 100\%$$

Where T_c is the number of parasites/ml in the control wells and T_p is the average number of moving parasites/ml (Mohamed et al., 2023; Sabt et al., 2023).

3.5.3. *In vitro* cytotoxicity testing

The cytotoxicity of the compound that which exhibited promising antileishmanial activity, was investigated *in vitro* against African green monkey kidney (VERO) cells. In a 96-well plate, VERO cells 1×10⁵ cells per well were incubated in triplicates with different concentrations of the most active compound (0–100 µM) for 72 hours at 37°C in an incubator with 95% humidity and 5% CO₂. The cell viability was determined by measuring the optical density (OD) of formazan. The CC₅₀ value, which is the concentration of the compound required to kill 50% of the fibroblast cells, was calculated and compared with the standard drug, miltefosine. Both the CC₅₀ and IC₅₀ values (the concentration of the most active compound required to kill 50% of *L.major*) were computed using the formula:

$$\text{Growth inhibition (\%)} = (OD_{\text{control}} - OD_{\text{test}} / OD_{\text{control}}) \times 100.$$

Additionally, SI was calculated using the formula:

$$SI = CC_{50} / IC_{50}.$$

3.6. *In silico* investigations

3.6.1. Molecular docking

The X-ray structures of *Lm*-PTR1 enzyme was retrieved from the Protein Data Bank (PDB) with PDB ID: 7PXX. The PDB structure was prepared using OpenEye's MakeReceptor GUI of OEDOCKING 3.5.0.4 of OpenEye Scientific Software^{31,32} at default levels. Redundant chains, non- essential ions, water molecules and ligands were discarded. The co-factor NADPH was kept. The search box around the X-ray co-crystal ligand was set 22.80 Å × 24.95 Å × 23.66 Å with outer contour volume of 1989 Å³. Site shape potentials were calculated via setting the binding site contours in a balance between the protein and the solvent-exposed area. The protein files were saved as OEDU format for FRED docking. The structures of the compounds were built, then different conformations were generated for FRED docking via OpenEye OMEGA conformer generation software. The docking was carried out using OpenEye's FRED docking software at default levels. The docking settings were confirmed via successful pose-retrieval experiment of the co-crystal ligand of *Lm*-PTR1 PDB ID: 7PXX. The 3-D and 2-D depictions of the docking poses and binding sites were generated via Molecular Operating Environment (MOE).

3.6.2. ADMET Predictions

ADMET of the most active compound were conducted using SwissADME (Daina et al., 2017) and ProTox 3.0 (Banerjee et al., 2024) web tools with its underlying methodologies to calculate descriptors for physicochemical parameters, drug-likeness parameters, medicinal chemistry friendliness parameters and toxicity.

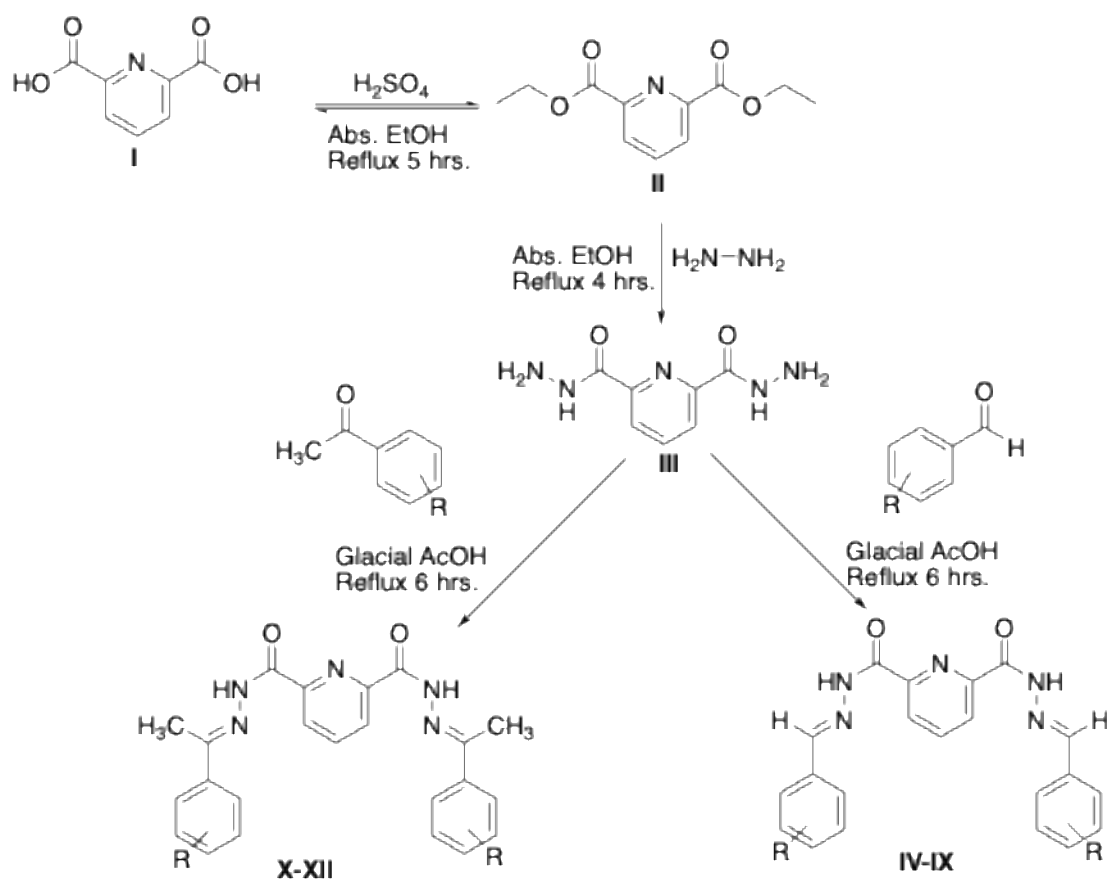
Chapter (4)

4. Results and discussion

4.1. Chemistry

4.1.1. Synthesis of the target compounds

Scheme 4 represents the synthetic scheme to obtain target compounds.



Scheme 4: the designed synthetic scheme to obtain target compounds.

4.1.1.1. *Synthesis of diethyl dipicolinate, II*

Compound **II** was synthesised in a typical Fischer-esterification method. The mechanism of reaction that is shown in Figure 14 starts with protonation of carbonyl group that would make its carbon more available for nucleophilic addition of ethanol. Then, ethanol attacks in a nucleophilic addition reaction. Next, another proton gets transferred from sulfuric acid that facilitate water molecule elimination and finally ester get formed by deprotonation.

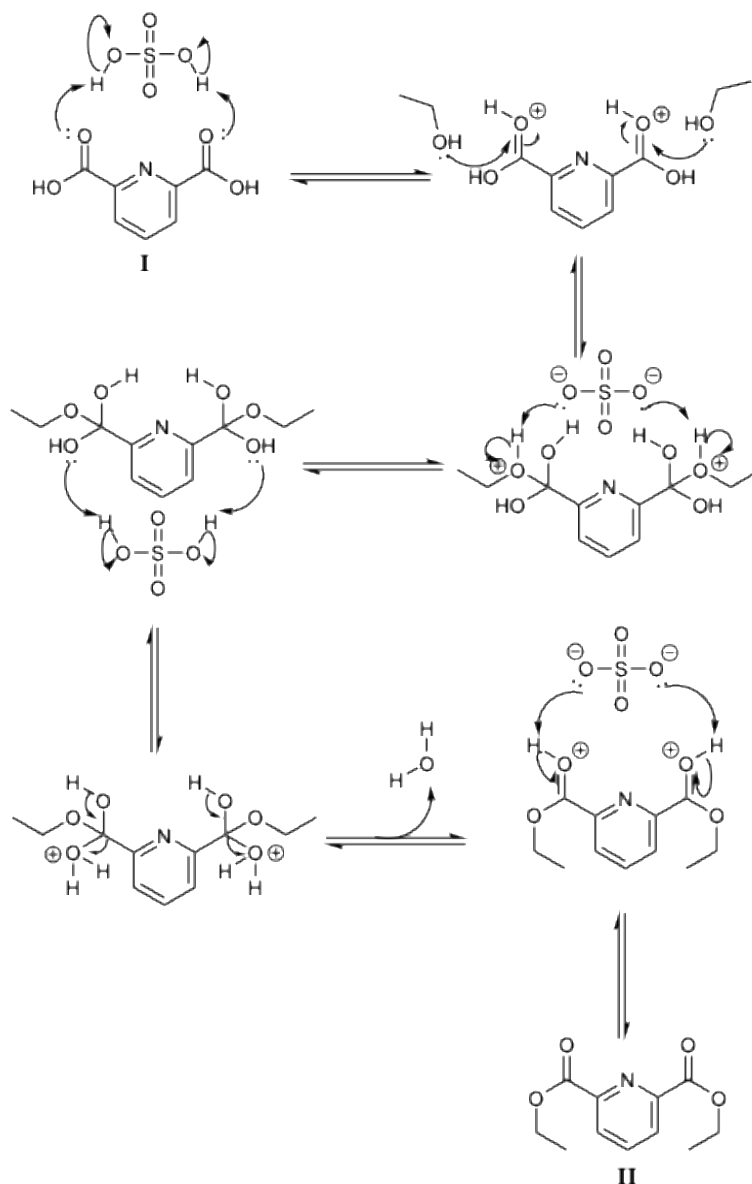


Figure 14: Fischer Esterification Mechanism for formation of 2:

Since the reaction is reversible, it is expected that part of the starting material is still available and thus to obtain pure product of **2**, DPA must be removed. The latter was achieved by acid-base extraction that is depicted in Figure 15 using 5% Na_2CO_3 solution. Extraction process was monitored by TLC plate. Eventually, organic layer was with a single spot on TLC plate with R_f of 0.86 in 10% Methanol/DCM system.

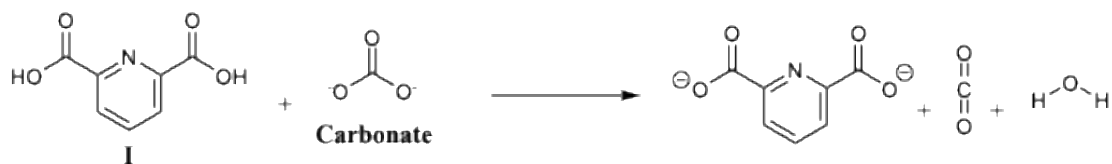


Figure 15: acid base reaction between DPA (**I**) and carbonate ion that was the basis for extraction to get pure product of **II**.

Organic layer was dried by adding Mg_2SO_4 until no more turbidity is observed and then solution was filtered. Filtrate was collected and excess DCM evaporated by rotatory evaporator to obtain crystals of **II**. Finally, product's melting point was determined, IR, ^1H and ^{13}C NMR, and elemental analysis were done to validate the obtained product.

4.1.1.2. *Synthesis of Dipicolinic hydrazide, II*

Dipicolinoylhydrazine, **3** was synthesized based on literature in which hydrazine hydrate is added to compound **2** that was dissolved in ethanol and heated under reflux for 4 hours (Amini et al., 2020; Bhattacharyya et al., 2016; Chakraborty et al., 2021; R. Kumar et al., 2018; Yadav & Singh, 2018; Zamani et al., 2023). The mechanism of reaction is a typical nucleophilic reaction, and it is shown in Figure 16. Ethanolate is a good leaving group thus hydrazine could replace it through nucleophilic addition.

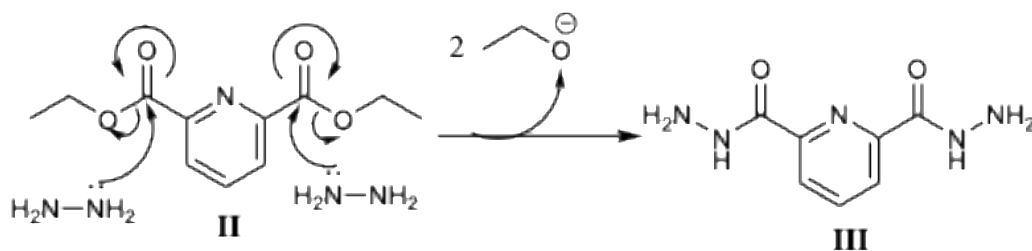


Figure 16: mechanistic representation of nucleophilic addition of hydrazine to compound **II** to form compound **III**.

Avoiding polymer formation or any other side reaction possible, the reaction was first cooled down using ice bath that allows rate of reaction to decrease. Then after the addition of hydrazine, the reaction was kept for 30 minutes on the ice bath and then, it was brought into room temperature. Upon reaching to room temperature, change in solution's colour was observed from colourless into greenish-white, which indicates that reaction is started. Had it been kept at room temperature from start, it would start immediately, and it would be difficult to control it in case of any side reaction. For 1 hour, reaction was kept at room temperature and white precipitate started forming. To increase rate of reaction, reaction pot was heated under reflux on an oil bath and allowed for 4 hours. Crude product was collected through filtration and recrystallised from water according to previous research (Li et al., 2007) to obtain needle-like white crystals. Crystals were collected after one week by filtration and washed several times by water to remove any hydrazine or ethanol attached to crystals. For 5 days, product was kept in desiccator over CaCl_2 drying agent.

4.1.1.3. Synthesis of bis(aldehyde/ketone) dipicolinoyl hydrazones, IV-XII

Scheme 1 was used to synthesise compounds **IV-XII**. First step was to activate aldehyde or ketone using glacial acetic acid rendering carbonyl being more available for nucleophilic addition as Figure 17 shows.

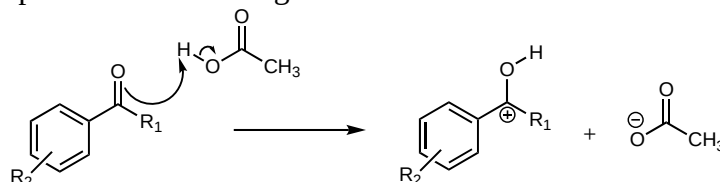


Figure 17: activation of aldehyde mechanism in which $R_1 = -H$ or $-CH_3$ and R_2 is based on Table 3.

Activation of aldehyde or ketone carbonyl group was observed by change in solubility of the added compound as it dissolved completely. The latter process took 15 minutes on average. Then, hydrazide (**3**) was added in portions to the mixture over 30 minutes period. The mechanism of reaction is illustrated in Figure 18. Mechanistically, the amine group will reach to the activated compounds and since huge amount of aldehyde/ketone is present compared to 2,6- dipicolinoylhydrazine (**3**), it is expected that both sides of will be converted into hydrazone. Acetic acid mediates the elimination of water molecule and formation of imine bond.

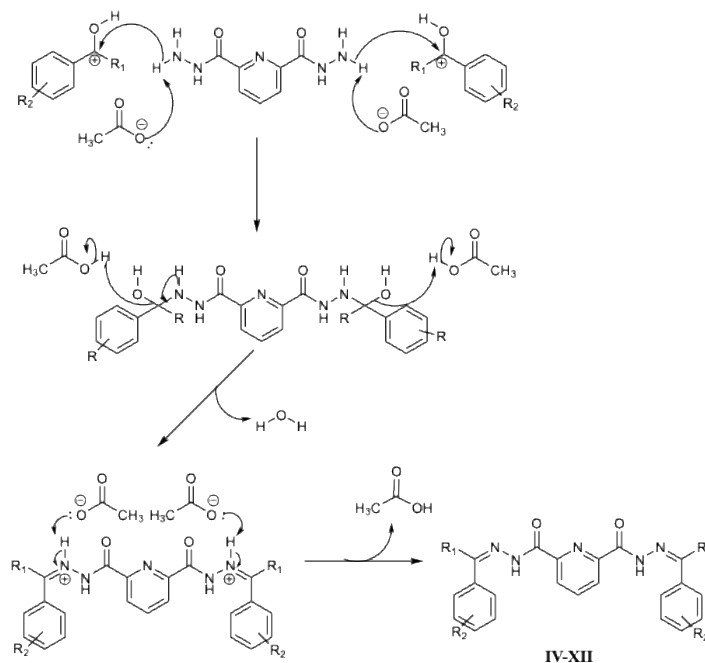


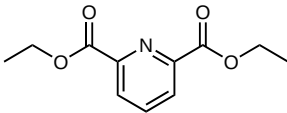
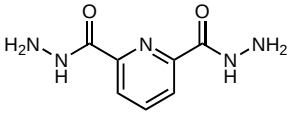
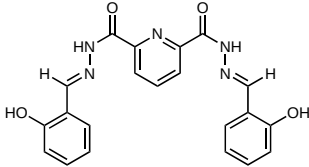
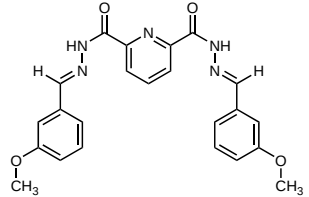
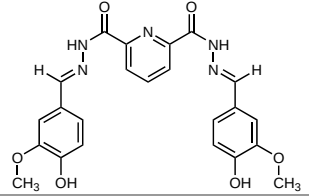
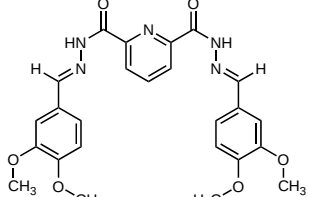
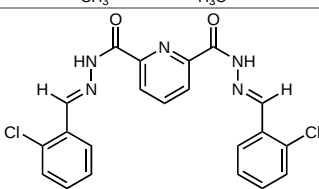
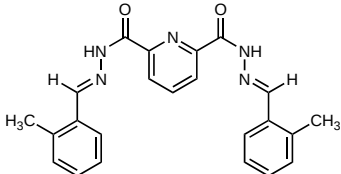
Figure 18: mechanism of formation of hydrazones (**VI-XII**) after the activation of aldehyde/ketone (Figure 17). $R_1 = -H$ or $-CH_3$ and R_2 is based on Table 3.

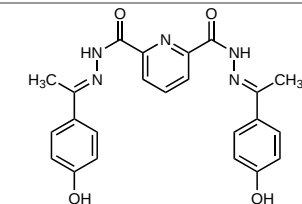
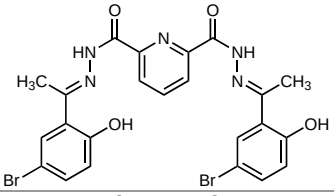
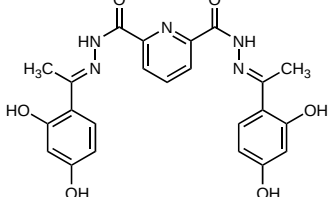
The reaction progression was monitored by TLC plates and after 6 hours no further progress was observed thus reaction was allowed to cool down to room temperature and crude product was obtained by filtration. Recrystallisation was done using different solvents as listed on Table 3 that suits the properties of each product. After structural validations, anti-leishmanial activity of compounds was screened.

4.2. Physical properties of the synthesized compounds

Initial stage analysis of was carried by determining melting point and calculating percentage yield of synthesized compounds as Table 4 shows.

Table 4: structure, molecular formula, molecular weight, calculated percentage yield and determined melting points of synthesized compounds.

Number	Structure	Molecular Formula	Molecular Weight (g/mol)	Yield (%)	Melting Point (°C)
II		C ₁₁ H ₁₃ NO ₄	223.08	60.4	35-37
III		C ₇ H ₉ N ₅ O ₂	195.08	85.46	295-297
IV		C ₂₁ H ₁₇ N ₅ O ₄	403.4	77.37	329-330
V		C ₂₃ H ₂₁ N ₅ O ₄	431.45	58.77	272
VI		C ₂₃ H ₂₁ N ₅ O ₆	463.45	82.46	265-267
VII		C ₂₅ H ₂₅ N ₅ O ₆	491.5	79.38	274-275
VIII		C ₂₁ H ₁₅ Cl ₂ N ₅ O ₂	440.28	70.89	324
IX		C ₂₃ H ₂₁ N ₅ O ₂	399.45	73.1	309-310

X		$C_{23}H_{21}N_5O_4$	431.45	66.3	330
XI		$C_{23}H_{19}Br_2N_5O_4$	589.24	33.1	292
XII		$C_{23}H_{21}N_5O_6$	463.45	95	296-297

Compounds were synthesised with a good yield of 33.1% to 95%. Melting points of compounds **VI**, **VIII**, **X**, and **XI**, have decomposed on the reported temperatures on table 4 which means that they are not thermally stable at these temperatures. The remaining compounds were melting in the range of 2°C, that shows that compounds are pure. Further, for the same compound there are variations among the reported temperatures, for example compound **III** melting point was reported to be 248°C by Amini et al. (Amini et al., 2020), also reported by Zamani et al. (Zamani et al., 2023) to be 299°C and 285°C by Gudasi et al. (Gudasi et al., 2006). Such variation gives indication that even though melting point is important to be determined yet it is not a decisive qualitative test -at least for these compounds- and that further analytical tools are required for identification of the synthesized compounds. The latter was done by employing spectroscopic and elemental analysis to validate synthesizing the aimed compounds.

4.3. Spectroscopic analyses of synthesized compounds

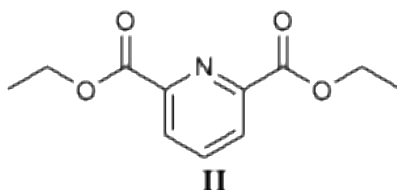
Structural elucidation through spectroscopic analysis was done using IR, and NMR spectroscopic techniques. NMR results were obtained after dissolving samples in DMSO and the chemical shifts are given in δ (ppm). Splitting patterns were designed as follows: *s*: singlet; *d*: doublet; *t*, triplet; *q*: quartet; and *m*: multiplet. Example spectra are attached in the appendix.

4.3.1. Diethyl dipicolinate, II

IR (KBr) (cm^{-1}): 3056.10 ($-\text{OCH}_2-$), 3050.11 ($=\text{CH}$), 2986.90 ($-\text{CH}_3$), 1741.02 ($\text{C}=\text{O}$), and 1585.26 ($\text{C}=\text{N}$, **3**).

^1H NMR (DMSO); $\delta(\text{ppm})$: 1.25 (*t*, 6H $-\text{CH}_3$), 4.3 (*q*, 4H, $-\text{O}-\text{CH}_2-$), 8.1 (*m*, 1H Pyridine-C4-**H**) and 8.22 (*d*, 2H Pyridine-C3-**H**).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 13 (**C** $_3$), 61 ($-\text{O}-\text{CH}_2-$), 128 (Pyridine-**C** $_3$), 140 (Pyridine-**C** $_4$), 148 (Pyridine-**C** $_2=\text{N}$), 163 ($\text{C}=\text{O}$).



IR spectrum of compound **2** showed strong bands at 3056.10, 3050.11, and 2986.90 cm^{-1} . It was expected to have three peaks in the range of 2850 – 3100 cm^{-1} that represent stretching of ethyl and pyridine protons. Such expectations were met by having first peak at 3056.10 cm^{-1} that represents the stretching of $-\text{O}-\text{CH}_2-$ due to electron withdrawing impact of oxygen. Then second peak at 3050.11 cm^{-1} , represents pyridine protons. Such protons are influenced by the pyridine ring and thus they must appear on spectrum between the peaks of ethyl protons. Methyl hydrogens were at 2986.90 cm^{-1} as they are far from any electron withdrawing atom or group. Ester carbonyl was at expected as strong band at 1741.02 cm^{-1} and finally $\text{C}=\text{N}$ group band was medium at 1585.26 cm^{-1} .

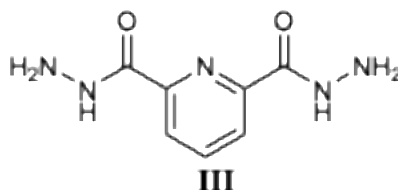
^1H -NMR and ^{13}C -NMR spectra validated structure of compound **II**. First, $-\text{CH}_3$ group protons were shown at 1.25 ppm as triplet, as expected with integration of 6H, and carbon of the same group was obtained at 13 ppm in ^{13}C -NMR spectrum. $-\text{CH}_2-$ group was deshielded due to oxygen attached and thus its protons were at 4.3 ppm as a quartet with integration of 4H. The same group's carbon was obvious at 61 ppm in ^{13}C -NMR spectrum. Carbon 3 (**C** $_3$) of Pyridine ring was obtained at 128 ppm and its proton was shown as doublet at 8.22 ppm with 2H integration. The other proton in the pyridine ring (**C** $_4$) was found at 8.1 ppm with integration of 1H as a multiplet. **C** $_4$ is more deshielded compared with **C** $_3$ and hence it was expected to be obtained at higher field. Finally, ester's carbonyl carbon was obtained at 163 ppm and pyridine's **C** $_2=\text{N}$ was at 148 ppm.

4.3.2. Dipicolinic hydrazide, **III**

IR (KBr) (cm^{-1}): 3265.20 & 3276.61 ($-\text{NH}_2$), 3185.40 ($-\text{NH}$), 3016.11 ($=\text{CH}$), 1691.29 ($\text{C}=\text{O}$), and 1639.03 ($\text{C}=\text{N}$).

^1H NMR (DMSO); $\delta(\text{ppm})$: 4.55 (s, 4H $-\text{NH}_2$), 7.0 (t, 3H Pyridine-C3,4-**H**), 10.6 (s, 2H **NH**).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 124 (Pyridine-**C3**), 140 (Pyridine-**C4**), 149 (**C2**=N Pyridine), 163 (**C**=O).



IR spectrum of compound **III** showed presence of $-\text{NH}_2$ functional group as two adjacent bands at 3265.20 and 3276.61 cm^{-1} that suggests that free amine is present as the aimed structure. Also, at 3185.40 cm^{-1} a strong band of secondary amine was recorded. The amide carbonyl was observed at 1691.29 cm^{-1} that also suggests obtaining the aimed structure. Pyridine ring's protons manifested at 3016.11 cm^{-1} and $\text{C}=\text{N}$ group of the same ring was at 1639.03 cm^{-1} . This primary screening of structure gives hints regarding the obtaining the aimed structure that would be the core for further synthesis. Therefore, NMR analysis was employed to validate the structure.

Structure was further analysed through ^1H -NMR and a singlet peak with chemical shift of 4.55 ppm and integration of 4 protons that is attributed to primary amine ($-\text{NH}_2$). Secondary amine was obtained at 10.6 ppm as expected. Pyridine protons are expected to appear between 6 and 8 ppm. A triplet signal at 7 ppm was recorded with integration of 3H that could be attributed to protons at position **3** and **4** on pyridine ring. Yet, a doublet is expected to be of proton **3** and for proton **4** it must be a multiplet; however, this trend might be resolved using a higher resolution NMR instrument or ^{13}C -NMR that would give a decisive conclusion regarding the structure.

^{13}C -NMR spectrum confirmed the structure with recording four carbon environments. First peak was at 124 ppm, and it was attributed to **C3** on pyridine ring. Second peak was of **C4** at 140 ppm, more deshielded. Such results could be understood going through resonance of pyridine ring. As it can be seen in Figure 19, position 4 is more deshielded due to localisation of a positive charge compared to position 3 that has localisation of electrons that would make it more shielded. Such nuance difference clarifies the previous dilemma as its impact is almost un-observable in ^1H -NMR yet too sharp in ^{13}C -NMR.

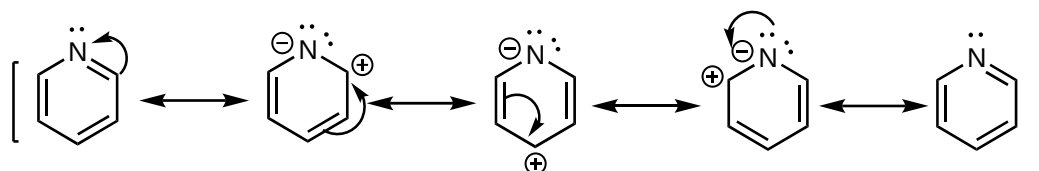


Figure 19: resonance of Pyridine ring:

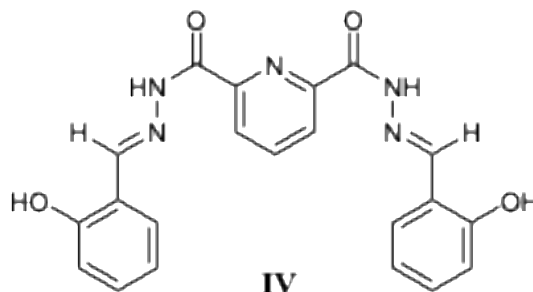
Further, a peak of C=N of pyridine ring was obtained at 149 ppm and finally carbonyl carbon was at 163 ppm. Confirming structure of core compound in this research brings more confidence in coming synthesis results that shall be assigned relatively.

4.3.3. *Bis(2-hydroxybenzaldehyde) dipicolinoyl hydrazone, IV*

IR (KBr) (cm^{-1}): 3448.72 (-OH), 3232.70 (-NH), 3055.11 (=CH), 2870.81 (N=C-H, 7), 1681.93 (C=O), and 1535.34 (C=N).

^1H NMR (DMSO); $\delta(\text{ppm})$: 6.95 (*m*, 4H Phenyl-C3,5-**H**), 7.25 (*m*, 2H Phenyl-C4-**H**), 7.25 (*m*, 2H Phenyl-C5-**H**), 8.20 (*m*, 1H Pyridine-C4-**H**), 8.30 (*d*, 2H Pyridine-C3-**H**), 8.85 (*s*, 2H N=C-**H**), 11.10 (*s*, 2H -OH), 12.40 (*s*, 2H NH).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 116 (Phenyl-**C3**), 119 (Phenyl-**C1**), 120 (Phenyl-**C4**), 127 (Pyridine-**C3**), 129 (Phenyl-**C5**), 133 (Phenyl-**C4**), 141 (Pyridine-**C4**), 148 (hydrazone-**C=N**), 149.5 (Pyridine-**C2=N**), 159.5 (Phenyl-**C2-O-H**), 160 (**C=O**).



The success in synthesizing compound **IV** was validated through analysis of the obtained product. First, IR spectrum showed disappearance of primary amine signal and appearance of new hydroxyl group band that is located around 3448.72 cm^{-1} and secondary amine at 3232.70 cm^{-1} compared to compound **III**. Most important band was at 2870.81 cm^{-1} that is characteristic of imide proton stretching. Such band might suggest the formation of hydrazone bond, yet they will not confirm obtaining the structure of aim, therefore, NMR was used.

A characteristic peak at 8.20 ppm of hydrazone bond N=C-**H** formation was obtained as a singlet and 2H integration value. Such peak along with deshielding of amide proton as it was shifted from 10.6 ppm (compound **4**) towards 12.40 ppm due to impact of C=N bond, aromatic ring peaks at expected places, and appearance of hydroxyl proton at 11.10 ppm were crucial to suggest that compound **4** was successfully obtained. Further validations were done using ^{13}C -NMR.

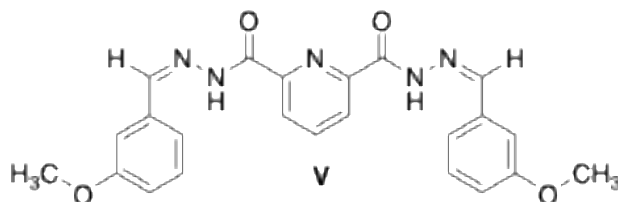
As it was expected, 11 carbon environments were recorded on ^{13}C -NMR spectrum, most important peak was at 148 ppm that validates the attachment of aldehyde to hydrazide forming hydrazone.

4.3.4. *Bis(4-methoxybenzaldehyde) dipicolinoyl hydrazone, V*

IR (KBr) (cm^{-1}): 3163.25 (NH), 3019.17 ($-\text{CH}_3$), 2995.97 ($=\text{CH}$), 2837.71 ($\text{N}=\text{C}-\text{H}$), 1671.29 ($\text{C}=\text{O}$), and 1579.45 ($\text{C}=\text{N}$).

^1H NMR (DMSO); $\delta(\text{ppm})$: 3.75 (s, 6H $-\text{O}-\text{CH}_3$), 7.0 (d, 2H Phenyl-C4-H), 7.4 (m, 6H Phenyl-C1,5,6-H), 8.25 (t, 1H Pyridine-C4-H), 8.32 (d, 2H Pyridine-C3-H), 8.75 (s, 2H $\text{N}=\text{C}-\text{H}$). 12.35 (s, 2H, NH).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 56 ($-\text{O}-\text{CH}_3$), 112 (Phenyl-C2), 117 (Phenyl-C4), 121 (Phenyl-C6), 126 (Pyridine-C3), 131 (Phenyl-C5), 137 (Phenyl-C1), 141 (Pyridine-C4), 149 (Hydrazone-C=N), 150 (Pyridine-C2=N), 160 (C=O), 160 (Phenyl-C3-O).



IR spectrum of compound **V** showed medium band at 3163.25 cm^{-1} that is attributed to secondary amine (N-H). Also, medium band due to of aryl methoxy ($-\text{O}-\text{CH}_3$) group stretching was observed at 3019.17 cm^{-1} . Aromatic protons were stretching around 2995.97 cm^{-1} . Aldehyde proton has a characteristic stretching around $2830\text{--}2650\text{ cm}^{-1}$ and since oxygen and nitrogen atoms are of relatively close electronegativity, a slight shift towards lower energy must be expected. A small band around 2837.71 cm^{-1} was observed and it could be attributed to imine's proton stretching ($\text{N}=\text{C}-\text{H}$). Further, such suggestion could be supported more by amide carbonyl band that was assigned at 1671.29 cm^{-1} and no observation of aldehyde carbonyl that shows up at around $1740\text{--}1720\text{ cm}^{-1}$. Finally, pyridine's $\text{C}_2=\text{N}$ was shown at 1652.49 cm^{-1} . For structural conformation, IR is not enough but it gives clue regarding found functional groups and thus structure could be investigated more through ^1H NMR and ^{13}C NMR.

^1H NMR results matched the expected signals as $-\text{O}-\text{C}-\text{H}_3$ signal was shown as a singlet and of integral of 6H at 3.75 ppm. Since two aromatic rings are present in proposed structure, and both must be in 6.5 – 8.2 ppm, aryl group is expected to be more shielded compared to pyridine due to presence of nitrogen in pyridinyl group and thus they could be differentiated. The latter was represented as aryl proton at position 4 was observed as a doublet at 7 ppm with 2H integral value and the other aryl protons were observed at 7.4 ppm with integral of 3H and multiplet splitting. A representative proton that is deshielded due electron density shift due to nitrogen is hydrazone proton. The latter proton was observed at 8.75 ppm as a singlet signal with integral of 2H that confirms the attachment of aldehyde to hydrazide amine. Finally, amide proton was manifested as a signal at 12.35 ppm.

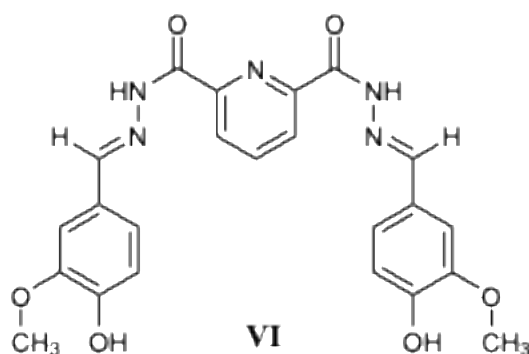
Further, structure of compound **V** was confirmed by ^{13}C NMR. The obtained spectrum showed 12 different carbons environments that matches the expectations regarding this compound. First signal at 56 ppm represents O-**C**-H₃. 7 signals between 110 ppm up to 142 ppm represent the aromatic carbons. At 149 ppm, hydrazone bond formation was confirmed due to observation of C=N bond signal manifestation. Further, pyridine ring C=N signal showed up at 150 ppm. Two overlapping signals at 160 are attributed to carbonyl and C=**C3**-O-, as they have similar characteristics due to electron withdrawing effect of oxygen in both cases, yet carbonyl carbon is expected to be more shielded because of localization as carbon Phenyl **C3** is under aromatic ring impact and thus it would be more de-shielded.

4.3.5. *Bis(3-methoxy-4-hydroxybenzaldehyde) dipicolinoyl hydrazone, VI*

IR (KBr) (cm^{-1}): 3448.72 (-OH), 3195.90 (-NH), 3000.57 (=CH), 2845.41 (N=C-H, **8**), 1666.50 (C=O), and 1512.19 (C=N).

^1H NMR (DMSO); $\delta(\text{ppm})$: 3.75 (s, 6H -O-**C**H₃), 6.82 (d, 2H Phenyl-C5-**H**), 7.20 (d, 2H Phenyl-C6-**H**), 7.45 (s, 2H Phenyl-C2-**H**), 8.20 (m, 1H Pyridine-C4-**H**), 8.30 (d, 2H Pyridine-C3-**H**), 8.60 (s, 2H -O**H**), 9.65 (s, 2H N=C-**H**). 12.15 (s, 2H N**H**).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 56 (-O-**C**H₃), 109 (Phenyl-**C2**), 116 (Phenyl-**C5**), 123 (Phenyl-**C6**), 126 (Pyridine-**C4**), 127 (Phenyl-**C1**), 141 (Pyridine-**C3**), 148 (Hydrazone-**C=N**), 149 (Phenyl-**C3**), 150 (-Pyridine-**C2=N**), 153 (Phenyl-**C4-O**), and 160 (**C=O**).



Compound **VI**'s IR spectrum showed a characteristic strong band of phenolic hydroxyl group stretching at 3448.72 cm^{-1} . Also, amide proton (O=C-N-**H**) stretching band appeared at 3195.90 cm^{-1} that matches what to be expected. Methoxy group is expected to be small, yet it has not appeared clearly in the spectrum due to overlapping with aromatic protons that did appear as small peaks at 3000.57 cm^{-1} . The differentiation between aromatic and methoxy protons was made based on the fact that electron withdrawing impact of oxygen exceeds that of aromatic ring and thus, protons of methoxy group must be shifting towards lower wavenumber i.e. below 3000 cm^{-1} , yet that region was occupied by broad bands of hydroxy and amide protons. A small peak at 2845.41 cm^{-1} of N=C-H group was observed, and it could suggest that hydrazone bond was formed, yet further analysis must be done. Finally,

amide carbonyl stretching was recorded at 1666.50 cm⁻¹ and C=N group at 1512.19 cm⁻¹.

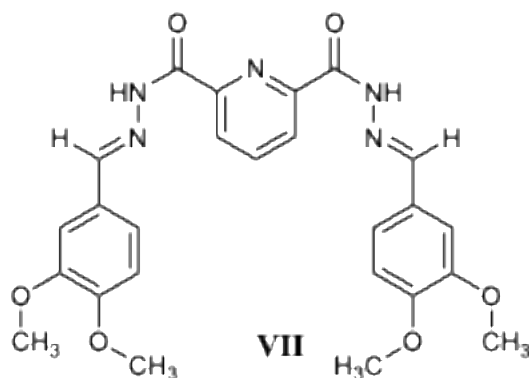
Elucidation of compound **VI** structure was done by analyzing ¹H- and ¹³C- NMR spectra. Compared to compounds **IV** and **V** has an extra hydroxyl group at position **4**, thus a slight change in chemical shift and appearance of new peak of -OH group in ¹H-NMR is expected. Such expectations were met by observing hydroxyl proton as a singlet at 8.60 ppm with integration of 2H. further, chemical shift of carbon **C4** on phenyl has changed from 117 to 153 ppm due to the deshielding impact of oxygen. Also, instead of having multiplet signal of phenyl's protons at positions **2**, **5** and **6**, doublet signal of proton at position **5** was observed due to single proton nearby with slight shift in signal.

4.3.6. *Bis(3,4-dimethoxybenzaldehyde) dipicolinoyl hydrazone, VII*

IR (KBr) (cm⁻¹): 3214.04 (-NH), 3002.19 (-OCH₃), 2944.04 (=CH), 2837.38 (N=C-H, **8**), 1674.43 (C=O), and 1537.49 (C=N).

¹H NMR (DMSO); δ(ppm): 3.75 (s, 12H -O-**C**H₃), 6.98 (d, 2H Phenyl-C6-**H**), 7.25 (d, 2H Phenyl-C5-**H**), 7.45 (s, 2H Phenyl-C2-**H**), 8.23 (m, 1H Pyridine-C4-**H**), 8.30 (d, 2H Pyridine-C3-**H**), 8.45 (s, 2H N=C-**H**). 12.2 (s, 2H, **NH**).

¹³C NMR (DMSO); δ(ppm): 56 (-O-**C**H₃), 107 (Phenyl-**C**5), 112 (Phenyl-**C**2), 123 (Phenyl-**C**6), 126 (Pyridine-**C**3), 127 (Phenyl-**C**1), 141 (Pyridine-**C**4), 148 (hydrazone-**C**=N), 149 (Pyridine-**C**2=N), 152 (Phenyl-**C**3-O), 153 (Phenyl-**C**4-O) and 160 (**C**=O).



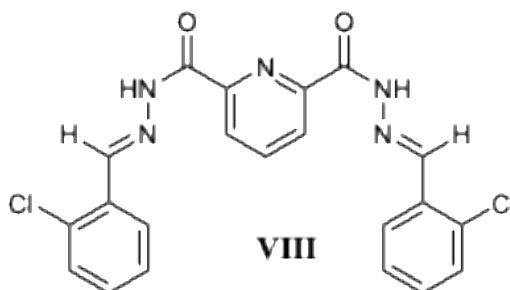
Structure of compound **VII** was confirmed by IR, ¹H- and ¹³C NMR spectra analysis. With reference to compounds **V** and **VI**, the overall shape of spectra was maintained yet a slight difference due to presence of extra methoxy group at position **4** on phenyl group was observed. This group presence was validated through increase in integration value of O-CH₃ group compared to **V** and **VI** from 6 to 12 hydrogens that represents both methoxy groups in compound **7**. Also, on ¹³C-NMR spectrum, two adjacent peaks at 152 and 153 ppm were obtained that show having two different types of C-O carbons i.e. carbon **C3** and **C4** on phenyl ring. Other bands and peaks in all spectra matched the expectations and thus, it could be concluded that aimed structure was obtained.

4.3.7. *Bis(2-chlorobenzaldehyde) dipicolinoyl hydrazone, VIII*

IR (KBr) (cm^{-1}): 3248.13 (NH), 3062.96 (=CH), 2920 (N=C-H) 1681.39 (C=O), and 1535.34 (C=N).

^1H NMR (DMSO); $\delta(\text{ppm})$: 7.45 (m, 4H Phenyl-C4,5-H), 7.55 (d, 2H Phenyl-C3-H), 8.1 (d, 2H Phenyl-C6-H), 8.20 (m, 1H Pyridine-C4-H), 8.35 (d, 2H Pyridine-C3-H), 9.1 (s, 2H C=N-H), 12.5 (s, 2H NH).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 126.1 (Pyridine-C3), 127.3 (Phenyl-C4), 128.4 (Phenyl-C6), 130.5 (Phenyl-C3), 131.9 (Phenyl-C4), 132.5 (Phenyl-C2-Cl), 132.6 (Phenyl-C1), 134 (Hydrazone-C=N), 146.5 (Pyridine-C2=N), 148.5 (Pyridine-C4), 160.5 (C=O).



Primary structural elucidations were done using IR spectrum. At 3248.13 cm^{-1} , a medium band of secondary amine N-H was recorded. Protons of aromatic rings (=C-H) were observed at 3062.96 cm^{-1} . A characteristic band of N=C-H was shown at 2920 cm^{-1} that suggests the attachment of aldehyde and amine to form hydrazone bond. Amide's carbonyl stretching was at 1681.93 cm^{-1} . Finally, C=N bond was manifested at 1535.34 cm^{-1} that could be attributed for both pyridine ring and hydrazone bond.

^1H -NMR spectrum showed a multiplet signal at 7.45 ppm integral of 4H. The latter indicates the resonance of aryl protons at position 4 and 5. Proton at position 3 was observed at 7.55 ppm with doublet signal and integral of 2H lastly with aryl ring, another doublet signal at 8.1 ppm with integral of 2H was recorded and attributed to proton at position 6. Most importantly, signal at 9.1 ppm that indicates the presence of N=C-H bond, was obtained as a singlet with 2H integral.

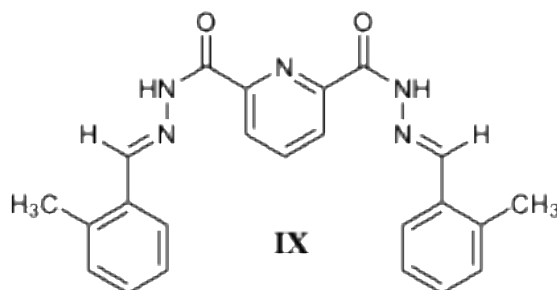
For ^{13}C -NMR spectrum, 11 different carbons were recorded that matches the expected number. Carbon 1 and 2 were influenced by adjacent chloro- group and two closely attached bands were obtained at 132.5 and 132.6 ppm. Furthermore, most important peak at 146.5 ppm of hydrazone carbon (HC=N) was obtained that confirms obtaining the aimed structure.

4.3.8. *Bis(2-methylbenzaldehyde) dipicolinoyl hydrazone, IX*

IR (KBr) (cm^{-1}): 3224.98 (NH), 3055.24 (=CH), 3000 (-CH₃), 2900 (N=C-H) 1681.93 (C=O), and 1589.34 (C=N).

¹H NMR (DMSO); $\delta(\text{ppm})$: 3.27 (s, 6H -CH₃), 7.26 (t, 6H Phenyl-C_{3,4,5}-H), 7.31 (t, 2H Phenyl-C₆-H), 8.25 (m, 1H Pyridine-C₄-H), 8.32 (d, 2H Pyridine-C₃-H). 9.1 (s, 2H C=N-H), 12.25 (s, 2H NH).

¹³C NMR (DMSO); $\delta(\text{ppm})$: 19 (Phenyl-C₂-CH₃), 126 (Pyridine-C₃), 126.5 (Phenyl-C₁), 127 (Phenyl-C₅), 130.5 (Phenyl-C₃), 131 (Phenyl-C₄), 132.5 (Phenyl-C₆) 137.5 (Phenyl-C₂-CH₃), 141 (Pyridine-C₄), 148.5 (hydrazone-C=N), 149.5 (Pyridine-C₂=N), 159.5 (C=O).



IR spectra of compound **IX** shows stretching bands of aromatic methyl (Aryl-CH₃) and aryl protons (Aryl-H) between 3100 and 3000 cm^{-1} . Amide carbonyl was obvious at 1681.93 cm^{-1} and C=N, too, at 1589.34 cm^{-1} . All over, a sharp conclusion could not be made at this stage and NMR results shall pave the way for more elucidations.

¹H-NMR showed all of chemical shifts, integral values, and splitting pattern going with expectations. Most important peak was at 9.1 ppm that confirms the attachment of aldehyde and hydrazone with integration value of 2H and a singlet splitting that could be attributed to C=N-H proton

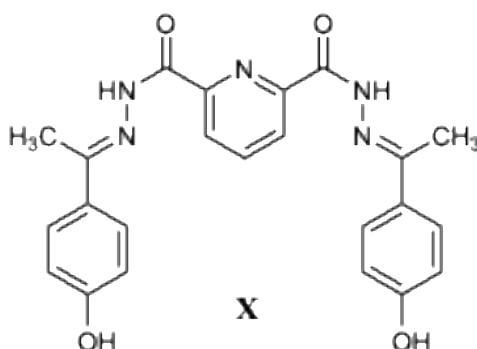
¹³C-NMR spectrum showed 12 different carbons environments that matches the expectations. The most decisive carbon, HC=N, was recorded at 148.5 ppm, as expected.

4.3.9. *Bis(4-hydroacetophenone) dipicolinoyl hydrazone, X*

IR (KBr) (cm^{-1}): 3314.99 (NH), 3056.81 ($-\text{CH}_3$), 2996.54 ($=\text{CH}$), 1688.85 ($\text{C}=\text{O}$), and 1506.34 ($\text{C}=\text{N}$).

^1H NMR (DMSO); $\delta(\text{ppm})$: 3.40 (s, 6H $-\text{CH}_3$), 6.75 (d, 4H Phenyl-C3,5-**H**), 7.70 (d, 4H Phenyl-C2,6-**H**), 8.20 (m, 1H Pyridine-C4-**H**), 8.30 (d, 2H Pyridine-C3-**H**). 9.80 (s, 2H $-\text{OH}$), 11.40 (s, 2H **NH**) .

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 15 ($\text{N}=\text{C}-\text{CH}_3$), 115 (Phenyl-**C3**), 126 (Pyridine-**C3**), 129.5 (Phenyl-**C5,6**), 140 (Phenyl-**C1**), 149 (Pyridine-**C4**), 157 (hydrazone-**C=N**), 159.5 (Pyridine-**C2=N**), 160 (**C=O**), 161.1 (Phenyl-**C4-OH**).



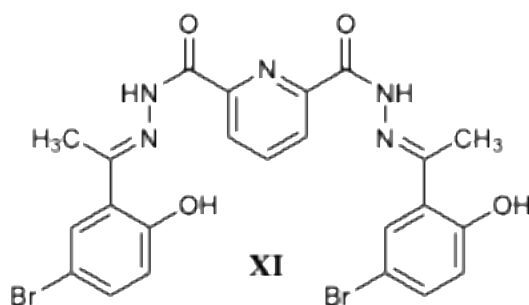
Structure of compound **X** was confirmed after analysing structure through IR and NMR spectra. First, strong band at 3314.99 cm^{-1} was obtained that represents the stretching of secondary amine group ($-\text{NH}-$). This group was validated too through ^1H -NMR as it appeared at 11.40 ppm as a singlet. Unlike the previous compounds, compound **9** cannot be validated from hydrazone proton. However, methyl hydrazone carbon shall give a clear conclusion regarding reaching to the aim. As with expectations, a peak at 157 ppm in ^{13}C -NMR spectrum was obtained that shows the latter carbon that suggests obtaining the aimed structure.

4.3.10. *Bis(2-hydro-5-bromo-acetophenone) dipicolinoyl hydrazone, XI*

IR (KBr) (cm^{-1}): 3441.01($-\text{OH}$), 3276.08 ($-\text{NH}$), 3062.50 ($-\text{CH}_3$), 2924.94 ($=\text{CH}$), 1697.36 ($\text{C}=\text{O}$), and 1512.19 ($\text{C}=\text{N}$).

^1H NMR (DMSO); $\delta(\text{ppm})$: 3.56 (s, 6H $-\text{CH}_3$), 6.70 (d, 2H Phenyl-C3-**H**), 7.44 (d, 2H Phenyl-C4-**H**), 7.75 (s, 2H Phenyl-C6-**H**), 8.30 (m, 1H Pyridine-C4-**H**), 8.35 (d, 2H Pyridine-C3-**H**). 11.75 (s, 2H **N-H**), 13.20 (s, 2H $-\text{OH}$).

^{13}C NMR (DMSO); $\delta(\text{ppm})$: 12 ($-\text{CH}_3$), 110 (Phenyl-**C5-Br**), 120 (Phenyl-**C3**), 122 (Phenyl-**C1**), 126 (Pyridine-**C3**), 131 (Phenyl-**C6**), 135 (Phenyl-**C4**), 140.9 (Pyridine-**C4**), 148 (Pyridine-**C2=N**), 158 (**C=O**), 159 (Phenyl-**C2-OH**), and 161 (Hydrazone-**C=N**).



IR spectrum of compound **XI** showed a strong and broad peak at 3441.01 cm^{-1} of -OH stretching. Such peak covered amine stretching region, yet secondary amine was observed as a shoulder at 3276.08 cm^{-1} . Also, -CH₃ group peak was obtained in its expected region and so it is with aromatic rings' protons. Amide carbonyl was obtained at 1697.36 cm^{-1} and finally C=N group was at 1512.19 cm^{-1} .

Since IR spectrum is not decisive, NMR spectra were analysed to get clear conclusion regarding compound. ¹H-NMR spectrum showed -CH₃ group protons were obtained at 3.56 ppm as a singlet with integration of 6H. Aryl and pyridinyl protons chemical shifts were at expected places. For amide nitrogen proton, it was recorded at 11.75 ppm with integration of 2 and -OH's proton was more deshielded due to electron withdrawing impact of -Br group at para- position (relative to -OH) and thus it has shifted to 13.20 ppm.

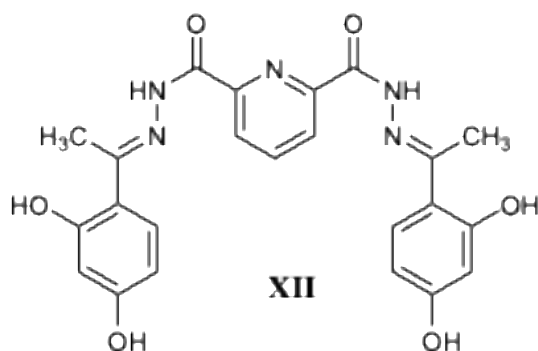
Finally, upon synthesizing compound **XI** successfully, it is expected to observe 12 peaks on ¹³C-NMR spectrum. Such expectations were met as 12 carbon environments were recorded on spectrum, at expected chemical shift values.

4.3.11. *Bis(2,4-dihydroacetophenone) dipicolinoyl hydrazone, XII*

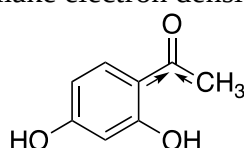
IR (KBr) (cm^{-1}): 3275.20 & 3276.61 (NH₂), 3185.40 (-NH), 3016.11 (=CH), 1691.29 (C=O), and 1639.03 (C=N).

¹H NMR (DMSO); $\delta(\text{ppm})$: 4.55 (s, 4H -NH₂), 7.0 (t, 3H Pyridine-C3,4-H), 10.6 (s, 2H NH).

¹³C NMR (DMSO); $\delta(\text{ppm})$: 124 (Pyridine-C3), 140 (Pyridine-C4), 149 (Pyridine-C2=N), 163 (C=O).



Results of NMR and IR of compound **XII** were out of expectations as they appear close enough to starting material i.e. compound **III**. First comparing IR spectra of both compounds, they appear exactly the same but of different intensities, and it was expected to observe -OH stretching between 3500 and 3200 cm^{-1} yet that region was almost clear. Therefore, it was NMR to confirm any conclusion. Qualitatively, ^1H -NMR and ^{13}C -NMR of compound **XII** and **III** were exactly the same and comparing chemical shifts, the obtained results were alike each other. Also, the high percentage yield of such compound (95%) and recrystallisation solvent that was determined to be water -that matches solvent for **III** recrystallisation. All lead to a definitive conclusion that reaction did not happen. Chemically, such results could be understood going through structure of ketone used. 2,4-hydroxyacetophenone is relatively not that much reactive due to the presence of electron donating groups (-OH) in meta and para positions and methyl group that make electron density over carbonyl high.



This high density of electrons would guard the compound from going into reaction in these conditions. To obtain compound **XII**, it could be suggested to change the reaction solvent to one that would provide sufficient energy and thus successfully activating the compound.

4.4. Elemental Analysis

Elemental microanalyses were performed in University of Ain-shams, Egypt. Results were found with $\pm 0.4\%$ of the theoretical values as Table 5 shows.

Table 5: Microanalyses of synthesized compounds:

Number	Molecular Formula	Molecular Weight (g/mol)	Composition %					
			Calculated			<i>Found</i>		
			C	H	N	C	H	N
II	C ₁₁ H ₁₃ NO ₄	223.08	59.19	5.87	6.27	58.92	6.15	5.98
III	C ₇ H ₉ N ₅ O ₂	195.08	43.08	4.65	35.88	42.82	4.32	36.06
IV	C ₂₁ H ₁₇ N ₅ O ₄	403.4	62.53	4.25	17.36	62.66	3.880	17.22
V	C ₂₃ H ₂₁ N ₅ O ₄	431.45	64.03	4.91	16.23	64.38	5.01	15.85
VI	C ₂₃ H ₂₁ N ₅ O ₆	463.45	59.61	4.57	15.11	59.72	4.78	14.86
VII	C ₂₅ H ₂₅ N ₅ O ₆	491.5	61.09	5.13	14.25	60.96	4.84	14.58
VIII	C ₂₁ H ₁₅ Cl ₂ N ₅ O ₂	440.28	57.29	3.43	15.91	57.32	3.28	16.08
IX	C ₂₃ H ₂₁ N ₅ O ₂	399.45	69.16	5.3	17.53	68.86	5.14	17.32
X	C ₂₃ H ₂₁ N ₅ O ₄	431.45	64.03	4.91	16.23	64.34	5.07	15.88
XI	C ₂₃ H ₁₉ Br ₂ N ₅ O ₄	589.24	46.88	3.25	11.89	47.16	3.72	12.25

4.5. Biological screening

4.5.1. *In vitro* screening of synthesized compounds

All synthesized compounds were evaluated *in vitro* against *L.major* for their anti-leishmanial activity in both forms as shown in Table 6. The activity is represented by IC_{50} , which is the concentration in μM of a compound required to achieve 50% growth inhibition. In anti-promastigote assay, IC_{50} values of synthesized compounds were ranging from 2.860-16.94 μM while reference drugs AmB-D and Miltefosine they were $0.04 \pm 0.001 \mu M$ and $7.830 \pm 0.3400 \mu M$, respectively. AmB-D reference drug was of the most superior activity that synthesized compounds are not comparable to it, yet compared with Miltefosine, compounds **I, II, IV**, and **X** have shown better activity with 7.062 ± 0.38 , 4.02644 ± 0.14 , 2.8628 ± 0.14 and $4.0782 \pm 0.18 \mu M$, respectively. Among these compounds, compound **IV** is superior to Miltefosine by almost 3-folds that renders it being a remarkable prospect with such a promising anti-leishmanial activity.

Table 6: Anti-promastigote activity (IC_{50}) of the test compounds and reference standards in μM .

Comp. No.	Antipromastigotes * IC_{50} , $\mu M \pm SD$
I	7.0624 ± 0.38
II	8.4048 ± 0.46
III	4.02644 ± 0.14
IV	2.8628 ± 0.14
V	13.9784 ± 0.66
VI	14.5928 ± 0.74
VII	16.9452 ± 0.95
VIII	8.6548 ± 0.44
IX	10.2816 ± 0.34
X	4.0782 ± 0.18
XI	8.2486 ± 0.36
AmB-D	0.04 ± 0.001
Miltefosine	7.83 ± 0.34

Further, Table 7 shows anti-amastigote assay that revealed the activity of synthesized compounds. IC_{50} of synthesized compounds was ranging from 3.840 to 22.26 μM , whereas AmB-D was of $0.150 \pm 0.01 \mu M$ and Miltefosine was of $8.070 \pm 0.24 \mu M$. Compounds **III**, **IV** and **X** showed comparable activity to Miltefosine with values of 5.843 ± 0.22 , 3.843 ± 0.16 and $6.143 \pm 0.14 \mu M$ as IC_{50} . Compound **IV** is better than miltefosine reference drug by almost two-folds. The latter result as well as results of anti-promastigotes assay make compound **IV** most active in this research and thus qualified to be further studied to deduce structure-activity relationship.

and -CH₃ at hydrazone bond, such occurrence decreased the activity. Also, upon having more than a functional group on the aryl ring, activity decreased too (X vs VI). Figure 21 shows a general conclusion that could be deduced from obtained results. In short, the inhibitory activity of this research's compounds increases with having ortho- hydroxyl group, no other substitution on aryl ring and starting with aldehyde rather than ketone (-H and -CH₃, respectively).

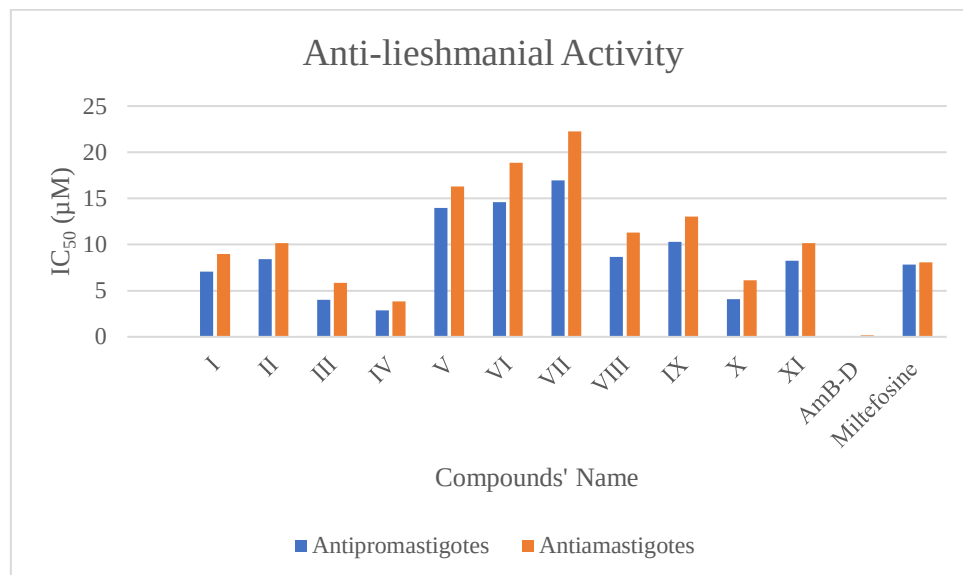


Figure 21: Synthesized compounds and reference drugs (AmB-D and Miltefosine) anti-leishmanial activities.

The latter results could be attributed to enhancement of diffusion rate as a compound having aryl group, aromatic nitrogen and hydrazone bond, would be capable of inhibiting *Lm*-PTR1 enzyme primarily due to better interaction with the backbone of target enzyme. (See Chapter 2.7.2).

4.5.2. Reversal anti-leishmanial activity via folic acid and folinic acid

To validate that mechanism of action of compound IV is through anti-folate mechanism, a method reported by Mendoza-Martinez et al. (Mendoza-Martínez et al., 2015) was used. *L. major* parasites were exposed to compound IV above its IC₅₀ and trimethoprim at 100µM (antifolate agent, positive control). Then, upon the addition of folic acid and folinic acid, the survival of parasites changed reaching to almost 100% in case of trimethoprim as Table 8 shows.

Table 8: *In vitro* evaluation of folate pathway inhibition expressed as percentage survival:

Entry	No Competitor added	Folic acid		Folinic acid	
		20µM	100 µM	20 µM	100 µM
IV	24%	76%	82%	84%	92%
Trimethoprim (100 µM)	72%	-	99%	-	-

When compound **IV** was incubated with *L.major* parasites and no competitor was added, percentage survival was 24% but when folic and folinic acid competitors were added, percentage survival increased abruptly reaching 76-92%. This observation could be explained by the concept of reversible inhibition of enzyme action. A competitive inhibitor would compete with the substrate hindering its approach to the active site of the enzyme as it would be occupied by the inhibitor, yet at high concentrations of the substrate, the substrate displaces the inhibitor and thus promoting the natural activity of the enzyme (Bjelakovic et al., 2002). Therefore, from the previous results it could be concluded that **IV** mechanism of action is mainly anti-folate.

4.5.3. *In vitro* cytotoxicity evaluation

Safety profile of compound **IV** was obtained by determining SI using method reported by Berhe et al. (Berhe et al., 2024). CC_{50} and SI values are shown in Table 9.

Table 9: CC_{50} values of the most active compounds against normal VERO cells and their selectivity index:

Comp. No.	CC_{50} µg/ml (µM)	<i>Antileishmanial</i>	
		IC_{50} µM	SI
IV	125 (309.87)	2.8628	108.2
Miltefosine	(99.7)*	7.83	12.7

*value obtained from Berhe et al. (Berhe et al., 2024).

The concentration required to inhibit the viability of VERO cells (CC_{50}) is higher than the concentration required to inhibit *L.major* promastigotes viability (IC_{50}) by far and this translates in obtaining SI of 108.2 that is better than SI value of Miltefosine reference drug by almost 9-folds. Therefore, lead compound **IV** is of superior safety profile.

4.6. *In silico* investigations

The aim of this section is to provide justifications of the obtained *in vitro* results and to predict the pharmacological properties and toxicity lead compound **IV**.

4.6.1. Molecular Docking

To rationalize *in vitro* results, molecular docking against *Lm*-PTR1 (PDB ID: 7PXX) was held using FRED software. Since *Lm*-PTR1 is NADPH dependent, all residues were removed keeping only this co-factor. After docking, 2D representation of most optimized pose (lowest in overall free energy) was depicted by MOE to identify interactions of ligand-protein complex as depicted in Figure 21. *Lm*-PTR1 was used as a presumed target for anti-folate pathways as the co-crystal structure of *Lm*-DHFR-TS has not been determined yet (Mohamed et al., 2023).

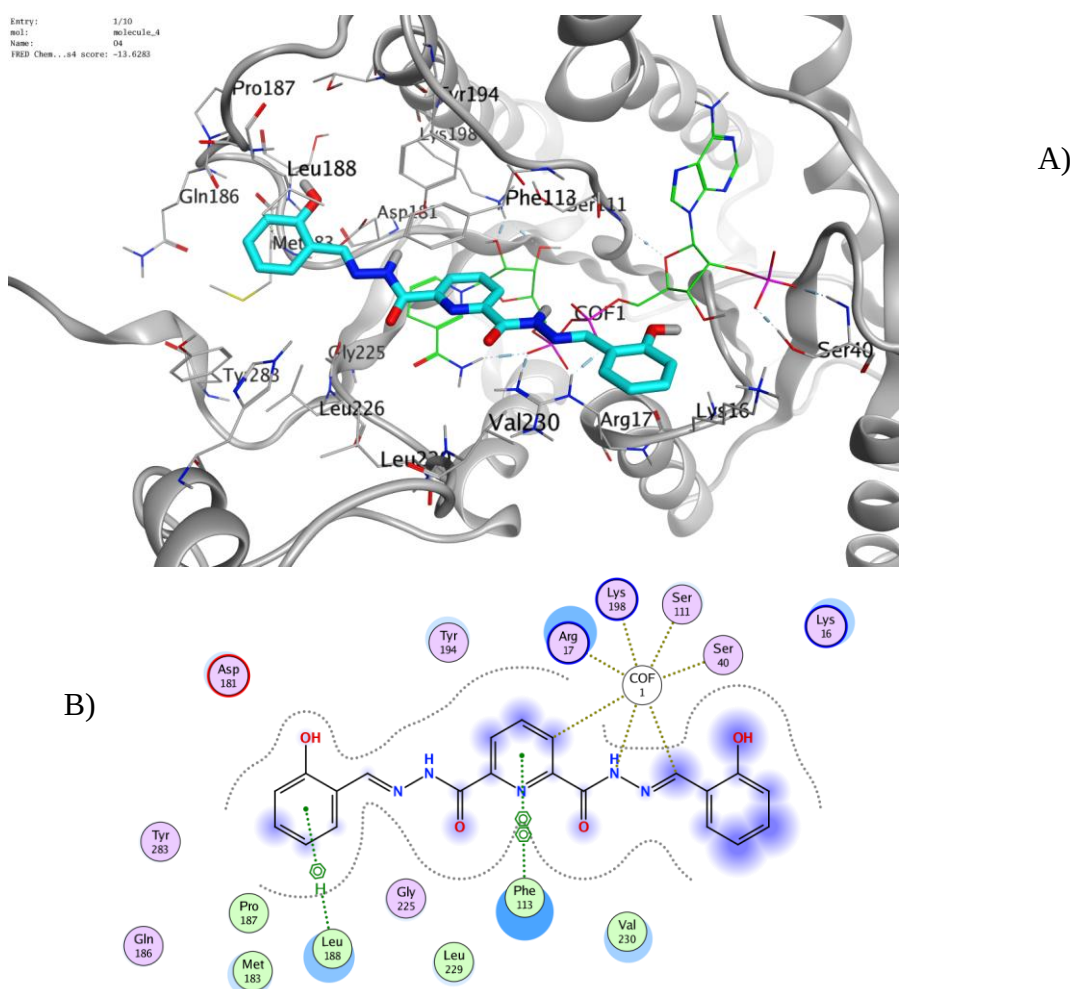


Figure 22: The docking pose of compound **IV**. Poses as cyan sticks in the binding site of the *Lm*-PTR1 (PDB ID: 7PXX) for (A) and (B), respectively, as 3 D and 2 D depictions. The Simon sticks representation is for NADPH co-factor (annotated as “COF” in the 2 D depiction).

The docking results justify the obtained *in vitro* anti-leishmanial activity. Compound **IV** showed good docking score (13.73 kcal/mol) compared with trimethoprim (-11.91 kcal/mol) and comparable score with other reported docked compounds using the same software and method as Table 10 shows.

Table 10: Docking score of compound **IV**, reported compounds and reference drug against *Lm*-PTR1:

Compound	Docking Score (kcal/mol)
4	-13.73
Trimethoprim	-11.91

It was reported by Schüttelkopf et al. (Schüttelkopf et al., 2005) that trimethoprim gains its antifolate activity against *Lm*-PTR1 enzyme by interactions with key residues that include Asp181, Phe113 and NADPH co-factor. From Figure 21 (B), compound **IV** showed similar interactions to that of trimethoprim as hydrogen bonding between hydrazone -NH moiety and NADPH, further, favourable hydrophobic interactions with NADPH and hydrazone C=N and pyridine ring. Also, π - π stacking was shown to be between pyridine ring and Phe113 residue and finally, hydrophobic interactions were shown between aryl ring and Leu188 residue. Alike trimethoprim, compound **4** established favourable interactions with key residues NADPH co-factor and Phe113 and structure of lead compound **IV** could be modified to increase its activity by conversion of -OH group on aryl ring into -NH₂ or any basic group and thus it would be able interacting with Asp181 as other research showed (Sabt et al., 2023). All in all, the obtained docking results verify the mechanism of action suggested by *in vitro* reversal anti-leishmanial experiment as compound **IV** mainly inhibits the catalytic function of *Lm*-PTR1.

4.6.2. ADMET predications

The aim of this section is to provide insights on pharmacological properties and toxicity of the most active compound in this research. The latter results shall be helpful to consider or neglect a compound as those compounds with poor bioavailability, adverse side effects and toxicity and do not meet drug-likeness standards frequently fail to be sound candidates for clinical tests. So, predication of these properties using SwissADME and Protox 3.0 will be crucial economically and time-wise.

Starting with ADME results, compound **IV** was predicted to have high passive gastrointestinal absorption (GIA) and it will fail in passing blood-brain barrier (BBB). Therefore, it could be made to anticipate that the occurrence of lead compound in the central nervous system will be low and so it is with adverse side effects on the same system. Permeability glycoprotein (P-gp) is involved in resistance mechanisms of drugs (Eldehna et al., 2019). For a compound to be a substrate in P-gp increases the chances of drug resistance mechanisms and lead compound in this research is predicted to be non-substrate. Inhibition cytochrome P (CYP) isoenzymes is correlated with toxic and adverse side of drugs (Eldehna et al., 2019). Fortunately, **IV** is non-active in inhibition any of these isoenzymes as Table 11 shows.

Table 11: *In silico* prediction of the pharmacokinetic properties of most active compound:

Pharmacokinetics of compound IV				
GIA	BBB	P-gp substrate	LogP	
High	No	No	2.63	
CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
No	No	No	No	No

Table 12 shows drug-likeness and medicinal chemistry properties of compound **4**. There are not violations to Lipinski rules that are summarised as lipophilicity (logP) ≤ 5 , molecular weight ≤ 500 , number of hydrogen bond donors ≤ 5 and number of hydrogen bond acceptors ≤ 10 (Lipinski et al., 2001). Also, there are no violations to Veber rules that state number of rotatable bonds should not exceed 10 and total polar surface area should be below or equal to 140 Å² (Veber et al., 2002). Nevertheless, PAINS (pan-assay interference compounds) alert was predicted due to presence of phenol-hydrazone group which implies that this scaffold can have interference in protein assay and thus biological assay results will not be robust. All over, from GIA value and the remaining results, it could be suggested that compound **4** has a good bioavailability profile.

Table 12: *In silico* prediction of drug-likeness and medicinal chemistry properties of most active compound:

Drug-likeness and Medicinal Chemistry		
Lipinski #violations	Veber #violations	PAINS #alerts
0	0	1

ProTox 3.0 predicted toxicity of most active compound over wide range of targets with assignation of either being active or inactive against the target and the probability of each predicted activity. Toxicity of compound falls into category “4” (toxicity ranges from 1 to 6 where 1 is most toxic and 6 is nontoxic) which implies compound being harmful upon swallowing with predicted LD₅₀ of 1000 mg/kg. compound **IV** demonstrated activity hepatotoxicity, nephrotoxicity, carcinogenicity, clinical toxicity, aryl hydrocarbon receptor, ATPase family AAA domain-containing protein 5, and transthyretin. On the other hand, the compound showed no activity in neurotoxicity, respiratory toxicity, cardiotoxicity, mutagenicity, cytotoxicity, ecotoxicity, nutritional toxicity, nuclear receptor signalling pathways, stress response pathways, molecular signalling events and metabolism. The latter results suggest that no adverse side effects are expected in these areas. Radar chart (Figure 23) shows the details of each toxicological endpoint of compound **IV** and results are attached in the appendix.

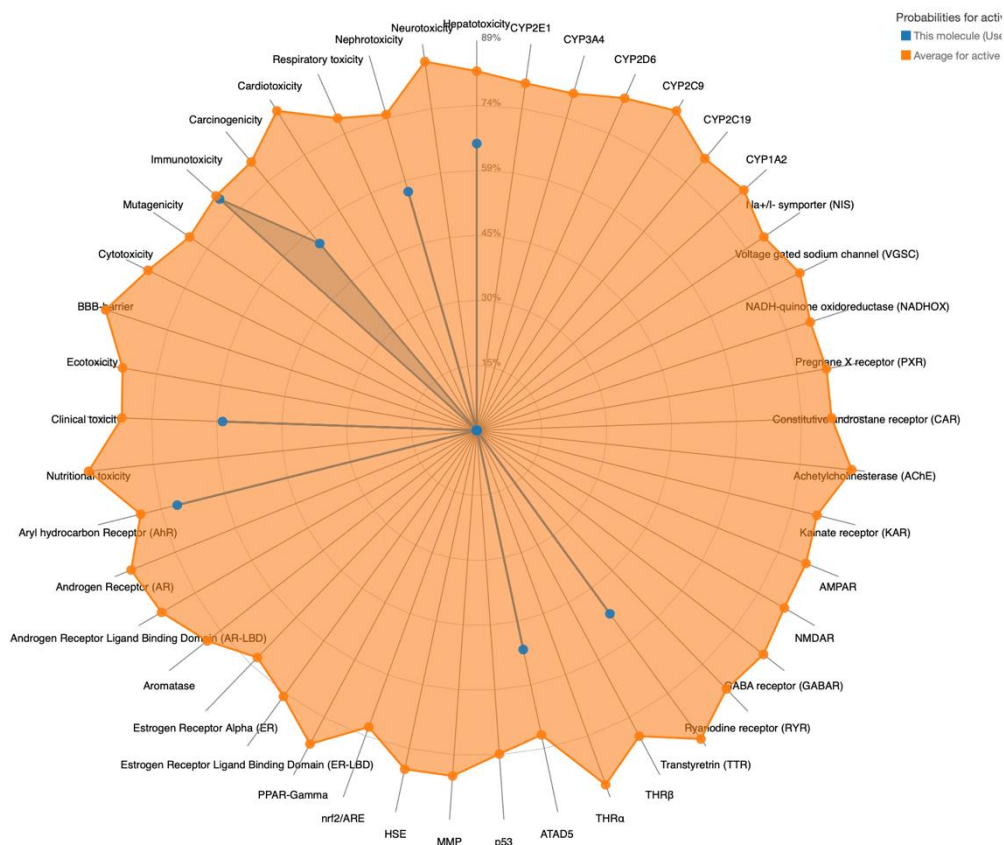


Figure 23: Toxicity radar chart for compound **IV** (Blue: probabilities for activity of compound **IV**, Orange: probabilities for activity - average for an active compound).

The obtained results from ProTox 3.0 highlight the importance of initial evaluation of toxicity of compound **IV** and thus aiding the process of decision making. Further, it helps researchers in optimizing the compound towards obtaining a safer profile.

Chapter (5)

5. Conclusion and recommendations

In conclusion, the objectives of this research were achieved by synthesizing dipicolinic acid derivatives with percentage yields ranging from 33.1%-85.46% . The synthesized structures were confirmed by spectroscopic and elemental analyses. Compounds were screened *in vitro* against the two forms of *L.major* parasites. All compounds showed activity against both forms of *L.major*, however compound **IV** was the most active with IC_{50} of $2.86 \pm 0.14 \mu M$ in anti-promastigotes assay and $3.84 \pm 0.16 \mu M$ in anti-amastigotes assay, which is superior to miltefosine reference drug by almost 3-folds. The mechanism of action of **IV** was revealed by anti-folate test that suggested that compound gains its anti-leishmanial activity by inhibition of folic acid pathway. Further this suggestion was supported by molecular docking results as the compound inhibited the catalytic action of *Lm*-PTR1 with docking score of -13.73 kcal/mol that is better than score of anti-folate reference drug trimethoprim with -11.91 kcal/mol. Safety profile of the compound was determined by *in vitro* cytotoxicity against VERO cells. The obtained IS value was 108.2 which is greater than reference drug miltefosine by 9-folds. Finally, *in silico* investigations suggested that the most active compound is of good bioavailability profile and that its toxicity profile could be enhanced.

It is recommended that synthesized compounds could be screened against other leishmania strains, malaria, and toxoplasmosis. Also, structural modifications on compound **IV** by changing bio-isosteres may enhance the anti-leishmanial activity. Finally, since other derivatives of dipicolinic acid showed anti-microbial activity and other bioactivities, it would be beneficial to evaluate the synthesized compounds to determine their potential.

6. References

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7. Appendix

Examples of spectroscopic results:

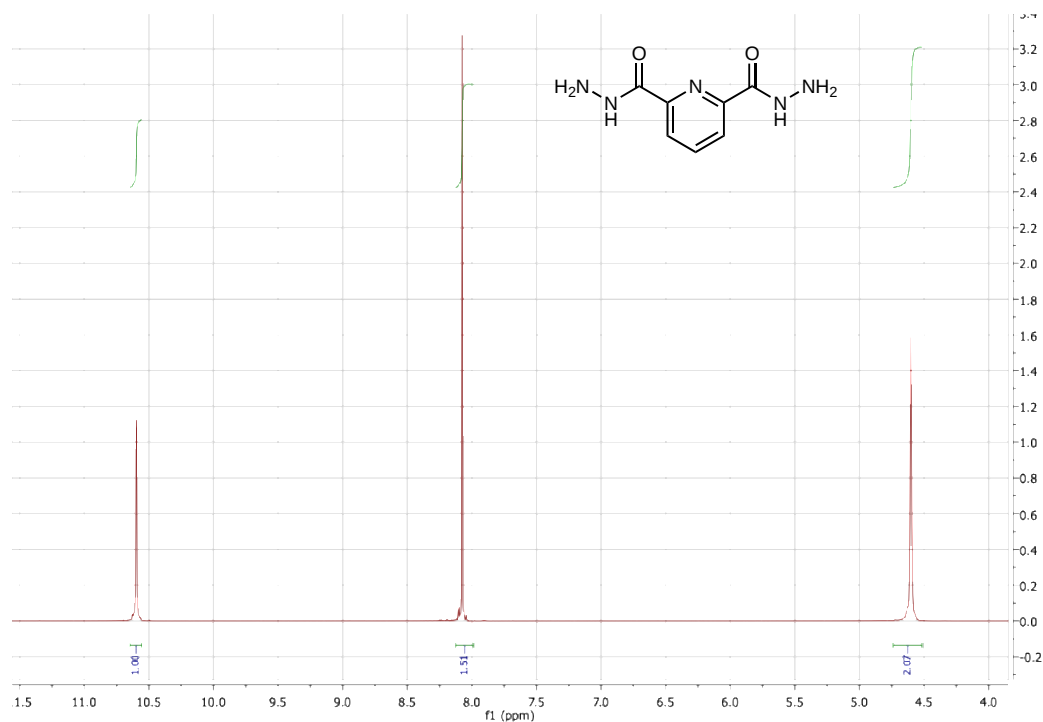


Figure 24: ^1H -NMR spectrum of compound III.

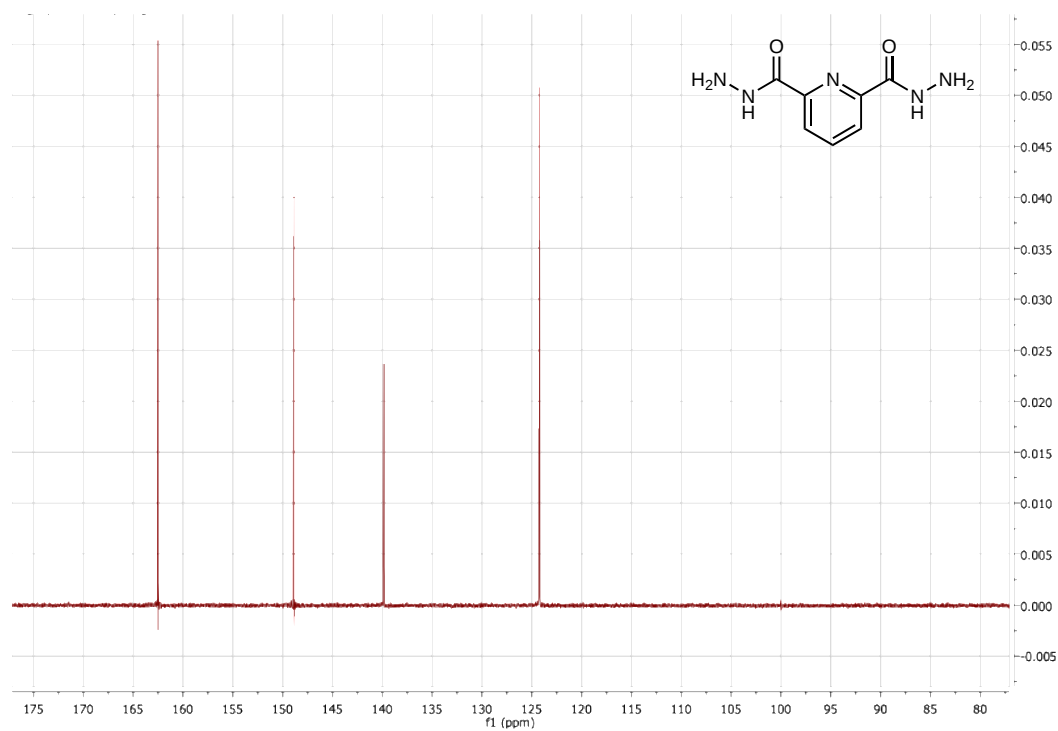


Figure 25: ^{13}C -NMR spectrum of compound III.

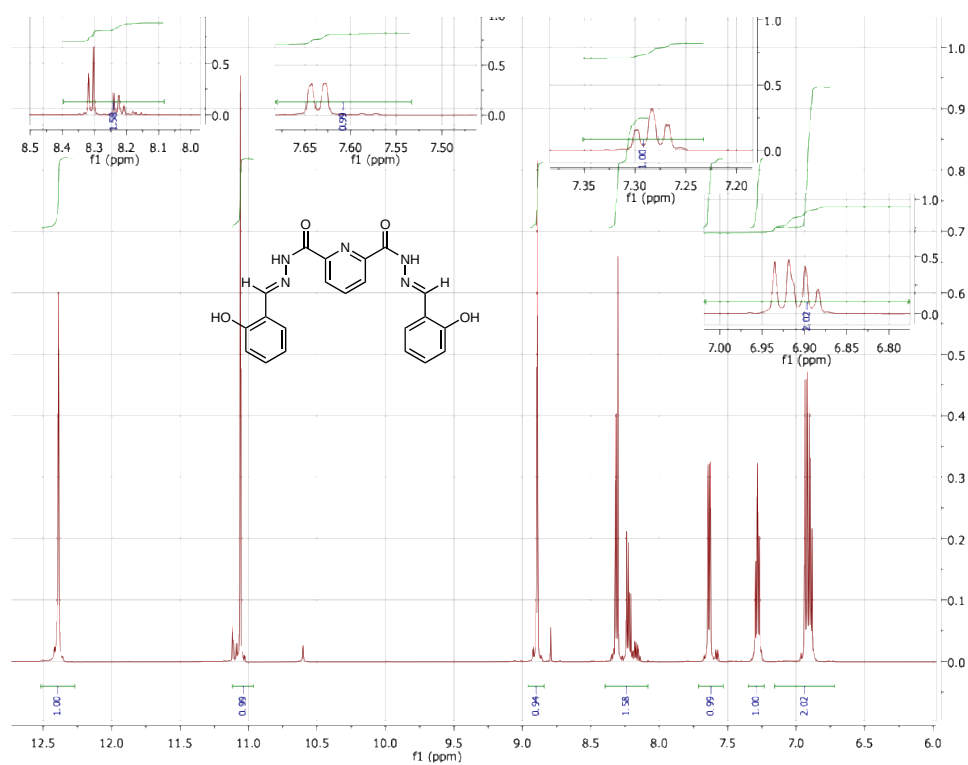


Figure 26: ¹H-NMR spectrum of compound IV.

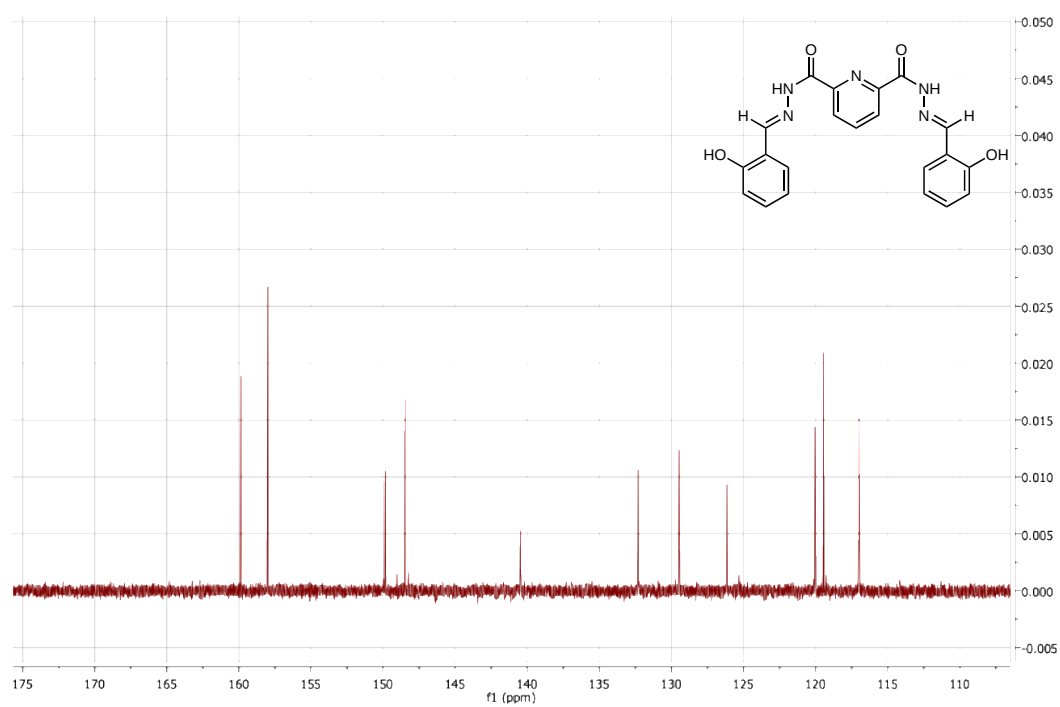


Figure 27: ¹³C-NMR spectrum of compound IV.

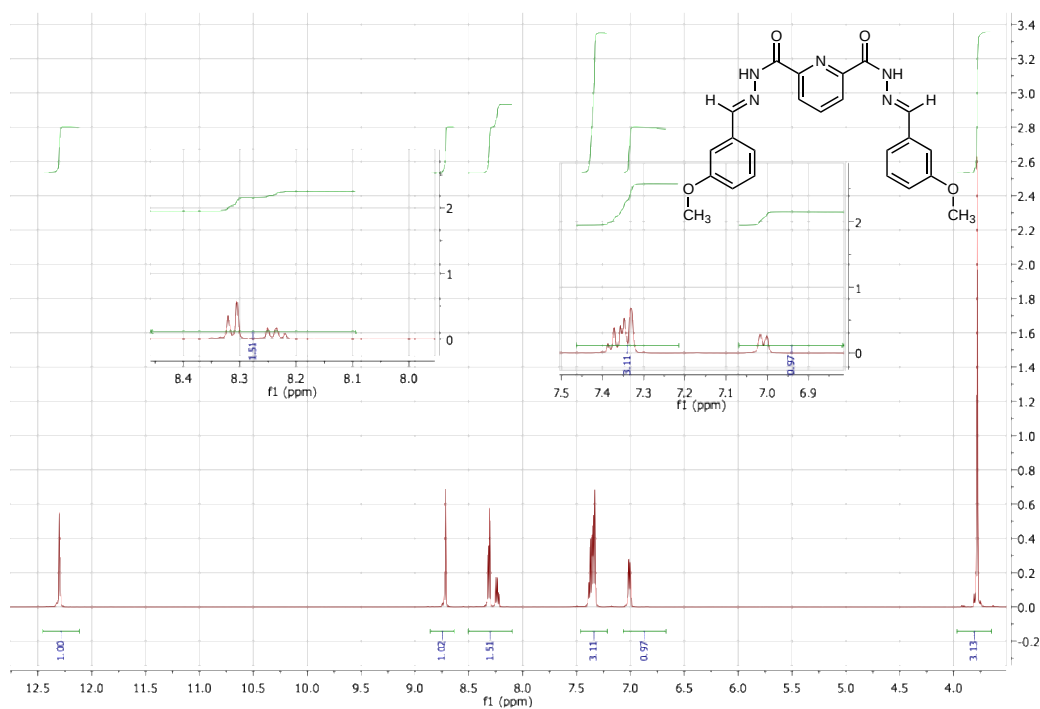


Figure 28: ^1H -NMR spectrum of compound V.

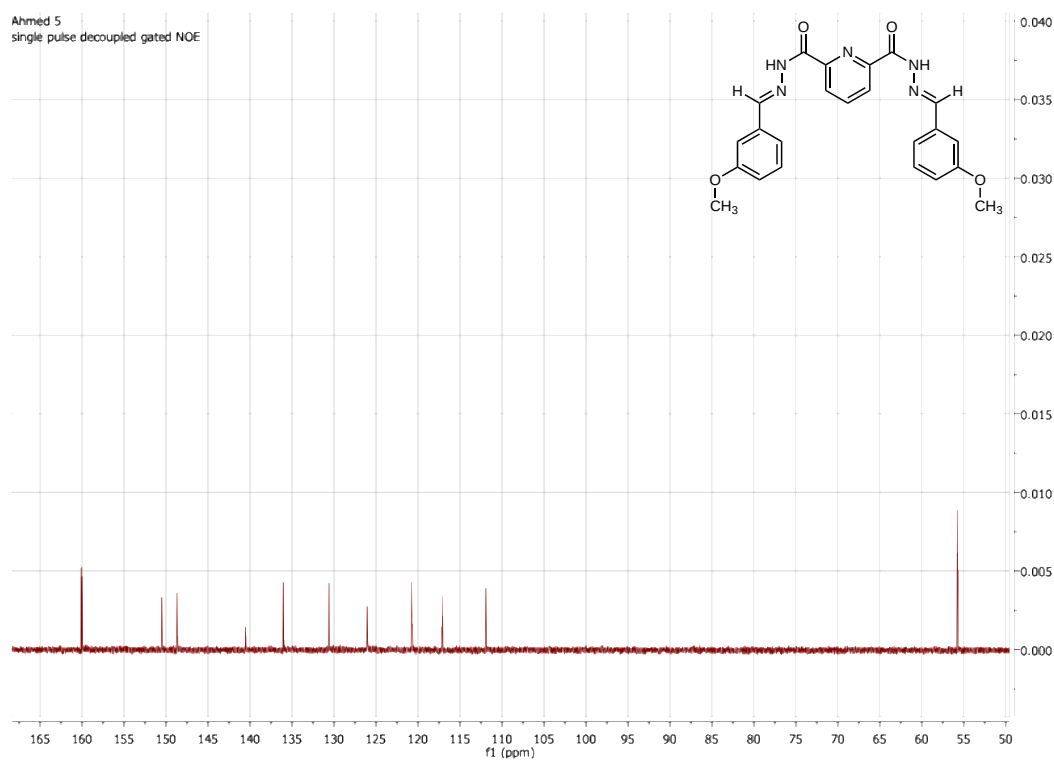


Figure 29: ^{13}C -NMR spectrum of compound V.

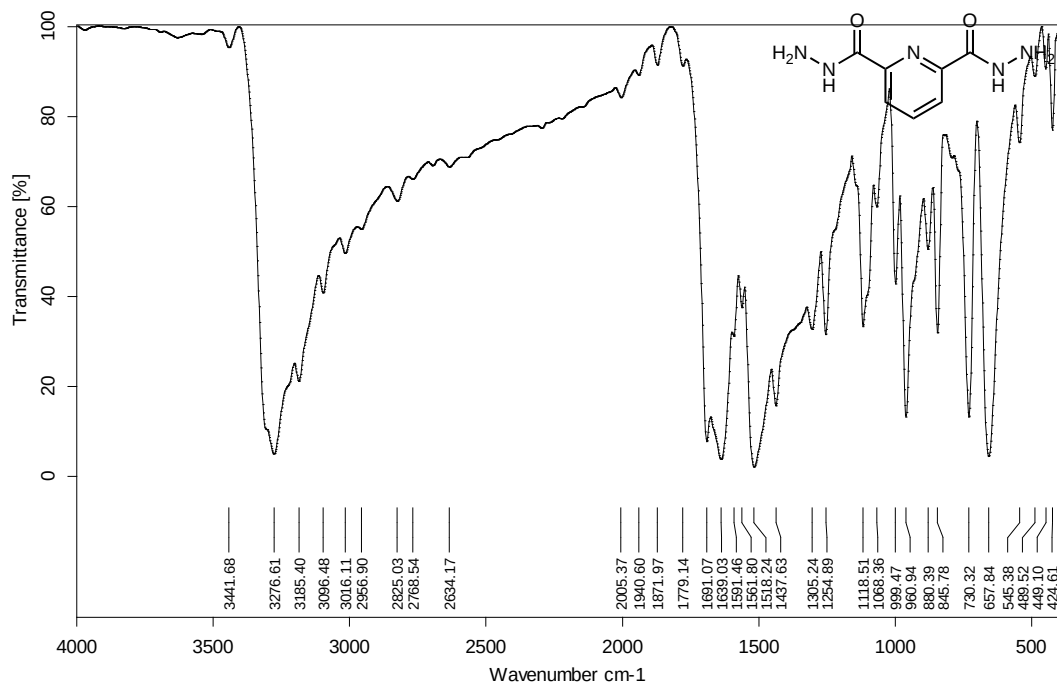


Figure 30: IR spectrum of compound III.

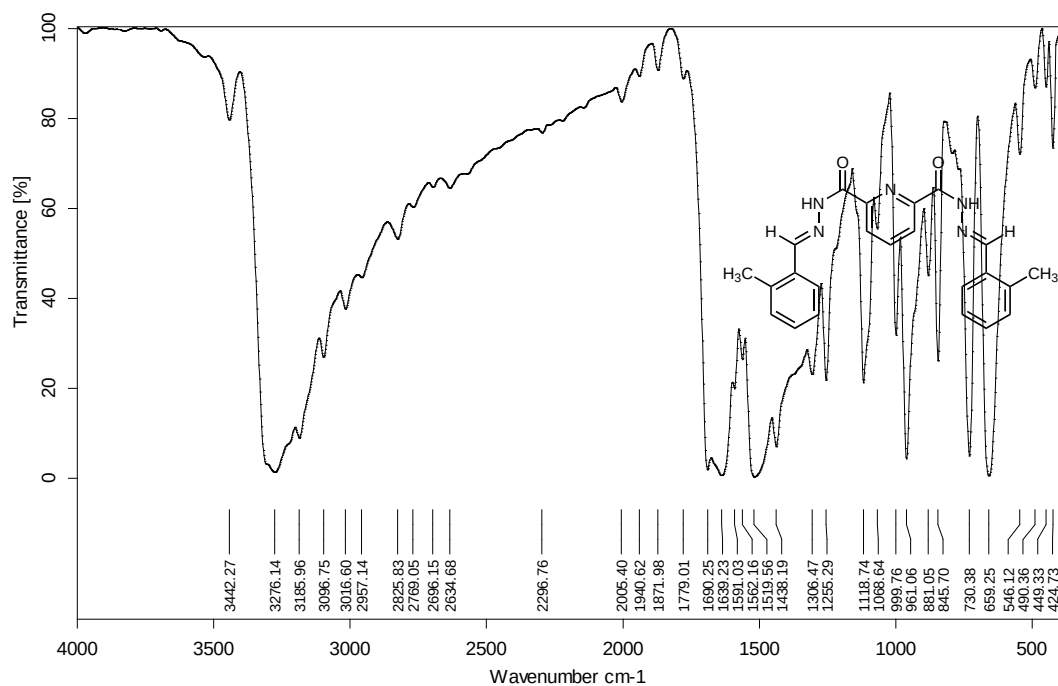


Figure 31: IR spectrum of compound IX.

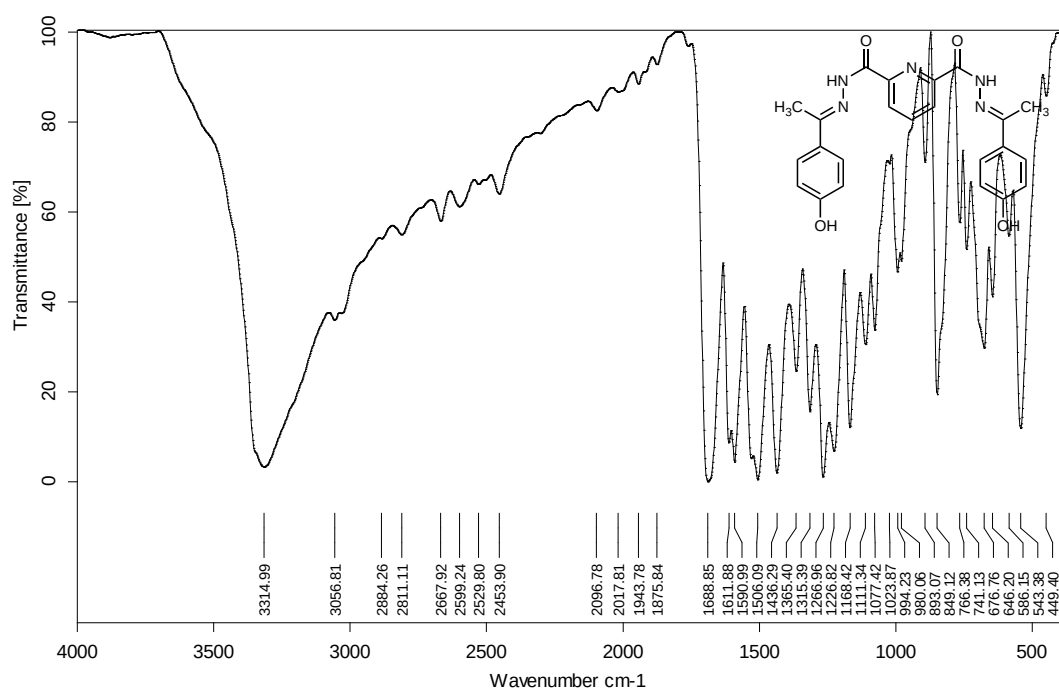


Figure 32: IR spectrum of compound X.

Table 13: ProTox 3.0 Results of compound **IV**

ProTox-3.0 - Prediction of TOXicity of chemicals			
Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Active	0.65
	Neurotoxicity	Inactive	0.56
	Nephrotoxicity	Active	0.56
	Respiratory toxicity	Inactive	0.52
	Cardiotoxicity	Inactive	0.78
Classification	Target	Prediction	Probability
Toxicity end points	Carcinogenicity	Active	0.55
	Immunotoxicity	Active	0.78
	Mutagenicity	Inactive	0.57
	Cytotoxicity	Inactive	0.75
	BBB-barrier	Inactive	0.54
	Ecotoxicity	Inactive	0.81
	Clinical toxicity	Active	0.58
	Nutritional toxicity	Inactive	0.63
Classification	Target	Prediction	Probability
Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Active	0.70
	Androgen Receptor (AR)	Inactive	0.97
	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	1
	Aromatase	Inactive	0.95
	Estrogen Receptor Alpha (ER)	Inactive	0.91
	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive	0.97
	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Inactive	0.98
Classification	Target	Prediction	Probability
Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.86
	Heat shock factor response element (HSE)	Inactive	0.86
	Mitochondrial Membrane Potential (MMP)	Inactive	0.68
	Phosphoprotein (Tumor Suppressor) p53	Inactive	0.90
	ATPase family AAA domain-containing protein 5 (ATAD5)	Active	0.51
Classification	Target	Prediction	Probability
Molecular Initiating Events	Thyroid hormone receptor alpha (THR α)	Inactive	0.59

	Thyroid hormone receptor beta (THRβ)	Inactive	0.68
	Transthyretin (TTR)	Active	0.51
	Ryanodine receptor (RYP)	Inactive	0.88
	GABA receptor (GABAR)	Inactive	0.81
	Glutamate N-methyl-D-aspartate receptor (NMDAR)	Inactive	0.83
	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPA)	Inactive	0.91
	Kainate receptor (KAR)	Inactive	0.99
	Achetylcholinesterase (AChE)	Inactive	0.96
	Constitutive androstane receptor (CAR)	Inactive	0.99
	Pregnane X receptor (PXR)	Inactive	0.86
	NADH-quinone oxidoreductase (NADHox)	Inactive	0.94
	Voltage gated sodium channel (VGSC)	Inactive	0.78
	Na ⁺ /I ⁻ symporter (NIS)	Inactive	0.97
Classification	Target	Prediction	Probability
Metabolism	Cytochrome CYP1A2	Inactive	0.78
	Cytochrome CYP2C19	Inactive	0.89
	Cytochrome CYP2C9	Inactive	0.58
	Cytochrome CYP2D6	Inactive	0.82
	Cytochrome CYP3A4	Inactive	0.84
	Cytochrome CYP2E1	Inactive	0.99

الملخص العربي

الليشمانيا هو مرض طفيلي ينتقل عبر لدغات ذباب الرمل. يمثل هذا المرض تحديًا كبيرًا للصحة العامة في العديد من المناطق الاستوائية وشبه الاستوائية، مما يؤدي إلى معدلات عالية من العدوى والوفيات. العلاجات الحالية، مثل الميلتيفوسين، محدودة بسبب السمية ومقاومة الطفيليات ومشكلات الوصول إلى العلاج. لذا فإن تطوير مركبات جديدة مع تحسين الفعالية وتقليل السمية أمر ضروري لمكافحة هذا المرض بفعالية وتوفير خيارات علاجية أفضل. تهدف هذه الدراسة إلى تشييد وتوصيف مشتقات حمض الديكولين، وتقييم أنشطتها المضادة للليشمانيا، ومناقشة علاقة التركيب بالنشاط لأكثر المركبات فعالية.

نجحت هذه الدراسة في تشييد مشتقات حمض الديكولين، حيث تم تحقيق نسب إنتاج تتراوح بين 33.1% و 85.46%، مع طريقة توليف سهلة نسبيا مما يجعل هذه المركبات المشيدة جذابة للدراسات المستقبلية. وقد تم تأكيد التركيب الكيميائي من خلال التحليل الطيفي والتحليل العنصري.

أظهرت الفحوصات في البايولوجية ضد شكلين من *L. major* أن جميع المركبات فعالة، ولكن أظهر المركب IV الفعالية القصوى (IC_{50}) بمقدار $2.84 \pm 0.14 \mu M$ ضد البروماستيغوتات و $3.84 \pm 0.16 \mu M$ ضد الأماستيغوتات، متفوقًا على الدواء المرجعي ميلتيفوسين بحوالي ثلاثة أضعاف. طريقة عمل المركب لتنشيط عمل الليشمانيا تمت دراستها في تجربة ضد حمض الفوليك والنتائج أظهرت أن المركب 4 يكسب فعاليته بأكبر صورة عن طريق تثبيط مسار حمض الفوليك. هذه النتائج تم تدعيمها عن طريق التمثيل الجزئي، الذي كشف عن درجة توجيه قدرها

$$-13.73 \text{ Kcal/mol} \text{ لـ } Lm\text{-PTR1}، \text{ متفوقًا بذلك على التريميثوبريم } (-11.91 \frac{\text{Kcal}}{\text{mol}})$$

بالإضافة إلى ذلك، أظهرت اختبارات السمية الخلوية للمركب IV أن المركب آمن نسبياً، متفوقاً على الميلتيفوسين. كما اقترحت التحليلات الحاسوبية مزيداً من التوافر البيولوجي وإمكانية تحسين ملفات السمية، مما يعزز من جدوى هذه المركبات كمرشحات واعدة للعلاج ضد الليشمانيا.

جامعة البحرين

قسم الكيمياء

كلية العلوم



تشديد مشتقات حمض الديكولين ذات النشاط المضاد للإشمانيا

أطروحة مقدمة كجزء من متطلبات الحصول على درجة الماجستير في
الكيمياء البيئية

إعداد

حسين عبدالحكيم صقر

20143110

إشراف

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قسم الكيمياء - كلية العلوم - جامعة
البحرين

جامعة البحرين

سبتمبر 2024