A Review on the Novel Heterocyclic Antileishmanial Drugs for the Treatment of Leishmaniasis

By

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Declaration

It is hereby declared that

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2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
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Abstract

New antileishmanialdrugs must be developed to make the treatment of leishmaniasis available for the people all over the world. It must be ensured that every single person has access to the medicines. Alternatives to the current antileishmanials are not only necessary for the lack of availability but also necessary for another crucial problem that is the toxicity level of the current medicines. In some cases, the current antileishmanials appeared to be ineffective because parasite resistance occurred. Therefore, search for alternatives should be done so that we can use them in different clinical situations like parasite resistance and toxicity. This review was done with the aim to study newer molecules that can be used as an alternative to the current antileishmanial agents.

Keywords: leishmaniasis; antileishmanials; novel; synthesis; evaluation; efficacy.

Dedication

To my mother and father for the enormous support till the end.

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Table of Contents

Declaration.....**ii**

Approval ..... **iii**

Abstract.....**iv**

Dedication ..... Error! Bookmark not defined.

Acknowledgement.....**vi**

Table of Contents.....**vii**

List of Tables ..... **viii**

List of Figures.....**xii**

List of Acronyms.....**xvi**

Chapter 1 Introduction.....**1**

1.1 Leishmaniasis.....1

1.2 Classification and Clinical picture.....1

1.3 Process of Infection.....3

1.4 Diagnosis, Treatment and Management 5

1.5 Drugs that are currently in use……...22

1.6 Monotherapy and Multitherapy…….26

Chapter 2 Results and Discussion **11**

Chapter 3 Conclusion and Future plan **93**

References.....**95**

List of Tables

Table 1: Species of parasites that are responsible for the treatment of Leishmaniasis 5

Table 2: Mechanism of action of the current antileishmanials 9

Table 3: Structural information of the 2-chloro-N-arylacetamides 11

Table 4: In Vitro activity of the 2-chloro-N-arylacetamides 12

Table 5: Molecular properties of the synthesized compounds 14

Table 6: Molecular information and In Vitro activity of the compounds 16

Table 7: In Vitro activities and molecular information of the synthesized compounds 18

Table 8: Molecular information and In Vitro activity of the synthesized thiophene derivatives 20

Table 9: Molecular information and In Vitro activity of the synthesized furan derivatives 20

Table 10: Molecular information, anti leishmanial activity and cytotoxicity of the synthesized compounds 23

Table 11: In Vitro activity of the synthesized compounds 24

Table 12: Molecular information of the synthesized compounds 25

Table 13: antileishmanial activity of the synthesized compounds against L. amazonesis 28

Table 14: antileishmanial activity of the synthesized compounds against L. braziliensis 28

Table 15: antileishmanial activity of the synthesized compounds against L. infantum 28

Table 16: Test for cytotoxicity of the synthesized compounds 30

Table 17: Molecular information of the synthesized compounds 31

Table 18: Antileishmanial activity of the synthesized compounds 33

Table 19: Molecular properties of the synthesized compounds 34

Table 20: Antileishmanial activity and cytotoxicity in THP1 and L929 cells 35

Table 21: Antileishmanial activity and cytotoxicity in VERO cells 35

Table 22: Molecular properties of the compounds 36

Table 23: Aqueous solubility of the synthesized compounds.....36

Table 24: In Vitro analysis of the compounds (Mäntylä, Garnier, et al., 2004).....36

Table 25: Molecular properties of the synthesized compounds.....39

Table 26: Antileishmanial activity and cytotoxicity of 1, 3-bis (aryloxy) propan-2amines....39

Table 27: Antileishmanial activity and cytotoxicity of the compounds 41

Table 28: antileishmanial activity of the compounds against different species of Leishmania parasites.....43

Table 29: In Vitro activity of the tetrahydro-β-carboline analogs 44

Table 30: Molecular properties of the compound.....45

Table 31: Molecular information of the 3-substituted-4-hydroxycoumarin 47

Table 32: Antileishmanial activity of the 3-substituted-4-hydroxycoumarin.....47

Table 33: Antileishmanial activity of different isolated compounds.....49

Table 34: Cytotoxicity (CC_{50}) and selective index (SI) of different isolated compounds.....49

Table 35: In Vitro antileishmanial activity and cytotoxicity of rhodacyanines.....51

Table 36: Inhibition of rhodacyanines in macrophages.....51

Table 37: Dosing regimen, inhibition percentage and confidence limit percentage of rhodacyanines 51

Table 38: Molecular information and antileishmanial activity of the compounds against different species of Leishmania parasites 53

Table 39: In Vitro antileishmanial activity 55

Table 40: Molecular information and In Vitro antileishmanial activity and cytotoxicity 57

Table 41: antileishmanial activity and cytotoxicity of th following compounds.....57

Table 42: Biological activity of Picramnia gracilis Tul.....58

Table 43: antileishmanil ativity of the synthesized compounds against different species of Leishmnia parasites.....60

Table 44: biological evalutation of the three compounds in comparison with Amphotericin B 62
Table 45: Molecular properties of compounds 63
Table 46: In Vitro antileishmanial activity and cytotoxicity of the synthesized compounds .. 64
Table 47: Structural information and biological evaluation against L. infantum 66
Table 48: Structural information and biological evaluation against L. donovani 66
Table 49: In Vitro antileishmanial activity and cytotoxicity of the synthesized compounds .. 68
Table 50: The effect of the compounds on intracellular amastigotes in a dose of 3 μM. 68
Table 51: Molecular information of the synthesized compounds 69
Table 52: Antileishmanial activity of the synthesized compounds 69
Table 53: Molecular properties and the antileishmanial activity of the compounds 71
Table 54: molecular properties of the pyridinium hydrazone derivatives 73
Table 55: Antileishmanial activity of the pyridinium hydrazone derivatives 73
Table 56: Molecular information of 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones 74
Table 57: Molecular information of the 3-substituted quinolines 76
Table 58: Antileishmanial activity and cytotoxicity of the quinoline compounds 77
Table 59: Molecular information and antileishmanial activity of pyrazole carbaldehydes 78
Table 60: Molecular information, antileishmanial activity and cytotoxicity of the synthesized compounds 79
Table 61: Antileishmanial activity and cytotoxicity of substituted quinolines 81
Table 62: Antileishmanial activity and lethal concentration of the synthesized compounds .. 82
Table 63: CC_{50} and LD_{50} of the most potent compounds of the series 82
Table 64: Antileishmanial activity and cytotoxicity 84
Table 65: Molecular information and percentage of inhibition of 2, 4, 6-trisubstituted pyrimidines and 1, 3, 5-triazines 84

Table 66: In Vitro and In Vivo antileishmanial activity 86

Table 67: Different properties of Sitamaquine 87

Table 68: Baseline characteristics of the included patients 89

Table 69: Therapeutic outcome 3 months – D90 – and 7 months – D210 – from the beginning of the treatment by treatment group (intention-to-treat analysis) 90

Table 70: Pharmacokinetic parameters after conventional linear and allometric dosing in separate groups of Miltefosine-treated Eastern African pediatric patients with visceral leishmaniasis 92

List of Figures

Figure 1: Lifecycle of leishmania parasite (Esch & Petersen, 2013).....4

Figure 2: Current antileishmanial drugs: sodium stibogluconate (1); meglumine antimoniate(2); Pentamidine(3); miltefosine(4); Amphotericin B (5); paromomycin (6).....8

Figure 3: General synthetic route of 2-chloro-N-arylacetamide derivatives 11

Figure 4: Synthesis of 1, 3, 4 thiadiazol – 2 – ylthio acetamides from 5- nitrofuran 13

Figure 5: General structure of 1, 3, 4 thiadiazol – 2 – ylthio acetamides 13

Figure 6: Synthesis of 4-(1H-pyrazole-1-yl) derivatives 16

Figure 7: Cytotoxicity test results 17

Figure 8: Synthesis of 5-(5-nitroaryl)-2-substituted-thio-1, 3, 4-thiadiazoles 18

Figure 9: General procedure for synthesis 20

Figure 10: General structure of compound 1 22

Figure 11: General procedure for synthesis 23

Figure 12: Synthesis of amino acid-coupled 1, 2, 4-triazoles 24

Figure 13: Compound 1 26

Figure 14: General structure for compound 2 27

Figure 15: General procedure for synthesis of pterocarpanquinones 27

Figure 16: General procedure for synthesis of Thiosemicarbaone and Thiazolidinone (Tenório et al., 2005) 29

Figure 17: General structure of tiosemicarbazone 30

Figure 18: General structure of thiazolidinone 30

Figure 19: Synthesis of β-carbolines 32

Figure 20: General procedure for synthesis of 4-Phenyl-1, 3-thiazole-2-amines 34

Figure 21: general structure of buparvaquone 36

Figure 22: Synthetic route of 1, 3-bis (aryloxy) propan-2amines.....38

Figure 23: General structure of Chalcones 40
Figure 24: Synthetic procedure for compound 6 40
Figure 25: Synthetic procedure for compound 22 41
Figure 26: Synthesis of compound 24 from compound 16 41
Figure 27: General procedure for synthesis of compound 14-26 (Glaser et al., 2014) 42
Figure 28: General procedure for synthesis of compound 27 (Glaser et al., 2014) 42
Figure 29: Synthesis of compound 9. 44
Figure 30: Synthesis of compound 11 44
Figure 31: General procedure for synthesis of 3-substituted-4-hydroxycoumarin 46
Figure 32: Compound (9) Flavopereirinee, isolated from Geissospermum vellosii 48
Figure 33: Synthesis of compound 8 50
Figure 34: compound 7 50
Figure 35: compound 8, 9 and 10 51
Figure 36: General procedure for synthesis of amodiaquine analogs 53
Figure 37: General structures of pyrimidine-β-carboline 54
Figure 38: Synthesis of conpound 6 and compound 7 56
Figure 39: Synthesis of compound 14, 17 and 20 56
Figure 40: Structure of 5, 3'-Hydroxy-7, 4'-dimethoxyflavanone isolated from Picramniracilisa gracilis 58
Figure 41: The Morita–Baylis–Hillman reaction (MBHR) 59
Figure 42: The difference between Chalcone and chalcone like structures 59
Figure 43: General synthetic route for compounds 1d, 1e, 1g 60
Figure 44: Compounds that were synthesized 60
Figure 45: Structure of safrole 61
Figure 46: Synthesis of Di-hydrodillapiole and isodillapiole 61

Figure 47: Test for cytotoxicity of Dillapiole on the 3T3 fibrobast cells 62

Figure 48: Synthesis of compound 4 63

Figure 49: synthesis of compound 9 and compound 10 63

Figure 50: Synthesis of derivatives from 9-methyl-1phenyl-9H-pyrido [3, 4-b] indole 65

Figure 51: General procedure for synthesis for a series of pyrazole derivatives 67

Figure 52: Synthesis of quinoline-4-carboxylic acid 69

Figure 53: General procedure for synthesis of Sulfonamide 4-methoxychalcone derivatives 70

Figure 54: Structure of compound 3e 71

Figure 55: Structure of compound 3f 71

Figure 56: Structure of 3i 72

Figure 57: General procedure for synthesis of the pyridinium hydrazone derivatives 72

Figure 58: General procedure for synthesis of 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones 74

Figure 59: Mechanism of formation of 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones . 75

Figure 60: General procedure for synthesis of 3-substituted quinolines 76

Figure 62: General procedure for synthesis of pyrazole carbaldehyde derivatives 78

Figure 63: General procedure for synthesis of Quinoxaline 1, 4- di- N- oxide 79

Figure 64: General procedure for synthesis of a-enolic dithioesters (2) 80

Figure 65: Synthetic approach for the differently substituted quinolines 80

Figure 66: General procedure for synthesis of 1, 4-bis (substituted benzal hydrazino) phthalazines 82

Figure 67: structure of (+)-Phyllanthidine 83

Figure 68: General procedure for synthesis of 2, 4, 6-trisubstituted pyrimidines and 1, 3, 5-triazines 85

Figure 69: structure of an 8 aminoquinoline analog, Sitamaquine 87

Figure 70: structure of Pentamidine (Bakunov et al., 2009).....88

Figure 71: Structure of tamoxifen.....90

Figure 72: Structure of Miltefosine.....91

List of Acronyms

CC_{50}	Cytotoxic concentration for 50% population
IC_{50}	Inhibitory concentration for 50% population
SI	Selectivity Index
EE	Ethanol Extract
NF	Neutral Fraction
NA	Not Active
ND	Not Determined
AF	Alkaloid Fraction
FrDcm	Dichloromethane Fraction
FrMeOH	Methanol Fraction
AmpB	Amphtericin B
Sb^V	Sodium Stibogluconate
AQ	Amodiaquine
DMFO	α-difluoromethyl ornithinex

Chapter 1

Introduction

1.1 Leishmaniasis

Leishmaniasis is the term which is used for a visceral anthropologic and zoonotic disease. It is distributed in about 89 countries all over the world. Every year, there are over 350 million people who are put at risk due to this parasitic disease. The estimated annual incidence worldwide is almost two million. It is one of the most neglected disease in both tropical and subtropical countries. Due to this there is an evaluation of the risk factors such as under reporting and asymptomatic infection post leishmaniasis. Due to the lack of knowledge about the disease, resistance against the current drugs are developing. Leishmaniasis generally occurs among the people with a poor immune system. Thus, it is mostly seen among people who suffer from malnutrition, living in poor household areas. Today, it is an issue of major public concern.The type of disease that usually occurs in a particular region, is usually determined by the global distribution of each of the *Leishmania* species. For example, *L. donovani* is responsible for visceral leishmaniasis in Africa as well as South Asia. On the other hand, people living in the Mediterranean or in the Middle East and Latin America and some other parts of Asia; they are infected by*L. infantum* species. Even then, the climate of an area and other environmental changes have the potential to modify the geographic range of both the vectors such as: sand fly and also the parasites in the world.

1.2 Classification and Clinical picture

Three clinical forms of leishmaniasis can be found in humans. They are cutaneous, that occurs in skin; mucocutaneous, occurring in the mucus membrane and visceral, that occurs in the visceral organs. Clinical manifestations may vary by the parasitic species, area of

infection, and factors related to the host. Cutaneous Leishmaniasis is the most prevailing type of Leishmaniasis. Any individual who is exposed to the parasites causing cutaneous leishmaniasis will develop sores on the skin. Skin lesions start to be visible in some parts of the body. Lesions usually forms at the site where the vector may bite. Crusted papules or skin ulcers will be on the exposed skin and there will be a sporotrichotic spread of the sores.Mucocutaneous leishmaniasis infection that causes the skin to look horribly disfiguring. It occurs from the local destruction of the tissues of the eyelids, oro- and naso-pharynx, mouth and the nose. It progresses and hampers the nutrition along with the respiratory function. The pathogens of MCL is a result of a complex interplay between the host and the parasite factors.The most severe type is Visceral Leishmaniasis.VL produces only one out of 30–100 infected cases. An average advancement to typical VL usually requires 2-8 months or can occur often sooner, but there has been cases that reported the advancement takes as long as two decades after infection. Visceral leishmaniasis has the highest fatality rate. Usually the person develops anorexia, weight loss, pallor, fever, pain, and hepatosplenomegaly; which is commonly known as splenomegaly, lymphaedenopathy and progressive deterioration.It is a rule that the patients who come for diagnosis must appear with fever, thrombocytopenia and hepatosplenomegaly. Children might suffer from growth retardation. Late results may include abdominal distension, epistaxis, ascites, gingival hemorrhage and also edema. The laboratory results may thrombocytopenia, neutropenia, normocytic – normochromic anemia hepatic anemia, hypergammalobulinemia and hypoalbuminemia. Death can occur by bleeding or even secondary infection. In VL, the parasite proliferation occurs in the infected macrophages that are in the liver, bone marrow and in the spleen of the patients. It also causes hepatosplenomegaly along with suppression of bone marrow of the infected person. When the patient is co-infected with HIV, he will suffer much more. CL may occur on the exposed flesh within weeks or even months of the

vector bite. It occurs with maybe one or more skin lesions. Lesions may appear months or even years later after the bite. Symptoms for CL varies from ulcerative skin lesions to devastating mucosal inflammation. It is often followed by local lymphadenopathy. Viscerotropic Leishmania infection usually leads to asymptomatic or mild infection followed by spontaneous resolution.

1.3 Process of Infection

Leishmaniasis is a parasitic disease that spreads through a vector. The genus *Leishmania* actually belongs to a family of *Trypanosomatidae*. The transmission of leishmaniasis occurs through hematophagus vectors e.g. female sand flies of *Phlebotomine* and *Lutzomyia* genera. It has a complex life cycle of two stages:

i) Extracellular stage: within the invertebrate host, where they exist in the promstigote form.

ii) Intracellular stage: within the vertebrae host, where they exist in the amastigote form.

The species of *Leishmania* remains in the digestive tract of the vector in the beginning and multiplies inside it. When the vector feeds on the blood of the mammalian host, and the parasites are then transmitted to them. The parasites are in the promastigote form when they are inside the vector and when they enter the vertebrate hosts, they are in amastigote form and they becomespherical in shape. Inside the vertebrates, the parasites engage in initiating infection. They do that by the receptor mediated binding of the infective promastigotes, which are delivered to the tissues of the host during the time the vector feeds on the host. The parasitophorous vacuoles, in which the parasites are housed, fuse with lysosomes and forms phagolysosomes. Within the phagolysosomes, the parasites replicate and turn into amastigotes. As the parasite burden increases, the infected hosts get physically disrupted. The

infected macrophages. The infected macrophages deliver the extracellular amastigotes into the surrounding tissues where uninfected macrophages engulf them. Eventually both the

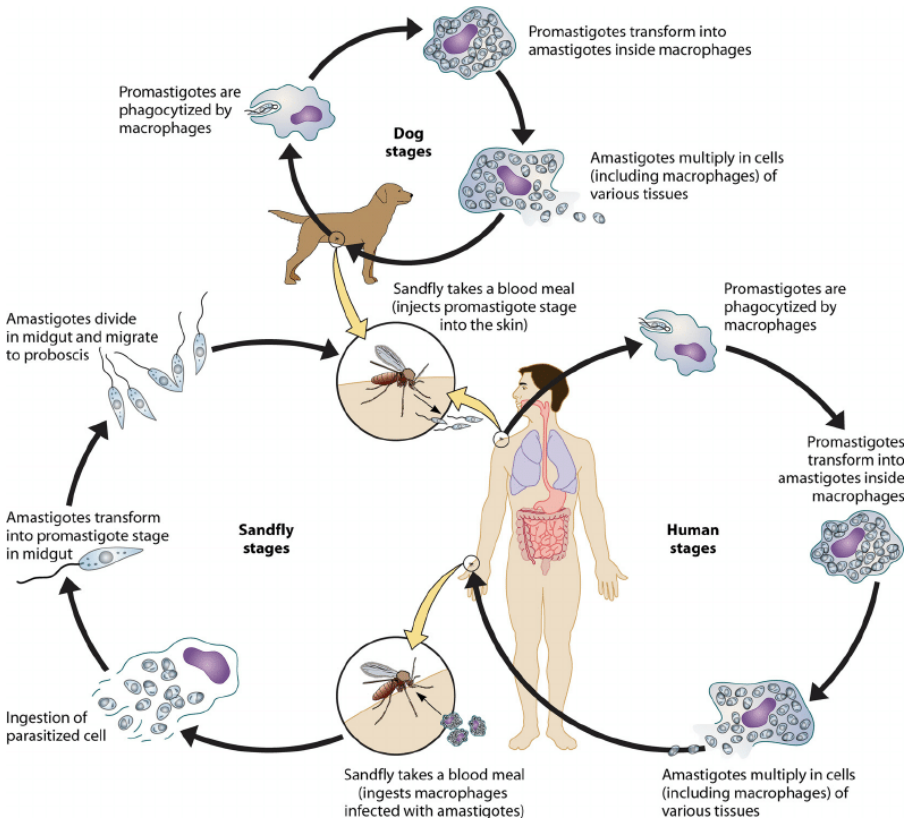


Figure 1: Lifecycle of leishmania parasite (Esch & Petersen, 2013)

parasites and the infected macrophages metastasize within the skin and visceral organs. There are more than twenty species of *Leishmania* parasites that causes infection. One of the most common vectors is the sand-fly. Parasites can be transmitted by the bite of a female sand fly if it is infected. Sandflies are of over 600 species. Even the ones that bite the humans are of various genera. The genera that are proven to spread Leishmaniasis in humans are *Lutzomyia and Phlebotomus.* Usually, each species of sand-fly transmits only one type of parasite into a host. Although it is rare, there is still a possibility for the parasites to be transmitted by other means like sharing the same needle, blood transfusions or even from mother to child during pregnancy.

Table 1: Species of parasites that are responsible for the treatment of Leishmaniasis

Species of *leishmania*	Diseases (in humans)	Geographical distribution	Vectors
L. infantum	VL, CL	Mediterranean basin; Middle East and Central Asia to Pakistan; China; Central and South America	Dog
L. major	CL	North Africa, Middle East and Central Asia, Sub-Saharan Africa and Sahel belt	*Gerbillidae* rodents
L. aethiopica	CL	Ethiopia, Kenya Rock hyraxes	Sandfly
L Mexicana	CL	Central America Various forest	Rodents
L. amazonensis	CL	South America, north of the Amazon	Forest rodents
L. donovani	VL	Far East	Sandfly
L. venezuelensis	CL	Venezuela	Sandfly
L. braziliensis	CL, ML	South America, Central America and Mexico	Numerous rain forest mammals
L. peruviana	CL	Peruvian Andes	Dog
L. donovani	VL	Ethiopia, Sudan, Kenya, India, China, Bangladesh, Burma	Sand fly

1.4 Diagnosis, Treatment and Management

A number of therapies are available for the different types of leishmaniasis. Based on the differences of practice in different regions, the preference of first line and second line treatment also differs. The current control strategies for a disease like is based on vector and reservoir control. Prevention strategies are being focused on currently but along with that new antileishmanial molecules are being studied on.

Diagnosis for cutaneous leishmaniasis is mainly done based on smears on the skin. Pentavalent antimonials are used in the treatment. A first line drug for visceral leishmaniasis is Pentavalent antimonials. Moreover, in the developed countries, second line drugs are used such as: Amphotericin B. but such drugs are only used in the developed areas because they are too costly. Diagnosis for cutaneous leishmaniasis is primarily based on skin smears and treatment is done by using Pentavalent antimonials. Other means of treatment include immunotherapy, heat therapy and cryotherapy.

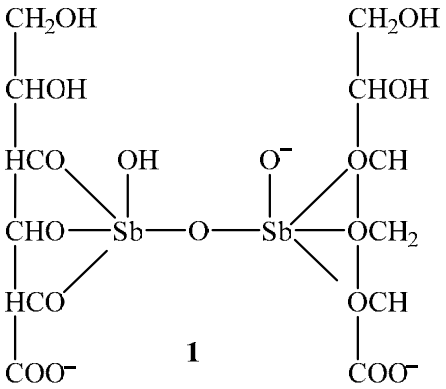
New antileishmanials must be developed to make the treatment a little less expensive and available for the people living in poor household areas. It must be ensured that every single people has the access to the medicines. Alternatives to the current medicines is also necessary for another crucial problem that is the toxicity level of the current medicines. For example, Pentavalent antimony has been considered a very important for therapy for leishmaniasis for such a long time. But eventually this agent had been causing multiple cases of toxicities. In some cases, they appeared to be ineffective because parasite resistance occurred. Therefore, alternatives to such medicines are necessary for being used in different clinical situations like parasite resistance and toxicity.

1.5 Drugs that are currently in use

At present, almost 25 compounds and formulations that can be used for its antileishmanial effects. But not all of them are of worth. The primary treatment for leishmaniasis is Pentavalent antimonial compounds, Glucantime and also Pentostam. Antibiotics such as amphotericin B is among the second line drugs. Another example of a second line compound is Miltefosine.

There were patients who showed resistance to the treatment that was done with antimonials. The solution to this problem was the use of Amphotericin B. it has been proven to have excellent antileishmanial activity but the limiting factor for this drug is its toxicity. This problem was solved with advent of lipid formulations. The deoxycholate in the amphotericin B molecule was replaced by other lipid groups. This masks the Amphotericin B molecule from the susceptible tissues that facilitates the uptake of the molecules by the reticuloendothelial cells and reduces toxicity Amphotericin B lipid associated formulations are:Amphotericin B colloidal dispersion, Amphotericin B lipid complex and liposomal Amphotericin B. this drug showed excellent activity when used against both visceral and

cutaneous leishmaniasis. Paromomycin is another antibiotic that is usually used to treat cutaneous leishmaniasis. The formulation that is currently in use has been proven to be very useful. Another very successful example of an antileishmanial drug is Pentamidine, which has been in clinical use against almost all forms of leishmaniasis. Althoughthis drug was proven to be a successful one at first, but the use of this drug was declined to a great extent due to its tendency to produce toxicity and its low efficacy. The latest in the list is Miltefosine. It is administered as an oral drug and is used for its leishmanicidal activities against visceral leishmaniasis.It is a very effective option for both immunocompetent and immunocompromised patients. Therefore, Miltefosine is definitely one of the most important of all of the recent advances made in antileishmanial therapy. Other clinically used alternatives for these leishmanicidals are: Sitamaquine, Allopurinol, some azoles like Fluconazole, Posaconazole, Ketoconazole and Itraconazole; and some other products that stimulates the immune system.



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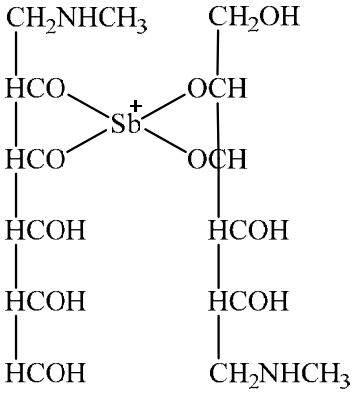

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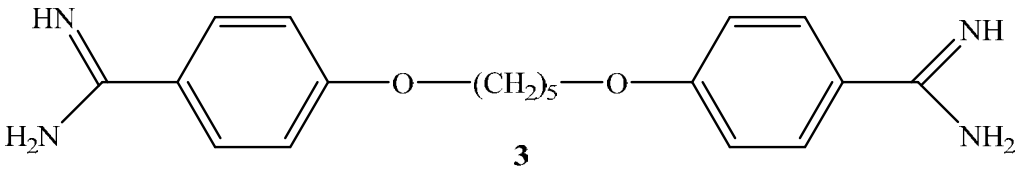

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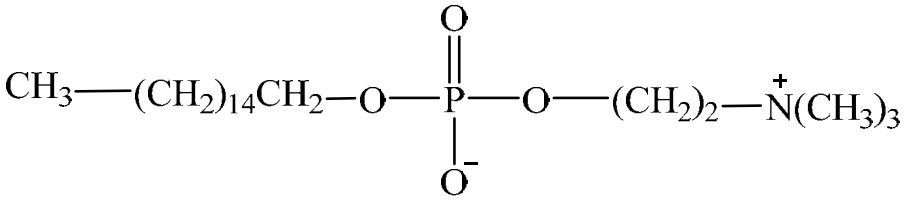

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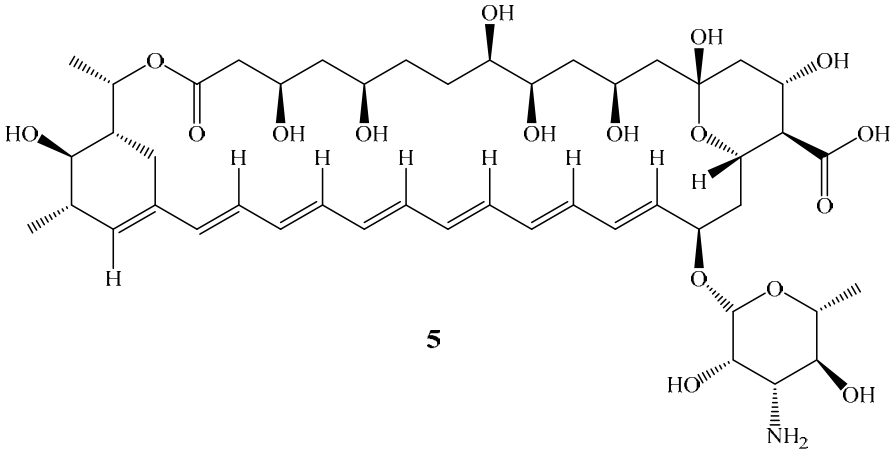

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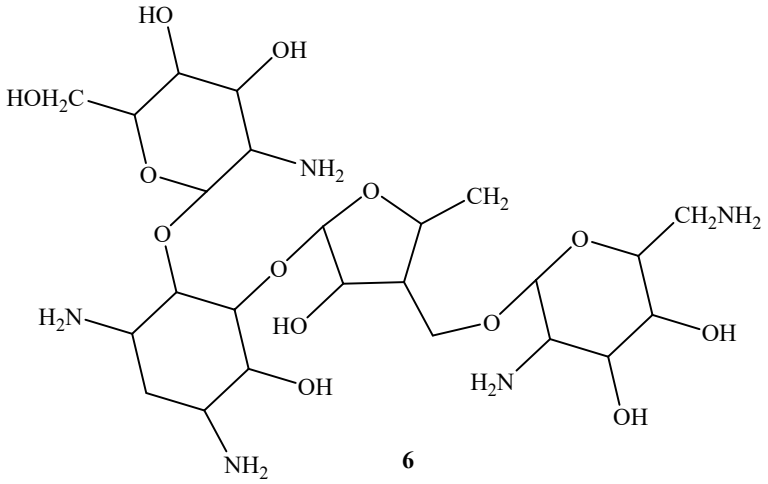


6

Figure 2: Current antileishmanial drugs: sodium stibogluconate (1); meglumine antimoniate(2);Pentamidine(3); miltefosine(4); Amphotericin B (5); paromomycin (6)

Table 2: Mechanism of action of the current antileishmanials

Drugs in use	Mechanism of action	Limitations
Pentavalent antimonial derivatives	Causes In Vivo reduction of Sb^V form to a more toxic SbIII form; also causes inhibition of glucolysis fatty acid beta-oxidation and inhibition of ADP phosphorylation. Along with nonspecific blocking of SH groups of proteins.	Toxicity Requires longer treatment Not effective against all species Resistance
Polyenic macrolide (Amphotericin B	Selective for 24 substituted sterols including ergosterol vis-a-vis cholesterol, the primary sterol counterpart to trigger cationic and anionic influx.	Toxicity
Alkylphospholipid	on mammalian cells, it causes modulation of the receptors of the cell surface inositol metabolism, phospholipase activation, protein kinase C and other mitogenic pathways eventually culminates in apoptosis	Only effective against VL
Aminoglycosides	It initiates protein synthesis and binds to the 30S ribosomal subunit. Also the promoted ribosomal subunit in association of cytoplasmatic and mitochondrial forms.	Toxicity Limited efficacy
Aromatic diamines	Acts on the pathogen genome related and affects the binding to the nucleic acids and DNA and produces disruptive effects like inhibition of the synthesis of DNA and RNA.	Toxicity Resistance

1.6 Monotherapy and Multitherapy

To choose between monotherapy or combination therapy, we at first need to consider the advantages of both of the processes. In case of the visceral form of Leishmaniasis, the researchers tend to choose multidrug therapy over anything. The idea is to use synergistic or additive activity at different sites. Using SSG and PM in a combination therapy was accidentally introduced during the 1980s when there was an epidemic of VL in Sudan. Since

then, several studies were done to establish a useful combination therapy for Leishmaniasis. Seifert and Croft in their experiment, showed that the highest potential of the drug Miltefosine was achieved when it was administered In Vivo with smaller concentrations of Amphotericin B and PM. Later on, different studies were done in Bangladesh, Africa, and India to reestablish the effectiveness of combination therapy. Regarding this, the American Society of Tropical Medicine and Hygiene (ASTMH) published guidelines in the year 2016.

Chapter 2

Results and Discussion

2-chloro-N-arylacetamides: synthesis, antileishmanial activity and QSAR studies.

There is a possibility that compounds such as 2-chloro-N-arylacetamides, alkylate the pivotal targets such as the thiol-based metabolism, Trypanosomatid, and can seriously damage the *Leishmania*parasites. This is a report on ten derivatives of 2-N-arylacetamide. In this report, the authors focused on the synthesis, biological evaluation, their half maximum inhibitory concentrations (IC_{50}) against *Leishmania amazonesis* (Stefânia Neiva Lavorato et al., 2008).

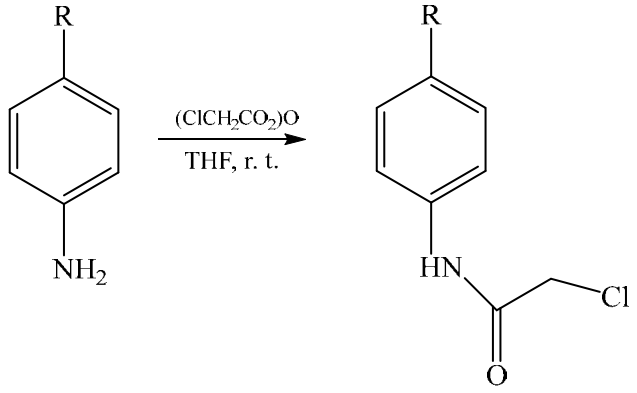


Figure 3: General synthetic route of 2-chloro-N-arylacetamide derivatives

Table 3: Structural information of the 2-chloro-N-arylacetamides

Compounds	R
2	CH_3CH_2COOH
3	COOH
5	$COCH_3$
8	NO_2

For this experiment, the synthesized compounds were tested against the strains of *Leishmania amazonesis.*Peritoneal macrophages used in this test were obtained from female BALB/c mice (8 weeks old). They were purchased from the Institute of Biological Sciences (ICB) from UFMG, and maintained under specific pathogen-free conditions.

Table 4: In Vitro activity of the 2-chloro-N-arylacetamides

Compounds	R	IC_{50}±SD (µM)	CC_{50}±SD (µM)	SI
2	$COOCH_2CH_3$	4.74±0.20	18.52±3.89	3.91
3	COOH	Inactive	ND	ND
5	$COCH_3$	5.39±0.67	34.28±16.66	6.36
8	NO_2	4.78±1.22	18.50±0.13	3.87
Amphotericin B	-	0.1±0.02	0.78±0.21	8.0

Amphotericin B was used as the positive control. All derivatives of the 2-chloro-N-arylacetamide showed very good antileishmanial activity except compound 3. Compounds 2, 5 and 8 were the most active ones with IC_{50} values below 6 µM. An electron-withdrawing aryl substituent present in the compounds might have contributed to improve their potential as alkylating agents. This is because the electron withdrawing aryl group increased the electrophilic character of the carbon alpha to chloro atom by the resonance effect.

1, 3, 4 thiadiazol – 2 – ylthio acetamides derived from 5- nitrofuran: Synthesis, antileishmanial activity and QSAR.

From the compound 5- (5- nitrofuran- 2- yl)-1, 3, 4 -thiadiazole, new compounds were synthesized to be tested for their antileishmanial activities. These compounds bear 2-mercaptoacetamide linker and they display In Vitro activity against the promastigote and amastigote forms of the parasite *Leishmania major*. This study contains the synthesis, antileishmanial activity, and the QSAR study of 2- (5- (5- nitrofuran- 2- yl)-1, 3, 4-thiadiazol-2-ylthio) acetamides(Vosooghi, Sabourian, Tahghighi, & Mahdavi, 2014).

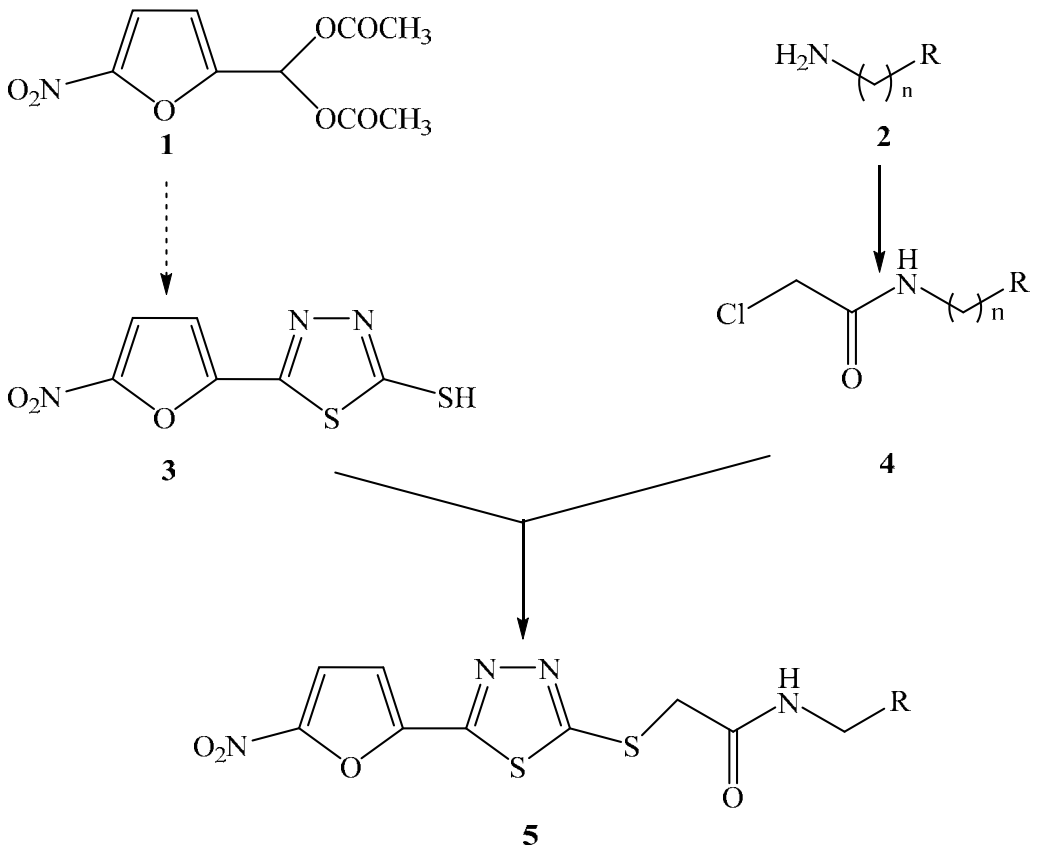


Figure 4: Synthesis of 1, 3, 4 thiadiazol – 2 – ylthio acetamides from 5- nitrofuran

At 25º C, the promastigotes were grown to be used for the assay. They were in their stationary phase when the promastigotes of *Leishmania major* was employed. The mouse peritoneal macrophages were used for the toxicity analysis of the compounds. MTT calorimetric assay was followed to determine the cell viability.

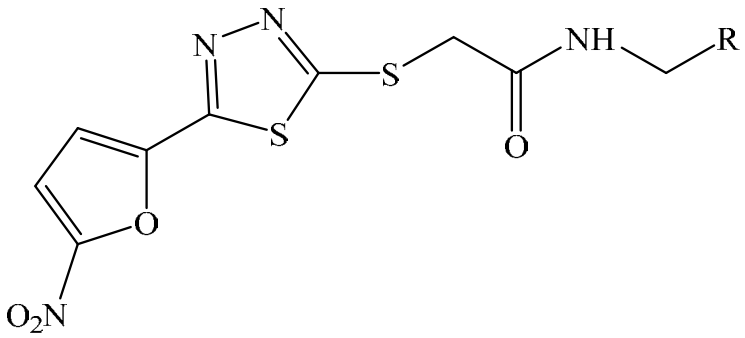


Figure 5: General structure of 1, 3, 4 thiadiazol – 2 – ylthio acetamides

Table 5: Molecular properties of the synthesized compounds

Compounds	**R**	**n**	**IC_{50}**	**CC_{50}**
5 a	Ph	0	46. 7	86.9
5 c	3-Cl-Ph	0	40. 6	74.29
5 f	4-MeO-Ph	0	59.9	64.36
5 h	3- NO_2-Ph	0	46. 7	83.93
5 i	2, 5-di-Ci-Ph	0	178	-
5 j	2 -F-4-NO_2 -Ph	0	559	-
5 k	5-Me-isoxazol-3-yl	0	114	-
5 m	Ph	1	66. 4	59.51
5 o	4-MeO-Ph	1	44. 6	91.35
5 q	3 , 4-di-MeO-Ph	2	19. 1	55.95
5 r	Me	2	19. 5	59.72

The reference drug was Glucantime. 5 q and 5 r appeared to be most potent with an IC_{50} value of 19.1 and 19.5 in 1M respectively. N-phenyl derivative 5a was compared to 5 c, the N-(3- or 4-halophenyl) analog. This comparison lead to the idea that a halogen substituent was responsible for its small increased anti-promastigote activity of the molecule. But the introduction of some other substituent, the 4-methoxyphenyl and 4-(trifluoromethyl) phenyl groups decreased its activity as an antileishmanial. 5i and 5j are di substituted - phenyl derivatives which showed a little lower activity. The compound 3-nitrophenyl, 5h exhibited a very similar activity to the compound N-phenyl derivative, 5a. The activity was reduced to a great extent when the N-phenyl group was replaced by the Nisoxazolyl or N-benzothiazolyl group. Moreover, it diminished the activity in the compound 5k in comparison to 5a. Then the IC_{50} values of the two compounds were compared, compounds 5a and 5m, revealed that introduction of different groups such as, N-benzyl group instead of the N-phenyl could not

enhance the inhibitory activity of the compounds against the promastigotes form of *L. major* strains. Interestingly, the compound 5o, a 4-methoxybenzyl derivative having IC_{50} = 44.6, exhibited better activity than 4-methoxyphenyl counterpart 5f having IC_{50} = 59.9. This study also revealed that the distance could be increased between the aryl ring and the nitrogen of acetamide residue. . It was clear that the 3, 4-dimethoxy phenethyl analog was the most potential one to be considered as an antileishmanial. Aside from the compound 5q, an N-propyl derivative 5r which had no aryl ring on pendent residue was among the most potent compounds. Therefore, the presence of the aromatic ring is not mandatory for the compounds of these series to possess an intrinsic antileishmanial activity.

Identifying new Active Antleishmanial compounds: 4-(1 H -Pyrazol-1-yl) Benzene sulfonamide Derivatives.

Pyrazoles are found to be active in both In Vitro tests as well as In Vivo tests against the parasites that cause leishmaniasis. This is a report where the authors described new derivative of pyrazole containing a sulfonamide group. The synthesis, the biological activity of the 4-(1H-pyrazol-1-yl) benzene sulfonamide derivatives along with their tendency to produce toxicity towards the mammalian cells, structure-activity relationship (SAR) is described as well(Marra et al., 2012).

Two species of parasites were used for this assay. They are: *Leishmania infantum* and *Leishmnia amazonesis.* Both the species of parasites were incubatedin metacyclic phase.The BALB/c mice were used to obtain peritoneal cells. These cells were used for infection of Leishmania parasites. Isolation of the parasites were also done by using these cells.

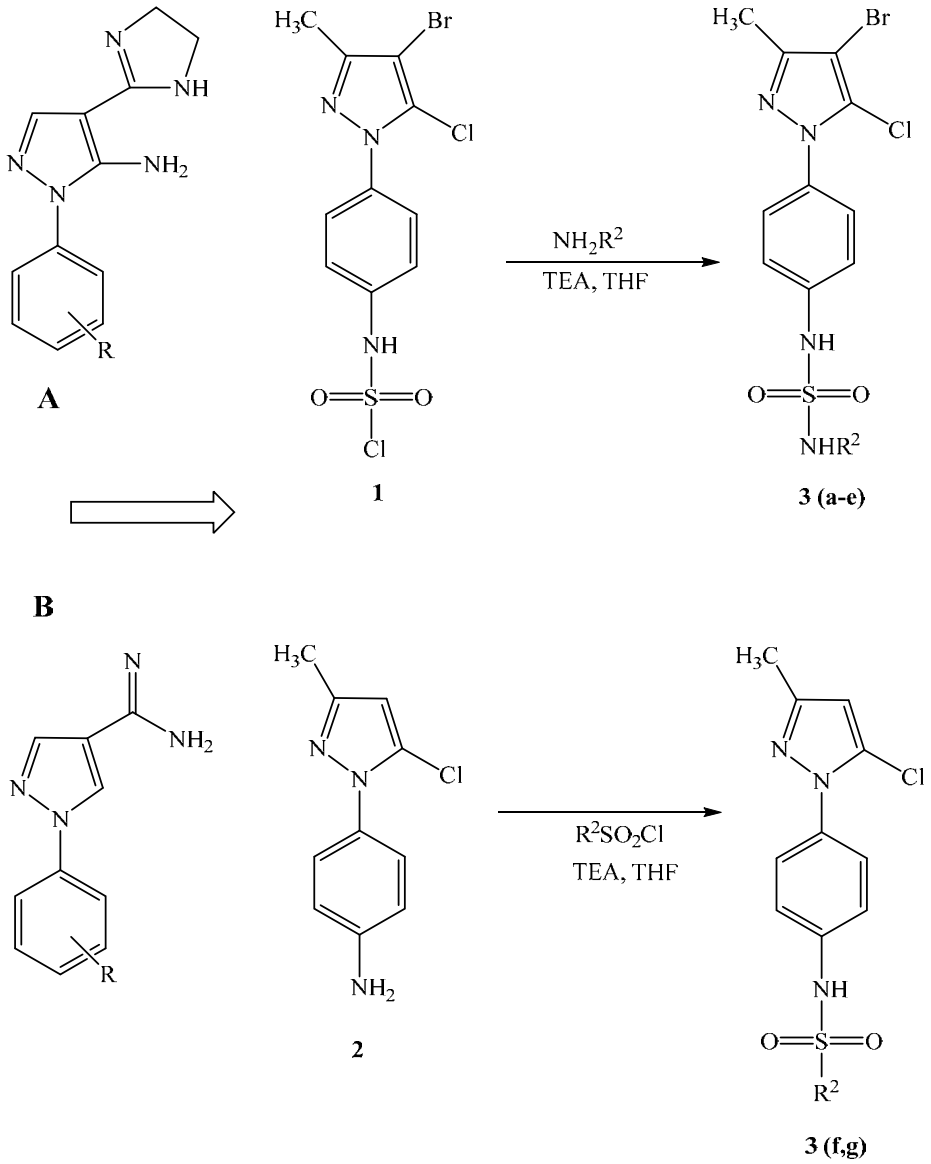


Figure 6: Synthesis of 4-(1H-pyrazole-1-yl) derivatives

Table 6: Molecular information and In Vitro activity of the compounds

Compounds	**R^2**	***Leishmania infantum*** **IC_{50}**	***Leishmania infantum*** **SI**	***Leishmania amazonesis*** **IC_{50}**	***Leishmania amazonesis*** **SI**
3a	H	0.228 ± 0.19	0.78	0.228 ± 0.33	0.78
3b	C_2H_5	0.059 ± 0.01	2.44	0.070 ± 0.02	2.05
3c	p-$CH_3C_6H_5$	0.123 ± 0.05	1.33	0.318 ± 0.59	0.51
3d	p-BrC_6H_5	0.099 ± 0.08	0.49	0.075 ± 0.01	0.65
3e	p-ClC_6H_5	0.065 ± 0.04	1.78	0.072 ± 0.05	1.61

The cytotoxicity of the compounds were also measured.The cytotoxicity profile is given in the following graph. It is expressed in IC_{50}/ 24 hr. the y axis shows cytotoxicity in mM. After this, the SI was calculated using the values in both the tables.The reference drug used for this assay was Pentamidine. Based on the overall results, it can be said that the compounds 3b – e

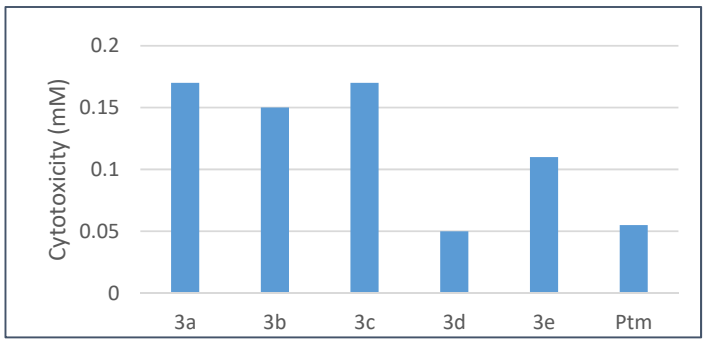


Figure 7: Cytotoxicity test results

showed outstanding activity on both of the promastigote forms of *Leishmania* parasites. The results were close to that of the reference drug, Pentamidine. The cytotoxic tests were done on murine peritoneal cells. In which, molecules 3b and 3c showed even better results than Pentamidine. Another molecule 3d shows results near to Pentamidine in terms of cytotoxicity. Therefore, these compounds are potential alternatives to be used as leishmanicidal agents.

5-(5-nitroaryl)-2-substituted-thio-1, 3, 4-thiadiazoles: Synthesis and antileishmanial activity.

1, 3, 4-thiadiazoles have anti parasitic properties which depends on their attachment to other heterocycles. It also depends on the type of substituent that is attached to it as well as the position it is attached to. This report focuses on the new structures of 5-(5-nitroaryl) 2-substituted thio-1, 3, 4thiadiazoles that were synthesized and their biological evaluation against the promastigote stages of *L. major* stains(Alipour et al., 2011).

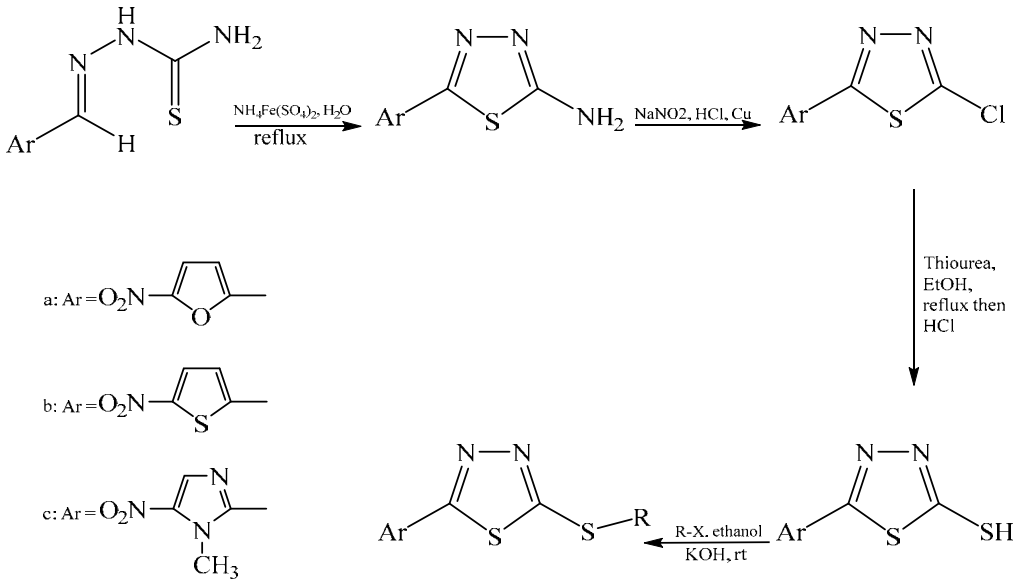
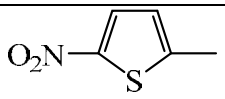
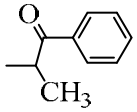
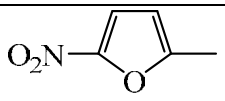
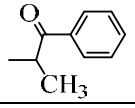
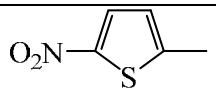
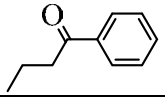
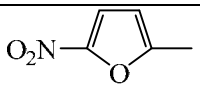
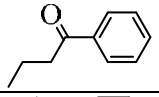
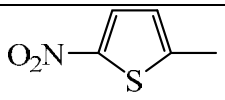
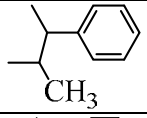
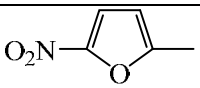
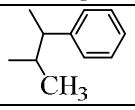


Figure 8: Synthesis of 5-(5-nitroaryl)-2-substituted-thio-1, 3, 4-thiadiazoles

The parasite in the promastigote form of *Leishmania major* were used. They were grown and maintained properly in blood cultures.Glucantime was the standard drug. The cytotoxicity of the drugs were measured in terms of IC_{50}. This helps to determine whether the new compounds have the potential to work as an antileishmanial or not.

Table 7: In Vitro activities and molecular information of the synthesized compounds

Compounds	Ar	R	IC_{50} (μM)
14			1.85
15			1.11
16			2.65
17			2.21
18			2.86
19			1.43

According to the data given above, all the compounds had IC_{50} values from 1.11 to 3.16 μM; which indicates that all they have excellent activity as antileishmanials as they were tested against the promastigotes of *L. major*. The two α-methyl benzyl derivatives are compound 18 and compound 19. α-methylphenacyl analogs are compounds 14 as well as 15. Both of these suggest that the carbonyl group are not necessary for the compounds to exert their optimum activity. Moreover, the replacement of the phenacyl group with a propiophenone homologue retained the activity for compounds 16 and 17. The structure-activity relationships for this series indicated that in all type of 5-(5-nitroaryl)-2thio-1, 3, 4-thiadiazoles, the S-pendant group have a high flexibility with the structural alteration, which retains a good antileishmanial activity.

The synthesis and biological evaluation of the 5-(nitroheteroaryl)-1, 3, 4-thiadiazols that contains acyclic amines at the C2 position.

The reason for the C2 position of 5-(nitroheteroaryl)-1, 3, 4-thiadiazoles to be chosen for modification is because it is the most flexible position.Modification of the molecule in C2 position affects its potency along with its other physical and chemical properties. The authors reported the synthesis of 5-(5-nitrofuran2-yl) - or 5-(5-nitrothiophen-2-yl)-1, 3, 4-thiadiazoles (II) that contain acyclic amine at their C2 position of the thiadiazole ring and the antileishmanial activity of the compounds were also determined. The promastigote form of the parasite*Leishmania major*was used for the analysis. It was grown and maintained at 25º C. The $IC_{50}$was determined.The toxicity of compounds such as compounds 6, 25, 29, 32,38 and others was assessed against mouse peritoneal macrophages.(Tahghighi et al., 2013).

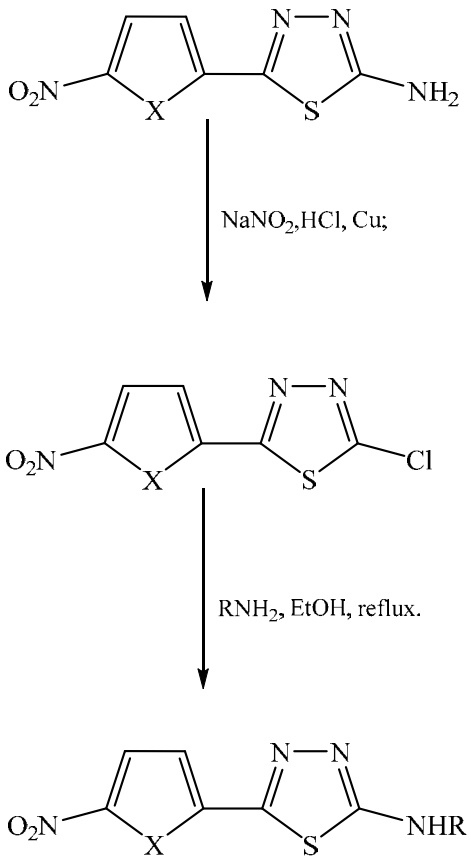


Figure 9: General procedure for synthesis

Table 8: Molecular information and In Vitro activity of the synthesized thiophene derivatives

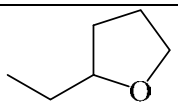
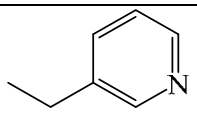
Compounds	R	Antipromastigote activity IC_{50} (µM)	Cytotoxicity CC_{50} (µM)	SI
27	Et	98.4 ± 0.12	-	-
28	c-Pr	97.3 ± 0.98	-	-
29	$(CH_2)_3OH$	3 ± 0.41	42.16	14.05
32	$(CH_2)_3OMe_2$	3 ± 0.5	37.80	12.60
38		35.8 ± 0.62	92.4	2.58
Glucantime	-	68.44[c]	-	-
Fluconazole	-	941.1 ± 4.98	-	-

Table 9: Molecular information and In Vitro activity of the synthesized furan derivatives

Compounds	R	Antipromastigote activity IC_{50} (µM)	Cytotoxicity CC_{50} (µM)	SI

3	Me	54 ± 0.17	-	-
4	Et	50 ± 0.8	-	-
6	$(CH_2)_2OH$	21 ± 0.65	122.11	5.81
7	$(CH_2)_3OH$	18 ± 0.2	62.33	3.46
9	$CH_2CH(OMe)_2$	13 ± 0.53	62.46	4.80
11	$(CH_2)_3N(Et)_2$	13 ± 0.43	104.60	8.05
12	$(CH_2)_2N(i\text{-}Pr)_2$	13 ± 0.23	42.18	3.24
15	$CH(Et)(CH_2OH)$	10 ± 0.66	83.29	6.94
25		26 ± 0.6	41.72	1.60
Glucantime	-	68.44[c]	-	-
Fluconazole	-	941.1 ± 4.98	-	-

The drugs that were used as a reference drugs are Meglumine antimonate (Glucantime®) and fluconazole.In the 5-nitrofuran series, better performance was exhibited by the compounds 9, 11, 12 and 15. While, 5-nitrothiophene derivatives 29 and 32 were the most active compounds. Again, replacement of O or S atom results in different responses by the compounds; as seen in the hydroxypropyl derivative of 5-nitrothiophene, compound 29; which appeared to be almost 6 fold more potent than its 5-nitrofuran counterpart, compound 7. It also revealed that oxyalkyl and aminoalkyl containing derivatives showed much better performances on an average if they are compared to the compounds containing simple alkyl side chain; comparing 3, 4, 27, 28 to 6-15, 29 and 32. α - branching can also improve the activity to a moderate level; such as the hydroxyethyl derivatives 6 and 15. When toxicity was analyzed against macrophages, Compound 6 exhibited low toxicity. But significant toxicity was exhibited by two of the most potent ones, 29 and 32. In conclusion, the n5-(5-

nitrothiophen-2-yl) 1, 3, 4-thiadiazole-2-amines that bears an acyclic amine are can be used as active antileishmanials.

5. The synthesis and In Vitro leishmanicidal activity of 5(Nitroheteroaryl)-1, 3, 4-Thiadiazols, which contains cyclic amine analogues; tested against the Iranian *L. major* strains.

The 5-(nitroheteroaryl)-1, 3, 4-thiadiazole containing cyclic amine at the C-2 position has been proven to have excellent antileishmanial activity. Moreover, different substituents when added to the molecule has affected both the potency of the compound as well as the physicochemical properties of that compound.Based on this, to discover novel antileishmanial agents, the authors have synthesized new derivatives of 5(Nitroheteroaryl)-1, 3, 4-Thiadiazols and introduced other groups on the C-2 position of the thiadiazole ring (Tahghighi, Foroumadi, Kabudanian-Ardestani, & Mahdian, 2017).Toxicity test was done upon the peritoneal macrophages of mice. MTT colorimetric assay was used to determine cell viability. CC_{50} was calculated to determine toxicity. The test was done against *L. major*.

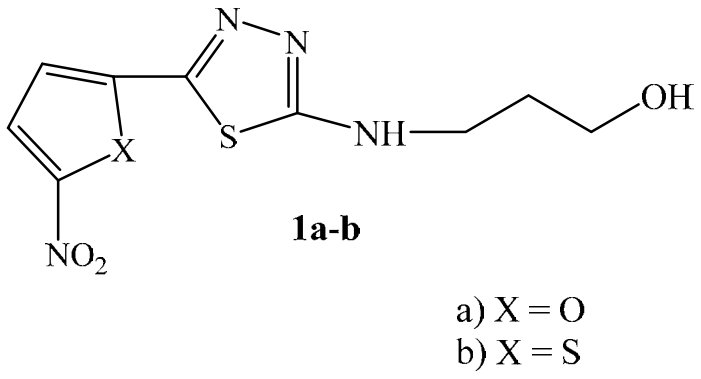


Figure 10: General structure of compound 1

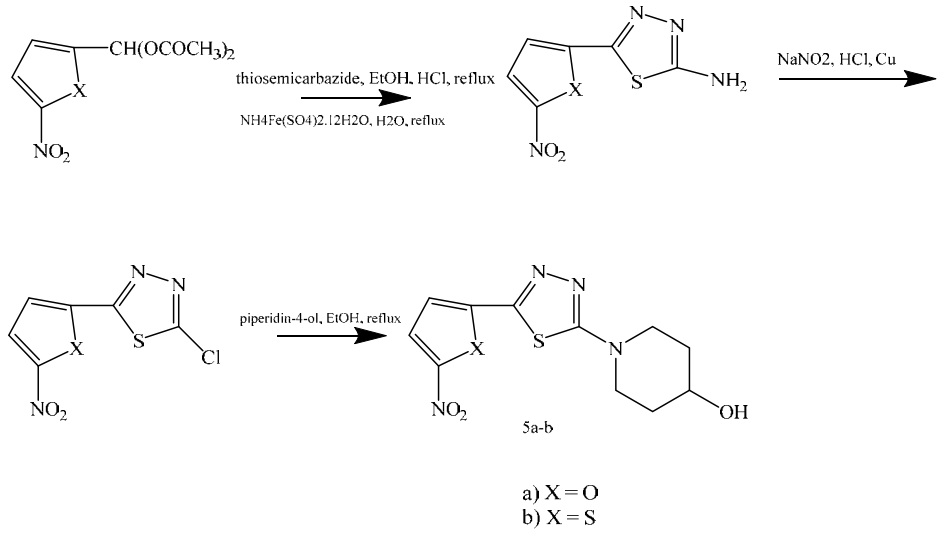
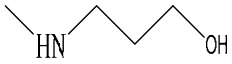
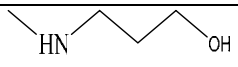
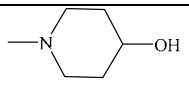
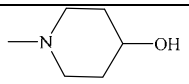


Figure 11: General procedure for synthesis

Table 10: Molecular information, anti leishmanial activity and cytotoxicity of the synthesized compounds

Compounds	**R**	**X**	**Anti-promastigote activity IC_{50} (µM)**	**Cytotoxicity CC_{50} (µM)**	**SI**
1a		O	18 ± 0.2	62.33	3.46
1b		S	3 ± 0.41	42.16	14.05
5a		O	68.9 ± 0.107	78.54	1.14
5b		S	27 ± 0.12	63.55	2.35
Glucantime	-	-	68.44[c]	-	-
Fluconazole	-	-	941.1 ± 4.98	-	-

The selectivity index, SI of the compounds were measured by CC_{50}/IC_{50}. The compounds 1a and 1b,exhibited excellent activity against the promastigote forms of *L. major*. The compound 1b was 6 times more potent than the compound 1a.This indicates that O and S replacements results in different responses. Different responses take place due to O or S replacement. Furthermore, compounds 1a-b proved to be toxic against the mouse peritoneal macrophages. The highest selectivity index was of 1b.the best substitutions at the C-2 and C-

5 positions of the thiodiazole rings are done in 1a and 1b. Their activity is much better than 5a and 5b.

Amino acid-coupled 1, 2, 4-triazoles: Synthesis, evalution an in silico studies.

In this experiment, compounds containing triazole amino acid derivatives were prepared. All of the synthesized compounds were evaluated for their In Vitro antileishmanial activity and were compared to Miltefosine andAmphotericin B deoxycholate (Bekhit, 2019).

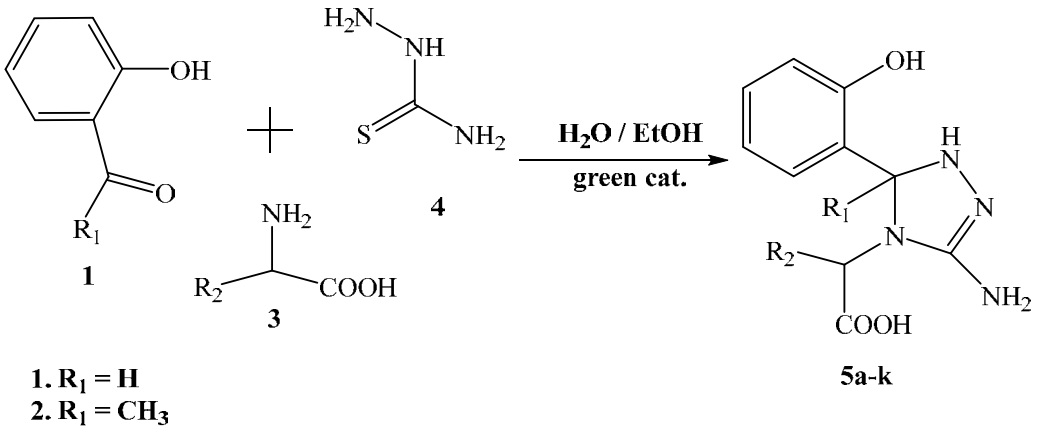


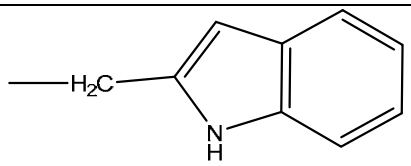
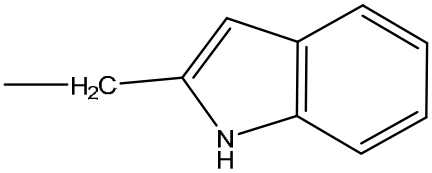
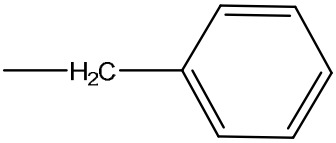
Figure 12: Synthesis of amino acid-coupled 1, 2, 4-triazoles

Only *L. major* promastigotes were used for the assay. The peritoneal macrophages f swiss mice were used in the assay.

Table 11: In Vitro activity of the synthesized compounds

Compounds	IC_{50}values (µg/mL)
5c	0.0516±0.28
5d	0.0312±0.21
5e	0.0866±0.04
5f	0.0484±0.06
5i	0.8644±0.04
5j	0.4662±0.05
5k	0.8668±0.02
Miltefosine	3.1924±0.14
Amphotericin B deoxycholate	0.0472±0.02

Table 12: Molecular information of the synthesized compounds

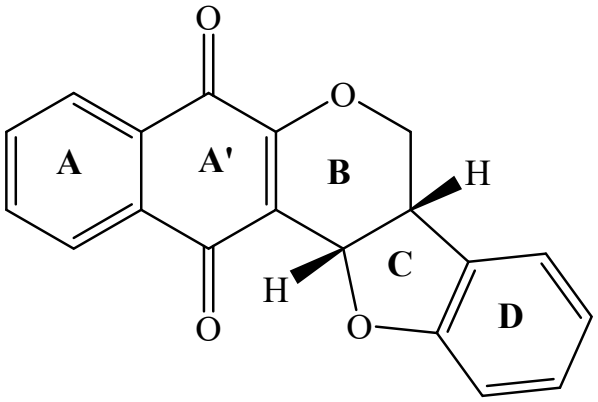
Compounds	R_1	R_2	Time (hrs)	Yield (%)
5c	H		3	81
5d	CH_3		3	85
5e	H	$-CH(CH_3)_2$	2	88
5f	CH_3	$-CH(CH_3)_2$	2	91
5i	H		3	84
5j	CH_3	$-CH_2-CH_2-S-CH_3$	2	79
5k	H	$-CH_2-COOH$	2	83

5c, 5d, 5e, 5f were found to be the most active ones. The test was done on mice. Even after administrating up to 250 mg/ kg orally, the mice did not show any signs of toxicity.Miltefosine and amphotericin B were used as a reference drugs. Some compounds showed IC_{50}values better than standard drugs. They are. Compounds 5c, 5e, 5i, 5j, 5f and 5k. The lead compound was 5d. 5d exhibited almost 200 times higher activity than a current drug of use, Miltefosine. Theeffectof varying R_1 between 2-hydroxy benzaldehyde and 2-hydroxy acetophenone was not much. But the varying of R_2 had an impact. The effect varied when different amino acid was placed inR_2. Such as when amino acid Tryptophan was placed in R_2 position, 5c and 5d was produced and showed highest activities. Another important observation was made. More hydrophobic the compound was, more antileishmanial activity it possessed. By observing the structure activity relationships of the compounds, it was

confirmed that the hydrophobic moieties such as isopropyl groups or indolyl groups played important roles in the antileishmanial effects of the compounds.

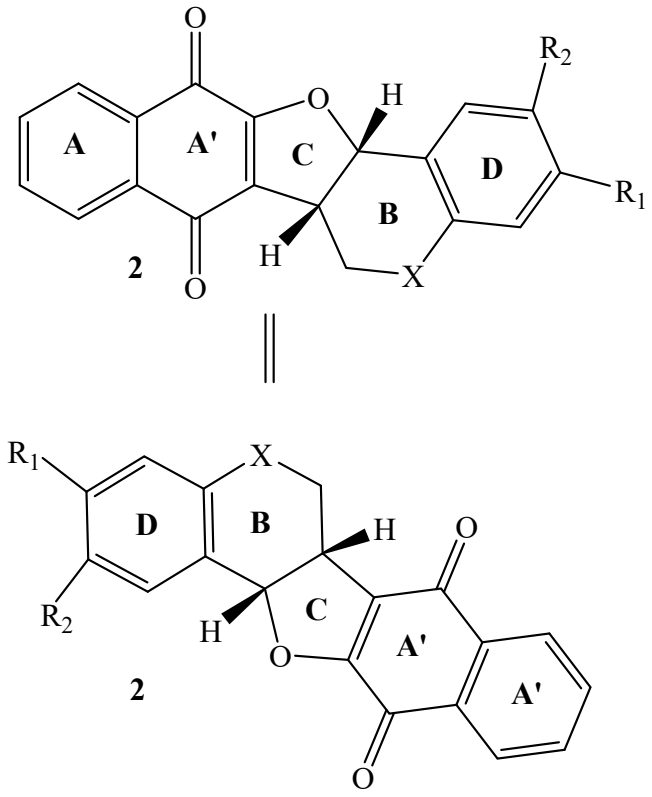
The synthesis and the antileishmanial activitities of a class of compounds called the pterocarpanquinones.

Due to the potential of the compound LQB-118 the authors synthesized pterocarpanoquinones of second generations. This was done based on the exchange of the positions between pyran, the ring A and furan, the ring C. This paper contains the synthesis of pterocarpanoquinones, the antileishmanial activity of the synthesized compounds upon the different species of Leishmania parasites of extracellular promastigote and intracellular amastigote stages(Faiões et al., 2018).



LQB-118
(1)

Figure 13: Compound 1



For 2	X	R_1	R_2
a	CH_2	H	H
b	O	H	H
c	O	H	Cl
d	O	OCH_2O	OCH_2O
e	CH_2	OCH_3	H
f	CH_2	H	OCH_3

Figure 14: General structure for compound 2

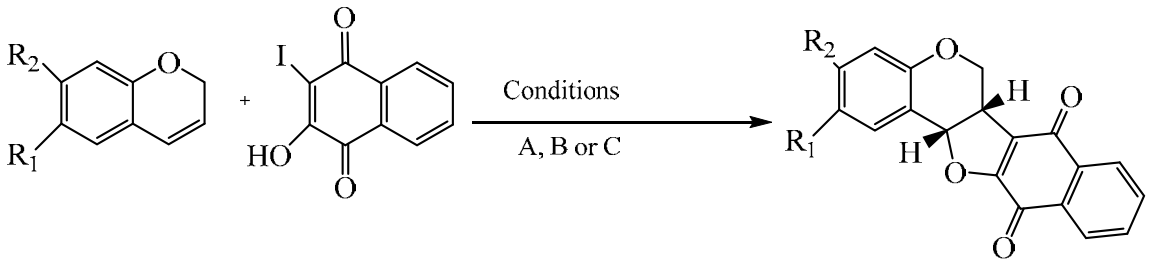


Figure 15: General procedure for synthesis of pterocarpanquinones

The three species of Leishmania parasites that were used are as follows: *L.amazonensis, L. braziliensis and L. infantum.*The peritoneal macrophages of swiss mice were used for the toxicity test. The cell were well maintained. For the test, the macrophages were incubated with the synthesized compounds for 72hrs at a temperature of 37°C in 5% CO_2.

Table 13: antileishmanial activity of the synthesized compounds against L. amazonesis

Compounds	**CC_{50} (µM) in Murine model**	**IC_{50} (µM) in promastigote**	**IC_{50} (µM) in Amastigote**	**SI**
LQB 182 (2b)	16.90±1.20	1.08±0.20	0.85±0.02	19.8
LQB 236 (2c)	43.90±0.60	1.15±0.18	0.60±0.13	73.16
LQB 168 (2d)	77.70±1.10	1.37±0.04	0.45±0.06	172.6
LQB-118 (1)	18.46	1.73	1.45	12.73
Pentamidine	8.50±1.25	4.80±0.09	1.90±0.10	4.47

Table 14: antileishmanial activity of the synthesized compounds against L. braziliensis

Compounds	CC_{50} (µM) in Murine model	IC_{50} (µM) in promastigote	IC_{50} (µM) in Amastigote	SI
LQB 182 (2b)	16.90±1.20	10.98±1.25	7.84±2.46	2.15
LQB 236 (2c)	43.90±0.60	17.85±1.12	8.34±1.47	5.26
LQB 168 (2d)	77.70±1.10	28.21±1.61	7.04±2.29	11.03
LQB-118 (1)	18.46	3.40 b	7.50 b	2.461.73
Pentamidine	8.50±1.25	13.0±0.04	7.70±2.40	1.1

Table 15: antileishmanial activity of the synthesized compounds against L. infantum

Compounds	**CC_{50} (µM) in Murine model**	**IC_{50} (µM) in promastigote**	**IC_{50} (µM) in Amastigote**	**SI**
LQB 182 (2b)	16.90±1.20	1.00±0.30	3.60±0.90	4.69
LQB 236 (2c)	43.90±0.60	1.80±0.30	>25	ND
LQB 168 (2d)	77.70±1.10	2.00±0.40	>50	ND
LQB-118 (1)	18.46	4.08 c	3.25 c	5.68
Pentamidine	8.50±1.25	5.70±0.12	0.40±0.20	21.25

The compounds that were synthesized appeared to be very active against the two species of *Leishmania* parasites: *L. infantum* and *L. amazonesis.* But their activity decreased to a great extent when they were used against the strain of *L. braziliensis.*The compound 2d, which had a methylenedioxy group in ring D, was the most potent. Among all of the compounds it had minimum IC_{50} values for *L. amazonesis* and *L. braziliensis.* However it did not show much

activity against the *L. infantum* species. The only compound which was able to exert action against all three of the *Leishmania* parasites is the compound 2b.

Thiosemicarbazones and thiazolidinones, SAR-studies and antiextra and Intracellular parasitic elimination of *Leishmaniasis amazonesis*

This report contains the synthesis, SAR studies and the In Vitro analysis of the Thiosemicarbazones and thiazolidinones.

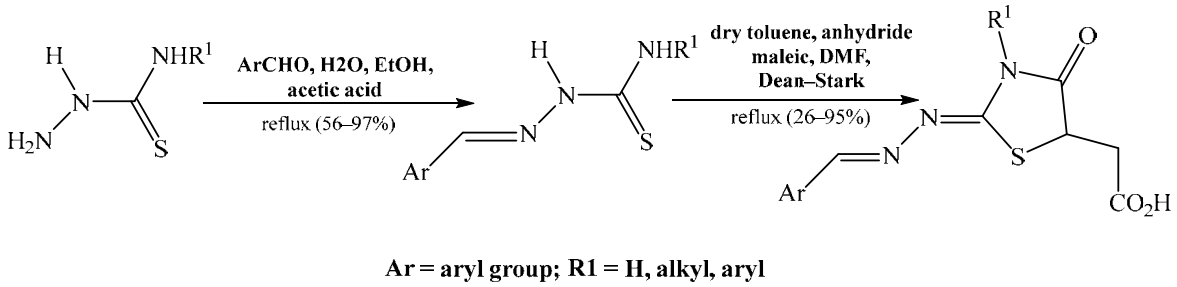


Figure 16: General procedure for synthesis of Thiosemicarbaone and Thiazolidinone(Tenório et al., 2005)

The species of *Leishmania amazonensis*were used in their promastigote stage. The culture in which they were maintained was made of 10% heat inactivated foetal bovine serum. It also had 0.01% folic acid and 0.4% hemin in it. The temperature was maintained at 28°C. An aliquot was transferred to a new medium every four days to maintain the exponential growth curve. Normal Suisse mice were sources of macrophages. It was obtained through peritoneal washing using Hank's solution at 4°C and cultivated in 24-well plates containing glass cover slips at a growth rate of 5 x 105. After 1 hour of culture, the Hank's solution was replaced by DMEM 1152 medium. The temperature was maintained at 37ºC. To analyze the toxicity profile of TSC and TZD, the CC_{50} against macrophages were determined.

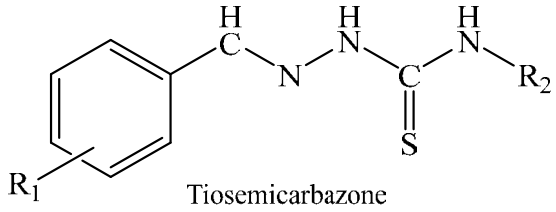


Figure 17: General structure of tiosemicarbazone

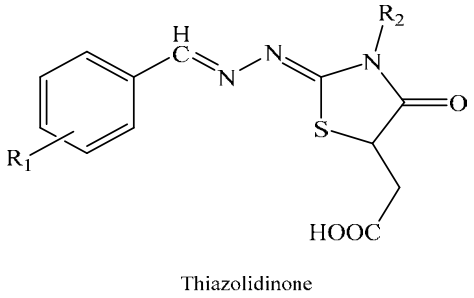


Figure 18: General structure of thiazolidinone

Table 16: Test for cytotoxicity of the synthesized compounds

Compound		**Infected cells (%)**				**Number of Intracellular parasites (Mean+SD)**					**CC_{50} (mM)**
TSC/TZD(mM)	control	0.1	1	5	10	Control	0.1	1	5	10	
1 (TSC)	67±3	75±5	23±3	13±3	0	486±58	519±86	126±33	46±14	0	0.3
6 (TSC)	70±2	65±6	6±1	6±2	0	861±119	799±115	46±8	10±7	0	0 0.2
9 (TZD)	69±6	62±5	20±4	17±2	13±4	558±116	542±111	147±52	77±19	51±19	0.4
12 (TZD)	67±2	71±2	10±2	6±2	0±0	1224±124	1216±75	47±12	8±4	0±0	0.2
14 (TZD)	84±3	78±2	11±2	11±4	0±0	1480±207	1041±129	85±25	23±9	0±0	0.2

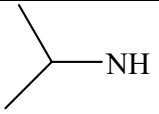
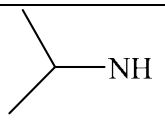
The compounds effects were dose and time dependent. The compounds 5 and 8 were similar to Hydroxyurea, the control drug; eliminated all parasites after 2 days of incubation at 10

mM. For a better evaluation, when the TSCs data at 1 mM concentration on 1, 3 and 6 days based on parasite survival were aligned, it reinforced that, 5, 6 and 8 exhibited higher antiparasitic effects than other compounds.However, in case of TZD treatments, compounds 12, 9 and 16 eliminated all promastigotes after 1, 2 or 3 days of incubation respectively. This pointed TZD as more effective than TSC.The comparison of TZD compounds, also revealed that compound 14 is capable of eliminating all parasites just after 3 days of treatment.

βcarboline-1, 3, 5-triazine hybrids: synthesis, biological activity and their mode of action:

β-carbolines which carries substituents at the C1 and C3 positions of β-carboline nucleus is very active against leishmaniasis. In this context, the authors described several classes ofβ-carboline alkaloids of both natural and synthetic of origin. Their synthesis and activity against the *Leishmania*parasites are briefly discussed as well (Paula, Stefanello, Ferreira, Vataru, & Sarragiotto, 2018).

Table 17: Molecular information of the synthesized compounds

Compound	**n**	**R_1**	**R_2**
8a	0	Ph	Cl
8d	0	2-Cl-Ph	Cl
9a	2	Ph	Cl
9d	2	2-Cl-Ph	Cl
9e	2	$3-NO_2-Ph$	Cl
16a	2	Ph	
16b	2	$4-OCH_3-Ph$	
Miltefosine	-	-	-

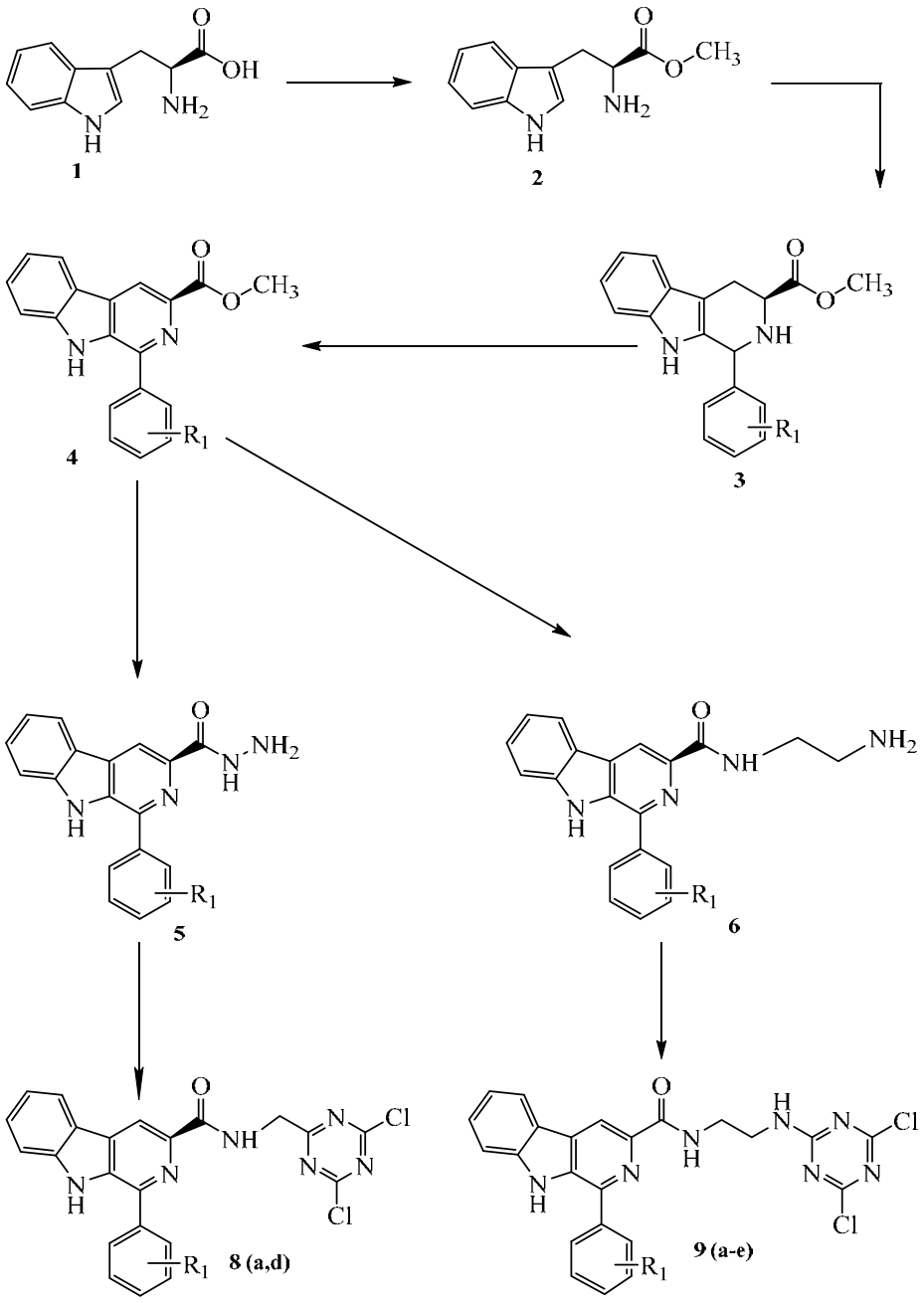


Figure 19: Synthesis of β-carbolines

Table 18: Antileishmanial activity of the synthesized compounds

Compound	Promastigotes IC_{50} (µM)	Amastigotes Promastigotes	J774A1 CC_{50} (µM)	Promastigotes IS	Amastigotes IS
8a	43.3 ± 10.3	1.1 ± 0.1	94.5 ± 7.8	2.2	85.9
8d	34.1 ± 10.6	n.t	22.0 ± 4.2	0.6	ND
9a	30.9 ± 0.9	1.9 ± 0.4	134.6±11.8	4.4	70.8
9d	7.6 ± 2.02	n.t	28.5 ± 3.6	3.8	ND
9e	5.1 ± 0.1	1.1 ± 0.2	83.1 ± 7.8	16.3	75.5
16a	7.5 ± 2.5	35.0 ± 0.5	98.1 ± 6.5	13.1	2.80
16b	6.2 ± 1.4	1.2 ± 0.5	145.7±10.1	23.5	121.4
Miltefosine	18.5 ± 1.1	2.4 ± 0.1	40.5 ± 1.7	2.2	16.9

For this assay, *L. amazonesis* species was used in their amastigote form.Macrophages that were used for the assay were used at its log phase. They were used at a concentration of about 5 x 10-5 cells mL-1.Among the hybrids compound 8a and compound 9a exhibited good antileishmanial activity. They were highly selective towards the *L. amazonensis*species. These two compounds demonstrated even better activities when a 2-chloro and a 3-nitro substituents was added at the 1-phenyl position of the compounds. The results were 9d, a 4 times stronger compound and 9e, a 6 times stronger compound respectively. Another active compound was the compound 16 a. it had an isopropylamino group in the 1, 3, 5-triazine ring. This being present, and the phenyl substituted groups at the β-carboline nucleus formed compound 16b with an even greater activity.

Scaffolds for new antileishmanial agent: 4-Phenyl-1, 3-thiazole-2-amines

The 2-aminothiazole ring has antiprotozoal properties. *L. amazonensis* is responsible for cutaneous leishmaniasis. In this report the authors mentioned the synthesis and the biological evaluation of eight 4-phenyl-1,3-thiazol-2-amines. For the assay, *L. amazonesis* was used as the parasite(Rodrigues et al., 2018).

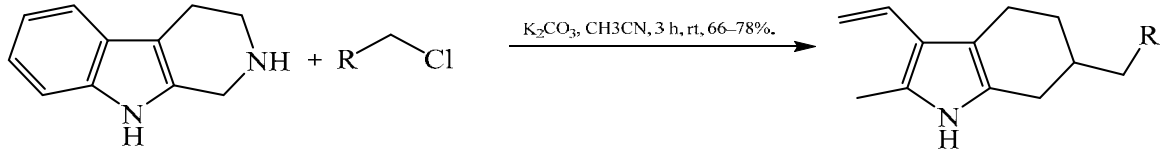


Figure 20: General procedure for synthesis of 4-Phenyl-1, 3-thiazole-2-amines

Table 19: Molecular properties of the synthesized compounds

Compound	R	CLogP[a]	MR[b]	Yield %
1	H	2.61	50.20	61
3	4-CH_2CH_3	3.47	59.85	59
4	3,4-Cl	3.64	59.81	53
5	4-CF_3	3.49	56.18	18
6	4-$C(CH_3)_3$	4.23	68.87	27

[a] Calculated n-Octanol/Water Partition Coefficient
[b] Molar Refractivity.

L929 and THP1 cell lines were used to measure the cytotoxicity. The cells that were used: Vero cells, a monkey kidney cells. The test foe cytotoxicity was done using the MTT method. In all cases, the CC_{50} was calculated. SI values were calculated by CC_{50} for each of the mammalian cell line against each mammalian c ine divided by IC_{50} for *L. amazonensis*. THP1 cells: human monocytic cell line. L929 cells: from fibroblasts from subcutaneous connective tissue. Vero cells: kidney epithelial cells extracted from African green monkeys.

Table 20: Antileishmanial activity and cytotoxicity in THP1 and L929 cells

Compounds	**IC_{50} (µM)**	**IC_{50}**	**CC_{50} (µM) THP1 cells**	**SI**	**CC_{50} (µM) L929 cells**	**SI**
1	957.56	3.02	143.57	0.15	198.26	0.20
3	46.63	4.33	117.27	2.51	95.45	2.05
4	53.12	4.27	84.65	1.59	121.12	2.28
5	53.37	4.27	92.21	1.73	106.08	2.00
6	20.78	4.68	45.73	2.20	27.07	1.30
Amphotericin B	16.23	–	ND	–	ND	–
Pentamidine	10.76	–	ND	—	ND	–

Table 21: Antileishmanial activity and cytotoxicity in VERO cells

Compounds	**IC_{50} (µM)**	**CC_{50} (µM) VERO cells**	**SI**
1	957.56	710.06	0.74
3	46.63	1217.51	26.11
4	53.12	255.03	4.80
5	53.37	511.76	9.59
6	20.78	118.17 5.69	5.69
Amphotericin B	16.23	ND	–
Pentamidine	10.76	ND	–

The THP1 cells used in the experiment are from human monocytic cell line. It was obtained from a patent suffering from acute monocytic leukemia. The L929 cells were taken from the fibroblasts of subcutaneous connective tissue. Four of total eight compounds exhibited activity against the promastigote stage of the parasite. Compound 6 was the most active one as it exhibited an IC_{50} value close to Amphotericin B, which is the standard drug. Among other very potent compounds from the series was compound 3, compound 4 and compound 5. However, none of the compounds appeared to possess better activity than the standard drug Pentamidine, another standard drug. Compound 6 exhibited a suitable cytotoxic profile with a

selectivity index of 2.20. Moreover, Compounds 3, 4 and 5 also showed suitable selectivity indexes. It was also observed that toxic dose for each of the compounds were nearly as high as twice the effective dose. In both the cells the results were this way.

Novel buparvaquone oxime derivatives: Synthesis and activity

This report contains the synthesis, SAR-studies, In Vitro antileishmanial activity, cytotoxicity of twenty one 1, 3-bis (aryloxy) propan-2-amine derivatives; these compounds differ in the nature and position of the substituents on their aromatic rings(Mäntylä, Rautio, et al., 2004).

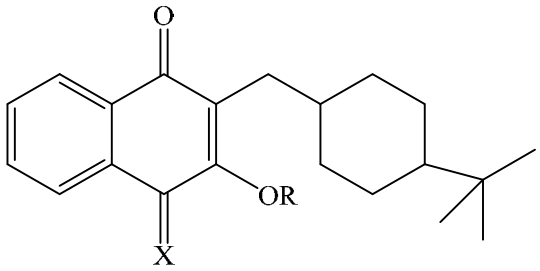


Figure 21: general structure of buparvaquone

Table 22: Molecular properties of the compounds

Compound	X	R
2	-NOH	-H
3	$-NOCH_3$	-H
5	-NOH	$-CH_3$

Table 23: Aqueous solubility of the synthesized compounds

Compound	Aqueous solubility (lg/mL)
BPQ	0.03±0.01
2	5.42±0.46
3	<0.03
5	ND

Table 24: In Vitro analysis of the compounds (Mäntylä, Garnier, et al., 2004)

Compound	% Inhibition (lg/mL)				ED_{50} (lg/mL)
	30	10	3	1	
BPQ	—	——	—	—	0.14a
2	86.9	12.2	0.0	—	17.52
3	86.1	1.9	0.0	—	21.16
5	3.6	0.0	—	—	>30
Sbv (control)[b]	66.1	39.7	v	—	15.89

The test was done on the species *L. donovani*. The In Vitro test was done with liver microsomes. Sodium stibogluconate (Sbv) was used as the standard control compound.The ED_{50} values of the oximes 2, 3 and 5 prove their activity is a little less than that of the buparvaquone (compound1), which have high antileishmanial activity against the promastigotes. Even then, the activity of the compounds 2 and 3 are close to the standard drug sodium stibogluconate. At the same time, the compound 5, O-methyl-buparvaquone oxime was nearly inactive. Another observation was made by the authors regarding the release of NO during the In Vitro analysis. It was assumed that compound 2, buparvaquone oxime; compound 3, buparvaquone-O-methyloxime and compound 5, O- methyl-buparvaquone oxime did not release NO during the experiments. Therefore, their activity can be considered as moderate against the parasite. Interestingly, the compound 2 had to undergo an enzymatic oxidative cleavage to buparvaquone. Therefore, these compounds can be administered as prodrugs of Buparvaquone. The test at the end also indicated that the oxime structure can be pretty useful to be used as a prodrug template.

1,3-bis (aryloxy) propan-2amines: synthesis and their potential to be an antileishmanial.

1, 3-bis (aryloxy) propan-2-amines are synthesized from epichlorohydrin. In this report, the authors focused on theantileishmanial activity of 21 of these compounds. Along with that, the synthesis and SAR studies are also explained. The compounds under analysis has different substituents on their aromatic ring (Stefânia N Lavorato et al., 2017).

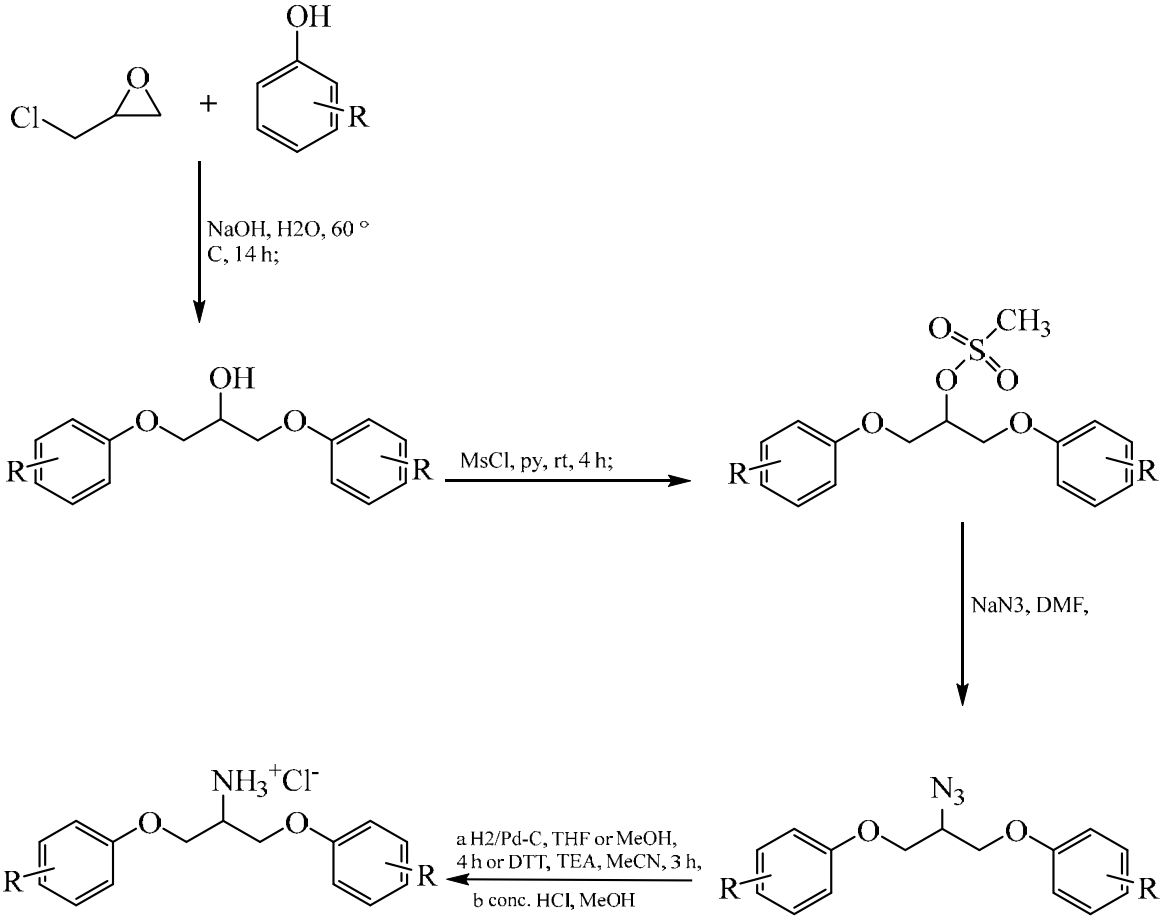


Figure 22: Synthetic route of 1, 3-bis (aryloxy) propan-2amines

Table 25: Molecular properties of the synthesized compounds

Compounds	R	ClogP	MW (Da)	HBD	HBA
4e	3-NO_2	2.55	333.30	2	9
4m	2-CH_3	3.40	271.36	2	3
4o	4-CH_3	3.40	271.36	2	3
4p	2-Cl	3.57	312.20	2	3
4q	3-Cl	4.14	312.20	2	3
4r	4-Cl	3.85	312.20	2	3

The parasite species that was used is *L. amazonensis*. Female BALB/c mice were the source of peritoneal macrophages. The mice were 8 weeks of age.

Table 26: Antileishmanial activity and cytotoxicity of 1, 3-bis (aryloxy) propan-2amines

Compounds	IC_{50} (µg/mL)±SD	IC_{50} (µM)±SD	CC_{50} (µg/mL)±SD	CC_{50} (µM)±SD	SI
4e	3.91±0.69	10.6±1.9	32±3	87 ±7	8.3
4m	4.10±1.24	13.3±4.0	16±1	51 ±2	3.8
4o	1.67±0.24	5.4±0.8	13±5	41 ±15	7.5
4p	4.26±1.21	12.2±3.5	24±4	69 ±11	5.7
4q	1.83±1.25	5.2±3.6	15±0	44 ±1	8.4
4r	1.02±0.04	2.9±0.1	9±1	24 ±4	8.4
Amp B	0.2±0.0	0.22±0.00	1±01	1±01	4.9

Amphotericin B, the drug which was used as positive control. In this assay, almost all the compounds were found to be potential antileishmanials except for a few. The bisarylamine compounds 4o, 4r and 4r demonstrated best performance as an antileishmanial agent. The common thing between these compounds are that they all hadIC_{50}values below 10µM.Usually, to ensure that a particular compound is safe to be administered as a drug, an SI value over 10 is preferred. These three compounds are considered safe according to their SI values; which is above eight. This indicates that the synthesized compounds havemoderate selectivity.Regarding para-substituted bisarylamines those having higher ClogP values; containing one or more substituents that are hydrophobic, were the most active para-

substituted compounds. The more lipophilic the compounds are the more easily it will get pass the parasite membranes. Which means, there will be higher concentrations of the compound at the site of action. This explains the reason for the lipophilic compounds to be more active the hydrophilic ones.Similar action was demonstrated by the compound 4m; which was ortho- substituted. Additionally, compounds such as 4e contains an electron withdrawing group m-NO_2; these compounds exhibited similar activity.

Chromenochalcones; Potential Antileishmanial Agents: SAR and biological studies

Chalcones have the ability to exhibit multi target profile. They are natural-like. This report contains the Structure Activity Relationship (SAR) studies along with the pharmacokinetics (PK) and mechanism of action of such compounds(Agnihotri et al., 2014).

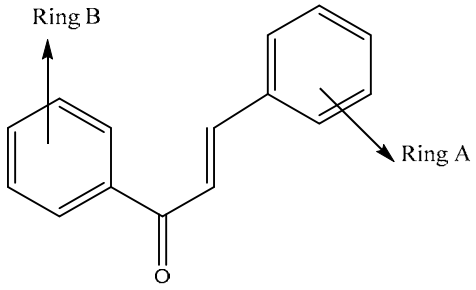


Figure 23: General structure of Chalcones

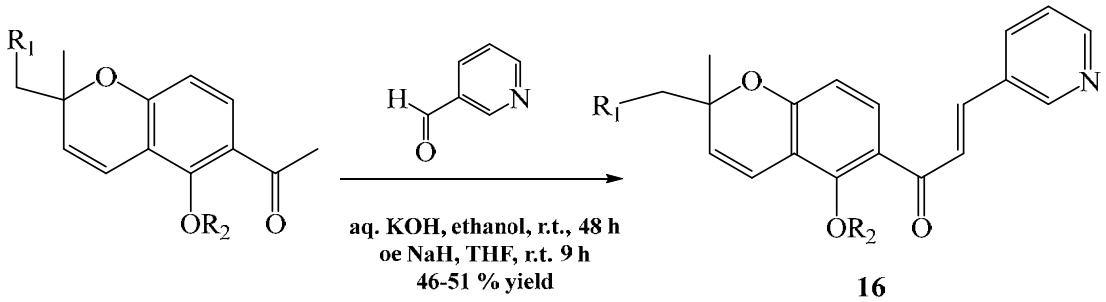


Figure 24: Synthetic procedure for compound 6

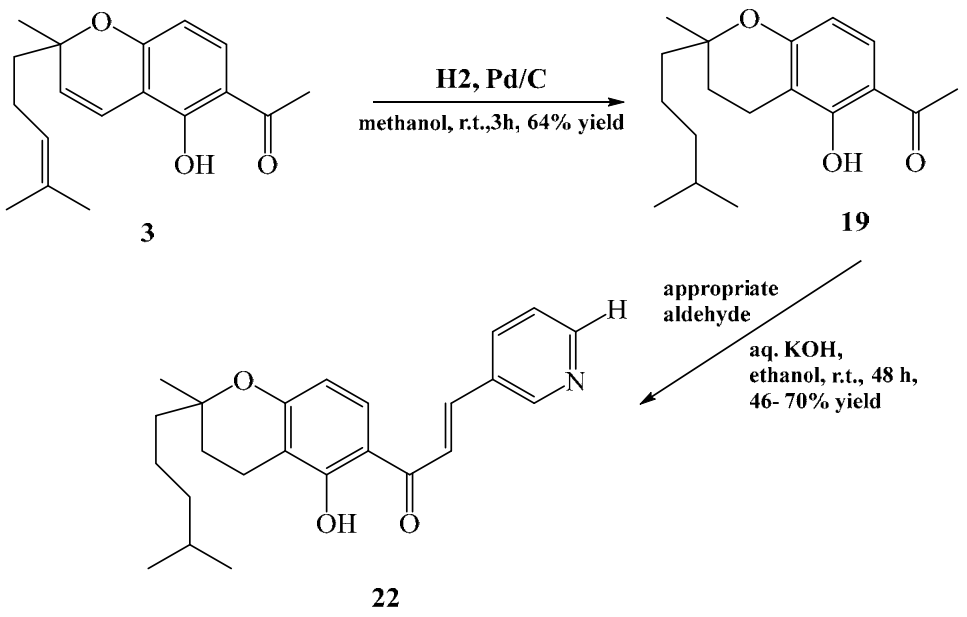


Figure 25: Synthetic procedure for compound 22

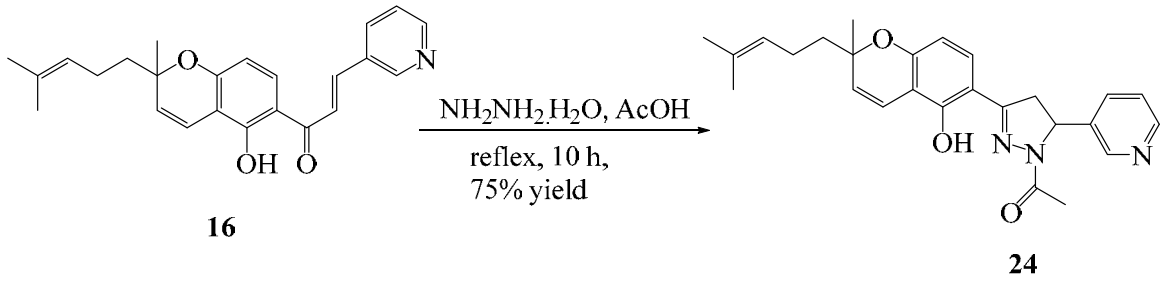


Figure 26: Synthesis of compound 24 from compound 16

Table 27: Antileishmanial activity and cytotoxicity of the compounds

Compound	**antipromastigote (% inhibition at 25 µM)**	**antiamastigote IC_{50} (µM)**	**cytotoxicity IC_{50}(µM)**	**(SI)**
11	99.5	5.8	>400	>68.9
14	99.5	6.3	201.2	31.9
16	99.8	5.4	40.7	7.5
17	99.8	1.7	65.2	38.3
22	99.8	5.7	156.3	27.4
24	99.5	6.4	88.5	13.8
Miltefosine	100	8.4	52.5	6.2

The parasites were tested in hamster model. For this assay, the strain of *L. donovani* was used.The compounds were compared to Miltefosine for their antileishmanial assay. Sodium Stibogluconate (SSG) was used as another reference. In the In Vitro studies, five of the synthesized compounds 11, 14, 16, 17, 22, and 24 exhibited more potency than the reference drugs themselves. The compound 16 exhibited activity at a constant level up to the 28th post treatment day. This compound proved to have a double characteristic. It acts as a killing agent towards the parasites as well as an immuno stimulant towards the host. Compound 16 is a very potential candidate to be used against the non-healing form of leishmaniasis.

Valeriana wallichii: **a source for Cinnamic Acid Bornyl Ester and its derivatives exhibiting antileishmanial effect.**

In this report, the authors determined the In Vivo effects of both compounds (-)-Bornyl cinnamate and (-)-bornyl 3-phenylpropionate as an alternative to the current drugs used in the treatment of Cutaneous Leishmaniasis(Masic et al., 2015).

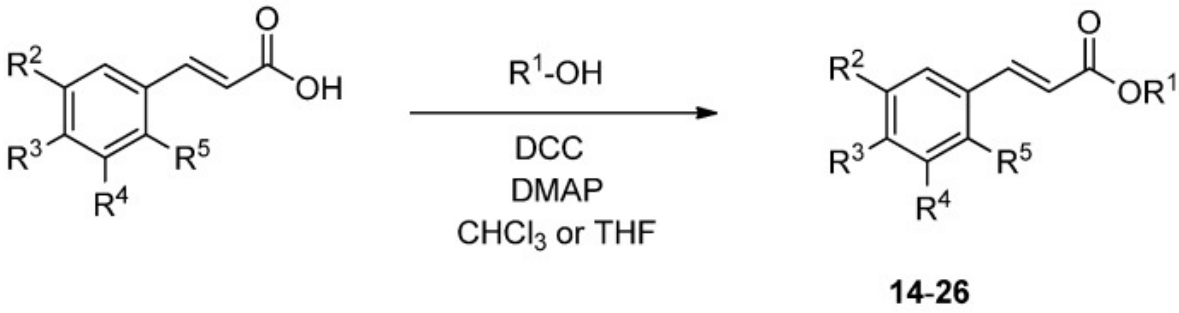


Figure 27: General procedure for synthesis of compound 14-26 (Glaser et al., 2014)

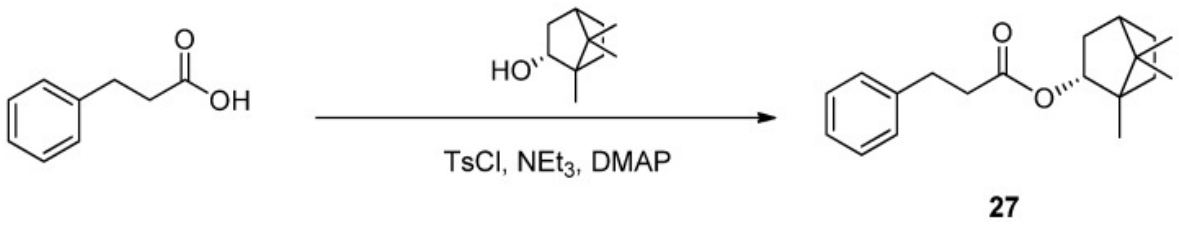
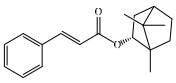
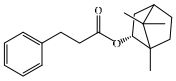


Figure 28: General procedure for synthesis of compound 27 (Glaser et al., 2014)

The *L. major* parasite was obtained from a patient who had an oriental sore. It was obtained in 1981. For In Vivo testing of the compounds, BALB/c mice weighting 16g to 18 g was used. When the test was started the mice were of 6-8 weeks old.The IC_{50} was calculated. $IC_{50}$is 50% inhibitory concentration on *L. donovani* promastigotes and *L. major* promastigotes and amastigotes. The IC_{50} values for positive control are as follows: Miltefosine 36.2µM (*L.major*), 33.0µM (*L. major* amastigotes) 65.5µM (BMDM). This drug was used to compare the antileishmanial activities of the test compounds to figure out their potential as an antileishmanial.

Table 28: antileishmanial activity of the compounds against different species of Leishmania parasites

Compounds	***L. donovani* promastigotes**	***L. major* Promastigotes**	***L. major* amastigotes**	**BMDM**	**HEP G2**	**HEK 293T**
 (-)-Bornyl cinnamate	15.6	39.6	10.9	54.3	>100	83.1
 (-)-bornyl 3-phenylpropionate	>100	50.2	89.1	>100	>100	>100

The best activity was observed in compound 1 against both *L. donovani* promastigotesand *L. major* amastigotes and promastigotes. The cytotoxicity of the compound was variable to the highest dose of 100 µ M. Compound 2, when given at a dose up to to100 µ M, inhibited growth of both the Leishmania parasites. The most astonishing part of the experiment was that there was no cytotoxicity demonstrated against any of the three types of cells. The treatment with compound 2 proved to be a successful one.

Synthesis of N-substituted tetrahydro-β-carbolines and their antileishmanial and antitrypanosomal actions

This report contains the synthesis of a group of N- substituted tetra hydro-β-carbolines. The compounds were tested for whether they had any leishmanicidal activity or not. The test was done through an In Vitro assay. This assay involved the promastigotes and axenic amastigotes of *Leishmania donovani*strains which is the causative agent for visceral leishmaniasis(Manda et al., 2014).

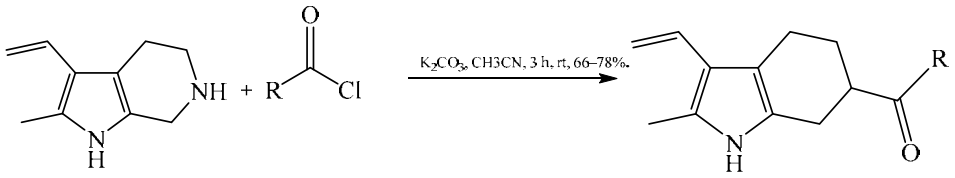


Figure 29: Synthesis of compound 9.

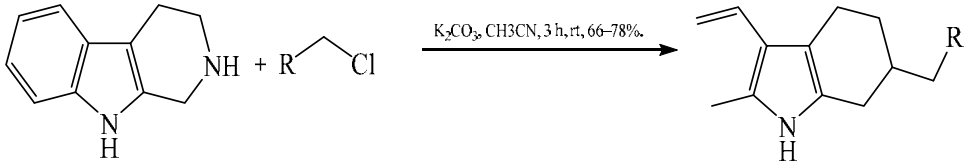
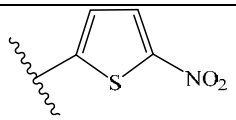
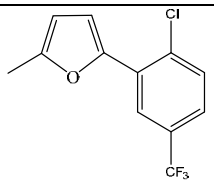
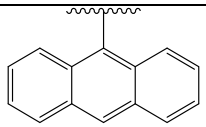
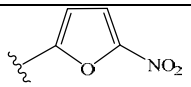
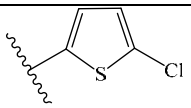
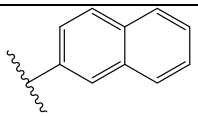


Figure 30: Synthesis of compound 11

Table 29: In Vitro activity of the tetrahydro-β-carboline analogs

Entry	IC_{50}/ IC_{50}(lM)			
	L.donovani			***T. brucei***
	Promastigotes	**Axenic amastigotes**	**Macrophage amastigotes**	**Trypamastigote**
9b	12.7/36.6	62.8/107.3	NA	NA
9i	39.6/69.1	37.1/76.0	NA	NA
11d	16.0/76.0	80.4/103.6	NA	11.0/18.8
11e	33.8/—	116.7/—	NA	1.0/28.1
11f	9.1/25.6	NA	NA	8.9/14.5
11h	22.1/28.1	87.59/—	28.3/>32	10.2/17.6
Pentamidine	4.8/6.	>29.4/>29.4	2.0/3.1	0.0041/0.0070
Amp B	0.3/0.4	0.3/0.4	0.2/0.5	NT
DMFO	NT	NT	NT	28.0/68.2

Table 30: Molecular properties of the compound

Entry	R
9b	
9i	
11d	
11e	
11f	
11h	

The In Vitro antileishmanial activity of the compounds were tested against the *Leishmania* parasite of the *Leishmaniadonovani*species.The parasites were in their promastigote form and then anexic amastigotes were used as well. In order to determine whether the compounds are harmful to the patient or not, the selectivity index had to be determined. It was determined to the concentration of 25 lg/mL. Three mammalian cells were used to determine the selectivity. Monkey kidney fibroblasts; the vero cells, HEPG2; the human hepatome cells and epithelial cells of the pig kidney; LLC-PK1.

The compounds were compared to Pentamidine, Amphotericin B and DMFO. The most potent one was 11e. The compounds that exhibited good antitrypanosomal activity are thiophen-2-yl linked analog, 11f and naphthyl linked analog 11h. Among the remaining ones, the anthracene and naphthyl linked analogs 11d and 11h appeared to be the compounds that had better antileishmanial effect. 11f had excellent activity that was as good as

Pentamidine.On the other hand, 11e, 11f and 11h happened to demonstrate much better activity than DFMO.

3-substituted-4-hydroxycoumarin derivatives: synthesis and activity as an antileishmanial

In this report, the authors focused on synthesizing compounds to be used against the enzyme called Adenine phosphoribosyl transferase which is found in *L. donovani*. The study was done to understand the features of the 3-substitured-4-hydroxycaumarine derivatives that may be responsible for their antileishmanial activity. The synthesis, SAR-studies, In Vitro antileishmanial activity, cytotoxicity of 3-substituted-4- hydroxycoumarin derivatives are described here.The assay was done on the culture of *L. donovani* promastigotes, the cytotoxicity test was carried out using HeLa cell line. The standard compounds used in this experiment are as follows: STD 1 is Pentamidine; STD 2 is Miltefosine; STD 3 is Butylated hydroxy toluene and STD 4 is Ascorbic acid (Zaheer, Khan, Sangshetti, & Patil, 2016).

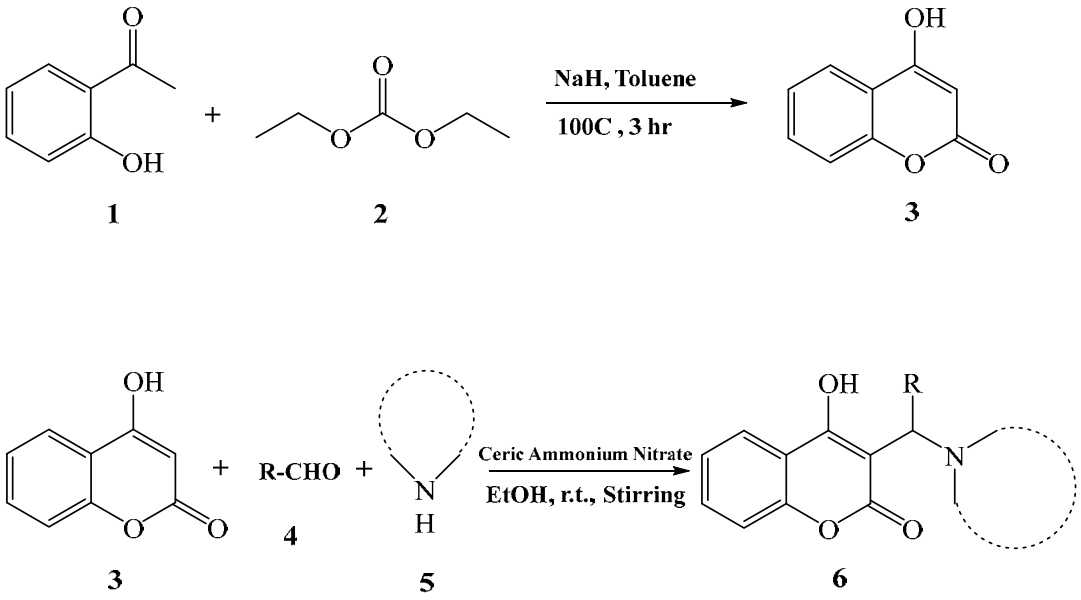


Figure 31: General procedure for synthesis of 3-substituted-4-hydroxycoumarin

Table 31: Molecular information of the 3-substituted-4-hydroxycoumarin

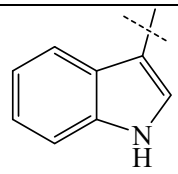
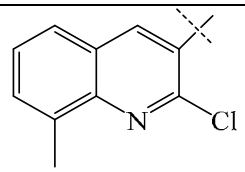
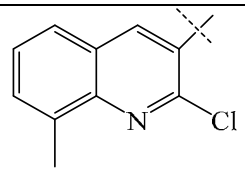
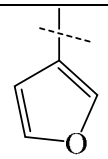
Entry	Molecular formula	R	Reaction time	Yield (%)	R_f value	MP (ºC)
6c	$C_{23}H_{23}N_3O_3$		20	92	0.54	158–160
6h	$C_{25}H_{23}ClN_3O_3$		20	91	0.86	134–136
6i	$C_{25}H_{24}ClN_3O_3$		15	98	0.73	156–158
6j	$C_{18}H_{17}NO_5$		20	90	0.37	110–114

Table 32: Antileishmanial activity of the 3-substituted-4-hydroxycoumarin

Entry	*L. donovani* (IC_{50}mmol/L)	Antioxidant (IC_{50}mmol/L)
6c	13.11±0.74	10.79±0.69
6h	9.90±0.33	10.60±0.48
6i	6.90±0.12	10.73±0.61
6j	14.67±0.98	15.58±0.83
STD 1	16.15±0.85	ND
STD 2	12.50±0.90	ND
STD 3	ND	16.47±0.18
STD 4	ND	12.47±0.85

The compounds 6h and 6i appeared to be much more potent than the standard drugs Pentamidineand Miltefosine. The synthesized compounds are categorized into three classes, the indole class, the quinoline class, and the furan class.It can be said that for the antileishmanial activity, the presence of the heterocycles and the secondary amines at the 3 position of the 4- hydroxycoumarin is essential. For example, compound 6c was produced when a 4-methylpiperazine group was introduced at position 3. The compounds of the indole class showed extra ordinary activity.In both the compounds 6h and 6i, to increase their

activity, it was important that the 2-chloroquinolinyl group was replaced with 2-chloro-8-methylquinolinyl. This resulted in the increase of the potency from 5 to even 70 folds.6i was the compound with most activity.In addition to that, antioxidant activity was present in all of the compounds that were being compared. Moreover, none of the synthesized compounds were proven to be cytotoxic towards the cells they are tested upon. Like:the HeLa cells.

An Alkaloid Flavopereirine: Derived from the plant *Geissospermum vellosii;* possesses Leishmanicidal Activity In Vitro

Chemotherapy, for the treatment of Leishmaniasis has been very limited. This is due to the toxic effects of the synthetic drugs, low efficacy of the safer compounds and of the alternative treatments, and resistance of the parasite. This study focuses on the In Vitro activity of flavopereirine, a natural alkaloid upon the promastigote cultures of *Leishmania amazonensis.*The antipromastigote activity of each of the extracts, fractions, and alkaloid were evaluated at 24, 48, and 72 h. *G. vellosii* cytotoxicity was evaluated against the modified THP-1 cells. This is actually Human monocytic leukemia cell line(da Silva e Silva et al., 2019).

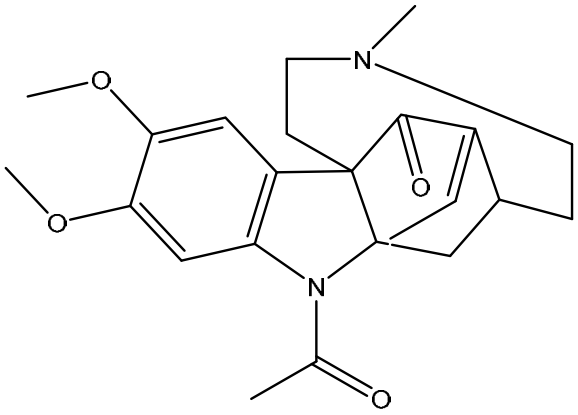


Figure 32: Compound (9) Flavopereirinee, isolated fromGeissospermum vellosii

Table 33: Antileishmanial activity of different isolated compounds

Samples	**IC_{50}(µg/mL) + SD**		
	24 h	48 h	72 h
FrMeOH	1.71±0.15	3.75±0.52	5.95±0.66
F6AF	1.56±0.16*	31.50±0.76*	1.24±0.15*
FrDcm	5.56±0.70	20.85±0.25	10.06±0.80
Flavopereirine	0.23±0.10* (0.93 µM)	2.34±0.50* 9.3 µM	0.15±0.06* 0.61 µM
Amphotericin B	0.42±0.09 (0.45 µM)	1.79±0.06 (1.94 µM)	0.35±0.01 (0.30 µM)

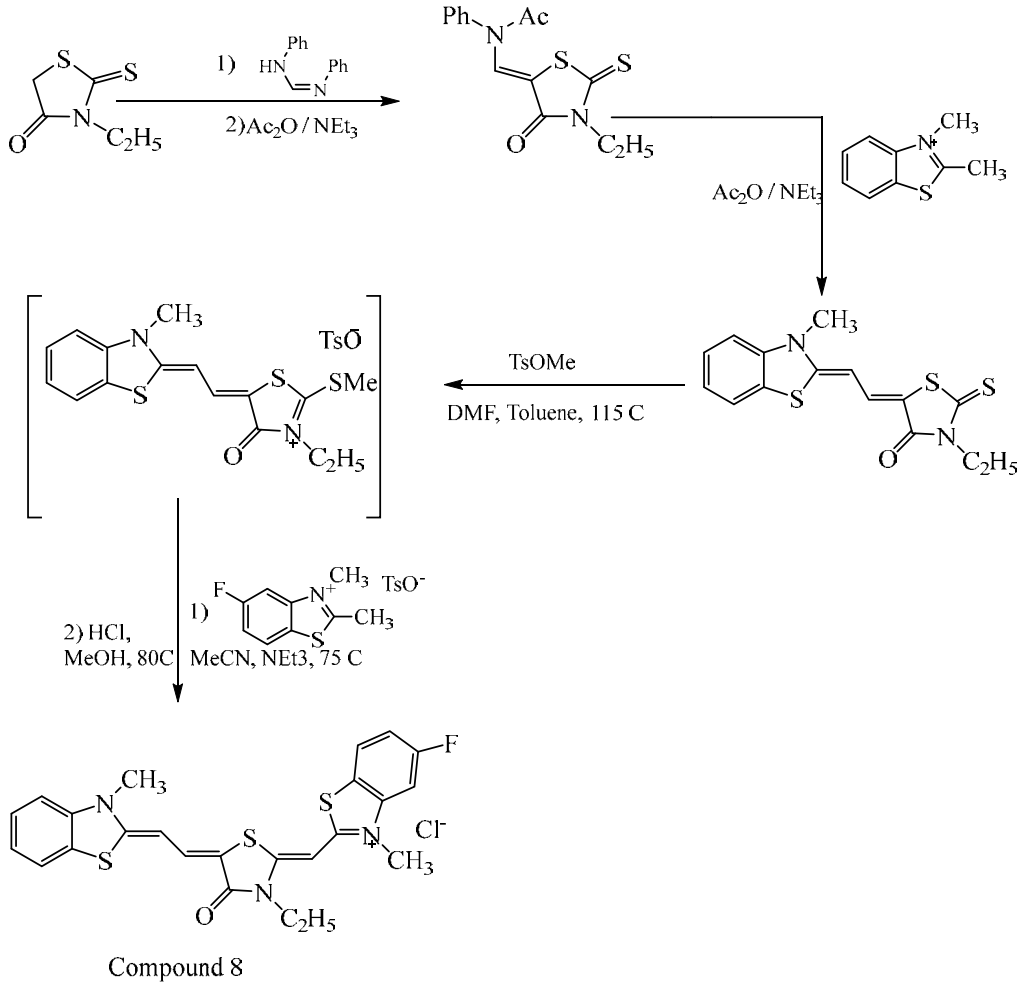
Table 34: Cytotoxicity (CC_{50}) and selective index (SI) of different isolated compounds

Samples	**24 h**		**48 h**		**72 h**	
FrMeOH	CC_{50}	SI	CC_{50}	SI	CC_{50}	SI
F6AF	443±0.45	147.8	625.7±0.34	19.9	629.4±0.91	508.0
Flavopereirine	225.5±0.9	976.2	533.3±0.15 (2156 µM)	228.2	734.0±0.86 (2968 µM)	4993.2
Amphotericin B	272.7±0.09 (295.1µM)	655.5	584.6±0.46 (632.6µM)	326.6	637.7±0.72 (69µM)	637.7±0.72 (690 µM)

Amphotericin B was used as a standard drug. Methanol proved to be active among all of the fractions, especially at 24 h. The fraction FrDcm also presented a better activity at 24 hr. but its anti promastigote effect reduced with increasing time. Sub fraction F6AF, at 24h, exhibited more activity than the alkaloid itself. No significant difference was observed at 72 h. Flavopereirine displayed great antileishmanial activity at all times. Cytotoxicity was with increased time and no significant toxicity was observed at 48 and 72 h. in terms of selectivity, the sub fraction F6AF, flavopereirine and amphotericin B proved to be more selective. Flavopereirine appeared to be more selective than amphotericin B, both at 24h and 72h.

Fluorine contained Rhodacyanine (SJL-01); which possess a High Efficacy for against Visceral Leishmaniasis (VL)

This report contains the synthesis, SAR-studies, In Vitro antileishmanial activity, cytotoxicity of Fluorinated Rhodacyanine(Yang et al., 2010).



Compound 8

Figure 33: Synthesis of compound 8

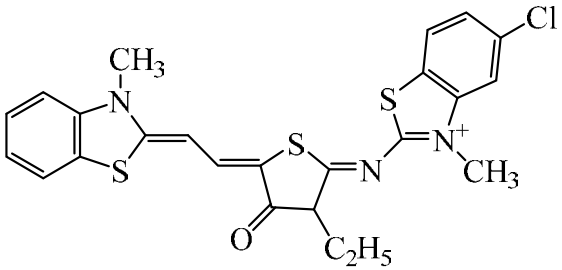


Figure 34: compound 7

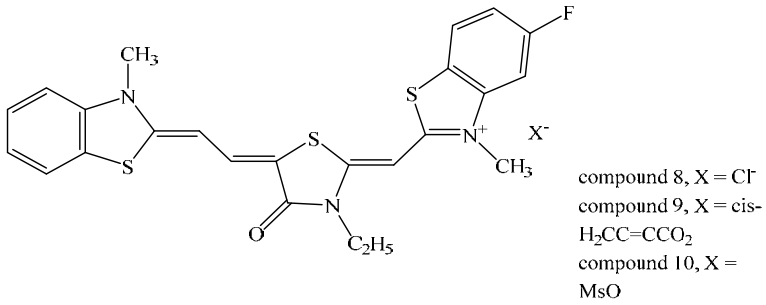


Figure 35: compound 8, 9 and 10

Pentostam sensitive *L. donovani*strains were employed for the analysis. Specific female pathogen-free BALB/c mice which were 6 to 8 weeks old and Syrian hamsters of the *Mesocricetus auratus* species were used. Amastigotes of *L. donovani* were isolated for the assay. It was done from the spleen of an infected donor hamster. Animals were infected intravenously through the tail vein.

Table 35: In Vitro antileishmanial activity and cytotoxicity of rhodacyanines

Compound	***L. donovani*** **IC_{50}(µM)[a]**	**L-6** **IC_{50}(µM)b**	**Selectivity**
7	0.028	21.2	757
8	0.011	>173.7	>15000
9	0.025	71.7	2870
10	0.02	84.5	4225

Table 36: Inhibition of rhodacyanines in macrophages

Compound	***L. donvani*** **IC_{50} (µM)**
6	4.691
7	0.08
8	0.353
Miltefosine	0.811

Table 37: Dosing regimen, inhibition percentage and confidence limit percentage of rhodacyanines

Compound	**dosing regimen**	**Inhibition (%)**	**95% confidence limit**
7	50 (ip)×5	18.2	19.2
8	50 (ip) ×5	31.43	12.2
Pentostam	15 (sc) ×5	62.04	14.7
Amphotericin B	0.5 (iv) ×3	78.6	7.6
liposomal Amphotericin B	1.5 (iv) ×3	95.53	3.2

In case of the In Vitro antileishmanial activity, the substitution of an amine functional group exhibited a better activity as well as a good selectivity in the In Vitro testing of compound 6.

Compound 7, with an increased activity was formed when a chlorine atom was introduced on the right hand ring. Another compound, compound 8 with excellent activity was formed when this chlorine atom was substituted with a fluorine atom. Compound 8 also showed a high selectivity factor and a better efficacy than the compound 8. However, no bioavailability was exhibited by the compound 8 and compound 10, the corresponding mesylate after SC administration. The fore, the In Vitro study had to be carried out by IV administration of the compounds. After 5 times administration of the compound 8, inhibition up to 95% was observed.

Synthesis and anti-leishmanial activity of Amodiaquine analogs

In this work, the authors described the synthesis of two different series of Amodiaquine analogs. They are: 4-aminoquinoline -aryl derivatives and 4quinolinylhydrazones. The aim was to evaluate them according to their antileishmanial activity. In the second series, 4-amine-linker is present, and in the first series, a 4-hydrazone-linker was present. This analog is used in medicinal chemistry to a great extent. This is because it has the ability to interact with the DNA by the process of intercalation, chelation of metals and the generation of the metal ion induced radical intermediates. These are intracellular processes that directly and indirectly affects the normal metabolic processes of the parasites and may be responsible for affecting their survival (Coimbra, Silva, Dias, Corrales, & L, 2011).

Four species of Leishmania promastigotes were used for the test. They are: *L. chagasi,L. braziliensis*, *L. major* and *L. amazonensis.*

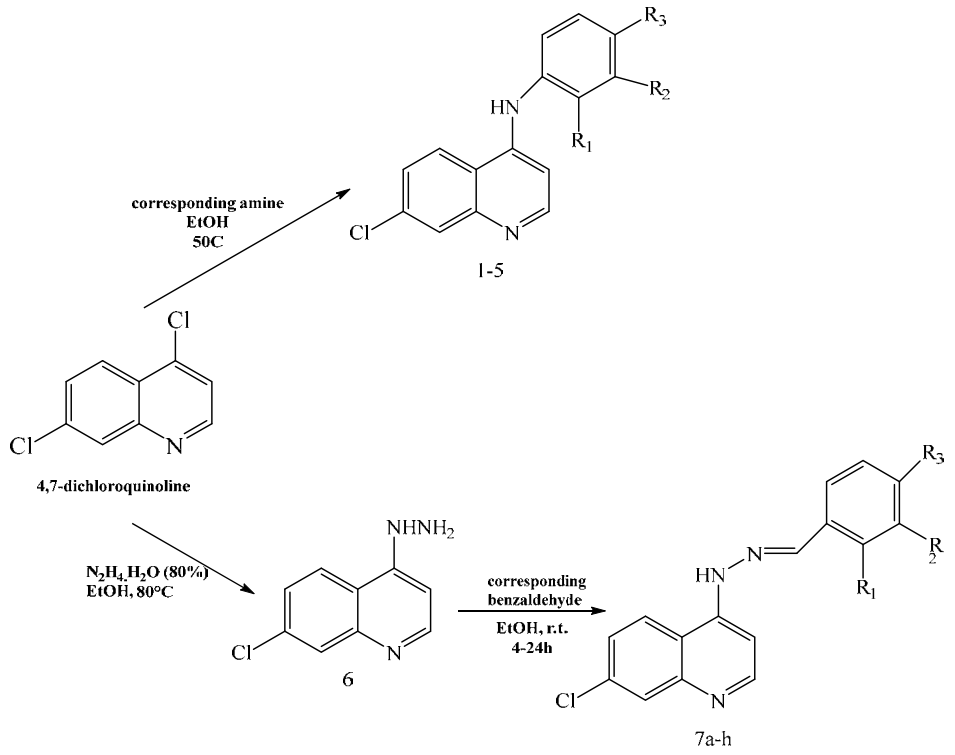


Figure 36: General procedure for synthesis of amodiaquine analogs

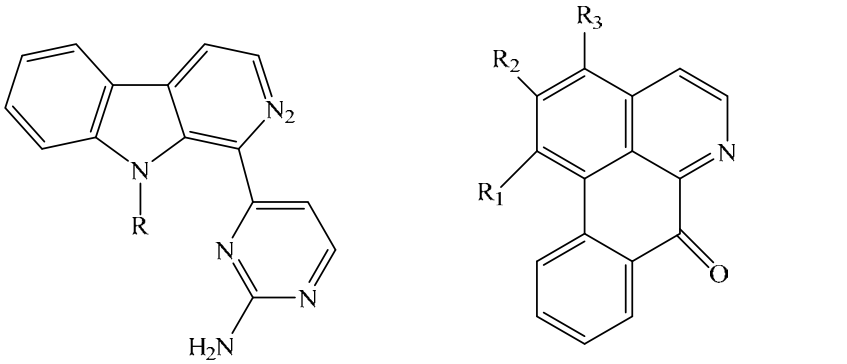
Table 38: Molecular information and antileishmanial activity of the compounds against different species of Leishmania parasites

Entry	Substituents			Antileishmanial activity				Macro-phages
	R_1	R_2	R_3	*L. amazonensis*	*L. braziliensis*	*L. chagasi*	*L. major*	
1	OH	H	H	20.1 ± 0.98	12.1 ± 1.36	7.9 ± 0.83	25.0 ± .52	>40
2	COOH	H	H	>40	>40	>40	>40	>40
3	H	H	OH	20.3 ± 0.93	12.9 ± 0.32	7.08 ± 1.45	14.6 ± .74	>40
4	H	OH	COOH	>40	>40	>40	>40	28.7 ± 0.28
5	H	H	H	10.1 ± 1.75	75 4.2 ± 0.65	9.8 ± 0.07	13.1 ± .64	24.7 ± 0.28
7f	H	H	NO_2	>40	>40	>40	>40	6.8 ± 2.51
7h	H	H	H	2.4 ± 0.496	4.1 ± 1.03	4.03 ± 1.65	19.4 ± 0.26	14.4 ± 7.56
AQ				14.5 ± 0.74	15.3 ± 0.56	7.5 ± 1.10	23.9 ± 0.04	-
AmB				0.4 ±0.05	0.3 ± 0.09	1.9 ± 0.25	0.3 ± 0.09	-

The synthesized compounds were compared to Amphotericin B. they were also compared to Amodiaquine in some factors. The compounds 1 and 3 contained a hydroxyl group in the aromatic ring in their structure. They displayed a good activity against almost all species of the promastigotes that were used for the analysis. In the second series, 7h displayed great activity against the promastigote forms of the *Leishmania* species. Both the compound 5 and 7compound 7h were more effective than the reference drug AQ and these compounds did not have any substituent in their phenyl ring. Only four compounds among the thirteen tested were cytotoxic against murine macrophages. They are 4, 5, 7f and 7h.

***Annona foetida*: a source of Pyrimidine-β-carboline and Other Alkaloids with Antileishmanial Activity**

The paper reports the method of isolation and the antileishmanial assay of the new pyrimidine-β-carboline alkaloids, N -hydroxyannomontine and annomontine along with two oxoaporphinic alkaloids O -methylmoschatoline and liriodenine (Costa et al., 2006).



(1) R = OH
(2) R = H

(3) $R_1 = R_2 = R_3 = OCH_3$
(4) $R_1 = R_2 = OCH_2O$, $R_3 = H$

Figure 37: General structures of pyrimidine-β-carboline

The bark of *A. foetida* was collected from the Brazilian Amazon. In Vitro screening of the isolated compounds were done against cultures of *Leishmania braziliensis* and *L. guyanensis* promastigotes. Four alkaloids were isolated from the sample. Two pyrimidine-β-carboline

alkaloids: N-hydroxyannomontine (1) and annomontine (2) and two oxoaporphine alkaloids: O-methylmoschatoline (3) and liriodenine (4). These compounds were subjected to antileishmanial and cytotoxic assay.

Table 39: In Vitro antileishmanial activity

Compound	**IC_{50}**	
	L. braziliensis	*L. guyanensis*
N-hydroxyannomontine (1)	252.7±2.2	437.5±2.5
annomontine (2)	34.8±1.5	>613.0
O-methylmoschatoline (3)	320.8±3.1	103.7±3.4
liriodenine (4)	58.5 ±1.8	21.5±0.4
Pentamidine	2.9±0,3	0.9±0.3

Pentamidine was used as positive control. All four of the compounds that were isolated exhibited more or less antileishmanial activity In Vitro against the promastigote forms of the *Leishmania braziliensis* strains. Against the strain called *L. braziliensis*, the compounds 2 and 4 showed much better activity than compounds 1 and 3. However, against this particular stain, the IC_{50} values were lower than 60 μM. In addition to that, Compound 2 showed no activity against *L. guyanensis*.But compound 2 was almost 6 times more active against the *L. braziliensis* strains. Compound 4 possesses a methylenedioxy moiety, which makes it about 8 times more active against both the species of promastigotes than 3. Therefore, compound 4, Liriodenine appeared to be the most active compound against *L. guyanensis* and compound 2 annomontine was the most active against *L. braziliensis* strains.

In Vitro and In Vivo Antileishmanial Activity of Novel Amino-pyrazole Ureas

In this report, contains the synthesis, SAR-studies, In Vitro antileishmanial activity, cytotoxicity of Amino-pyrazole Ureas(Mowbray et al., 2015)**.**

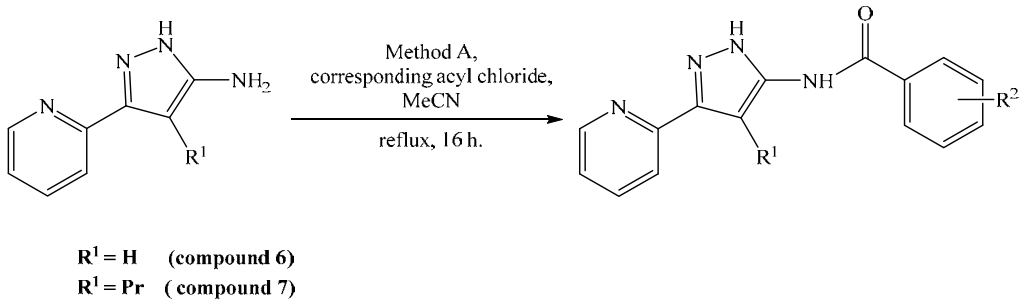


Figure 38: Synthesis of conpound 6 and compound 7

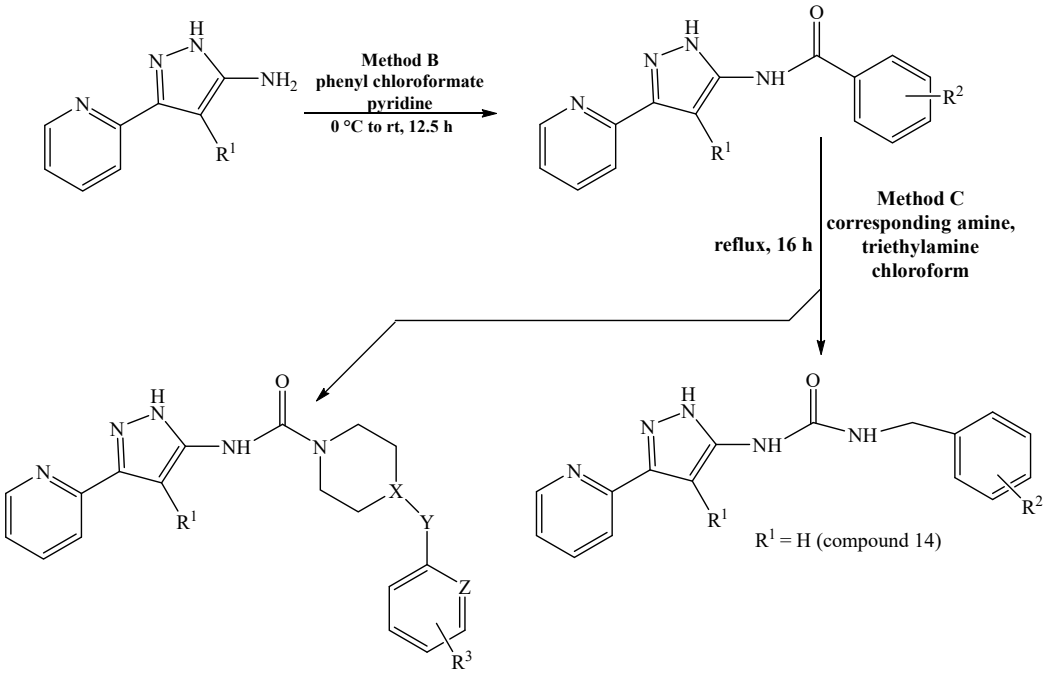


Figure 39: Synthesis of compound 14, 17 and 20

Cell cultures: for this assay, the Primary peritoneal macrophages (PMM) of mice were used. For the assay that was done In Vitro, The MRC5SV2 cells were cultured. These are diploid human embryonic lung fibroblasts. The test was done against the strains of *L. donovani* and *L. infantum*. The parasites were obtained from the Syrian golden hamsters of the *Mesocricetus auratus.* The Amastigotes used in the test were collected from the spleen of an infected donor. For the In Vivo test, the female golden hamsters were purchased.

Table 40: Molecular information and In Vitro antileishmanial activity and cytotoxicity

Compound	R^1	R^2	axenic *L. donovani* amastigote IC_{50} (µM)	intramacrophage *L. infantum* amastigote IC_{50} (µM)	PMM CC_{50} (µM)
6	-	OMe	0.738	>40.3	>40.3
7	Pr	4-OMe	-	2.02	>64
14	H	3-Cl	-	0.450	>64

Table 41: antileishmanial activity and cytotoxicity of the following compounds

Compound	X	Y	Z	R^3	intramacrophage *L. infantum* amastigote IC_{50} (µM)	PMM CC_{50} (µM)	MRC5	
							CC_{50} (µM)	SI
14	-	-	-	-	0.450	>64	>64	>142
17	CH		CH	H	0.296	>64	>64	>242
20	CH		CH	4-Cl	1.105	>55	>62	>74
Miltefosine	-	-	-	-	7.26	33.1	24.7	3.26

Miltefosine was used to compare the synthesized compounds with. In case of pyrazole derivatives, the compound 7 was formed by adding the cycloproppyl group at the position 4 of the pyrazole ring of the structure. This compound appeared to be much more active than the compound 6, which is the unsubstituted counterpart of the compound 7. Along with that compound 8 also showed excellent potency with 2-methoxy benzamide. These compounds did not show any cytotoxicity. However, there was still an issue concerning poor metabolic stability.The benzyl urea analogues showed submicromolar potency with almost no sign of toxicity although here still was high level of instability. The opposite was observed in case of amide subseries. Here, compound 14 showed that it was possible to synthesize a very active benzyl urea analogue without any substituent on its pyrazole core. On the other hand, Phenyl piperidine urea compound 17 was considered a lead compound. The In Vitro potency of the compound was good. It also had a good selectivity index (SI) and a good cytotoxicity. It was of about CC_{50}> 64µM; SI > 242 in value. In addition to that, the compound 17 showed equivalent antileishmanial activity against the strain of L. *donovani*. No activity in hamster

plasma was showed. Among the analogues that resulted from the new design strategy, compound 20 had improved stability.

5, 3'-Hydroxy-7, 4'-dimethoxyflavanone from *Picramniracilisa gracilis* Tul. (*Picramniaceae*) Fruit: Antileishmanial activity

In search of novel candidates for antileishmanial drugs, the authors focused on the In Vitro studies of 5, 3'-Hydroxy-7, 4'-dimethoxyflavanone which they extracted from the *Picramniracilisa gracilis* Tul. The potency of the isolated compound is described in the report(Robledo et al., 2015).

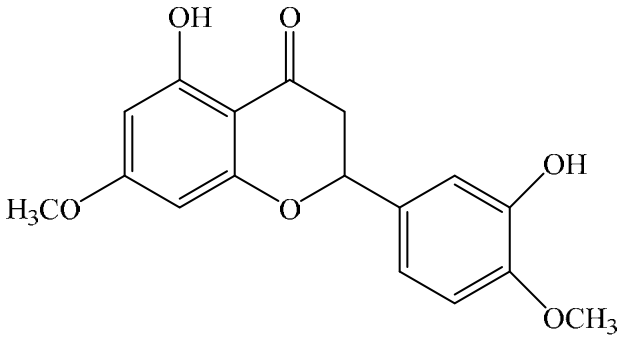


Figure 40: Structure of 5, 3'-Hydroxy-7, 4'-dimethoxyflavanone isolated from Picramniracilisa gracilis

The compound to be tested was isolated from the dried leaves and the dried fruits of *P. gracilis Tul*. This test was done against *L. panamensis.* The cytotoxicity test was done on human U937 cell line. The MTT method was followed. These cells were infected promastigotes in stationary phase of *L. panamensis.*

Table 42: Biological activity of Picramnia gracilis Tul.

Isolated compound	**LC_{50}**	**EC_{50}**	**IS**
5,3'-Hydroxy-7,4'-dimethoxyflavanone	>200.0	17.0 ± 2.8 (53.7μM)	>11.8
Amphotericin B	37.5 ± 7.6	0.06 ± 0.004	625.0
Meglumineantimoniate	>1000.0	6.8+0.5	>147.1

5,3'-hydroxy-7,4'-dimethoxyflavanone, separated of *P. gracilis,* was found to be a biologically active compound it was also proved to be highly selective. The extracted compound was tested In Vitro. It showed no toxicity on macrophages. The compound 5, 3'-hydroxy-7, 4'-dimethoxyflavanone showed excellent potency against the *L. (V.) panamensis.* It also showed very low toxicity on human U-937 cell line. By the In Vivo studies it was revealed that if the compound is administered in solution or as a cream, it improves the condition and it had absolutely no toxicity in the hamsters which had cutaneous leishmaniasis.

chalcone-like compounds: A molecular hybridization approach with syhtesis and antileishmanial evaluation.

This report contains the description of a new series of chalcone like compounds. The authors focused on the design, synthesis, and biological evaluation of these compounds (Barbosa et al., 2011).

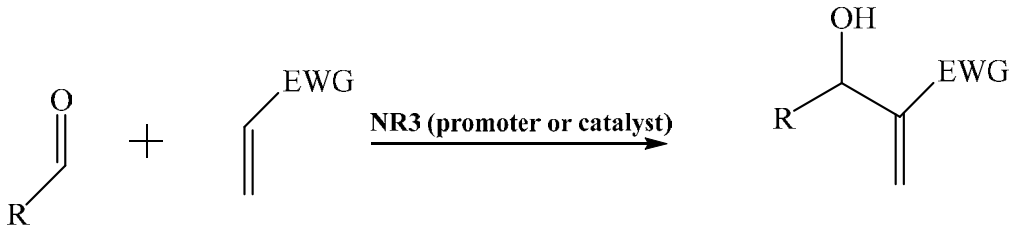


Figure 41: The Morita–Baylis–Hillman reaction (MBHR)

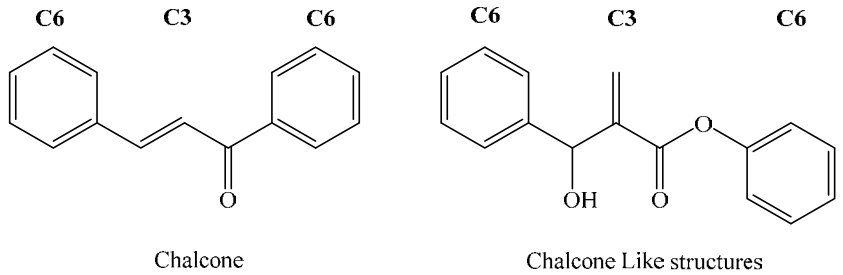


Figure 42: The difference between Chalcone and chalcone like structures

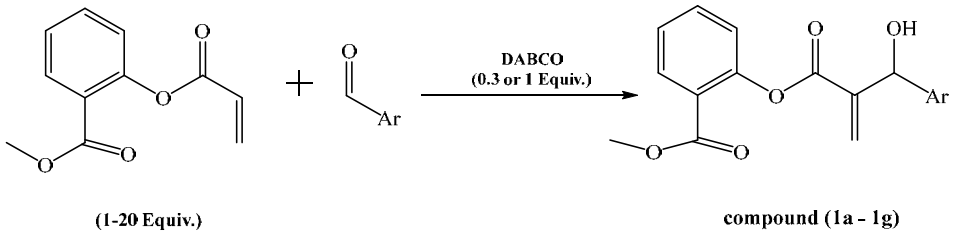


Figure 43: General synthetic route for compounds 1d, 1e, 1g

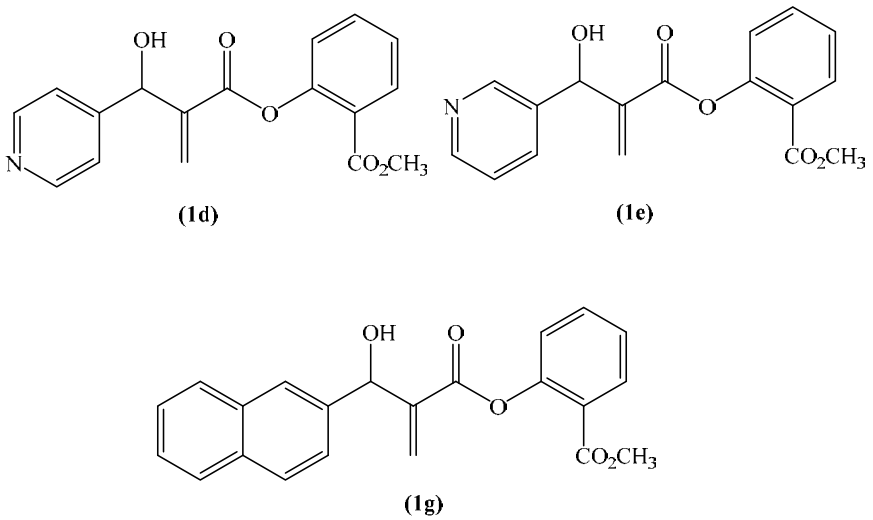


Figure 44: Compounds that were synthesized

Table 43: Antileishmanialactivity of the synthesized compounds against Leishmania parasites

Compound	**IC_{50} (lg mL1)** ***L. amazonensis***	**IC_{50} (lM)** ***L. amazonensis***	**IC_{50} (lg mL1)** ***L. chagasi***	**IC_{50} (lM)** ***L. chagasi***
1d	7.46	23.83	10.44	33.35
1e	9.75	31.15	12.11	38.69
1g	3.26	9.00	14.98	41.38
6	9.25	22.45	20.22	49.08
Glucantime	>4000	Variable	>4000	Variable
Amphotericin B	0.11	0.12	0.64	0.69

In this assay, the reference drugs were Glucantine and Amphotericin B. *L. amazonensis* and *L. chagasi* were used for the antileishmanial assay. Almost all of the synthesized compounds proved to be active against both *L. amazonesis* and *L. chagasi*. These compounds exhibited better activity than the previous compounds. The observation made on this assay was that the presence of salicylate moiety played a role in increasing the leishmanicidal activity of the compounds. The compounds 1d and 1e nearly twenty times more active than that of their

corresponding compounds. Dimer 6 had a very important against the *L. amazonensis* and *L. chagasi* as well. Second most active compound was 1g. 1g was also the most lipophilic of all.

Dillapiole: Discovery, Cytotoxicity SAR Studies of the Analogues as antileishmanials

In this study, the authors focused on the evaluation of the antileishmanial activity of dillapiole and its semi-synthetic analogues which are isodillapiole and di hydrodillapiole. These compounds were also tested for cytotoxicity. Along with that, the molecular properties of the compounds and the structure–activity relationships (SAR) were also described (Parise-Filho et al., 2012).

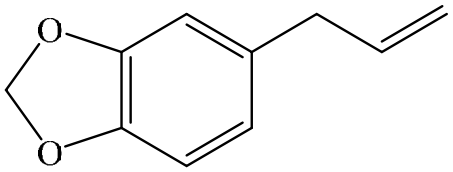


Figure 45: Structure of safrole

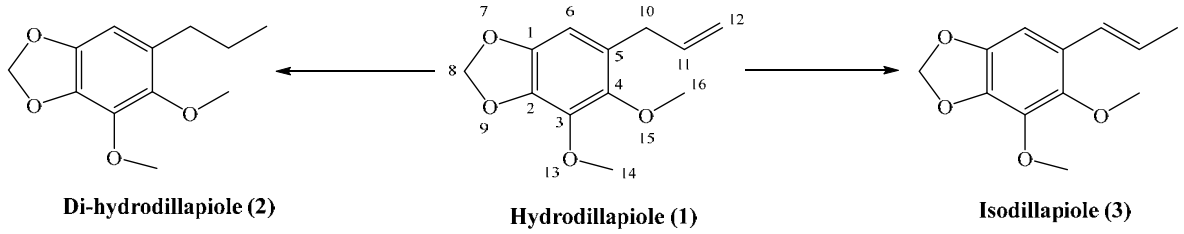


Figure 46: Synthesis of Di-hydrodillapiole and isodillapiole

The extraction was done from *P. aduncum* leaves. Dillapiole, compound 1 and the semi-synthetic analogues of the compound 1: di hydrodillapiole compound 2 and isodillapiole, compound 3 were tested against the strains of *Leishmania brasiliensis* and *Leishmania amazonensis* in their promastigote forms. The test was done against mouse fibroblast cells.

For the antileishmanial assay, Amphotericin B was used as standard. The MTT colorimetric assay was used in the evaluation of the cytotoxicity of the dillapiole and compounds derived from it in normal cells.

Table 44: Biological evaluation of the three compounds in comparison with Amphotericin B

Compounds	IC_{50} (mM)	
	Leishmania braziliensis	*Leishmania amazonensis*
1	69.3±4.9	59.4±4.0
2	99.9±10.4	90.50±8.6
3	122.9±13.9	109.8±9.5
Amphotericin B	0.054±0.0	0.033±0.0

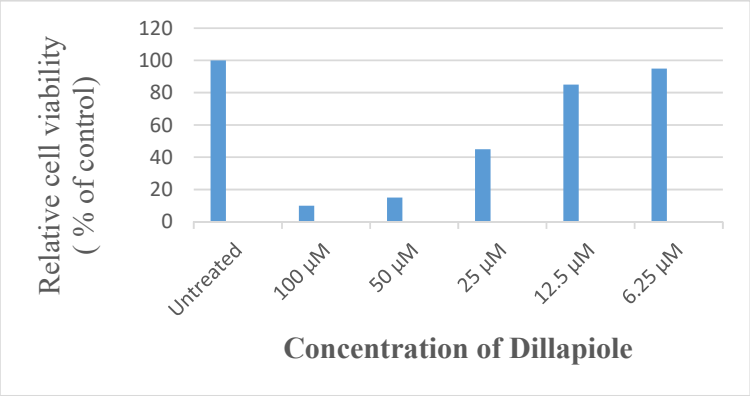


Figure 47: Test for cytotoxicity of Dillapiole on the 3T3 fibrobast cells

Standard drug for this assay was Amphotericin B. In this assay, the Compound 1, dillapiole was found to be most active against both the strains. Both the analogues 1 and 2 showed good activity as well but the activity decreased about 1.5- to 1.8-fold. Dillapiole was however more good against *L. braziliensis* than against *L. amazonensis*. This indicated to the fact that the antileishmanial effect of the compounds can be species dependent.

Synthesis, antileishmanial activity and cytotoxicity of 2, 3-diaryl- and 2, 3, 8-trisubstituted imidazo [1, 2-a] pyrazines

The authors explored the synthesis, SAR-studies, activity as an antileishmanial, cytotoxic effect of the compounds 2, 3-diaryl- and 2, 3, 8-trisubstituted imidazo [1, 2-a] pyrazines in this study(Marchand et al., 2015).

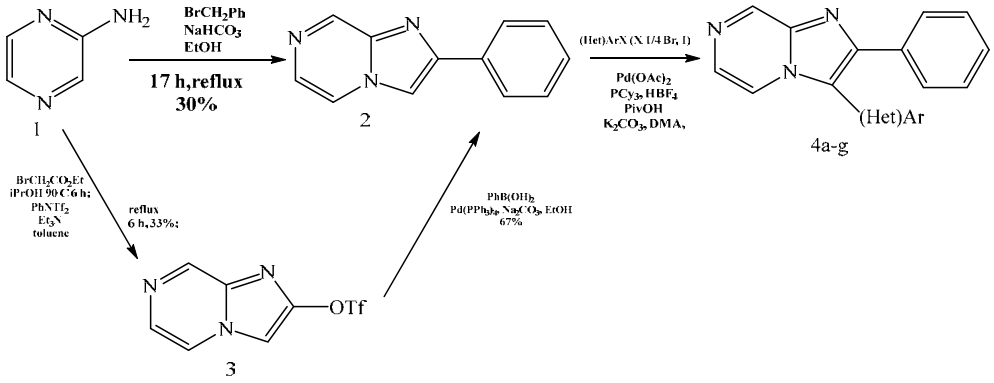


Figure 48: Synthesis of compound 4

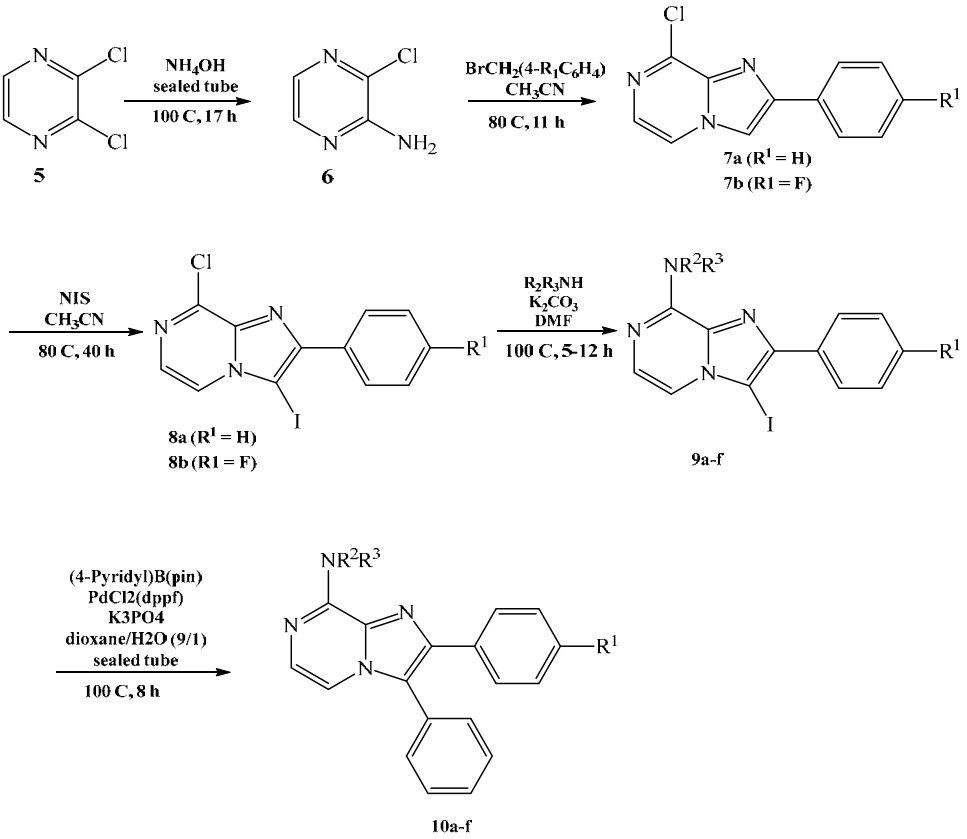
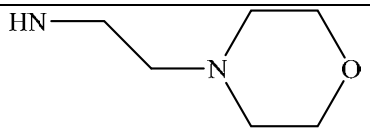


Figure 49: synthesis of compound 9 and compound 10

Table 45: Molecular properties of compounds

Compound	R^1	R^2	R^3
4g	H	4-Pyridyl	*H*
9f	F	I	
10e	F	4-Pyridyl	$N(CH_3)_2$

The antileishmanial assay was done against the promastigote stage of *Leishmania major*, the amastigote stage of *Leishmania major* and the axenic amastigote stage of the strains of *Leishmania amazonensis*. (MTT) micro method was used to determine the 50% inhibitory concentration (IC_{50}) of the compounds. The assay for cytotoxicity of the synthesized compounds was measured and evaluated in both different tumor cell lines as well as in the normal cell lines using the assay method of sulforhodamine B (SRB). Cell lines that were tested in this way include BALB/3T3 cells, which are non-tumorogenic cells from BALB/c mouse embryo cells along with others.For macrophages, female mice were used. Murine macrophages were harvested from the peritoneal cavities of the female BALB/c mice which were around 6 to 8 weeks old.

Table 46: In Vitro antileishmanial activity and cytotoxicity of the synthesized compounds

Compound	***L. major* promastigotes IC_{50} ± SEM (mM)**	**Cytotoxicity on macrophages IC_{50} ± SEM (mM)**	**Selectivity index**	**Cytotoxicity on 3T3 cells IC_{50} (mM)**	**Selectivity index**
4g	20.1 ± 12.2	153.7 ± 12.3	7.7	ND	ND
9f	2.8 ± 0.4	93.2 ± 9.5	33.3	>267.5	>95.6
10e	6.4 ± 0.2	42.0 ± 4.5	6.6	>375.0	>58.6

For this assay, Pentamidine was used as the standard drug. Two sub-series showed a very good antileishmanial activity. They also showed good therapeutic index, mainly against amastigote stage of the parasite. Compound 9f contains an iodine atom at the C3 position of the imidazo [1, 2-a] pyrazine ring in place of the 4-pyridyl group exhibits high protozoa selectivity.A significant increase in the activity is observed for the N, N dimethylamino derivative 10e. It exhibited the exact same level of activity as the standard, Pentamidine. Compound 9f exhibited very promising activities. Compounds 4g and 10e however appeared to have a moderate safety profile and 9f a high safety profile.

9-methyl-1phenyl-9H-pyrido [3, 4-b] indole derivatives: biological evaluation and SAR studies as an anti-leishmanial agents

In this study, the authors focused on designing new β-carboline and piperazine hybrid molecules. They included the biological evaluation of the synthesized molecules as antileishmanials.The amastigote and promastigote forms of *Leishmania infantum* and *Leishmania donovani* strains were used to evaluate the antileishmanial activity of the synthesized compounds. These molecules were tested for cytotoxicity upon HeLa cells(Barbosa et al., 2011).

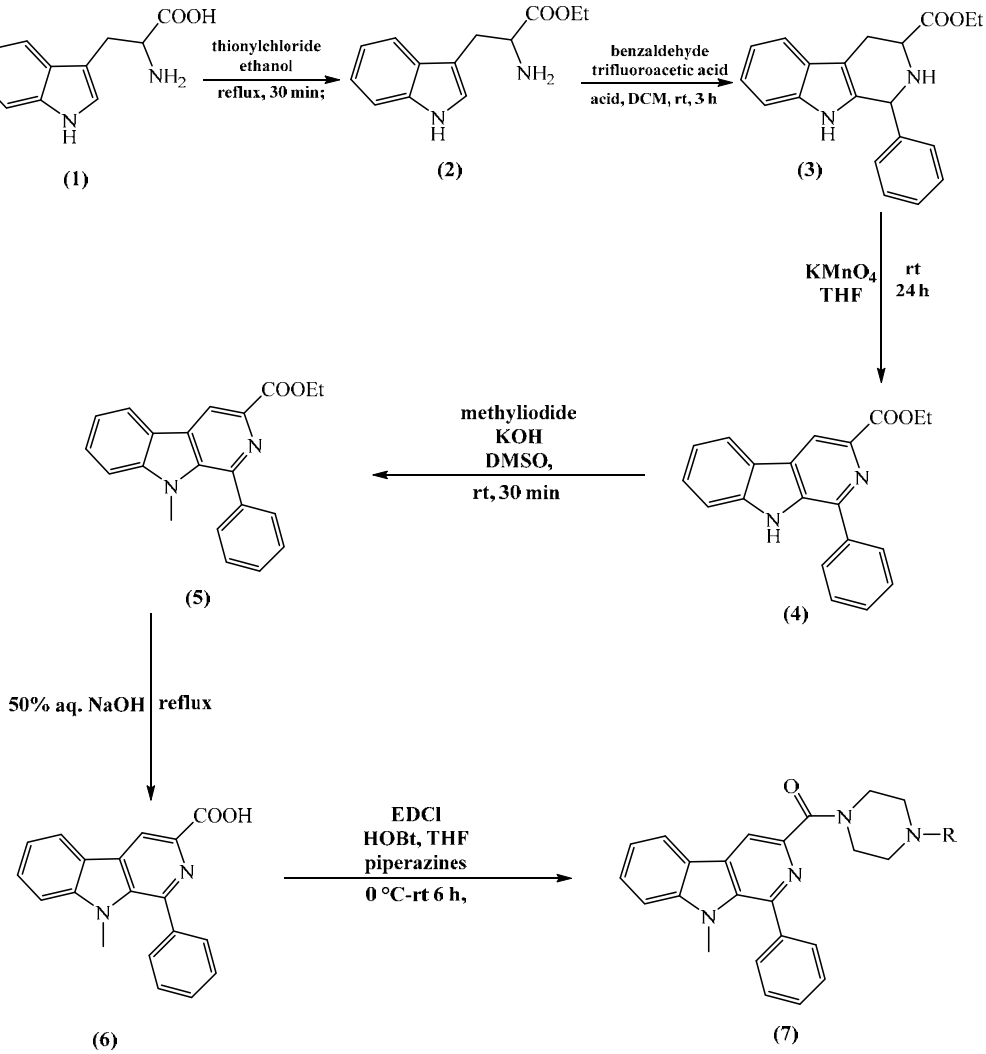


Figure 50: Synthesis of derivatives from 9-methyl-1phenyl-9H-pyrido [3, 4-b] indole

Table 47: Structural information and biological evaluation against L. infantum

Compound	R	CC_{50}(µM)	*L. infantum* promastigote EC_{50}(µM)	SI	*L. infantum* Axenic amastigote EC_{50}(µM)	SI
7c	$-2CH_3C_6H_4$	>500	3.73 ± 0.28	>134.0	2.6 ± 0.2	>192.3
7d	$-4OCH_3C_6H_4$	>500	1.59 ± 0.11	>314.5	1.4 ± 0.1	>357.1
7g	$-4ClC_6H_4$	>500	1.47 ± 0.32	>340.1	1.9 ± 0.2	>263.2
7o	$-4C_5H_4N$	29.7 ± 1.5	61.1 ± 5.4	0.48	ND	–
7p	$-2C_5H_4N$	273 ± 21.6	45.7 ± 3.3	5.97	ND	–
Pentamidine	–	–	8.31 ± 0.18	–	2.7 ± 0.4	–
Miltefosine	–	–	12.6 ± 1.1	–	4.8 ± 0.8	–

Table 48: Structural information and biological evaluation against L. donovani

Compound	CC_{50}(µM)	*L. donovani* promastigote EC_{50}(µM)	SI	*L. donovani* Axenic amastigote EC_{50}(µM)	SI	*L.donovani* intracellular amastigote EC50 (µM)	SI
7c	>500	9.45 ± 0.72	>52.9	5.3 ± 0.4	94.3	9.4 ± 1.3	53.2
7d	>500	0.91 ± 0.09	>549.5	0.9 ± 0.1	555.6	1.3 ± 0.1	384.6
7g	>500	5.02 ± 0.36	>99.6	3.8 ± 0.5	131.6	6.3 ± 0.3	79.4
7o	29.7 ± 1.5	19.5 ± 2.8	1.52	13.9 ± 0.4	2.1	7.2 ± 0.5	4.1
7p	273±21.6	8.5 ± 0.75	45.73	12.3 ± 0.6	22.2	7.9 ± 0.3	34.6
Pentamidine	-	6.40 ± 0.11	-	1.6 ± 0.1	-	23.7 ± 1.8	-
Miltefosine	-	3.12 ± 0.16	-	2.8 ± 0.4	-	6.4 ± 0.3	-

Miltefosine and Pentamidine are the two drugs that were used as standards for comparison. In the cytotoxicity assay, compounds 7o and 7p appeared to be the β-carboline derivatives which were the most cytotoxic ones against the HeLa cells.In the in-vitro evaluation, compounds 7g, 7d, and 7c exhibited the most potency against the promastigote forms of *L. infantum.* Among which 7d and 7g were almost 5 times more active than the standard drugs. The SAR studies demonstrated an amazing fact. The para substitution with ortho-para directing groups such as methoxy and chloro, results in a much greater activity. Whereas, the replacement of the phenyl ring with benzylandpyridyl ring was not necessarily helpful. The in-vitro evaluation also revealed that the compounds 7d, 7g and appeared to be very potent

against the amastigotes as well. They acted better than both the standard drugs. This study also brought another fact to light. It said that the β-carboline derivatives were much active against the axenic amastigote forms of *L. infantum.* Similar results were observed in case of *L. donovani* strains. 7d exhibited potent inhibition of *L.donovani* promastigotes, even better than standard drugs along with other compounds like 7h.

Pyrazole derivatives: Synthesis, molecular modeling and biological screening as leishmanicidals

In this report, the authors designed synthesized and evaluated novel candidates to be used as an antileishmanial. These compounds are open chain and cyclized derivatives containing pyrazole scaffold (Bekhit, Saudi, Hassan, Fahmy, & Tamer, 2018).

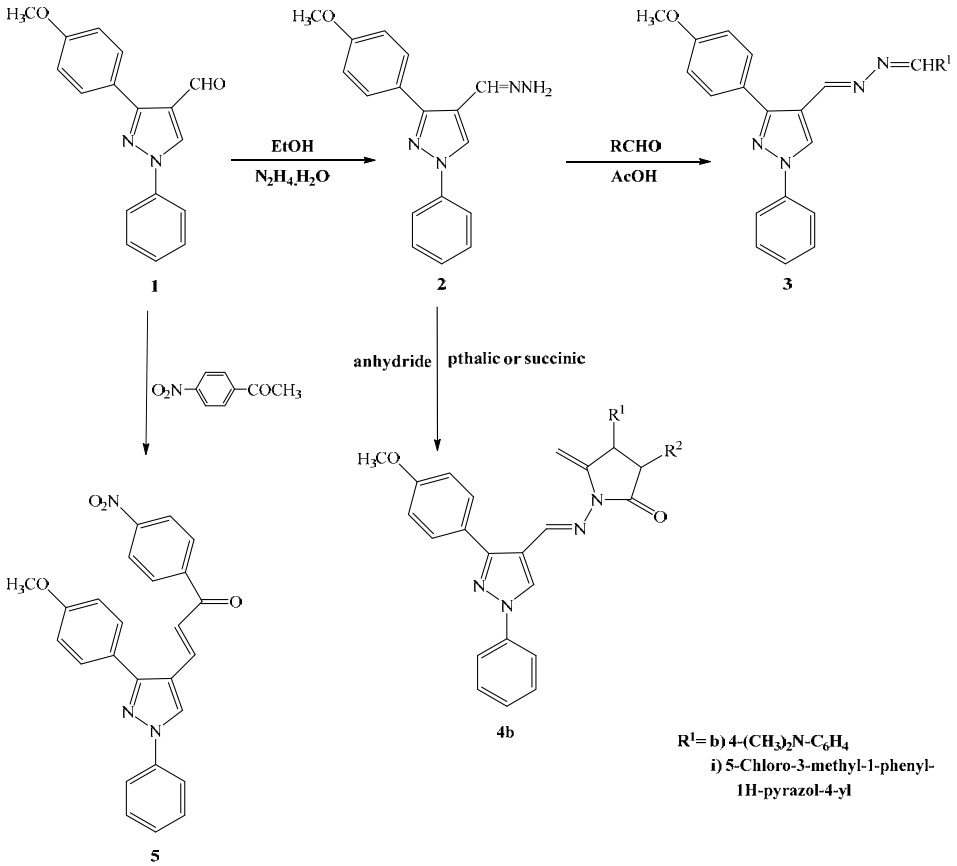


Figure 51: General procedure for synthesis for a series of pyrazole derivatives

L. major was used for antileishmanial assay.A quantitative colorimetric assay named as Alamar blue reduction assay was done to measure cytotoxicity of the targeted compounds. The obtained results wereanalyzed and IC_{50}values were calculated; shown in table 1. Pteridine reductase (PTR1) inhibition assay was also done. For the toxicity studies, RBC hemolysis assay,White blood cell cytotoxicity assessment was also done. The Peritoneal blood mononuclear cells were used as well.

Table 49: In Vitro antileishmanial activity and cytotoxicity of the synthesized compounds

Compound	**IC_{50} (µM) on promastigotes**	**IC_{50} (µM) on amastigotes**	**CC_{50} (µM) on PBMC**
3i	0.74±0.10	1.45±0.08	376.14±23.5
5	1.02±0.37	2.30±0.09	223.86±19.5
4b	2.64±0.33	5.04±0.04	167.07±26.4
9	5.70±0.05	6.76±0.34	ND
Miltefosine	7.81±0.34	8.09±0.09	ND
Amphotericin B deoxycholate	0.04±0.01	0.15±0.01	ND

Table 50: The effect of the compounds on intracellular amastigotes in a dose of 3 µM.

Compound	**0 day**	**3 days**
Control (no compound)	0.0	20±0.8
3i	0.0	8±0.6
5	0.0	12±0.6

For reference, Miltefosine and Amphotericin B was used. The compounds that showed the highest potency are compounds 3i and 5.Schiff bases, such as 3i, shows better activity against promastigotes. Usually, the nitro hetero compounds act as a pro drug. It gets activated when they come in contact of the nitro reductase enzyme inside the pathogen. This rationalizes the high antileishmanial activity of compound 5. The phthalimide derivative, 4b displayed higher antileishmanial activity. The direct attachment of the different rings such as oxadiazole, to the pyrazole ring, forms compound 9; exhibits moderate activity.

Quinoline-4-carboxylic Acids: Synthesis and biological screening

The authors described the synthesis of a group of the quinolone -4-carboxylic acids and they confirmed the chemical structures of these compounds. They also evaluated the antileishmanial activities of each of these compounds. The synthesis of the quinolone -4-carboxylicacids were done by using Pfitzinger reaction (Abdelwahid et al., 2019).

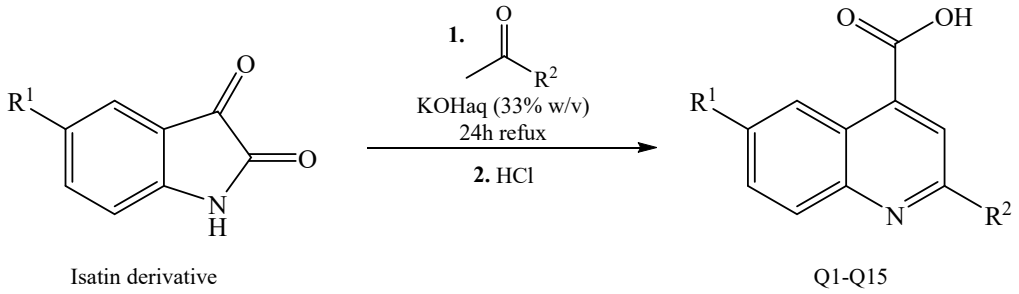


Figure 52: Synthesis of quinoline-4-carboxylic acid

Table 51: Molecular information of the synthesized compounds

compound	R^1	R^2
Q1	H	Me
Q4	Br	4-Methoxyphenyl
Q12	NO_2	4-Bromophenyl

The compounds were tested against *L. donovani* promastigotes in different concentration levels. A patient who was confirmed to be positive of VL was used for the isolation of the parasites by lymph node aspiration.For the evaluation of the activity as an antileishmanial, the IC_{50} values of each of these compounds were measured. The oral bioavailability of these compounds were determined as well.

Table 52: Antileishmanial activity of the synthesized compounds

Compound	IC_{50} (µg/mL)
Q1	1.49
Q4	27.03
Q12	17.19
Sodium stibogluconate	8.06
Amphotericin B	14.7

The drug Sodium stibogluconate along with another drug Amphotericin B were used as positive control drug for the biological evaluation. 2- methylquinoline -4-carboxylic acid, compound Q1 was proven as the most active one among the synthesized compounds. It proved to be 5 times more effective than the one called Sodium stibogluconate and ten times more effective than Amphotericin B. along with that compound 12 showed activity comparable to Amphotericin B. compound 4 showed moderate activity as well.

Sulfonamide4-methoxychalcone derivatives: Synthesis, biological evaluation and SAR studies as potential antileishmanials

This is a report based on the rational approach that the authors followed to synthesize a novel series of antileishmanial molecules of sulfonamide 4- methoxychalcone analogs and to develop new antiparasitic compounds. The authors also discussed the structure activity relationships (SAR) of the compounds of the novel series. This was done in order to determine the structural features as well as the electronic properties of the sulfonamide 4-methoxychalcone analogs that might lead to the discovery of new antileishmanials along with their biological evaluation(Andrighetti-fro, Sim, Rodrigues, Nunes, & Castro, 2009).

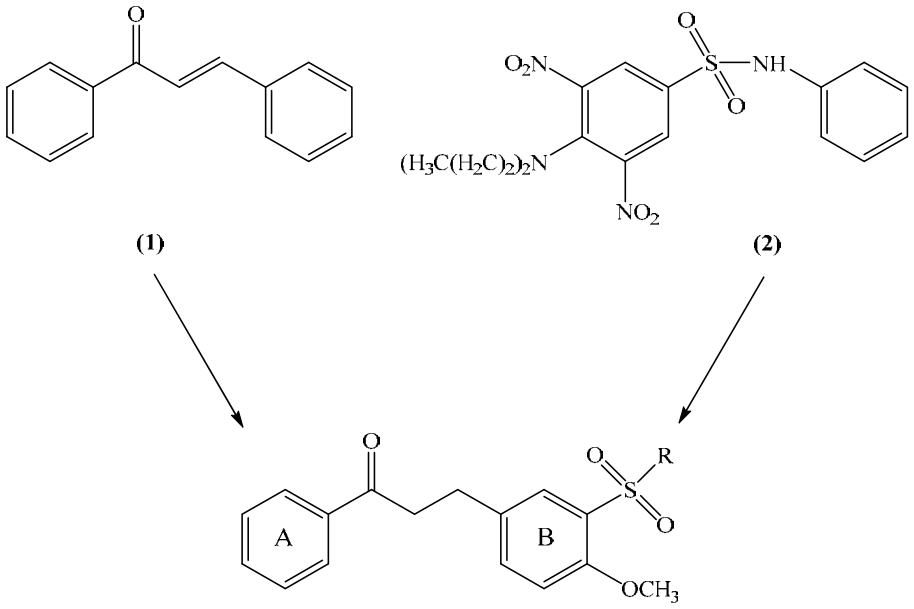
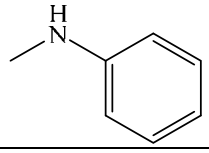
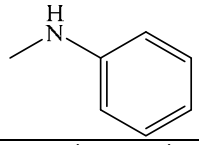
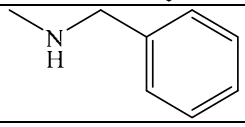


Figure 53: General procedure for synthesis of Sulfonamide 4-methoxychalcone derivatives

Both the promastigote and amastigote forms of *L. braziliensis* were used for the antileishmanial assay.The test for cytotoxicity was conducted with the peritoneal macrophages derived from mice. These mice were harvested from the peritonea of healthy mice.

Table 53: Molecular properties and the antileishmanial activity of the compounds

Entity	**R**	**IC_{50}(mM)**
3e		69.0 ±3.7
3f		4.6±1.3
3i		3.5±0.6
Amphotericin B	-	0.3±0.02
Pentamidine isothionate	-	19.6

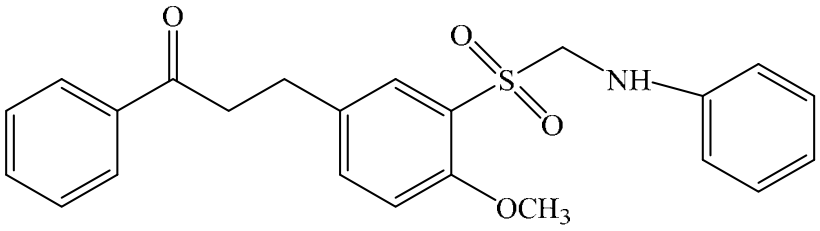


Figure 54: Structure of compound 3e

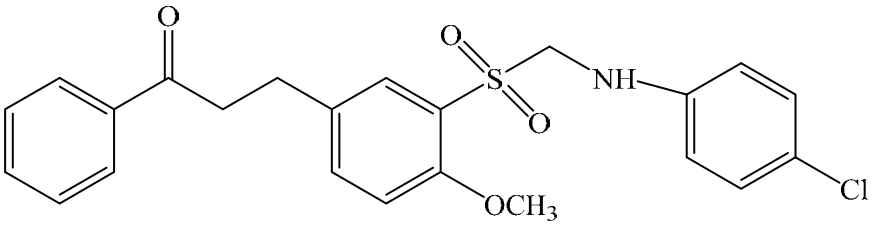


Figure 55: Structure of compound 3f

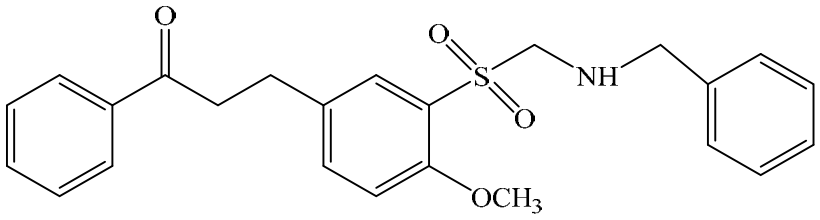


Figure 56: Structure of 3i

The positive control drug in this assay was Amphotericin B. This study revealed that the addition of sulfonamide generated derivatives to the lead compound results in better inhibition against the L. *braziliensis*species than the 4-methoxychalcon. The compounds were proven to be less active than Amphotericin B, but more active than Pentamidine isothionate, a current antileishmanial drug. Among all the compounds that were synthesized, the compound 3i had best activity compared to the other synthesized molecules against promastigotes of *L. braziliensis* species; the benzyl amino group present in its structure contributes greatly in developing this property. Thus it is almost 20 times more potent than another compound 3e.

Novel Pyridinium hydrazone derivatives: Synthesis and antileishmanial activity

Pyridinium skeleton is important for the antiprotozoan activity. This report contains the synthesis, SAR-studies, In Vitro antileishmanial activity, cytotoxicity of new Pyridinium hydrazone derivatives. These phenylethylidenehydrazinyl pyridinium salts bear different alkyl side chains on the pyridinium nitrogen. Promastigotes of *Leishmania tropica* were used for the assay(Alptuzun et al., 2013).

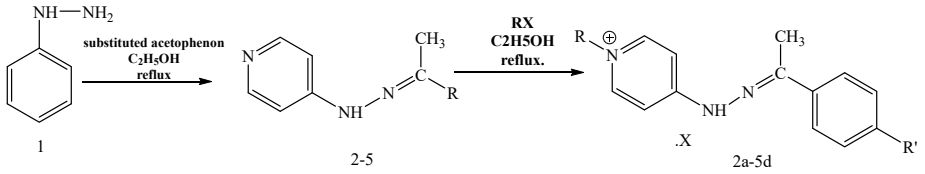


Figure 57: General procedure for synthesis of the pyridinium hydrazone derivatives

Table 54: molecular properties of the pyridinium hydrazone derivatives

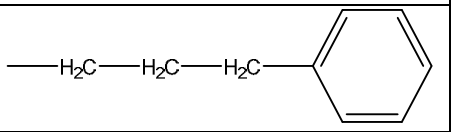
Compound	R′	R
3b	$-CH_3$	$-CH_2CH_3$
3c	$-CH_3$	$-CH_2CH_2CH_3$
3d	$-CH_3$	
5c	-Cl	$-CH_2CH_2CH_3$

Table 55: Antileishmanial activity of the pyridinium hydrazone derivatives

Compound	IC_{50} (µM)*L. tropica*
3b	11.69
3c	12.03
3d	6.90
5c	9.92

The compound 4- [2- (1-[4-methylphenyl]ethylidene)hydrazinyl]- 1- (3-phenylpropyl) pyridinium bromide, compound 3d appeared to be the most active one among all the other compounds. The study proved that those compounds, which have methyl substituent on the phenyl rings are more active than the other compounds which do not contain a methyl substituent. We can also come to the conclusion that almost all compounds were active after a certain extent. The antileishmanial activity of these compounds also varied based on the length of the chain on the Nitrogen orient. Yet not all of these compounds can be considered as potential antileishmanials. The compounds that are most likely to be considered as potential candidates for leishmanicidal effects are compound 3b, compound 3c, compound 3d and the compound 5c.

Novel chalconoids with antileishmanial properties: 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones; synthesis and biological evaluation

Chalcones present in plants like *Glycirrhyza glabra* and *Piper aduncum* are of natural origin and has been proven to have antileishmanial activity. Since then, researchers have been modifying the structures of chalcones to invent mopotent antileishmanial agents. This report contains the synthesis, SAR-studies, In Vitro antileishmanial activity, cytotoxicity of a series

of new chalconoids, which contains a 6-chloro-2H-chromen-3-yl group in its structure (Nazarian et al., 2010).

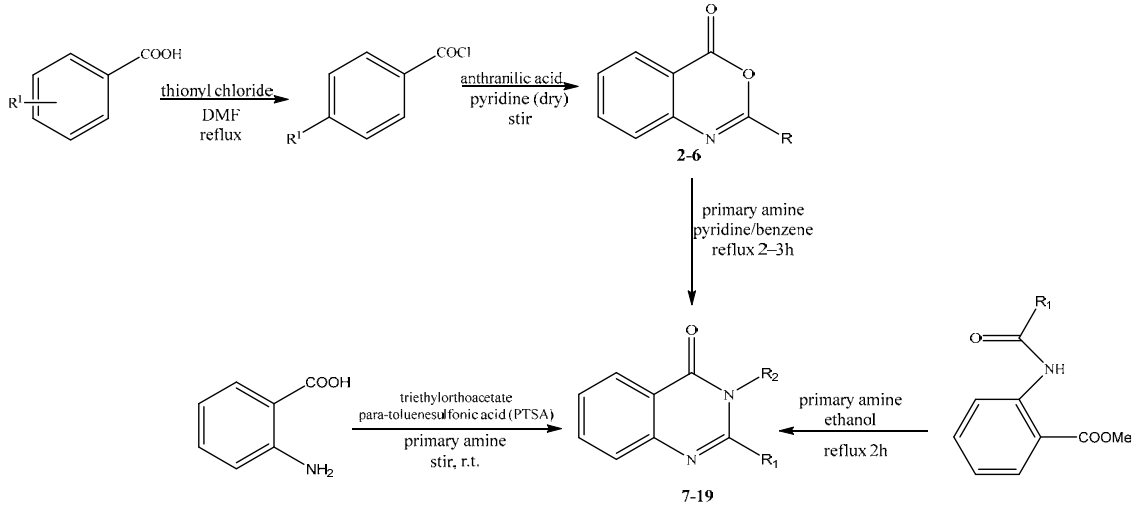
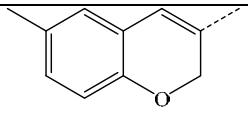
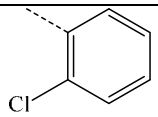
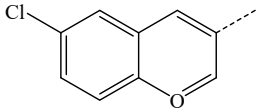
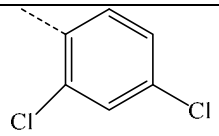
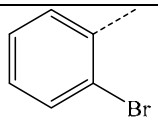
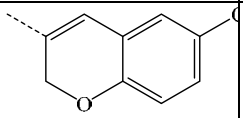


Figure 58: General procedure for synthesis of 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones

Table 56: Molecular information of 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones

Compound	Ring A	Ring B	Yield (%)	Molecular formula	IC_{50} (mM)
3b			45	$C_{18}H_{12}Cl_2O_2$	1.22±0.31
3c			88	$C_{18}H_{11}Cl_3O_2$	1.91±0.35
4a			98	$C_{18}H_{11}BrClO_2$	1.33±0.52
Glucantime	-	-	-	-	30±0.189

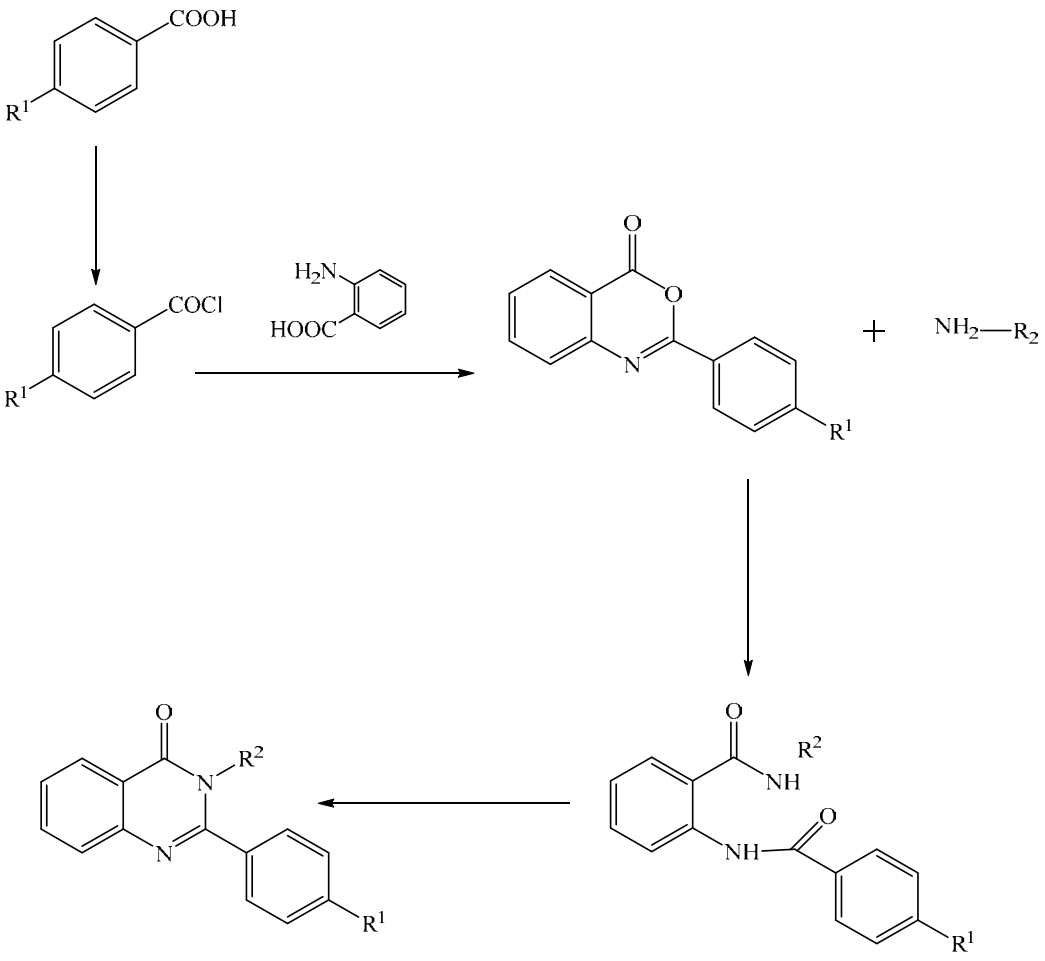


Figure 59: Mechanism of formation of 1- or 3-(6-chloro-2H-chromen-3-yl) propen-1-ones

Glucantime was used for reference. For the antileishmanial assay the *L. major* species of parasites were used; In Vitro toxicity was measured against mouse peritoneal macrophages.All the synthesized compounds proved to exhibit high activity against *L. major.* Among which the compounds 3b which contains 2-chlorophenyl, and compound 4a which contains 2-bromophenyl the most potent compounds. After the cytotoxicity assessment was done, each of the compounds appeared to be non-cytotoxic antileishmanials at lower concentrations. Therefore, (6- chloro-2 H- chromen- 3-yl) propenone could be an excellent candidate to be used as a lead compound from which effective agents for chemotherapy of leishmaniasis can be developed.

Novel 3-Substituted Quinoline: Synthesis and antileishmanial activities

This report contains the synthesis of four novel 3-substituted quinolines with allyl and cinnamyl motifs covalently attached to the quinoline entity of the structure. In Vitro parasite models are used in order to evaluate the antileishmanial activity of the compounds and to ensure that the compounds synthesized are potential antileishmanials (Tempone et al., 2005).

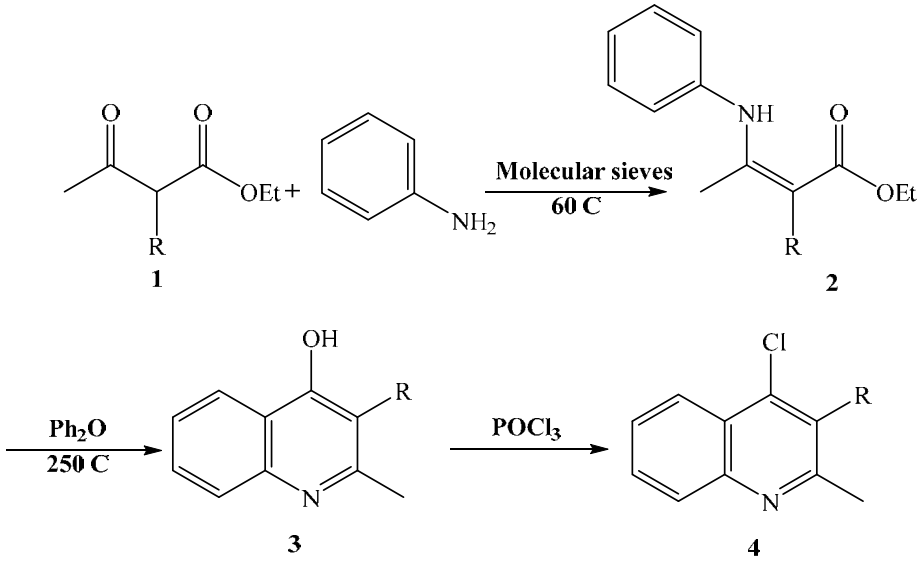
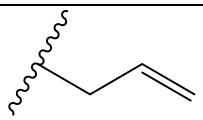
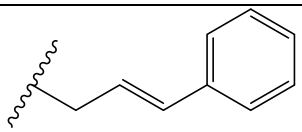


Figure 60: General procedure for synthesis of 3-substituted quinolines

Table 57: Molecular information of the 3-substituted quinolines

Entity	R
A	
B	

For this assay, the parasite group that was used is *Leishmania chagasi.* The animal used in the assay was golden hamsters. The amastigotes were obtained from an infected hamster spleen. The promastigotes were taken care of with calf serum 10% and 0.25% hemin serum 0.25% at a temperature of 24 ºC.

Table 58: Antileishmanial activity and cytotoxicity of the quinoline compounds

Compound	IC_{50} (µg ml^{-1}) (95% C.I.) for		Cytotoxicity; IC_{50} (µg ml^{-1}) (95% C.I.)	SI
	Promastigote	Amastigote		
3a	0.79 (0.45 – 1.37)	> 15	31.60 (19.82–50.38)	
3b	0.091 (0.06 - 0.13)	3.55 (3.34 – 3.78)	26.89 (20.47–35.34)	7.57
4a	18.78 (8.02 – 44.00)	> 15	13.77 (10.75–17.62)	
4b	1.79 (0.73 – 4.23)	> 15	22.00 (16.24–29.80)	
Sb^V		29.55 (28.09 - 31.09)	100	3.38
Pentamidine	2.02 (1.75 – 2.35)	-	2.57 (2.23–2.96)	-

Among all the compounds that were synthesized, the compound 3b had the lowest value of IC50. This proved that the compound 3b was of better activity than the other ones. Pentamidine was used for the drugs to be compared to. The compound 3b appeared to be of 22 times greater in activity than Pentamidine. The macrophages that were infected by *L. chagasi* were incubated were these new quinolone compounds. Only the compound 3b was active and the other compounds could not show activity even at their highest concentrations. It was almost 8.3 times more potent than the Pentavalent antimony itself.Simultaneously, it caused 7.6 times more harm to the mammal cells. The increase in the antileishmanial activity of these compounds may be a result of the introduction of a –OH group into the quinolone ring. Any other atom in position 4 did not contribute to the activity of the drug.

Pyrazole carbaldehyde derivatives: Antileishmanial screening, physicochemical properties and drug likeness

The following report contains the synthesis and the antileishmanial activity of the pyrazole carbaldehyde derivatives(Alodeani, Izhari, & Arshad, 2015).

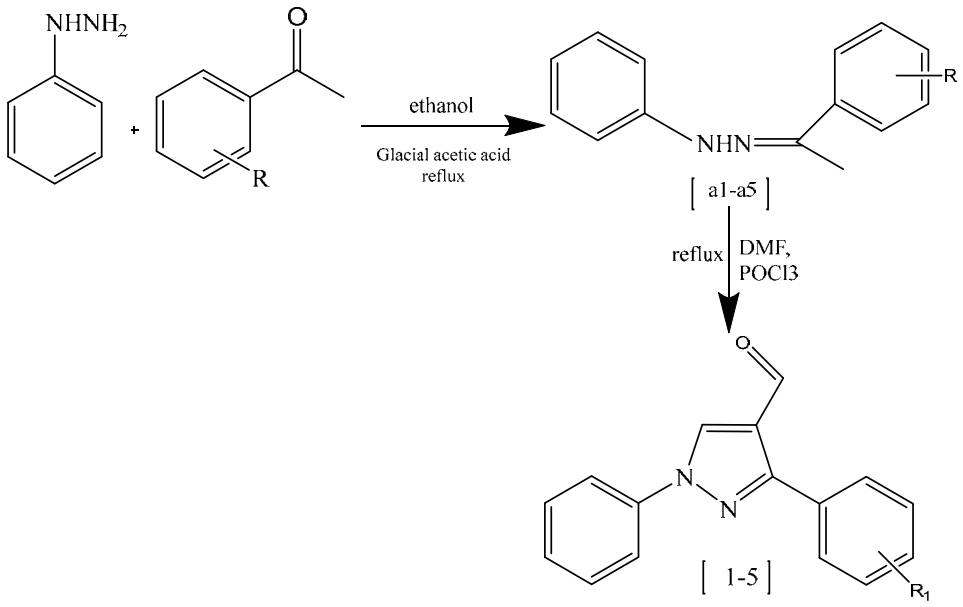


Figure 61: General procedure for synthesis of pyrazole carbaldehyde derivatives

Table 59: Molecular information and antileishmanial activity of pyrazole carbaldehydes

Compound	Molecular formulae	IC_{50} (µg/ml)
2	$C_{17}H_{14}N_2O$	30
3	$C_{187}H_{14}N_2O_2$	5.5
4	$C_{16}H_{11}ClN_2O_2$	15
Amphotericin B	$C_{47}H_{73}NO_{17}$	0.15

A culture of *Leishmania donovani* of promastigote stage were used for the antileishmanial assay. Amphotericin B was used as the standard antileishmanial agent. Among all the derivatives that were used for the evaluation of their leishmanicidal activity, the compound 3 and compound 4 were found to possess good activity; along with that the other compounds were found to be of moderately active. The bioavailability was good for almost all of them.

Quinoxaline 1, 4- di- N- oxide: Salicylamide along with sulfonamide derivatives

The 1, 4- di- N- oxide of Quinoxalines increase the pharmacological activity of the compounds immensely. This report contains the synthesis of the salicylamide and sulfonamide derivatives of quinoxaline derivatives. They were synthesized by adding different substituents at the 2, 3, 6 and the 7 position of the quinoxaline ring in their structure(Barea et al., 2011).

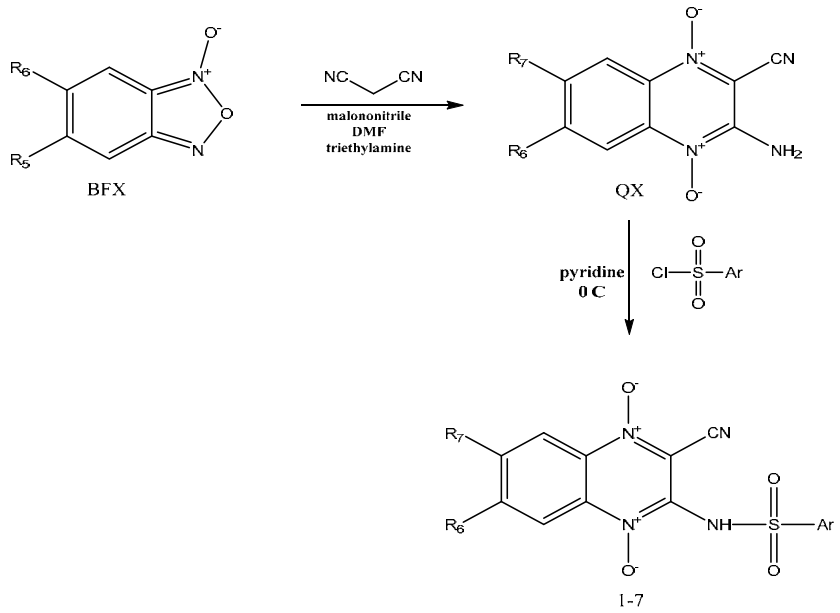


Figure 62: General procedure for synthesis of Quinoxaline 1, 4- di- N- oxide

Table 60: Molecular information, antileishmanial activity and cytotoxicity of the synthesized compounds

Compound	R_6	R_7	Ar	IC_{50} (µM)	IC_{50} Amas (µM)	CC_{50} (µM)	SI
2	H	CH_3	2-Naptyl	>24.6	16.3 ± 0.8	>245.6	>15.1
4	H	Cl	o-Nitrophenyl	17.4 ± 0.5	3.1 ±0.1	6.8 ± 0.8	2.2
5	H	Cl	p-Nitrophenyl	>23.7	2.1 ±0.1	7.9 ± 0.3	3.8
6	H	H	p-Nitrophenyl	18.7 ±	15.9 ± 1.3	89.1 ± 6.4	5.6
9	H	Cl	-	10.8 ± 1.8	33.6 ± 1.3	NT	-
14	H	F	-	7.4 ± 0.6	7.3 ± 0.1	13.6 ± 1.6	1.8
Amp B	-	-	-	-	0.2 ± 0.01	4.4 ± 0.1	22

The compounds that were synthesized were compared to a current drug Amphotericin B. The *Leishmania* parasite *L. amazonesis* was used for the assay. The Murine Peritoneal Macrophages (MPM) were used for the cytotoxicity test. Among all the compounds, compound 4 and compound 5 were active against *Leishmania* parasites. Both inhibited 50% parasite growth. But they were almost 15% less active than Amphotericin B. The lower activity may be a result of the lack of electronegative atom in the R_7 position. These compounds also had very low selectivity index, much low than that of the drug that was used

as standard. Compounds 2 and 6 were less active than compounds 4 and 5 but appeared to be far less toxic than the active ones. Compound 2 was more selective than compound 6. The SI value for compound 2 was nearly as good as Amphotericin B. the activity of the compound is supposed to drop if the R_7 position is occupied with either Cl orCH_3O. The compound will be active as a leishmanicidal compound if there is a $CH_3$of F in the position. Compound 14 as the most active one in series 2, was yet very less active than compounds 4 and 5. The compounds 4, 9, 5 and 14 exhibited mid-level of absorption.

2-Mercaptoquinoline Analogues: A Potent Antileishmanial Agent

Quinoline and its derivatives has always been very significant to the researchers because of its availability. The quinoline derivatives are available almost in a wide range of natural sources. Also it constitute a lot of biological properties(Koley, Tiwari, Singh, & Shankar, 2018).

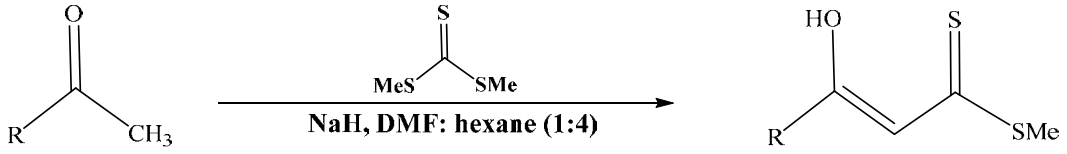


Figure 63: General procedure for synthesis of a-enolic dithioesters (2)

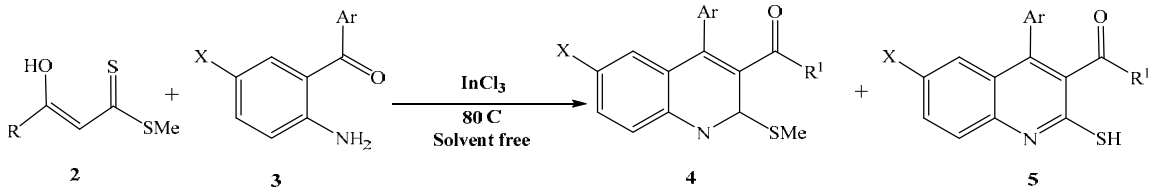


Figure 64: Synthetic approach for the differently substituted quinolines

Table 61: Antileishmanial activity and cytotoxicity of substituted quinolines

Compound	IC_{50} SD (mg/mL) Promastigote *L. donovani*	IC_{50} SD (mg/mL) intramacrophage amastigotes *L. donovani*	CC_{50} SD (mg/ml) RAW264.7 macrophage (mg/mL)	SI intra-macrophage amastigotes
5a	1.340.14	1.700.15	48.213.78	28.35
5c	1.520.59	2.101.23	45.332.47	21.58
5d	3.260.48	4.093.62	42.833.16	10.47
5f	1.920.40	2.300.58	49.581.16	21.55

Miltefosine was used to compare the synthesized compounds for assessing their biological activity. For the cytotoxicity assay, mouse microphages are used. The promastigote and amastigote forms of *L. donovani* strains were employed for the biological evaluation. The compounds 5a, 5c, 5d and 5f exhibited very good activity against the *Leishmania* parasites. The compounds were also subjected to cytotoxicity tests. Compound 5a had the highest value for selectivity followed by the compound 5c. By reacting ortho - aminoaryl ketons to the α-enolic dithioesters a series of quinolines was synthesized. They appeared to be very potential candidates to be used as antileishmanials.

1, 4-bis (substituted benzal hydrazino) phthalazines: activity as a leishmanicidal and molecular docking

In this report, the series on which the authors focused on is the 1, 4-bis (substituted benzal hydrazino) phthalazines. The specialty of this series is that all of the compounds of this series follow the Lipinski's rules. There is a hydrazinyl chain present in the structures of these compounds. Although the non-nitro derivatives were positively influenced by this group but the effect it had on the nitro derivatives was much negative.Eight of the derivatives were tested against the parasite strains *L. braziliensis* and *L. Mexicana* (Romero, Rodríguez, Oviedo, & Lopez, 2019)

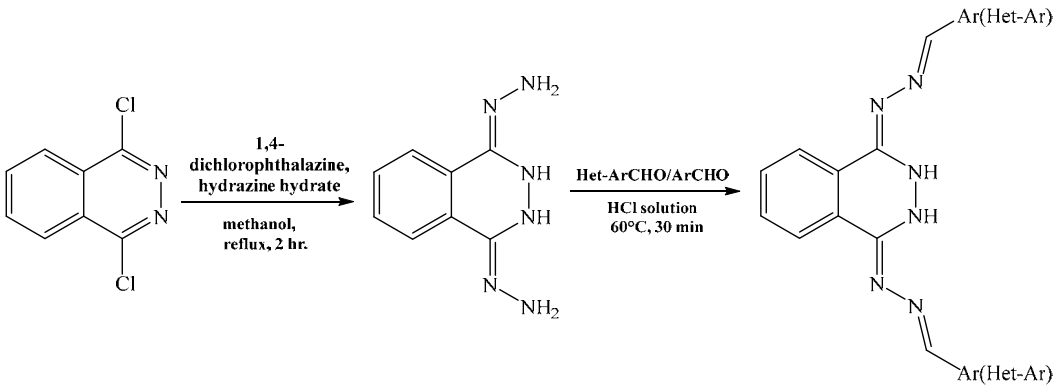


Figure 65: General procedure for synthesis of 1, 4-bis (substituted benzal hydrazino) phthalazines

Table 62: Antileishmanial activity and lethal concentration of the synthesized compounds

Compounds	**Ar**	**Antipromastigote IC_{50} (µM)**		**Antiamastigote LD_{50} (µM)**
		L. braziliensis	*L. mexicana*	
3a	C_6H_5	>50.0	>50.0	>50.0
3b	4-F- C_6H_5	2.37 ± 0.21	9.93 ± 0.87	4.71 ± 0.30
3f	2-OH-C_6H_5	7.90 ± 0.67	16.69 ± 1.32	8.95 ± 0.78
Glucantime	-	21.60 ± 1.78	-	32.0 ± 2.12

This series of di-substituted phthalazines contains highly conjugated nitro derivatives this results in a significant antileishmanial response. The most active compounds were the compound 3b and the compound 3f. the increased activity of these compounds are the evidence of the fact that if an extra aryl / heteroarylhydrazinyl is incorporated to the pthalazine core of the compounds of these series, there will be a significant improvement in the biological response of the compounds.

Table 63: CC_{50} and LD_{50} of the most potent compounds of the series

Compound	**Toxicity CC_{50} (µM)**	**Antiamastigote activity LD_{50} (µM)**			
	Macrophage	infected	reference	resistant	Clinical isolate
3b	30.00 ± 2.24	1.82±0.12	4.71±0.30	19.62±1.56	35.30±3.01
3f	42.51 ± 3.02	4.56±0.42	8.95±0.78	40.96±3.86	40.54±2.81
Glucantime	ND	ND	32.00±2.12	>50.0	>50.0

The most active compounds of the series were tested for toxicity. The results were compared with Glucantime. The compounds appeared to have a much better toxicity profile than the reference molecule. As they are active and also less harmful, they can be considered as excellent candidates to be used as an antileishmanial agent.

(+)-Phyllanthidine from Margaritaria nobilis: Leishmanicidal Activity and its Phytochemical Profile

(+)-Phyllanthidine and a group of other securinega alkaloids could be found in *Margarita nobilis*. In this report, the antileishmanial properties of the compound (+)-phyllanthidine was discussed. The alkaloids that were separated from *M. nobilis* contained a pyperidine or pyrrolidine ring, a 6- azabicycle[3.2.1] –octane and a α,β-butenolide which are considered as ring A, B, C and D respectively. The major isolated compound was (+) – Phyllathidine(Moraes et al., 2015).

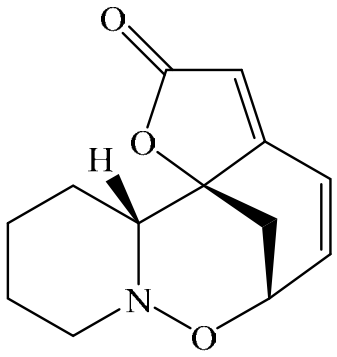


Figure 66: structure of (+)-Phyllanthidine

The antileishmanial activity of the compound was tested against *L. amazonesis*. The IC_{50} value for the compound was determined to be 82.37 µg/mL. Which indicates that it has the potential to be used against *Leishmania* parasites.

Table 64: Antileishmanial activity and cytotoxicity

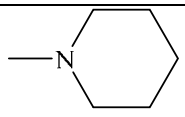
Factors Determined	Values
IC_{50}	82.37 µg/mL
CC_{50}	1727.48 µg/mL
CC_{50}	5268 µM

The cytotoxicity was tested by MTT assay. The assay indicated that the compound had no cytotoxic effect on the macrophages. The macrophages were obtained from the peritoneal cavity of the male mouse BALB/c. The results for the compounds were compared to Amphotericin B and the compound was proven to be a very potential candidate to be developed as an antileishmanial drug.

2, 4, 6-trisubstituted pyrimidines and 1, 3, 5-triazines: Synthesis and antileishmanial activity

In order to target the trypanosomal dihydrofolate reductase, pyrimidine compounds were developed in the beginning. In this study, the authors focused on the synthesis and the biological evaluation of a class of pyrimidine with a triazine moiety within to be used as a trypanosomal dihydrofolate reductase inhibitor(Sunduru, Palne, Chauhan, & Gupta, 2009)

Table 65: Molecular information and percentage of inhibition of 2, 4, 6-trisubstituted pyrimidines and 1, 3, 5-triazines

Compound	R_1	R_2	% Inhibition at 10 mg/ml Promastigote
13	$-OCH_3$		90.8
32	-	-	95.10
33	-	-	84.4

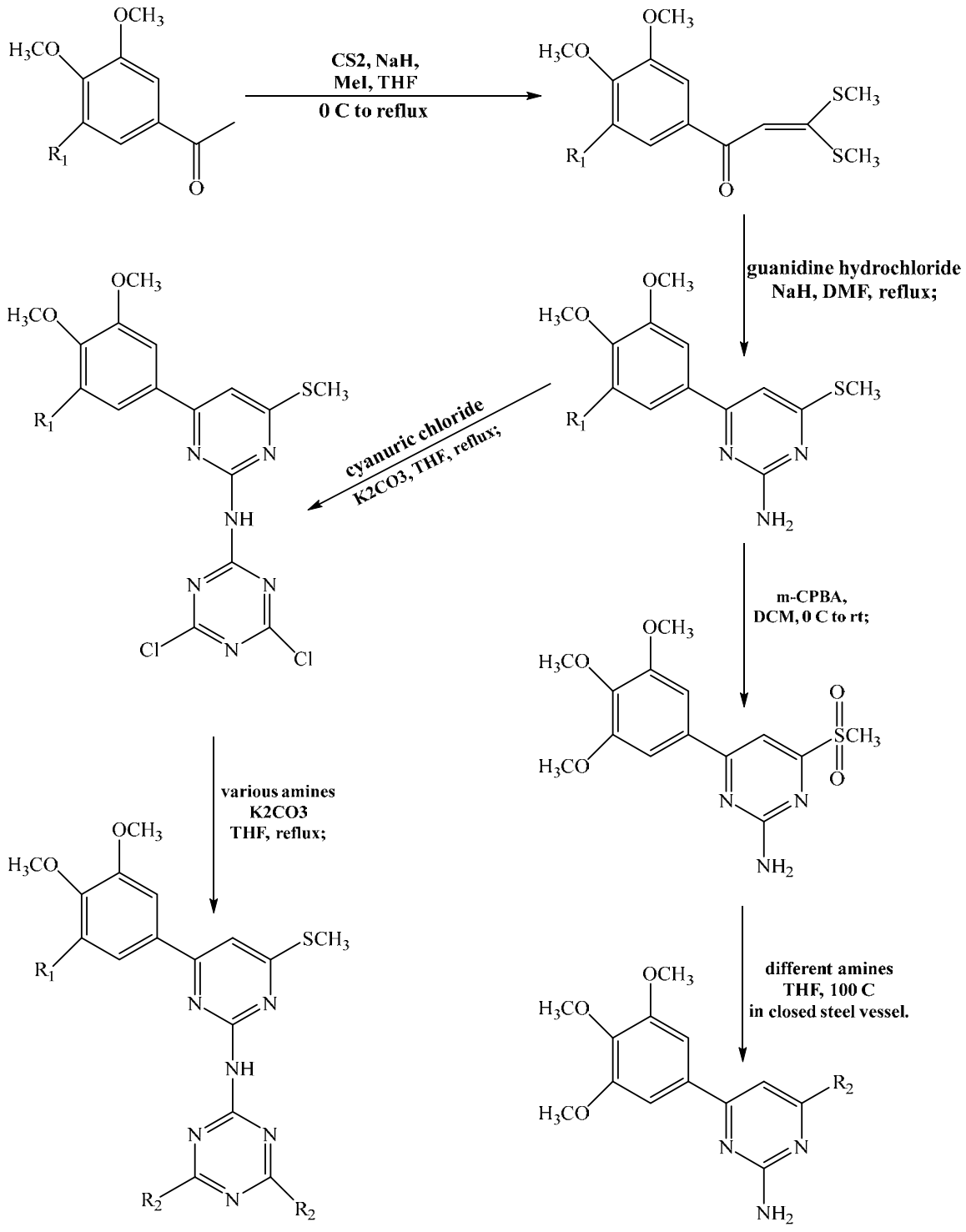


Figure 67: General procedure for synthesis of 2, 4, 6-trisubstituted pyrimidines and 1, 3, 5-triazines

The parasite *L. donovani* was used to evaluate the biological activity of the synthesized compounds. The golden hamster cell line was infected with the parasite for In Vivo testing.

Table 66: In Vitro and In Vivo antileishmanial activity

Compound	In Vitro antiamastigote activity IC_{50} (mg/ml)	Cytotoxicity against J774A.1 cell lines CC_{50} (mg/ml)	S.I. CC_{50}/IC_{50}	In vivo % inhibition 50 mg/kg 5 5 days in hamsters
13	1.80	51.67	28.70	56.58
32	5.12	81.52	15.92	48.46
33	4.99	49.93	10.00	54.10
Pentamidine	12.11	31.31	2.58	84.10 (20 mg/kg)
SSG	53.62	297	5.53	92 (40 mg/kg)

The activity of the synthesized compounds were compared with Pentamidine and SSG. Among all the synthesized compounds, the compound 13 appeared to be very active against the parasites of L. donovani. It had a 3, 4, 5 -trimethoxyphenyl group at the C 4 position of its pyrimidine ring. At the 4, 6 position of the piperidine ring 1, 3, 5-triazine. It showed great activity and was less toxic and with a CC_{50} value of 51.67 mg/ml. another active compound was the compound 32, consisting of a 3,4,5-trimethoxyphenyl group at position 4. Compound 33 contained a N,Ndiethylethylenediamine and butylamine at position 6. This is another active one amongst the synthesized compounds. Both of these compounds showed a lower toxicity and a higher selectivity index. After comparing these three compounds with Pentamidine and Sodium stibogluconate, it can be said that compound 13, 32 and 33 are potential candidates to be developed as an antileishmanial drug.

A putative antileishmanial drug candidate: Sitamaquine; its mechanism of action and drug resistance.

Although the molecular target of the drug was yet to be discovered, Sitamaquine underwent clinical trials this is because it has a short elimination half-life. For that there is a very less possibility for drug resistance to occur. The target was to find an antileishmanial for visceral leishmaniasis which can be given orally and has minimum possibility to cause drug resistance(Loiseau, Cojean, & Schrével, 2008).

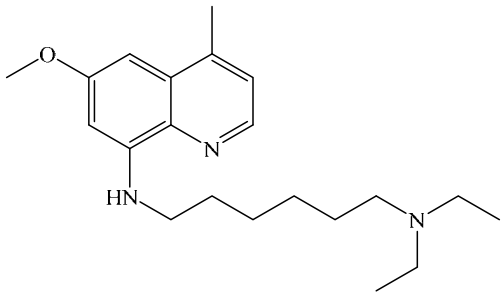


Figure 68: structure of an 8 aminoquinoline analog, Sitamaquine

Table 67: Different properties of Sitamaquine

Properties of Sitamaquine	
Chemical formulae	$C_{21}H_{33}N_3O.2HCl$
Solubility	Dihydrochloride: Water soluble (>100 mg/ml at 25°C) Ethanol soluble
Bioavailability	Plasma half-life: 26.1 hrs
Clinical trials phase II	Major urinary metabolite: 4-CH_2OH
Resistance	At risk
Toxicity	Headache Vomiting Abdominal pain Methemoglobinemia Renal adverse effects Glomerulonephritis

In Vitro, In Vivo activities and clinical trials of Sitamaquine:

Although the topical application of Sitamaquine hydrochloride could not cure the topical lesions cause by L. major, but in a lot of cases, it was proven to be the best option to treat Visceral Leishmaniasis. Such an example is when 8-[[6- (diethylamino) hexyl] amino]-6-methoxy-4-methylquinoline exhibited a high activity being seven hundred eight times more active than Meglumine Antimonate.The result of the very first clinical assay, done in Kenya showed results so good that it encouraged the future clinical trials. Later on in India, with 120 and in Kenya with 95 patients of Visceral Leishmaniasis phase II trial was done. Side effects such as cyanosis, abdominal pains and headaches were seen in both cases but in India cases of Methemoglobinemia occurred. Whereas, in Kenya, Renal adverse effect was observed. Because of the lack of activity on In Vivo models, clinical development for cutaneous leishmaniasis was not done.

Sitamaquine has been proven to have a shorter half-life several times. Therefore the possibility of accumulation in the host cells and causing toxicity becomes bare minimum. In order to evaluate Sitamaquine Resistance 160μM of the drug was administered In Vitro to murine peritoneal macrophages and the result was less ineffective for Balb/C mice. There was no sign of cross resistance. This ensures that even if Sitamaquine resistance does occur, alternative drugs can still be used.

An open label randomized clinical trial comparing the safety and effectiveness of one, two or three weekly PentamidineIsethionate doses (seven milligrams per kilogram) in the treatment of cutaneous leishmaniasis in the Amazon Region

The target for this study to be done was to ensure that the intramuscular administration of the drug in either single or two or three doses was safer and effective. It was a randomized clinical trial. That consisted on 159 patients in total. These patients were suffering from Cutaneous Leishmaniasis. They aged from 16 to 64 years old. The patients had minimum 1 to maximum of 6 lesions. The patients that were not include in the study were excluded based on their history of diabetes, any hepatic or renal diseases or even any cardiac diseases. The pregnant women were also excluded from this study(Gadelha et al., 2018).

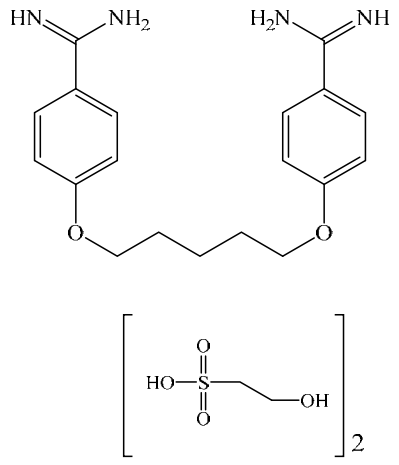


Figure 69: structure of Pentamidine(Bakunov et al., 2009)

The patients were separated in three groups: i) single IM dose of 7mg/kg body weight: 53 patients; ii) two IM doses in the interval of seven days 7mg/kg body weight: 53 patients; iii) three IM doses in the interval of 7 days 7mg/kg body weight. The lesions of the patients were photographed. Then the diameter of the largest lesion was measured. The patients were considered cured if their ulcers were completely cured by the end of the six months of the study. When there was a 50% reduction of the lesions, within the duration of 2 months it was considered as the secondary outcome. If there was a 50% increase of lesions or an appearance of a new lesion, it was considered as clinical failure. The parasites that are mostly responsible for causing leishmaniasis in 120 of the patients were identified as follows: *L. guyanensis, L. naifi* and *L. braziliensis.*

Table 68: Baseline characteristics of the patients included in the study

Genre	**One dose**	**Two dose**	**Three doses**
Female	17 (32, 1)	12 (22, 6)	8 (15, 1)
Male	36 (67, 9)	41, (77, 4)	45 (84, 9)
Age			
< 18	1 (1, 9)	1 (1, 9)	2 (3, 8)
18 |— 36	30 (56, 6)	21 (50, 9)	29 (54, 7)
36 |— 54	20 (37, 7)	17 (37, 7)	20 (37, 7)
> = 54	2 (3, 8)	4 (9, 4)	2 (3, 8)
No. of lesions (%)			
1	33 (62, 3)	26 (49, 1)	25 (47, 2)
3	8 (15, 1)	6 (11, 3)	8 (15, 1)
5	-	3 (5, 7)	2 (3, 8)

The results of the study made it clear that the use of Pentamidie Isethionate (PI) was effective and safe. But this result varied with the number of doses.

A randomized trial: combined therapy of Tamoxifen and meglumine antimoniate for the treatment of cutaneous leishmaniasis

The aim of this study was to evaluate the efficacy of Tamoxifen and Meglumine Antimonate when administered in a combination. This was a phase II clinical trial that occurred in the Bahia state of Brazil (Machado, Ribeiro, Franc, Carvalho, & Uliana, 2018).

The trial was done using 38 patients in total. They were all suffering from cutaneous leishmaniasis. The criteria for inclusion of the subjects were that they must have an untreated CL foe 1-3 months caused by *L. braziliensis.* The age range was minimum 18 up to 65.

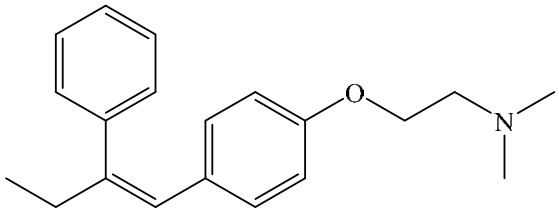


Figure 70: Structure of tamoxifen

The lesions must have a diameter of 1 to 5 cm. pregnant of breastfeeding women along with women wanting to avoid contraception were excluded. Patients with a history of pulmonary disease, cancer, tuberculosis and cardiac disease were also excluded.The subjects were separated in three groups. Among which, the first 15 were treated with standard Sb^{v}.Oral Tamoxifen in combination with Sb^{v} was given to the next group and the rest received topical Tamoxifen in combination with standard Sb^{v}. the lesion of these patients were measured across the ulcers in its lager diameters. Other hematological parameters and urea, creatinine levels were also measured.

Table 69: Therapeutic outcome 3 months – D90 – and 7 months – D210 – from the beginning of the treatment by treatment group (intention-to-treat analysis)

Factors observed	SbV (n = 15/ 39%)	SbV+TT (n = 11/29%)	SbV +T (n = 12/32%)	P-value
Cure rate at D90	8 (53%)	5 (45%)	8 (67%)	0.58
Cure rate at D210	6 (40%)	4 (36.4%)	7 (58%)	0.82
Relapse at D210	2 (25%)	1 (20%)	1 (12%)	0.81

In just a month, most of the patients ended up with only one lesion (74%, 28/38). But the overall results varied among the groups. Lymphadenopathy in association with the cutaneous lesion was observed in 93% of the patients treated with only Sb^{v}. and 64% and 33% were observed in patients treated with Tamoxifen topically and orally, respectively. Only 2 cases of relapses were identified after the whole 6 months period of treatment. The results indicated that the efficacy was higher in the group that was treated with oral Tamoxifen than the other

two.The adverse effects were very mild. Mostly there were complains about arthralgia and myalgia. But this is a common side effect that is associated with the administration of Sb^{v}. but there were also cases of like patient dropping out of treatment due to palpitation and headache in just two days. The results indicated that even if the drug Tamoxifen is used in much higher doses, and Sb^{v} at a lower dose, it will work.

An Open-label, Phase II Clinical Trial of Allometric Miltefosine: In the Treatment of Visceral Leishmaniasis in Eastern African Children; Pharmacokinetics, Safety, and Efficacy

Miltefosine is an alkylphosphocholine analogue. This study was conducted with the aim to come up with a safer and effective treatment for Visceral Leishmaniasis for the children living in the Eastern Africa. It was a phase II trial, which took place in two clinical sites in Eastern Africa; one in Kenya and in Uganda. 30 children between the ages of 4 to 12 years were used as subjects. They weighted below 30 kgs. These subjects did not receive any leishmaniasis therapy within 6 previous months. Another criteria for inclusion was that none of the subjects suffered from malnutrition, any other disease or any other severe infection. The subject underwent 28 days treatment.The problem with Miltefosine is that it has the tendency to accumulate in the body during treatment. Total drug plasma exposure and plasma maximum concentration were the two endpoints of the study (Mbui et al., 2019).

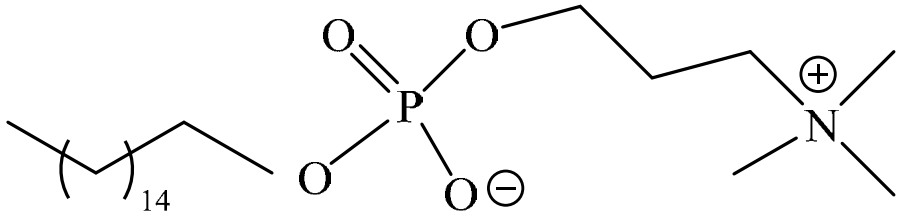


Figure 71: Structure of Miltefosine

Table 70: Pharmacokinetic parameters after conventional linear and allometric dosing in separate groups of Miltefosine-treated Eastern African pediatric patients with visceral leishmaniasis

Factors	Conventional dosing	Allometric dosing
Number of patients	21	27
Female %	24%	27%
Age median (range)	10 (7–12)	7 (4–12)
Height median (range)	1.35 (1.07–1.53)	1.25 (0.99–1.45)
Weight median (range)	24 (16–34)	22 (13–30)
Cmax<17.9µg/mLtarget,n(%) (mediun)	6/21 (28.6%)	4/27 (14.8%)
AUC0-210, µg*day/mL (mediun)	539	582

Total of 19 adverse effects were caused by the treatment during this time. They include blood and lymphatic system disorders, Infections and infestations, Nervous system disorders, Skin and subcutaneous tissue disorders, hypothermia, puncture site pain etc. Three of the patients experienced a treatment failure. They required Ambisome rescue treatment. The absence of clinical signs and symptoms of VL and the absence of parasite within the spleen or bone marrow after 28 days period was considered as cure of the disease. The 28 days cure rate was 96.7 % and the 210 days cure rate was 90 %.

This study indicated that Miltefosine is a very effective drug to be used in combination therapy against Visceral Leishmaniasis. Allometric dosing for patients weighting below 30 kg must be developed. A phase III trial must be envisaged in Eastern Africa.

Chapter 3

Conclusion

In our report we attempted on evaluating new antileishmanial compounds comprehensively that are currently being worked upon. Leishmaniasis is a disease that needs to be taken under control immediately. It is considered as a huge threat.The occurrence of the disease actually depends on the presence of the vectors and the susceptibility of the people living in that particular area. It targets people of every age. Theones with a weaker immune system are more at risk. At the same time, this horrific disease is much neglected. If we take a look at the statistics, in the Indian subcontinent alone, almost 2000000 to 4000000 cases come up each year. 90% of these cases are of Bangladesh, India, Brazil, Ethiopia, Sudan and South Sudan. What makes it more dangerous is that it is multisystemic.It is a parasitic disease and has very few treatment options. The existing antileishmanial drugs such as Pentamidin, Miltefosine are not very potent and there is a tendency of forming resistance against these drugs. Some of the very potent ones cause toxicity to the macrophages. Thus, newer more potent and safe dugs are needed to control the outbreak of leishmaniasis. In this review, we have studied new compounds and their derivatives that the researchers have found that might have the potential to be used as antileishmanial agents. We have discussed the synthetic procedure of the synthetic compounds and the sources of the natural ones. The In Vitro and In Vivo studies, bioavailability of the compounds were discussed along with it. Some of the compounds appeared to have potential to be used as an antileishmanial. Some appeared to be useless. We also mentioned which one of these compounds are most likely to be toxic for the host cells and at which concentration the toxicity of the compounds appear. We did the study with the hope that it pave the way for discovering newer, unique, and potent, less toxic and cost effective and safer drugs used against Leishmaniasis; that helps in eradicating this disease.

Future plan

Having done the review studies, we want to develop new molecules active against *Leishmania* parasites, which can be further developed as antileishmanial drugs. We would also want to study the In Vitro and In Vivo tests evaluating the activity and possibility of causing cytotoxicity of the potential antileishmanials. We look forward to discover more potential and safer antileishmanial drugs.

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