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**Antiprotozoal Quinolines Containing
Electrophilic Moieties**

By

Mlungiseleli Macdonald Ganto

**A thesis presented for the degree of
MASTER OF SCIENCE**

**in the subject
CHEMISTRY**

Supervisor

Associate Professor Kelly Chibale

Department of Chemistry

University of Cape Town

June 2004

EXPERIMENT IS THE INTERPRETER OF NATURE.
EXPERIMENTS NEVER DECEIVE. IT IS OUR JUDGEMENT
WHICH SOMETIMES DECEIVES ITSELF BECAUSE IT
EXPECTS RESULTS WHICH EXPERIMENT REFUSES.

LEONARDO DA VINCI

1452 - 1519

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DECLARATION

I declare that “**Antiprotozoal quinolines containing electrophilic moieties**” is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete reference.

Mlungiseleli Macdonald Ganto

University of Cape Town

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ABSTRACT

Compounds containing the quinoline moiety have been the mainstay of antimalarial chemotherapy. However, the emergence of resistant strains of *Plasmodium falciparum*, the causative agent of malaria, has compromised the efficacy of these antimalarial quinolines. Therefore the development of new efficient drugs is of critical importance. Extensive research has identified the cysteine proteases in malaria and other parasitic diseases as potential targets for new chemotherapy due to their critical roles in the life cycles of the causative agents.

Due to their role in the antimalarial activity of clinically available drugs, quinolines were used as scaffolds to which electrophilic groups, such as thiosemicarbazone, α,β -unsaturated ketone and pyrazoline moieties were appended. A series of quinoline 2-, 3-, 4- and 8-thiosemicarbazones were prepared by condensation of quinolinecarboxaldehydes or acetylquinolines with thiosemicarbazide. N1 NH₂-substituted thiosemicarbazones were also prepared by reaction of thiosemicarbazone thioesters with primary and secondary amines. The product yields ranged from low, moderate to excellent. Another series of compounds, the chalcones were prepared *via* the Claisen-Schmidt condensation of aromatic aldehydes and methyl ketones. These were further converted into pyrazoline derivatives by reaction with hydrazine hydrate. Pyrazoline derivatives were further reacted with substituted aromatic sulfonyl isocyanates to produce *N*-substituted pyrazole sulfonyl ureas. The product yields ranged from low, moderate to excellent. All compounds were characterized by spectroscopic and analytical techniques.

The thiosemicarbazones were then evaluated for inhibition of parasitic cysteine proteases falcipain-2 (from *Plasmodium falciparum*), cruzain (from *Trypanosoma cruzi*) and rhodesain (from *Trypanosoma brucei*) and also for *in vitro* activity against the chloroquine resistant strain (W2) of *P. falciparum*. Most of the compounds were not effective against falcipain-2, but some were weak inhibitors, with N'-quinolin-2-yl-ethylene-thiosemicarbazone (**59**) showing modest activity against the W2 strain. On the other hand, N'-quinolin-2-yl-methylene-1'-(3-dimethylamino-1-propylamine)-thiosemicarbazone (**49**) and N'-quinolin-3-yl-methylene-1'-(3-dimethylamino-1-propylamine)-thiosemicarbazone (**52**) were weak inhibitors of the growth of W2 strain, but had no potency against falcipain-2 at the tested concentration. Generally,

there was no correlation between the ability of the compound to inhibit falcipain-2 and antiplasmodial activity against W2 *in vitro*. This suggests that falcipain-2 is not the target of these compounds. Structure activity relationship (SAR) data against cruzain and rhodesain is interesting, because a number of compounds (thiosemicarbazones) that were not effective against falcipain-2 were potent in the low micromolar range. Compounds such as N'-quinolin-4-yl-methylene-thiosemicarbazone (**38**) and N'-(2-Chloro-8-methyl)-quinolin-3-yl-methylene-thiosemicarbazone (**48**) were the most effective against cruzain. Against rhodesain, more compounds were effective as compared to cruzain. Compounds such as **38**, N'-quinolin-2-yl-methylene-thiosemicarbazone (**42**), N'-(2-chloro-6-methoxy)-quinolin-3-yl-methylene-thiosemicarbazone (**44**), N'-(2-chloro-8-methyl)-quinolin-3-yl-methylene-thiosemicarbazone (**48**) and N'-quinolin-2-yl-ethylene-thiosemicarbazone (**59**) were the most effective against rhodesain. N1 NH₂-substitution was found to lower the potency of the thiosemicarbazones against both cruzain and rhodesain.

The chalcones and pyrazolines were evaluated against a chloroquine sensitive strain (D10) of *P. falciparum*. All these compounds lacked potency against the D10 strain at the chosen concentrations, and the biological data obtained was inconsistent to make any meaningful interpretation.

ABBREVIATIONS

ABC	Adenosine 5'triphosphate Binding Cassette
ADP	Adenosine 5'diphosphate
Anal.	Analytic
AQ	Aminoquinoline
Ar	Aromatic
ATP	Adenosine 5'triphosphate
ATPase	Adenosine 5'triphosphatase
α	Alpha
β	Beta
Br	Bromo
$^{\circ}\text{C}$	Degrees celcius
Calc	Calculated
Cd	Cadmium
CF_3	Trifluoromethane
CH_3	Methyl
$-\text{CH}_2\text{CH}_3$	Ethyl
Cl	Chloro
cm^{-1}	wavenumbers (in IR)
Cmpd	Compound
CQ	Chloroquine
Cu	Copper
δ	Chemical shift (in NMR)
d	doublet (in NMR)
dd	double of doublets (in NMR)
ddd	double double doublet or doublet of doublet of doublets (in NMR)
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
dppp	1,3-bis(diphenylphosphino)propane
EI	Electron impact

E64	L-trans-epoxysuccinyl-leucylamido (4-guanidino) butane
equiv.	equivalent
Et ₃ N	Triethylamine
EtOH	Ethanol
F	Fluoro
FAB	Fast atomic bombardment
FT	Fourier transform
g	grams
H	Hydrogen
His	Histidine
hr	hour
HRMS	High Resolution Mass Spectrometry
Hz	Hertz (in NMR)
I	Iodo
IC ₅₀	Inhibitory concentration to inhibit 50 % of enzyme activity or parasite growth
IR	Infrared
<i>J</i>	coupling constant (in NMR)
k	kilo
KBr	Potassium bromide
K ₂ CO ₃	Potassium carbonate
kDa	kilodaltons
Ln	n ligands
M	Moles per cubic decimeter (mol/dm ³)
m	multiplet (in NMR)
MDR	Multidrug resistance
MeMgBr	Methylmagnesium bromide
MeOH	Methanol
μ	Micro
MgSO ₄	Magnesium sulfate
MHz	Megahertz (in NMR)
mL	milliliter
mmol	millimole(s)
mp	Melting point

MS	Mass Spectrometry
MW	Molecular weight
m/z	mass to charge ratio (in mass spectra)
n	nano
Na	Sodium
Na ₂ CO ₃	Sodium carbonate
NaOH	Sodium hydroxide
NaOMe	Sodium methoxide
ND	Not determined
NH ₂	Amine
N ₂ H ₄ .H ₂ O	Hydrazine hydrate
NMR	Nuclear Magnetic Resonance
O	Oxygen
OAc	Acetate
OH	Hydroxyl
OMe	Methoxyl
<i>P.</i>	<i>Plasmodium</i>
Pd	Palladium
Pgh1	P-glycoprotein homolog 1
PPh ₃	Triphenylphosphine
ppm	parts per million (in NMR)
2-PrOH	Isopropanol
q	quartet (in NMR)
R _f	Retention factor (in chromatography)
RT	Room temperature
s	singlet (in NMR)
S	Sulfur
SAR	Structure-Activity Relationship
SCH ₃	Thiomethyl
SiO ₂	Silicon dioxide
S _N	Nucleophilic substitution
SO ₃ .Pyr	Sulfur trioxide pyridine complex
t	triplet (in NMR)
<i>T.</i>	<i>Trypanosoma</i>

THF	Tetrahydrofuran
tlc	thin layer chromatography
TMS	trimethylsilane (in NMR)
UV	Ultraviolet
Zn	Zinc

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CHAPTER 1

INTRODUCTION

1. MALARIA

1.1. Disease

Malaria is a protozoal disease transmitted by the *Anopheles* mosquito, caused by minute protozoa of the genus *Plasmodium*. It occurs in most tropical and subtropical regions. Malaria is a very old disease, which is reported to have originated from Africa. It then spread to other regions of the world such as the Mediterranean, India and South East Asia through human migration. A large number of fatalities and death rates due to malaria are reported in Africa.¹⁻³

It is estimated that malaria affects 300-400 million people worldwide, with close to 2 million deaths reported annually; most of which are children under the age of five years from Africa. Human malaria is caused by four species of the *Plasmodium* namely; *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium falciparum*. Of the four species of *Plasmodium*; *Plasmodium falciparum* is the most virulent and deadly malaria parasite and it is responsible for the most malaria cases that lead to death.^{1,2} The malaria parasite requires two hosts; the human and a female *Anopheles* mosquito in order to start its complex life cycle.²

1.2 *Plasmodium falciparum* life cycle

The *Plasmodium* complex life cycle begins within the female *Anopheles* mosquito, where it undergoes a development stage which requires a blood meal to mature its eggs. Therefore the *Plasmodium* life cycle really begins when the infected female mosquito bites a human host, withdrawing blood while injecting sporozoite-containing salivary glands.^{2,3} The sporozoites first circulate in the blood for a short period of time, before settling in the liver. In the liver they enter the liver cells (parenchymal cells) and multiply to form merozoites. This stage of the life cycle is called the pre-erythrocytic schizogony. After about 5 days the parenchymal cells rupture and release the merozoites into the red blood cells (erythrocytes). The red blood cells provide the parasite with a safe environment from the human host's immune system.^{2,3}

P. vivax and *P. ovale* have a continuing liver cycle, while on the other hand *P. falciparum* does not have a continuing liver cycle.³ In the red blood cells the parasites develop into two forms, the sexual and an asexual. The asexual form produces schizonts which contain merozoites. The merozoites cause the infected red blood cells to rupture. The merozoites are then released from the infected red blood cells and they invade new red blood cells. The merozoites develop through the so-called ring, trophozoite and schizont stages.²⁻⁴ On the other hand, the sexual form produces the male and female gametocytes in the blood stream, and these are ingested by the female mosquito when it feeds from the infected human host.²⁻⁴ The male and female gametocytes fuse in the mosquito's gut and pass through the gut wall where they develop into oocysts. The oocysts develop over a period of days and release sporozoites that migrate to the salivary glands which are passed onto another human host when the mosquito takes another meal.^{2,3} This is illustrated in figure 1.

In *P. vivax*, *P. ovale* and probably *P. malariae*, all stages of development subsequent to the liver cycle can be observed in the peripheral blood. In the case of *P. falciparum* only ring forms and gametocytes are observed in the peripheral blood. The developing forms appear to stick in the blood vessels of the large organs such as the brain and restrict blood flow with serious consequences. *P. falciparum* invades red blood cells of all ages whereas *P. vivax* and *P. ovale* prefer younger red cells. *P. malariae* on the other hand prefer matured red cells.³

PLASMODIUM LIFE CYCLE

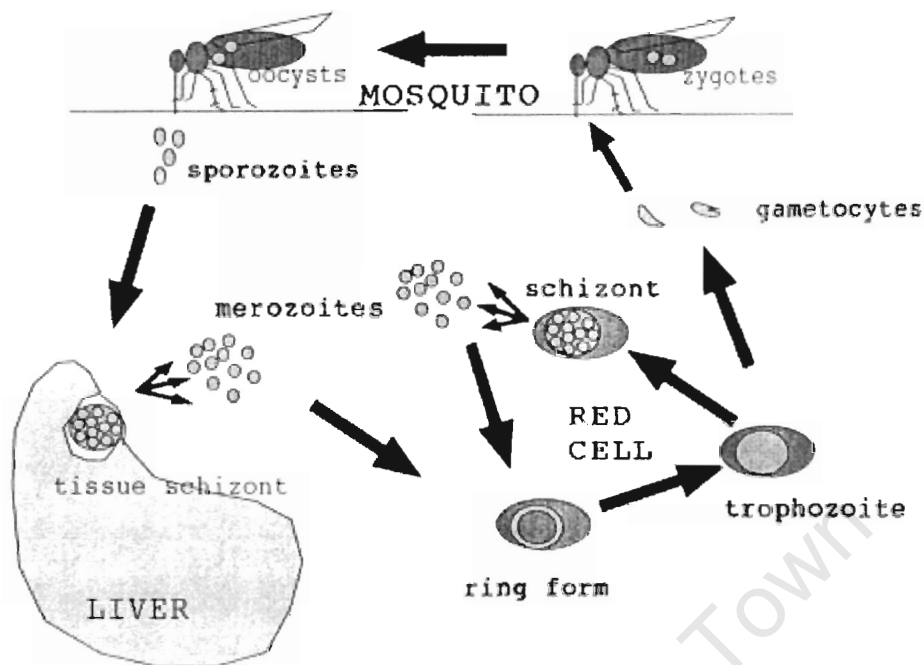


Figure 1. Life Cycle of *Plasmodium falciparum*⁸

Parasite growth in the red blood cells is supported by the ingestion of host hemoglobin.⁵ Hemoglobin comprises 95 % of the cytoplasmic protein of the red blood cells, and the malaria parasites degrade 60-80 % of the hemoglobin for nutrition during the intraerythrocytic cycle.⁶ In *P. falciparum* hemoglobin degradation occurs predominantly in trophozoites and early schizonts, stages where the parasites are highly metabolically active. Hemoglobin degradation is an ordered, complex process. Hemoglobin is taken by trophozoites into the parasite's acidic food vacuole, analogous to lysosomes.^{5,7} In the food vacuole it becomes a substrate for numerous proteolytic enzymes, which break it down into heme, a major component of the malaria pigment (hemozoin), and globin which is hydrolyzed into its amino acids for the parasite's nutrition.^{5,7,8} The degradation pathway is illustrated in figure 2 below.

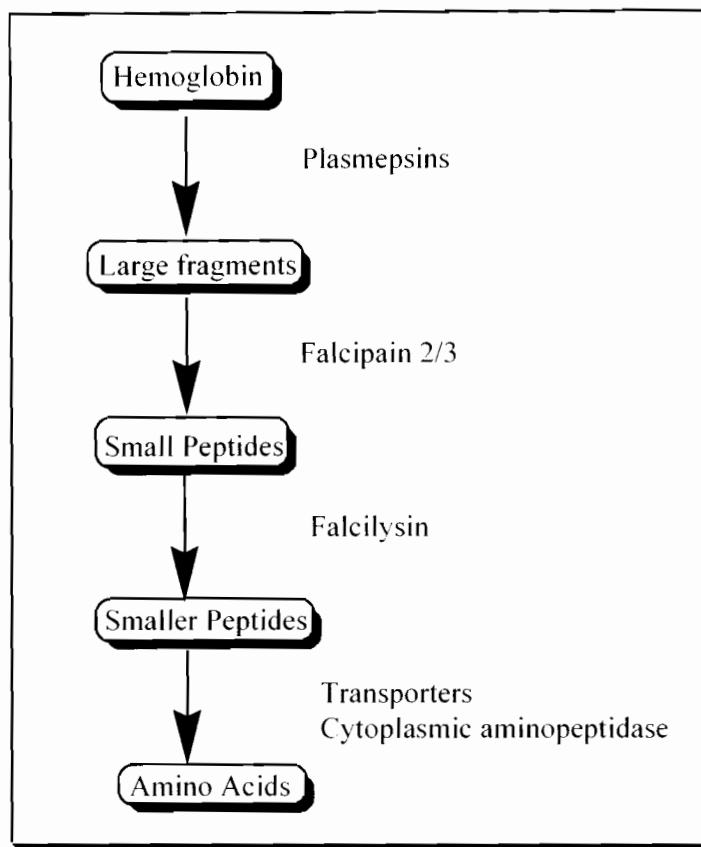


Figure 2: Schematic representation of degradation of hemoglobin for the parasite's nutrition

The amino acids produced from globin are incorporated into parasite proteins. On the other hand the four equivalents of reduced heme, ferriprotoporphyrin IX [Fe(II)PPIX] are produced with oxygen. Ferriprotoporphyrin contains iron, a metal that can be oxidized to a ferric (Fe^{3+}) state or reduced to a ferrous (Fe^{2+}) state. It is the reduced heme that can transport oxygen. The molecular oxygen accepts an electron from the reduced heme, Fe(II)PPIX to initiate a chain of oxygen radical metabolism. The Fe(II)PPIX is then oxidized to Fe(III)PPIX (hematin), and this is toxic to the microorganisms of the parasite in its free form. Therefore the parasite detoxifies the Fe(III)PPIX by forming a malaria pigment, also known as hemozoin. Hemozoin appears to contain dimerized heme. It is the hemozoin formation that is the target of chloroquine and related 4-aminoquinolines.⁸⁻¹¹

1.3 The *Plasmodium* food vacuole

The *Plasmodium* food vacuole is a special organelle optimized for hemoglobin degradation. It is acidic; hence it is a site for a number of processes such as acidification, hemoglobin proteolysis, peptide transport, heme dimerization, detoxification of oxygen radicals and quinoline action. A number of proteins in the food vacuole are known, and these include aspartic, cysteine and metalloproteases, an ATP-binding cassette (ABC) transporter, an ATPase, a heme polymerase, and several oxidant enzymes.¹²

There are four distinct proteases in the food vacuole, which act in an ordered fashion in hemoglobin degradation as depicted in figure 2. Two aspartic proteases, plasmepsins I and II appear to initiate the degradation process. A cysteine protease, falcipain-2, plays a vital downstream role after the initiation by plasmepsins. A metalloprotease, falcilysin, functions further downstream to the above-mentioned proteases.¹² Plasmepsins have a pH optima near 5, and this is consistent with their environment in the acidic food vacuole.^{13,14} While it is only aspartic protease inhibitors that effectively block the initial steps of hemoglobin degradation, a cysteine protease is also implicated in the initial stages of hemoglobin degradation. This implication is due to the observation of swollen food vacuoles filled with undegraded globin when the parasites are treated with cysteine protease inhibitors.¹⁵⁻¹⁸ It is possible that there could be an accumulation of erythrocyte glutathione in the food vacuole and a highly reducing environment that would rapidly denature hemoglobin, thus causing the falcipain-2 to function early in the process.^{19,20}

Falcilysin, a zinc metalloprotease, is determined to function downstream of the plasmepsins and falcipain-2 in the hemoglobin degradation pathway. Falcilysin does not cleave hemoglobin or globin, but it degrades fragments of hemoglobin that are generated by the plasmepsin II.²¹ The acidic conditions of the *Plasmodium* food vacuole are most likely maintained by an ATP-driven proton pump, homologous to the lysosomes.²² ATPase activity has been found to be enriched in the food vacuole membranes.^{23,24} Several genes homologous to the ABC transporter family have been found in the *P. falciparum*.^{25,26} One of these genes, *mdr1*, encodes a 160kDa protein called P-glycoprotein homolog 1 (Pgh 1) which

is in the food vacuole membranes. Pgh 1 looks like a typical ABC transporter, with twelve transmembrane domains and two hydrophobic domains with nucleotide binding capabilities. Pgh 1 has been shown to be capable of binding ATP, ADP, and GDP.²⁷ Pgh 1 is presumed to mediate transport across the food vacuole, because similar mammalian ABC transporters can act as energy-dependent transporters that efflux drugs in multidrug resistance (MDR) tumor cells.²⁸

The *Plasmodium* food vacuole is much like the nuclear reactor. It generates food for the parasite by means of amino acids from the globin, yet on the other hand it produces toxic products such as heme which is toxic to the parasite. Thus a valid approach for the development of antimalarial chemotherapy, is the inhibition of the hemoglobin degradation within the food vacuole.¹² Therefore for the design and development of inhibitors, the acidic environment of the food vacuole must be considered for the accumulation of inhibitors inside the food vacuole.

1.4 Chemotherapy

Antimalarial drugs have been used for centuries. The first antimalarial drugs were alkaloids extracted from the bark of the Cinchona tree.^{2,3} Quinine **1** is one of the drugs extracted from the Cinchona tree, and it has been in use for more than three centuries. It was the only effective drug for the treatment of malaria. It is the only drug that has remained largely effective for treating malaria for over a long period of time. Lately, quinine is only used for the treatment of severe *falciparum* malaria, partly due to undesirable side effects.³

In the 1920s methylene blue **2** (figure 3) was shown to have some antimalarial activity and attempts at the design and preparation of synthetic antimalarial drugs were initiated. Analogs of methylene blue were then prepared by replacing one methyl group with a basic side chain, and this enhanced the activity against malaria. It was then believed that the basic side chain was critical for antimalarial activity, and this led to the synthesis of plasmoquine, which was later called pamaquine **3**. Pamaquine was first used in 1926 and it was capable of radical cure. However, later it was found to be toxic, and a less toxic analog primaquine **4** was synthesized and developed. Primaquine, which is an 8-aminoquinoline, is still

used to eradicate the refractory hypnozoites (liver reservoirs) of *P. vivax* and *P. ovale*.²

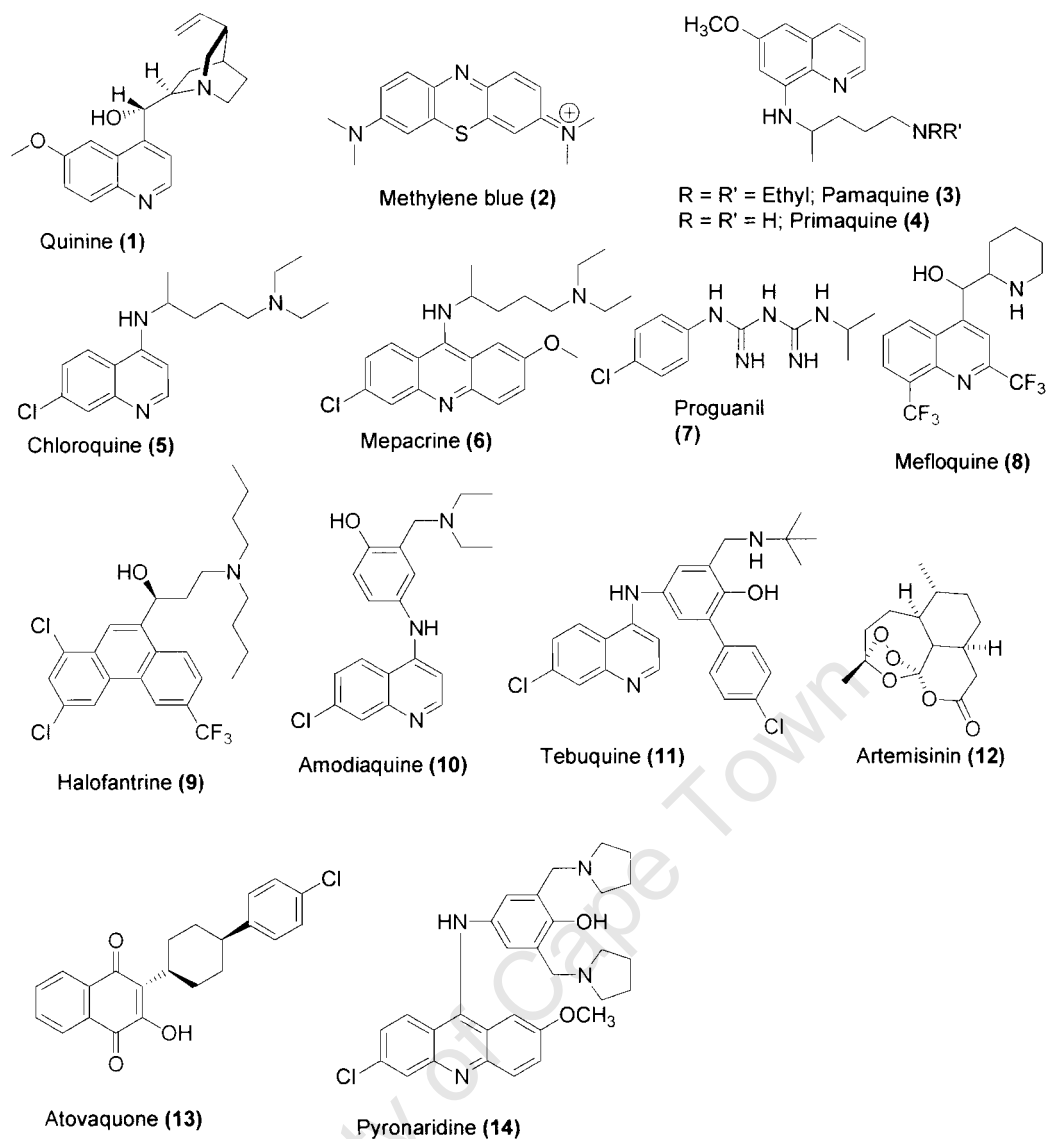


Figure 3: Structures of some antimalarial drugs

Following the discovery of quinine, extensive efforts including the screening of thousands of compounds have led to the development of a number of effective synthetic antimalarial drugs. The most important of these drugs is chloroquine 5, a 4-aminoquinoline that is effective for both treatment and prophylaxis. Chloroquine was first used in the 1940s shortly after the Second World War. It was effective in curing all forms of malaria, such that it was thought that malaria can be

eradicated. It has advantages such as minimal toxicity to the human host, and it is widely available at low cost throughout the world.^{2,3} Unfortunately nowadays, most strains of *falciparum* malaria are resistant to chloroquine, and more recently strains of resistant *vivax* malaria have been reported. Malaria resistance to chloroquine has rendered its use for presumptive treatment of *falciparum* malaria and chemoprophylaxis inappropriate. No other antimalarial is as safe and effective as chloroquine. Quinine remains the best antimalarial drug for the treatment of chloroquine-resistant *falciparum* malaria.²

In the 1930s another antimalarial drug mepacrine **6**, a 9-amino-acridine derivative was developed. It was used as a prophylactic on a large scale during the Second World War, and then it was considered a safe drug. However, now it is considered to have too many undesirable side effects, such that its use has been stopped. In 1946, proguanil **7**, a drug which belongs to the biguanide class of compounds was synthesized. It has a biguanide chain attached at one end to the chlorophenyl ring. Proguanil is a folate antagonist and it destroys the malarial parasite by binding to the enzyme dihydrofolate reductase. In some countries it is used as a prophylactic.³

To address the widespread chloroquine-resistant strains of malaria, a few more antimalarial agents were developed and synthesized. Among these antimalarial agents, mefloquine **8**, a quinoline methanol derivative that is structurally related to quinine was introduced in 1971. It was effective against malaria, and because of its long half life it was an effective prophylactic. However, widespread resistance, together with undesirable side effects has limited its use. In addition it cannot be used with quinine because of the structural relationship.^{2,3}

In the 1940s, halofantrine **9** was identified, but it was only introduced in the 1980s. It belongs to the class of compounds called the phenanthrene-methanols and it is not related to quinine.³ It has been used for the treatment of chloroquine-resistant strains of malaria, but due to its short half life it is not a good prophylactic. Its use is now restricted, due to reports of serious cardiotoxicity and variable oral absorption.^{2,3}

Efforts to address the burden of chloroquine-resistant malaria were continued, and another 4-aminoquinoline, amodiaquine **10** was synthesized. It is a Mannich base of chloroquine together with tebuquine **11**. These were introduced as alternatives to chloroquine and have been used as prophylactics for *falciparum* malaria for over 40 years. One of the most promising developments in malaria chemotherapy was the discovery of artemisinin **12**. It was isolated from the *Artemisia annua*, a plant that is widely used in China to treat fever.³

Atovaquone **13** which is approved for the treatment of patients with Pneumocystis infections appeared to be effective against malaria when combined with proguanil. However, its use has been limited by recrudescence after treatment. Pyronaridine **14**, an acridine derivative used to treat malaria in China, has shown efficacy against *falciparum* malaria.²⁹⁻³¹ The structures of all these antimalarial drugs are shown in figure 3.

1.5 Mode of action of chloroquine

Despite its long period in use, the mechanism of action of chloroquine is still a subject of intense debate, thus it is still far from being resolved. However, the commonly accepted hypothesis is that chloroquine and related quinoline-containing antimalarial drugs act primarily on heme disposal, a process in which intraerythrocytic-stage malaria parasites detoxify heme in the food vacuole.³² Chloroquine is proposed to interfere with hemozoin/ malaria pigment formation, by complexing with free heme; thereby creating toxic complexes that result in parasite death.^{2,33} Studies on the crystal structure of β -hematin, a synthetic equivalent of hemozoin suggest that chloroquine is chemisorbed onto the actively growing crystal faces thereby inhibiting hematin sequestration and contributing to the toxic levels of chloroquine-hematin in the cell.^{8,34}

Numerous theories about the mode of action of chloroquine have emerged, but they appear to agree in that chloroquine selectively affects the stages of the parasite life cycle after hemoglobin is digested. They also suggest that chloroquine selectively accumulates in the food vacuole. Chloroquine is a weak base ($pK_{a1} = 8.1$; $pK_{a2} = 10.2$), and this would explain its selective accumulation in the food vacuole. At neutral pH, outside the food vacuole; chloroquine is uncharged and

can diffuse freely through the membranes. Inside the acidic food vacuole, chloroquine becomes doubly-protonated, membrane-impermeable, and thus is trapped inside the food vacuole.^{32,35}

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CHAPTER 2

CYSTEINE PROTEASES AND THEIR INHIBITORS

2.1 Parasitic cysteine proteases

Proteases, also termed peptide hydrolases, catalyze the cleavage of amide linkages in macromolecular proteins and oligomeric peptides. They have been identified in biological systems, from viruses to vertebrates. It is estimated that it could be very difficult to hydrolyze a peptide bond without proteases as biological catalysts, and hydrolysis could take centuries, whereas a protease can degrade as many as one million peptide linkages per second.³⁶ Proteases range from monomers of 10 kDa to multimeric complexes of several hundred kDa. They can initiate their catalytic activity either within a polypeptide chain (endoprotease activity), or from the amino or carboxyl ends (exopeptidase activity).^{36,37}

The proteases have been divided into groups based on the catalytic mechanism used during the hydrolysis of peptide bonds. The main catalytic types are serine, threonine, aspartate, metallo and cysteine proteases.^{36,37} Cysteine proteases of parasitic organisms are divided into two main groups referred to as clans, CA and CD.^{36,38} In 1879, the first cysteine protease was purified and characterized from *Carica papaya*, the papaya fruit, and was thus named papain. Since its discovery, numerous proteases that have sequences similar to papain have been called papain-like. Clan CA, or papain-like proteases are further divided into families. Important parasite proteases are located to family C1 (cathepsin B and L-like), and family C2 (calpain-like). Other clans and families of pathogenic organisms include clan CB and clan CC (viral proteases) and clan CD (family C13; legumain-like).³⁶

The majority of parasite cysteine proteases belong to the family C1 within clan CA. Members of the CA clan are either targeted to intracellular vesicle compartments (these include cathepsins B, C, H, K, L and S), or are secreted and thus possess a leader peptide. Clan CA proteases are also characterized by their sensitivity to the general cysteine protease inhibitor E64 (figure 4) and having substrate specificity.³⁶

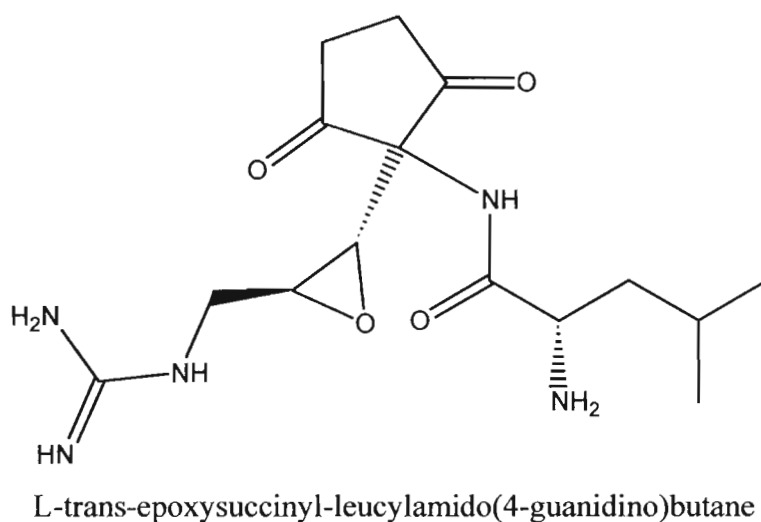


Figure 4: Structure of a general cysteine protease inhibitor, E64

Proteases bind peptide substrates in their active site cleft through backbone and side chain interactions that lie around several defined pockets in the enzyme termed S1, S2, S3, S4 for those pockets that bind to the corresponding amino acids that are N-terminal to the scissile amide bond while the S1', S2', S3', S4' pockets bind to the amino acids C-terminal to the scissile bond. The corresponding side chains in the peptide substrate are termed P1, P2, P3, P4 and P1', P2', P3', P4'.^{36,39} This is illustrated in figures 5A and 5B.

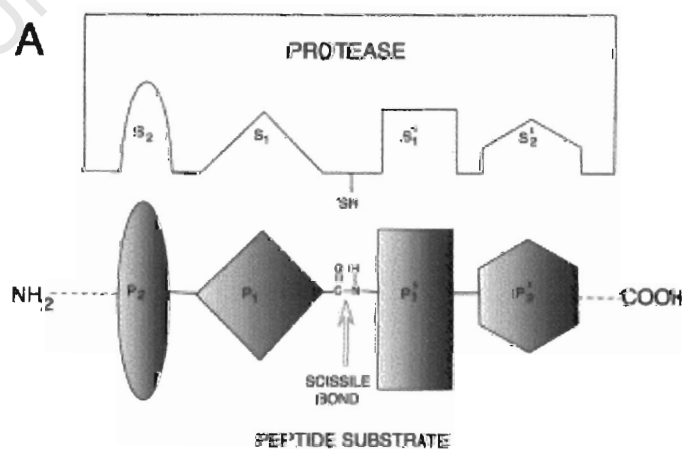


Figure 5A: Schematic presentation of the peptide substrate interaction with the active site pockets of the cysteine protease. Amino acids from the peptide are denoted by “P” and the subsites within the cysteine protease are denoted by “S”.³⁶

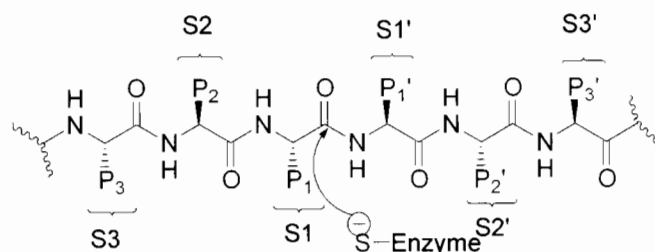


Figure 5B: Standard nomenclature for protease substrate cleavage. P_n, P₃, P₂, P₁, P₁', P₂', P₃', P_n', designate the amino acid side chains of a peptide substrate. Cleavage occurs between the P₁ and P₁' residues. The corresponding binding sites in the protease active-site are designated as S_n, S₃, S₂, S₁, S₁', S₂', S₃', S_n'.³⁹

The chemical nature of the protease S₂ pocket, and in particular the amino acid residue 205 (papain numbering), is thought to be critical for substrate preference. Cathepsin B activity can be differentiated from the cathepsin L by hydrolysis of small peptides that contain arginine at P₂ by cathepsin B. The cathepsin B and L subfamily can be further delineated by the length and sequence similarity within the respective pro-regions as well as the number and order of cysteine residues involved in disulfide bond formation.³⁶

Cysteine proteases are recognized to be critical to the life cycle or pathogenicity of many parasites, such that they are promising targets for antiparasitic chemotherapy. They play important roles in immunoevasion, enzyme activation, virulence, tissue and cellular invasion, as well as excystment, hatching and moulting. Cysteine proteases are mechanistically similar to serine proteases, but they are better nucleophiles, due to the extra shell of electrons present in the sulfur of the thiol group. The nucleophilicity of the thiol group is enhanced by the close proximity of an active site histidine which acts as a proton donor/general base.^{36,37}

Cysteine proteases are functionally diverse due to their unique nucleophilicity, adaptability to different substrates and stability in different biological environments. Parasitic cysteine proteases function in a broader chemical environment when compared to their homologous host enzymes. Mammalian lysosomal cysteine proteases are relatively unstable at neutral pH when compared to the parasite cysteine protease. The instability of mammalian cathepsins may be a regulation mechanism to minimize unwanted proteolysis in inappropriate

cellular or tissue compartments. The lysosomal pH has been estimated to be as low as pH 4.0, and low pH can activate lysosomal enzymes as well as denature protein substrates. On the other hand parasite cathepsins are stable and active at low pH, in addition they remain stable and even more active at neutral pH.³⁶

A peptide substrate or protein must bind to the protease's active site before the hydrolysis of the peptide bonds. The binding efficiency is a function of both the respective chemical environments that the protease subsites create, and the chemical nature of the peptide that interacts directly with the active site groove. Important factors affecting the interactions include; size, polarity, charge, hydrophobicity and acceptability. Although a single peptide bond is cleaved during catalysis, a number of amino acids on either side of the site of cleavage are critical to fix the catalytic register and specificity of an enzyme (figure 5A). Sequences that directly flank the active site cysteine and histidine residues are also highly conserved to maintain the catalytic register.³⁶

The sulfhydryl or -SH group of the cysteine side chain and the imidazole of histidine give rise to a thiolate-imidazolium charge relay diad (figure 5C). This is frequently but not always, stabilized by a highly conserved asparagine. A highly conserved glutamine forms the oxyanion, a crucial element in forming an electrophilic centre to stabilize the tetrahedral intermediate during hydrolysis. The two ionisable groups of the thiolate-imidazolium diad allow a broad pH range of enzymatic activity, consistent with a pKa of cysteine ~ 4.0 and pKa of histidine ionization ~ 8.5. The charge relay diad is stabilized by the chemical environment of the active site. It is thought that the main interactions of papain-like cysteine proteases with their substrate occur at S1, S2 and S1' (figure 5A).³⁶

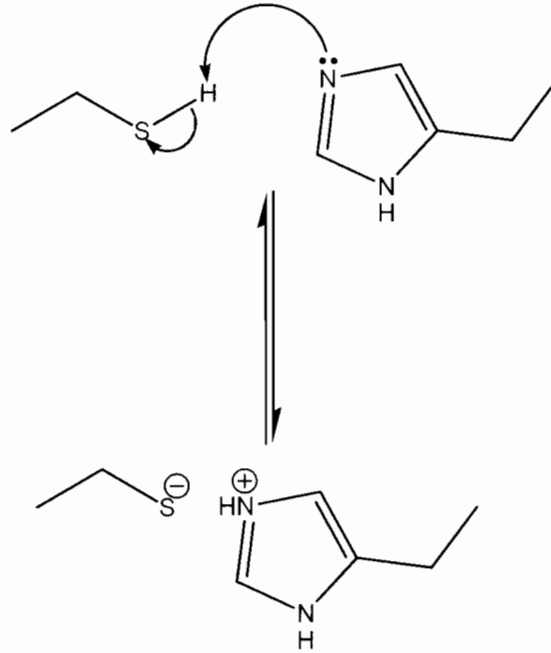
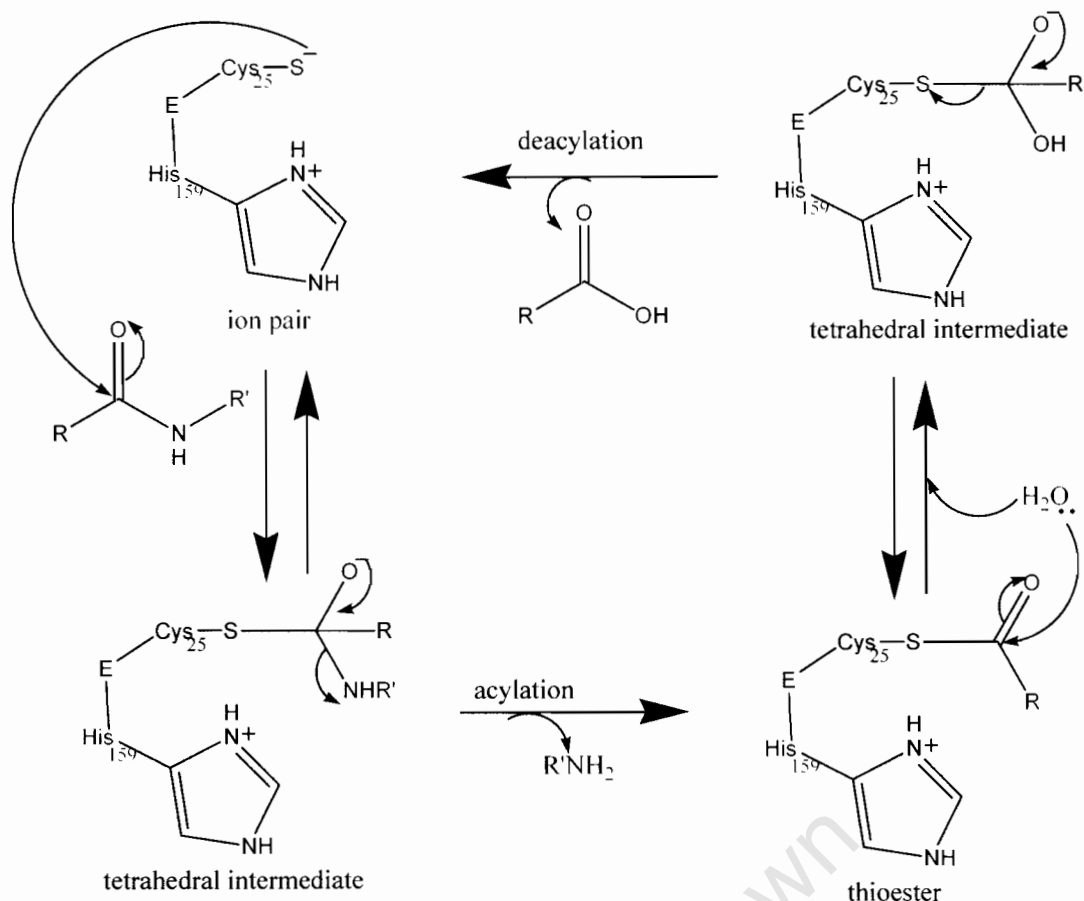


Figure 5C: Schematic representation of the active site showing: proximity of sulfhydryl of cysteine to the imidazole group of histidine, and the equilibrium with the thiolate-imidazolium charge relay diad.³⁶

The mechanism of hydrolysis of the peptide bonds has been elucidated and documented (scheme 1). During peptide hydrolysis, the nucleophilic thiolate cysteine attacks the carbonyl carbon of the scissile amide bond of the bound substrate, and forms a tetrahedral intermediate which is stabilized by the so called oxyanion. The tetrahedral intermediate transforms into an acyl enzyme (enzyme substrate thiol ester) with the simultaneous release of the C-terminal portion of the substrate (acylation). This step is followed by the hydrolysis of the acyl enzyme with water, forming a second tetrahedral intermediate which finally splits into the free enzyme and the N-terminal portion of the substrate (deacylation).³⁷



Scheme 1: Mechanism of substrate hydrolysis by cysteine proteases.³⁷

2.2. Malarial and Trypanosomal cysteine proteases

It is now clear that the malaria parasite has three cysteine proteases, falcipain-1, falcipain-2 and falcipain-3. Falcipain-1 is not the most important contributor to hemoglobin degradation. Current evidence suggests that falcipain-1 is involved in invasion of the host cell by the malaria parasite.⁴ The malarial cysteine proteases that play a critical role in hemoglobin degradation are falcipain-2 and falcipain-3.³⁶ Falcipain-2 and -3 are predominantly expressed in the merozoite acidic vacuole where hemoglobin hydrolysis occurs.³⁸⁻⁴⁰ Falcipain inhibitors that inhibit hemoglobin degradation also block parasite development. Thus falcipain is considered a promising target for antimalarial chemotherapy. In actual fact, drug development should consider the inhibition of falcipain-2 and falcipain-3 in order

to effectively inhibit parasite hemoglobin hydrolysis, and thus block parasite growth.⁴¹

Trypanosoma cruzi, the causative agent of American trypanosomiasis (Chagas' disease) is transmitted to humans either by triatomine vectors or infected blood products. It is found as an intracellular form, the amastigote, and as a trypomastigote form in human blood. Chagas' disease affects primarily the heart and the nervous system. Presently available chemotherapy (nifurtimox, benznidazole) has limited potency and severe side effects for the treatment of chronic forms of the disease. Efforts to characterize novel chemotherapeutic targets in *T. cruzi* identified a papain-like cysteine protease, called cruzain. Cruzain is found in all stages of the life cycle of *T. cruzi*. Inhibition of cruzain has been shown to impair *in vitro* cell invasion and to block amastigote replication as well as trypomastigote-amastigote differentiation, thereby arresting intracellular development.³⁷

T. brucei rhodesiense is one of the causative agents of human African trypanosomiasis (sleeping sickness), which is transmitted by the bite of the tsetse fly. Infections with *T. brucei rhodesiense* and other trypanosomes result in severe clinical consequences if left untreated. Clinical manifestations of African trypanosomiasis are intermittent fever, headaches, maniacal behavior, transient edema, and excessive sleepiness. Rhodesain is the major cysteine protease of *T. brucei rhodesiense*, and has been identified in all life-cycle stages of the parasite, especially during infection and parasite development. Inhibition of rhodesain by synthetic inhibitors has been shown to arrest *T. brucei rhodesiense*. This has further shown that rhodesain is important for the life cycle of the parasite.³⁷

2.3 Inhibition of cysteine proteases

As alluded to earlier, cysteine proteases catalyze the hydrolysis of amide bonds in peptides and proteins through nucleophilic attack by the active site cysteine thiol on the amide carbonyl. This mechanism has been exploited by many cysteine protease inhibitors, which have an electrophilic moiety, such as a carbonyl or Michael acceptor moieties that react with the active-site cysteine residue.⁴² The advantages of targeting the cysteine proteases include the fact that they are found

in almost all protozoan parasites, and also possess a highly reactive thiol group which is a target of the electrophilic moieties of the inhibitors developed.^{43,44}

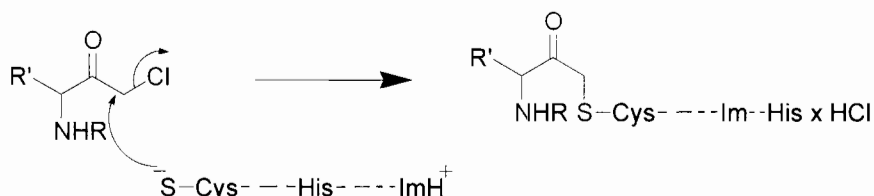
Previously, it was not clear as to whether cysteine protease inhibitors could arrest parasitic infections without toxicity to the human host due to the fact that cathepsin B and L are major lysosomal proteases found in almost every cell in humans and domestic animals. Also, cathepsin S is another enzyme that is thought to play a role in peptide hydrolysis. Some inhibitors of cysteine proteases such as vinyl sulfones, are very effective inhibitors of parasitic cysteine proteases, and there was an initial concern about their toxicity towards the homologous host cathepsins B, L and S. However, to date no toxicity has been reported against the homologous host enzymes.⁴⁵ There are possible explanations as to why these inhibitors are not toxic to the host enzymes. Firstly, the concentration of proteases like cathepsins B, L and S in mammalian cells may be considerably higher than in parasites. Secondly, mammalian cells have a much larger array of cysteine proteases, as well as other classes of proteases which may provide a level of redundancy not found in parasitic systems.^{44,45} In any case, there are no autoimmune phenomena or laboratory and histologic abnormalities reported with some of the tested inhibitors. In addition, safety studies show little toxicity against the homologous host enzymes.⁴⁵ Studies have thus proved that cysteine protease inhibitors can selectively arrest parasite infections without undue toxicity to the host.⁴⁴

2.3.1 Classification of cysteine protease inhibitors

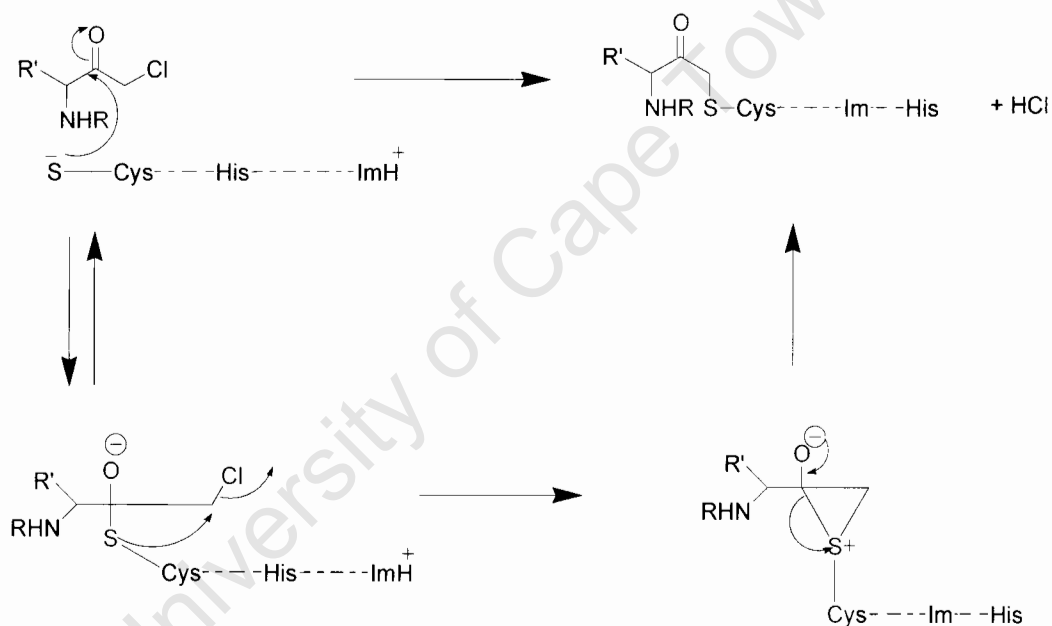
A variety of cysteine protease inhibitors designed and synthesized thus far are divided into three mechanistically distinct groups, which are;

- (i) A series of active site titrants, whose mechanism of inhibition is based upon irreversible alkylation of the thiol moiety of the active site cysteine residue. The first specific irreversible inhibitors for proteases (serine, cysteine and threonine) were designed by attaching a reactive warhead to a good substrate structure. The early warheads used were alkylating agents such as diazo compounds or haloketones. Examples of irreversible inhibitors include peptidyl chloromethyl ketones⁴⁶⁻⁴⁸. They alkylate the

active site cysteine residue to form an irreversible thioether adduct. Their mechanism of inhibition is not fully resolved. However, it is generally agreed that there are two possible mechanisms of inhibition, one mechanism involves direct displacement of the halide group of the inhibitor by the thiolate anion of the cysteine residue to form a thioether adduct (scheme 2a). While the other mechanism involves a thiohemiketal and a three-membered sulfonium intermediate. The intermediate then rearranges to give the final thioether adduct (scheme 2b).⁴⁷⁻⁴⁸

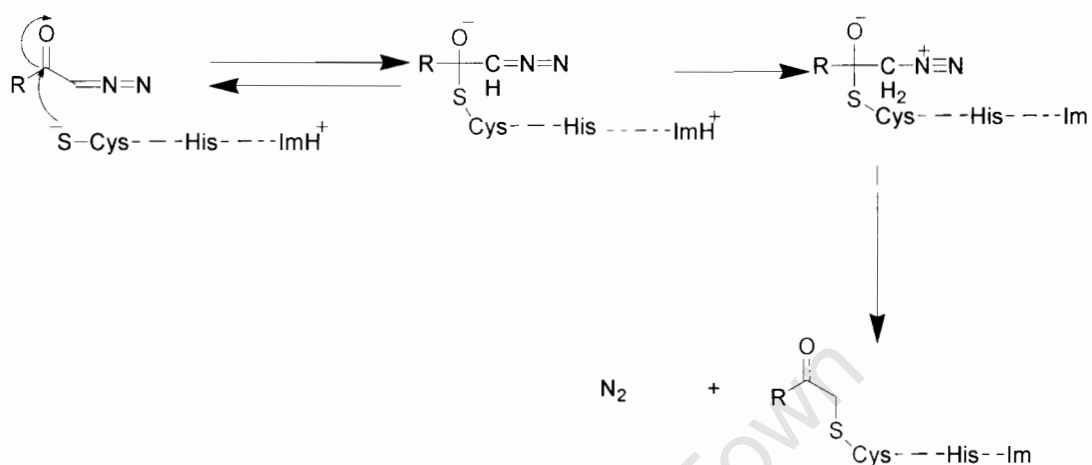


Scheme 2a: Irreversible inhibition of cysteine protease by chloromethyl ketones: direct displacement of the halide group.



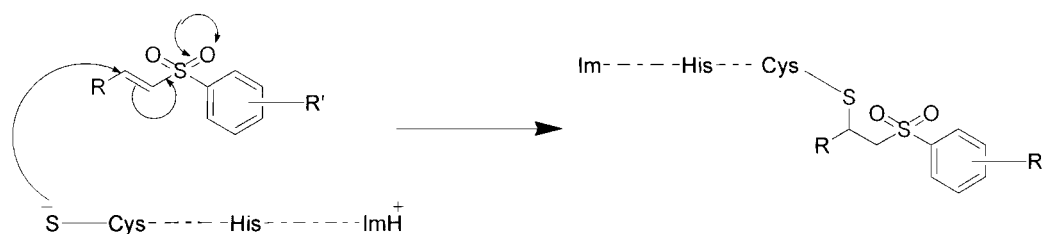
Scheme 2b: Irreversible inhibition of cysteine protease by chloromethyl ketones: involving thiohemiketal and a three-membered sulfonium intermediate.

Peptidyl diazomethyl ketones are also irreversible inhibitors of cysteine proteases, and they also alkylate the active site thiol group. Their mechanism of inhibition is thought to involve a proton transfer from the active site histidine (Im = imidazole of histidine) residue to the methylene carbon of the inhibitor with the loss of N_2 and alkylation of the active site cysteine residue. The other postulated mechanism involves addition of the cysteine residue to the carbonyl group of the inhibitor to give a tetrahedral adduct, which then rearranges to form an irreversible thioether adduct (scheme 3).⁴⁶⁻⁴⁸



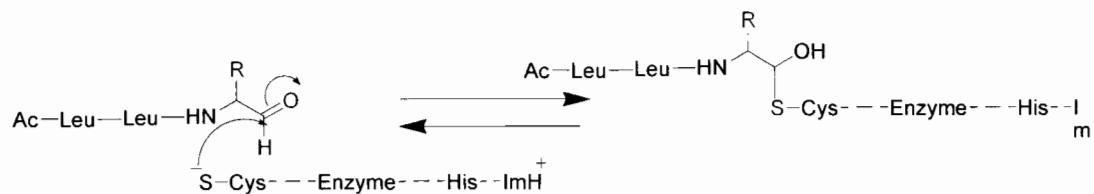
Scheme 3: Irreversible inhibition of cysteine protease by diazomethyl ketones

Peptide and amino acid derivatives that contain a Michael acceptor are specific irreversible inhibitors of cysteine proteases. This class includes the vinyl sulfones and α,β -unsaturated carbonyl derivatives.⁴⁶⁻⁴⁸ The mechanism of inhibition by vinyl sulfones and α,β -unsaturated esters and other α,β -unsaturated carbonyl derivatives involves a Michael addition. The active site cysteine residue attacks the β -carbon, followed by the protonation of the α -carbon to form an irreversible thioether adduct (scheme 4). Irreversible inhibitors are also called alkylating agents because their mechanism of inhibition involves alkylation of the active site cysteine residue.^{47,48}

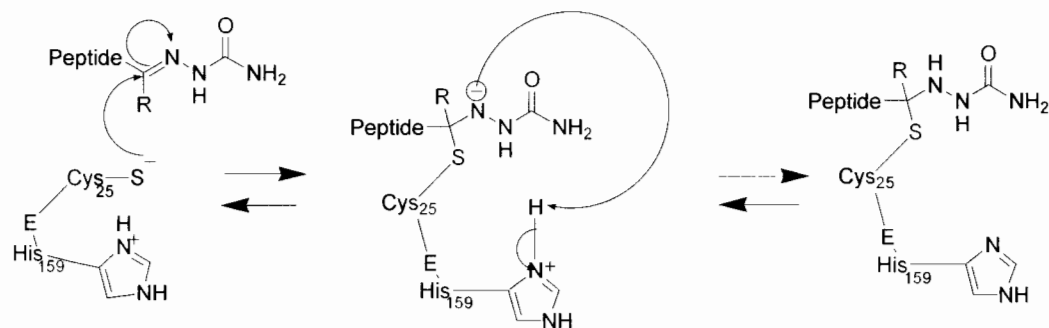


Scheme 4: Irreversible inhibition of cysteine proteases by vinyl sulfones

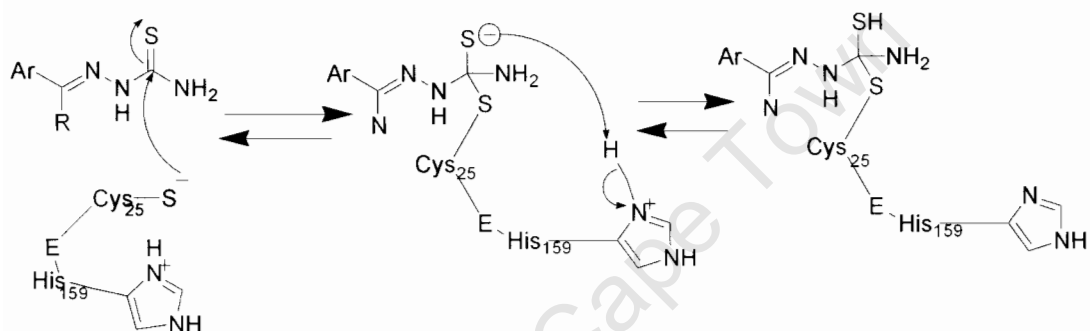
- (ii) Another series of inhibitors forms a covalent, yet reversible transition state-like intermediate with the active-site cysteine residue. Examples of this series include carbonyl based peptidyl aldehydes (scheme 5), ketones and α -keto esters and amides. Reversible inhibition usually involves the noncovalent interaction between the enzyme and the inhibitor. However, there are cases whereby covalently bound inhibitors result in reversible inhibition because of hydrolytically labile bonding. Some reversible inhibitors bind to the enzyme with high affinity, resulting in slow dissociation of the enzyme-inhibitor complex, such that they appear irreversible, thus they are therefore termed tight-binding reversible inhibitors. There are also some reversible inhibitors that inhibit the cysteine protease very slowly due to conformational changes following enzyme-inhibitor complex formation, and these are termed slow-binding reversible inhibitors. Peptidyl semicarbazones represent an example of reversible inhibitors. They have been used previously as intermediates for the synthesis of peptidyl aldehydes, which are also reversible inhibitors.⁴⁸ Their mechanism of action involves the formation of a reversible tetrahedral adduct. The cysteine residue thiolate anion attacks the protected carbonyl carbon (C-5 carbon, scheme 6a).^{44,48,49} Another group of reversible inhibitors that are structurally related to semicarbazones are the thiosemicarbazones. Their mechanism of inhibition involves attack of the thiocarbonyl carbon (C=S) of the inhibitor by the thiolate anion of the cysteine residue (scheme 6b). They are non-peptidic and are better inhibitors of cruzain than their respective semicarbazones.⁴⁹



Scheme 5: Reversible inhibition of cysteine proteases by peptidyl aldehydes



Scheme 6a: Reversible inhibition of cysteine proteases by peptidyl semicarbazones



Scheme 6b: Reversible inhibition of cysteine proteases by thiosemicarbazones

- (iii) The last series of inhibitors forms a stable thioacyl-enzyme complex with the active site cysteine which, by nature of this intermediate is slow to hydrolyze. Thus, they are called slow turnover inhibitors, and examples of this series include peptidyl carbamate esters **15** and acyl-bis-hydrazides **16** (figure 6).⁴⁶

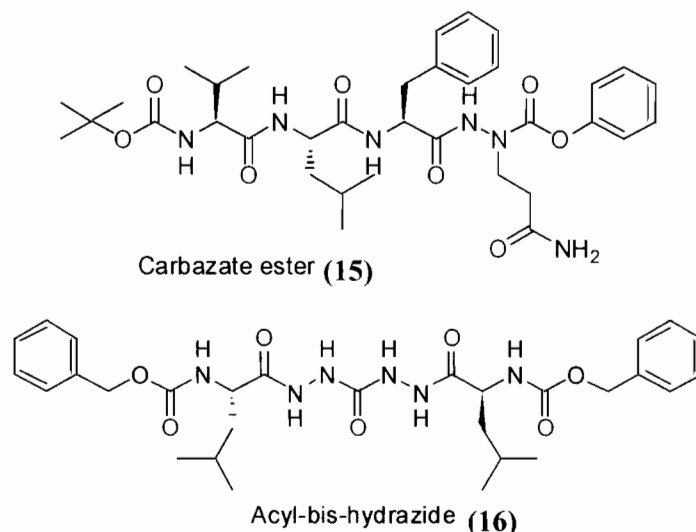


Figure 6: Examples of slow turnover inhibitors

2.4 Inhibitors of malarial and trypanosomal cysteine proteases

Extensive research to identify newer targets for chemotherapy has led to the identification of protease inhibitors as an attractive alternative. The disruption of the hemoglobin degradation in the malaria parasite seems to be a logical approach to antimalarial chemotherapy. It has been demonstrated previously that fluoromethyl ketone-derivatized dipeptides could cure a murine infection of malaria.⁵⁰ Then vinyl sulfone-derivatized dipeptides were also shown to be potent against the murine models of malaria.⁵¹ They were reported to potently block hemoglobin degradation and parasite growth in malaria.⁴¹ Several independent studies have confirmed the efficacy of vinyl sulfone-derivatized dipeptides to rescue mice from an acute lethal infection of *T. cruzi*.⁵² Preliminary studies have also confirmed that cysteine protease inhibitors can arrest the replication of *T. brucei* in mice.⁴⁴ Vinyl sulfones, peptidyl chloromethyl ketones, diazomethyl ketones and fluoromethyl ketones have all been examined against *T. brucei* (figure 7).⁵³

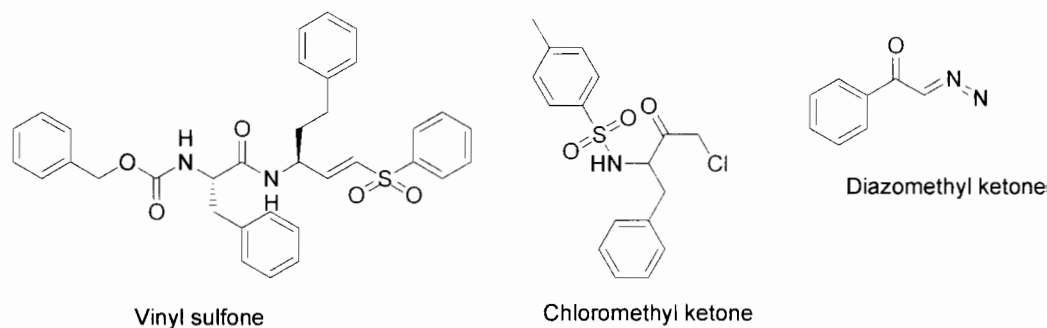


Figure 7: Examples of irreversible inhibitors of cysteine proteases

Chalcones have also been extensively studied against the malarial cysteine protease, falcipain. For example chalcone **17** (figure 7) was reported to have IC_{50} values of 0.51 and 0.72 μM against chloroquine resistant and chloroquine sensitive *P. falciparum* respectively.⁵³ Acyl hydrazones and α,β -unsaturated amides exemplified by compound **18** are also reversible inhibitors of cysteine proteases. Isatins, are also well known reversible inhibitors of cysteine proteases⁵⁴, and these as well as their thiosemicarbazone derivatives **19** have been shown to inhibit cruzain, falcipain-2, and rhodesain.⁵⁵ Another class of compounds that have been evaluated against cruzain and rhodesain are the aryl ureas, represented by compound **20** that were found to be a promising class of compounds with acceptable IC_{50} values.⁵⁶ Lately, thiosemicarbazone derivatives represented by the general scaffold **21** ($X = \text{CH}, \text{N}$) have also been identified as inhibitors of cysteine proteases; cruzain, falcipain-2 and rhodesain.⁵⁷ A recent review has summarized a full range of parasitic cysteine protease inhibitor scaffolds.⁵⁸

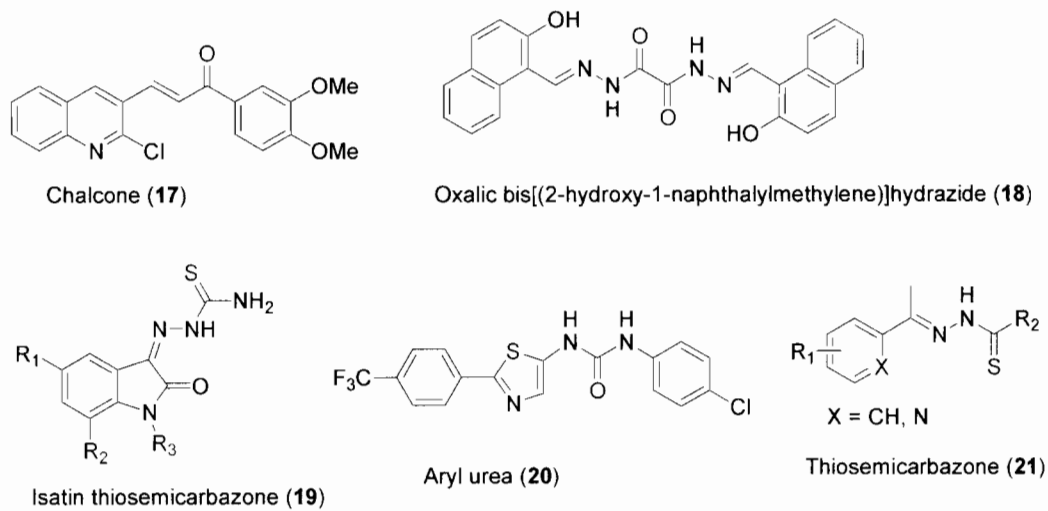


Figure 8: Structures of a few cysteine protease inhibitors

CHAPTER 3

SYNTHESIS AND CHARACTERIZATION OF TARGET COMPOUNDS

3.1 QUINOLINE THIOSEMICARBAZONES

3.1.1 BACKGROUND AND RATIONALE

3.1.1.1 Background on Quinoline compounds

Recent efforts aimed at circumventing chloroquine and general aminoquinoline resistance have focused mainly on structure-activity relationship (SAR) studies of chloroquine and related 4-aminoquinolines. These studies included the removal of the 7-Cl group, which abolished antiparasmodial activity. Thus it was then established that the 7-Cl is important for chloroquine activity, even though its specific role is not fully understood.⁵⁹ Further studies included the substitution of the 7-Cl group with various substituents such as F, Br, I, CF₃ and OMe. 7-Iodo- and 7-bromo-aminoquinolines (AQs) with short side chains were generally active against both chloroquine-susceptible and chloroquine-resistant *P. falciparum*. In addition, 7-iodo-AQs with long (10-12 carbons) diaminoalkane side chains were also found to be active against both strains of *P. falciparum*. On the other hand, 7-fluoro-AQs and 7-trifluoromethyl-AQs were generally less active than 7-Cl, 7-I, and 7-Br-AQs against both strains of *P. falciparum*. 7-OMe-AQs were inactive against both strains of *P. falciparum*.⁵⁹

Further SAR studies included substitutions on other positions of the aminoquinoline rings, which resulted in reduced antiparasmodial activity. The length of the diaminoalkane side chain was also varied in order to investigate the effect it might have on the antiparasmodial activity. It was then observed that aminoquinoline derivatives with short (2-3 carbons) and long (10-12 carbons) diaminoalkane side chains were active against both chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*. On the other hand, aminoquinolines with intermediate (4-8 carbons) diaminoalkane side chains were only active against chloroquine-susceptible *P. falciparum*, but not active against chloroquine-resistant *P. falciparum*.^{59,60}

SAR studies carried out by Egan et al. on chloroquine and related aminoquinolines suggest the following;⁶¹

- (i) Chloroquine and related antimalarial drugs act by complexing ferriprotoporphyrin IX (Fe(III)PPIX), inhibiting its conversion to β -hematin (hemozoin) and hence its detoxification.
- (ii) The basic amino side chain is also essential for antiplasmodial activity and drug accumulation in the food vacuole of the *P. falciparum*.
- (iii) The presence of the 7-chloro group in the 4-aminoquinoline ring is required for β -hematin inhibition.

Hence these studies suggest that there is still merit in pursuing quinolines for antimalarial activity. These SAR studies are summarized in figure 9 below.

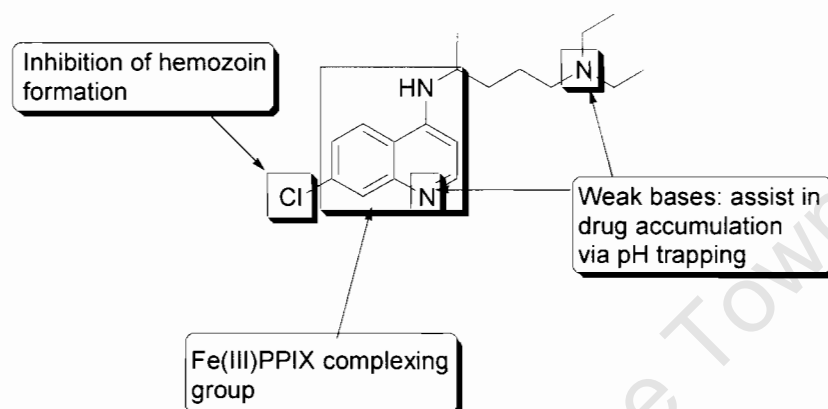


Figure 9: Proposed structure-activity relationship studies in chloroquine

3.1.1.2 Background on Thiosemicarbazones

Thiosemicarbazones are a class of compounds possessing a wide spectrum of medicinal properties. They have been studied and evaluated among other diseases, against tuberculosis⁶², and trypanosomiasis⁶³, as well as anticancer therapeutics⁶⁴, as well as for their parasitocidal activity against *Plasmodium falciparum*.⁶⁵ Currently, a thiosemicarbazone, Triapine, is being evaluated in human phase II trials as an antineoplastic therapeutic.⁶⁶

Recently Du and coworkers⁴⁹ reported a series of thiosemicarbazones possessing inhibitory activity against cruzain, the cysteine protease of *Trypanosoma cruzi*.

Another series of thiosemicarbazones based on the isatin scaffold was reported by Chiyanzu and coworkers⁵⁵. Inhibitory activity against cruzain, falcipain-2 and rhodesain was studied and promising scaffolds identified. Thus based on these reports, thiosemicarbazones represent a promising class of compounds on which to base antiprotozoal cysteine protease inhibitors.

Work by Du and coworkers⁴⁹ on thiosemicarbazones generated the basic structure-activity relationships (SAR) against cruzain. This SAR is summarized in figure 10. Firstly, the authors examined the nature of halide substituents on the aromatic ring, which were tolerated. Secondly, the variation of an alkyl group (R²) at position 5 of the thiosemicarbazone scaffold was studied, and the trend of activity was observed to increase with the size of the substituent; H < methyl < ethyl. Thirdly, substitution at the N1 nitrogen was evaluated, with monosubstitution on the N1 nitrogen, resulting in reduced activity. Fourthly, the effect of sulfur in the C=S double bond was also investigated by substitution of sulfur with oxygen, and this resulted in poor activity of the compounds, leading to the conclusion that sulfur is important for activity. This led the authors to conclude that the logical site of attack by the active site's cysteine thiol is the C=S double bond. Lastly the C=N4 double bond was reduced to a C-N4 single bond resulting in poor activity.

Similar studies were carried out by Klayman and coworkers⁶⁵, in this case for antimalarial activity. With regard to the C=S double bond it was found that the sulfur is indeed critical for the activity of the compounds, as the C=O double bond diminished antimalarial activity. In addition, structure-activity relationship studies with regard to substitution at the N1 nitrogen were carried out with a variety of substituents. It was observed that *N1,N1*-disubstitution by both alkyl groups resulted in varying activities. For example the *N1,N1*-diethyl compound showed no antimalarial activity *in vitro*. On the other hand, an *N1,N1*-disubstitution with a methyl and a cycloalkyl group resulted in cures *in vivo* at dosages of 80-320 mg/kg, and higher doses resulted in toxicities. Compounds with N1-nitrogen atom incorporated into 4-9-membered rings were also evaluated in this study, and optimum activity was found in the 6-membered rings *in vitro*.



Figure 10: Summary of Known Thiosemicarbazone Structure Activity Relationship Studies with Cruzain⁴⁹

Recent studies carried by Greenbaum and coworkers⁵⁷ on thiosemicarbazones against the parasitic cysteine proteases; cruzain, falcipain-2 and rhodesain revealed that substituents at the 3' or 4' position on the aryl ring are most favored to bind protease targets and therefore merit expansion. On the other hand, the parasite SAR studies revealed that the pyridine thiosemicarbazones do not inhibit the target cysteine proteases, but are excellent parasitocidal compounds, especially compounds **22** and **23**. Furthermore compounds **24**, **25** and **26** were shown to be effective against both *T. brucei* in culture and rhodesain, and this is the only case where enzyme inhibition was also matched with parasitocidal activity in culture. Lastly, N1 NH₂-substitution lowered the potency against the target proteases, but enhanced killing of *T. brucei* and *P. falciparum*, especially in compounds with a protonatable terminal nitrogen like compound **27**. The authors suggest that this may be due to the increased uptake of the compound across cell membranes or enhanced binding to another target protein.

Other scaffolds were also discovered during this study, which represent more general parasitocidal compounds capable of killing all parasites. Two of the scaffolds **27** and **28** are bidentate metal chelators (*via* the hydrazinic nitrogen and

S atoms), while two scaffolds **22** and **23** are potential tridentate metal chelators. Their mechanism of action is not very clear, but it seems that compounds **27** and **28** can inhibit proteases, although **28** only inhibits rhodesain, while **27** inhibits rhodesain and cruzain. Yet both **27** and **28** can kill the organisms. On the other hand compounds **22** and **23** do not inhibit the target proteases, but are very effective at killing at least two or all the three tested parasites.⁵⁷

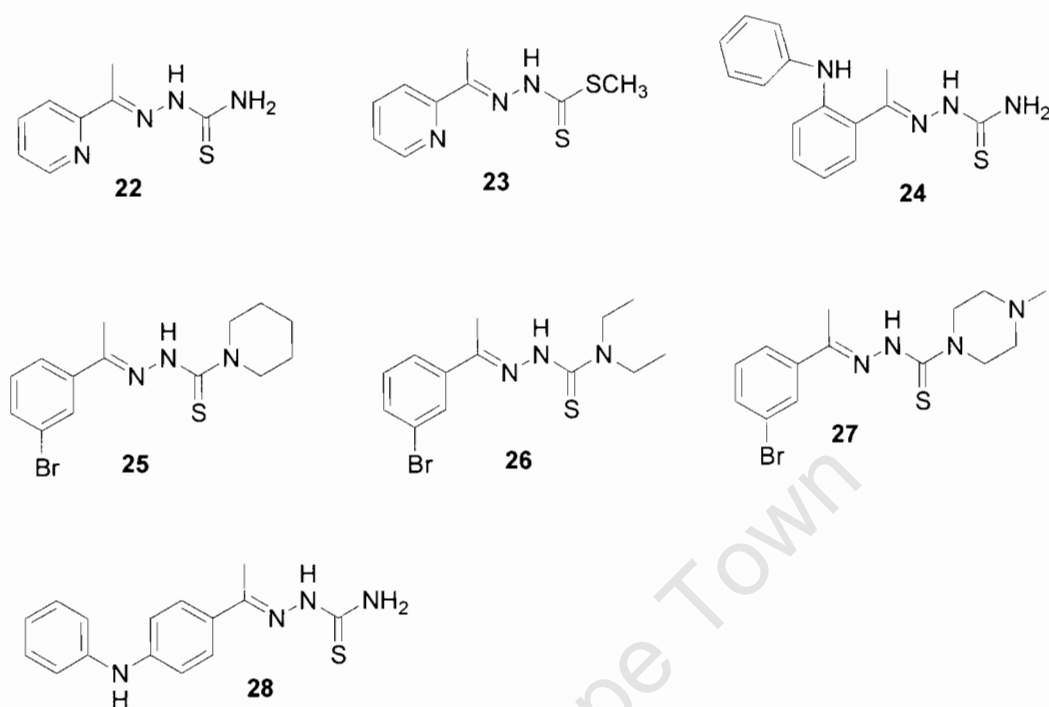


Figure 11: Structures of thiosemicarbazone scaffolds with activity against multiple cysteine proteases and parasites.⁵⁷

The detailed mechanism of action of thiosemicarbazones has not been clear. The antimalarial 2-acetylpyridine thiosemicarbazones may be acting as tridentate iron chelators in *P. falciparum*. There are two known ways for metal chelators to inhibit growth of *P. falciparum*: withholding iron from plasmodial metabolic pathways or forming complexes with iron that are toxic to the parasite.⁵⁷

3.1.1.3 Rationale on Quinoline thiosemicarbazones

In view of the background presented in sections 3.1.1.1 and 3.1.1.2 above, we decided to design and synthesize quinoline thiosemicarbazones primarily directed at *Plasmodium falciparum*. The rationale in this regard is based on a multi-therapeutic strategy summarized in figure 12. It is noteworthy that compounds such as thiosemicarbazones with multiple mechanisms of action could be useful in slowing down the emergence of antimalarial drug resistance or even overcoming it. Compounds aimed at a single target are more likely to be rendered ineffective due to the emergence of resistant parasites compared to compounds aimed at multiple targets.

Initially a strategy was envisaged in which the thiosemicarbazone moiety might inhibit the cysteine proteases *via* multiple mechanisms, while the quinoline nitrogen along with the thiosemicarbazone hydrazinic nitrogen would be important for accumulation in the acidic food vacuole of the parasite. In addition, metal chelation involving the sulfur of the thiocarbonyl (C=S) group and the N4 hydrazinic nitrogen atom of the C=N double bond was envisaged. Inhibition of some metalloenzymes such as falcilysin was also envisaged by the aforementioned metal chelation property of the thiosemicarbazone moiety. Metal chelation was envisaged for the iron in the red blood cells, because many enzymes in the malaria parasite in the erythrocytes depend on iron. Therefore it can be inferred that if iron can be withheld from the parasite by the iron chelating thiosemicarbazone moiety, this may disrupt the metabolism of the parasite by, for example preventing DNA synthesis (through the inhibition of the iron-dependent enzyme, ribonucleotide reductase), inhibiting *de novo* synthesis of heme and interfering with normal mitochondrial function and electron transport.⁶⁷⁻⁶⁹

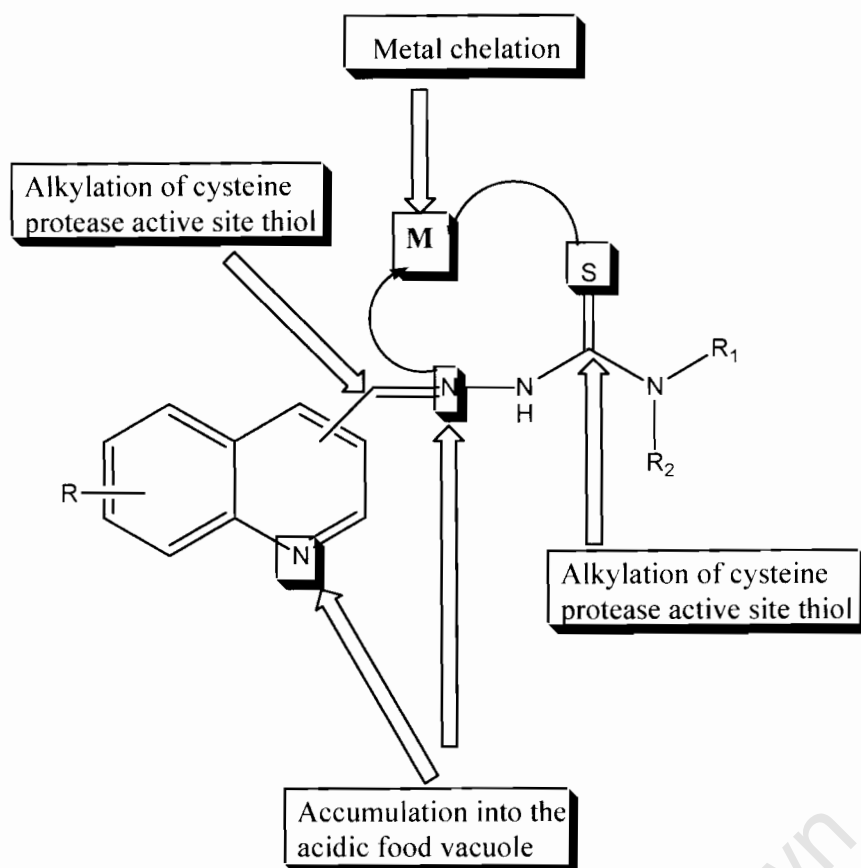


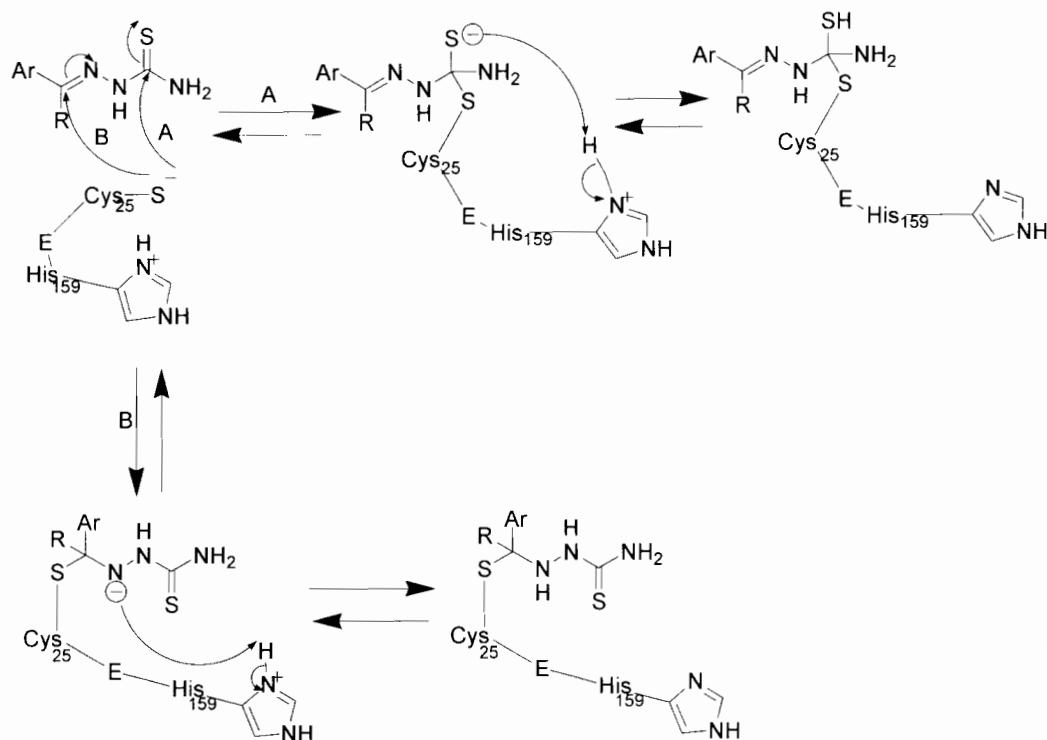
Figure 12: Envisaged Multitherapeutic strategy of quinoline thiosemicarbazones against *Plasmodium falciparum*.

Several classes of iron-chelating compounds have been reported to inhibit the growth of *P. falciparum* in the red blood cells. Numerous studies indicate that the degree of antimalarial activity of iron chelators correlates with the degree of lipophilicity, or the ability to cross the cell membranes, of the compounds.⁶⁹⁻⁷² The antimalarial iron chelators can be categorized based on the predominant mechanism of inhibition of parasite growth. There are two categories; those that withhold the iron from the plasmodial metabolic pathways, or those that form toxic complexes with iron.⁶⁹

The mechanism of antimalarial activity of Fe(III) chelators appears to be sequestration of iron necessary for plasmodial replication. On the other hand, Fe(II) chelators seem to have an antiparasitic effect other than the withholding of iron. An example of an Fe(II) chelator is the aromatic 8-hydroxyquinoline, which appears to complex with iron extracellularly, before subsequently entering the red

blood cells to produce a rapidly lethal free-radical-mediated intracellular reaction.^{68,73}

We also envisaged that the thiosemicarbazones could inhibit the target cysteine proteases by two independent mechanisms that utilize two unique chemistries of the active site cysteine thiol namely; nucleophilicity and metal binding properties. With regard to nucleophilicity, we envisaged alkylation of the active site thiol *via* addition to either the imine C=N double bond or the C=S of the thiol carbonyl group (Scheme 7). Regarding metal binding properties of the active site thiol, we reasoned that thiosemicarbazones might be metal-interactive inhibitors of cysteine proteases upon intracellular formation of a metal complex to which the active site thiol could coordinate. This metal-interactive inhibition of a cysteine protease has recently been demonstrated for metal chelating biguanides.⁷⁴ Various biguanides were reported to be metal-interactive inhibitors of cathepsin B from mammals and falcipain-2 from *P. falciparum*. Before this disclosure, antimalarial and antidiabetic biguanides had been unrecognized cysteine protease inhibitors, revealing protease inhibition as a contributing therapeutic mechanism in both diseases. Some biguanide derivatives might co-bind metals such as Zn²⁺, Cu²⁺ and Fe²⁺, and substrate recognition sites so as to enhance the natural inhibitory action of endogenous metals against protease catalytic sites.⁷⁴

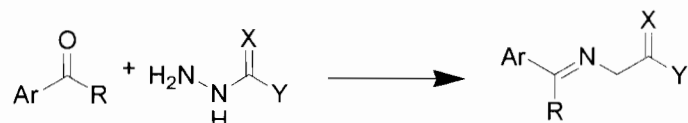


Scheme 7: The proposed mechanism of action of thiosemicarbazones against cysteine proteases from *Plasmodium falciparum*.

3.1.1.4 Synthesis and Characterization

General synthesis and characterization of (thio)semicarbazones

A range of thiosemicarbazones and semicarbazones were synthesized by the simple treatment of appropriate aromatic aldehydes or aromatic methyl ketones with a thiosemicarbazide or semicarbazide in alcoholic solvents such as; methanol, ethanol or isopropanol under reflux (Scheme 8). A minimum amount of acetic acid (1%) was used in the case of methyl ketones.⁶⁵ Reaction times varied between three hours for aldehydes and up to 24 hours for methyl ketones. The product yields were usually moderate to good. Work-up of the reaction mixtures was not necessary as the products usually precipitated out of the reaction solution. The corresponding structures of the products were readily characterized with the following spectroscopic techniques; ¹H-NMR, ¹³C-NMR, MS, IR spectroscopy and elemental analysis.

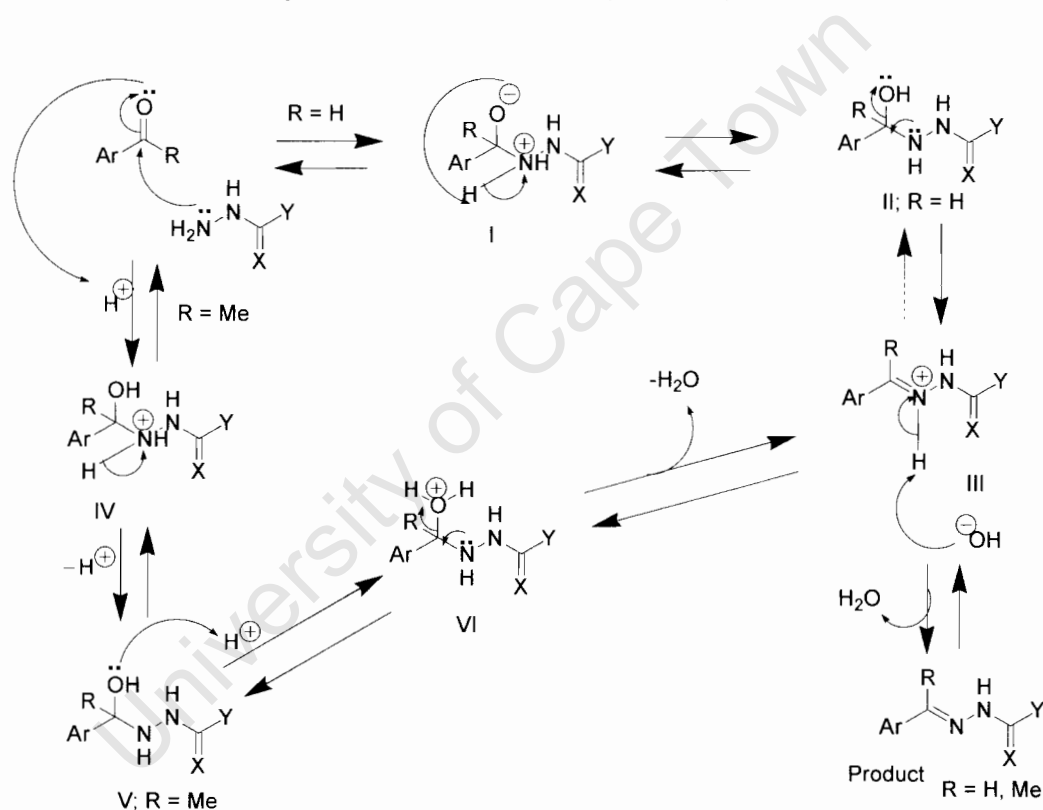


Ar = Aromatic ring; R = H, Methyl; X = S, O and Y = NH₂, SCH₃, NRR'

Scheme 8: General synthesis of (Thio)semicarbazones

Mechanism of the reaction

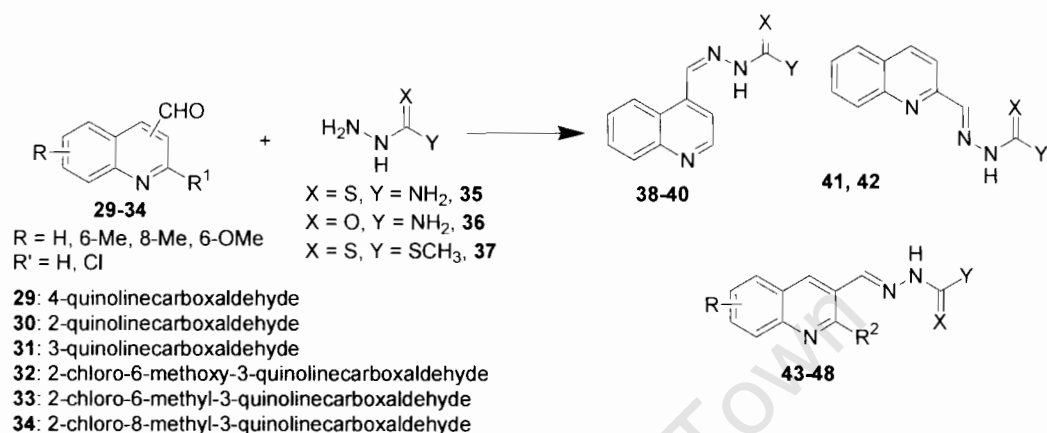
The nucleophilic thiosemicarbazide (or semicarbazide) attacks the electrophilic carbonyl group of the aromatic aldehyde (or ketone) to produce intermediate II, from which iminium ion (in case of ketones) III is formed, and eventually the product after loss of a proton from III. For ketone reactions, the added acid catalyzes the reaction by first protonating the carbonyl oxygen to generate intermediate IV. The proton lost from intermediate IV is used to convert the hydroxyl (OH) group in intermediate V into a good leaving group (water molecule) whose elimination from intermediate VI generates iminium ion III. It is noteworthy that the steps involved are reversible (scheme 9).



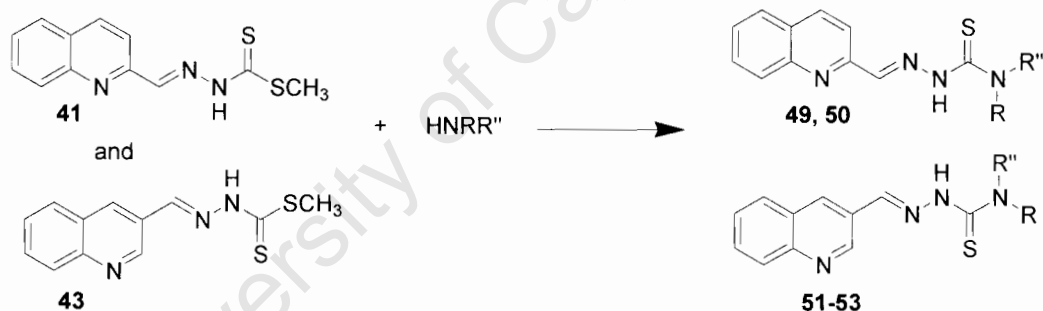
Scheme 9: Mechanism of thiosemicarbazone formation

Synthesis and characterization of specific target (thio)semicarbazones

(Thio)semicarbazones **38-48** were prepared from the treatment of quinolinecarboxaldehydes **29-34** with scaffolds **35-37** (scheme 10). *N,N*-Disubstituted and *N*-monosubstituted derivatives **49-53** were synthesized by reaction of thiosemicarbazone thioesters **41** and **43** with selected primary and secondary amines (scheme 11). The selected amines were piperidine, 3-dimethylamino-1-propylamine, and *N*'-(7-chloroquinolin-4-yl)-propane-1,3-diamine. The reactions were carried out in methanol under reflux with a minimum amount of a co-solvent (THF) as the thiosemicarbazone thioesters **41** and **43** were insoluble in methanol.



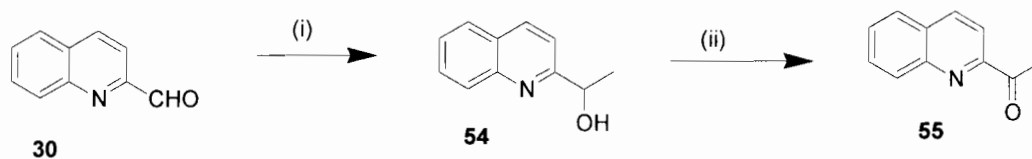
Scheme 10: Reagents and conditions: MeOH/EtOH/2-PrOH, reflux, 3hrs.



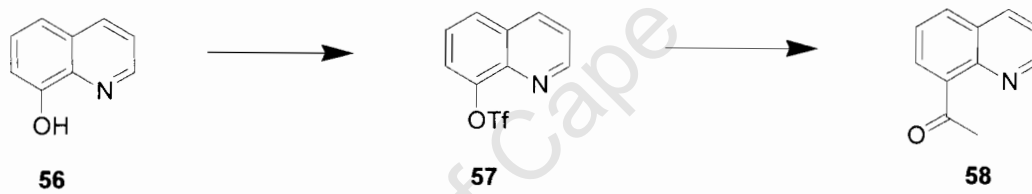
Scheme 11: Reagents and conditions: MeOH (THF, co solvent), reflux 12 hrs

For structure-activity relationship studies, only two acetylquinolines were synthesized and converted into (thio)semicarbazones. First, the 2-quinolinecarboxaldehyde **30** was converted to 2-acetylquinoline **55** via a two step reaction sequence involving a Grignard addition of methylmagnesium bromide to

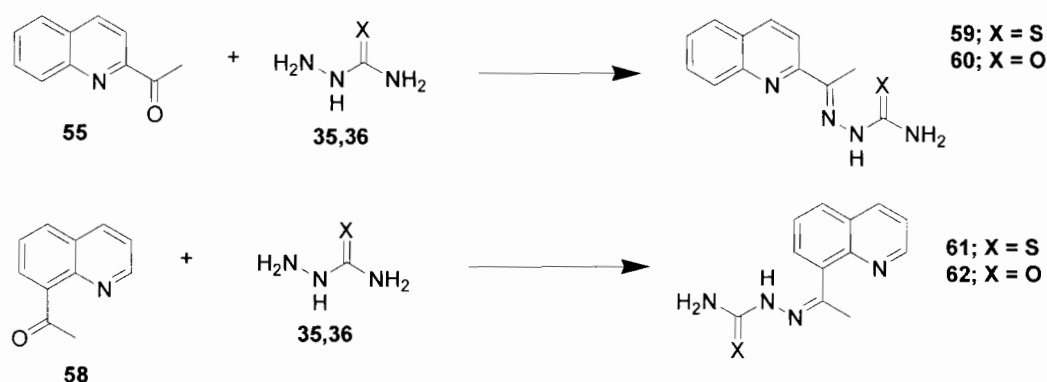
30 to form intermediate **54**, followed by oxidation using the modified Moffat oxidation protocol of sulfur trioxide. pyridine complex (scheme 12).⁷⁵⁻⁷⁷ The product yields for the intermediate **54** and 2-acetylquinoline **55** were 58 % and 99 % respectively. For the synthesis of 8-acetylquinoline **58**, commercially available 8-hydroxyquinoline **56** was transformed into triflate **57** by reaction with trifluoromethanesulfonic anhydride in the presence of pyridine (base and solvent). The reaction proceeded in 87 % yield. Triflate **57** was then coupled to butyl vinyl ether using the Heck reaction catalyzed by palladium (0) to give 8-acetylquinoline **58** after acid hydrolysis in 86 % yield (scheme 13).^{78,79} Both of these acetylquinolines **55** and **58** were treated with thiosemicarbazide **35** and semicarbazide **36** to yield the desired thiosemicarbazones or semicarbazones **59-62** (Scheme 14, table 1).



Scheme 12: Reagents and conditions: (i) MeMgBr (2.0 equiv.), THF, 58%; (ii) Et₃N (5.0 equiv.), SO₃.Pyr. (4.0 equiv.), DMSO/DCM (1/4), 99%.



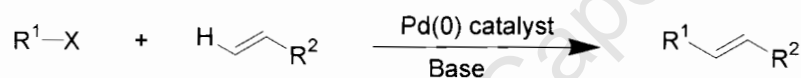
Scheme 13: Reagents and conditions: (i) Pyridine, Trifluoromethanesulfonic anhydride (1.10 equiv.), 87%; (ii) Pd(OAc)₂ (0.05 equiv.), dppp (0.055 equiv.), Et₃N (3.0 equiv.), Butyl vinyl ether (5.0 equiv.), DMF, 48 hrs, 86%.



Scheme 14: Reagents and conditions: EtOH, Acetic acid (1%), reflux, 24 hrs.

The Heck Reaction^{80,81}

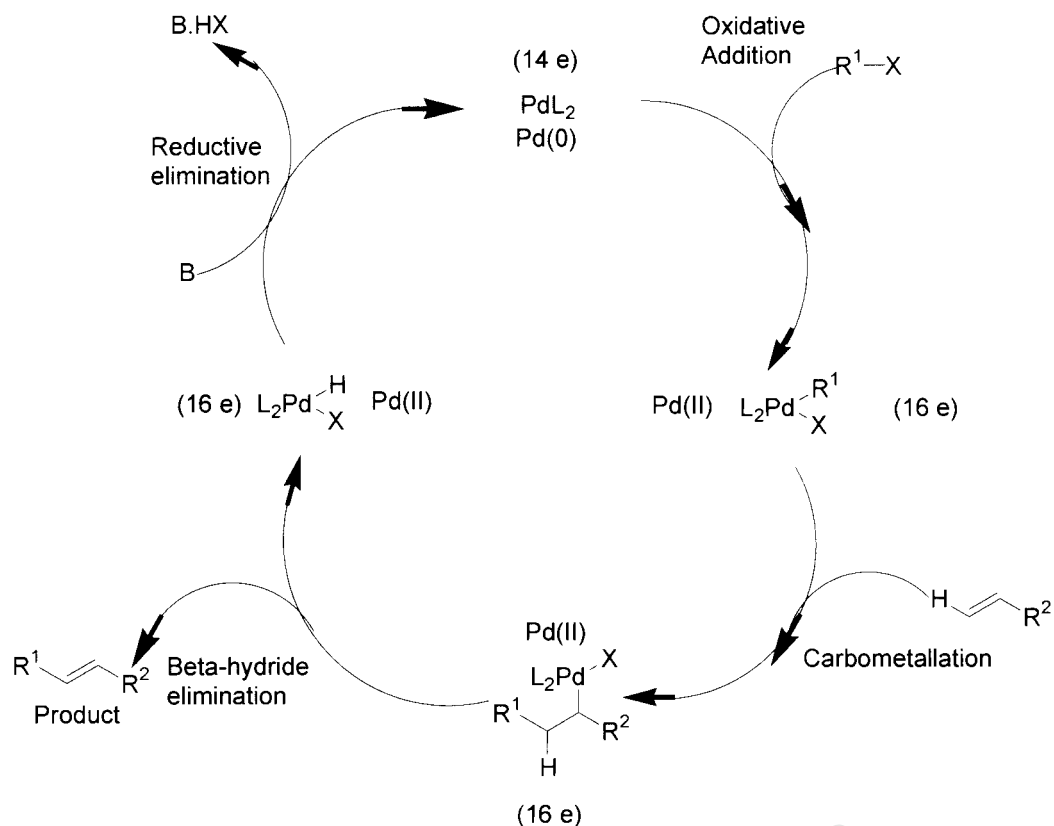
This is one of the reactions in organic chemistry based on transition metals. The Heck reaction is one of the most synthetically useful palladium-catalyzed reactions. It couples together an alkene with a halide or triflate to form a new alkene. Catalytic palladium Pd(0) is needed for this reaction (scheme 15). The method is very efficient, and carries out a transformation that is difficult by more traditional techniques. The alkene can be mono- or disubstituted and can be electron-rich, poor or neutral. The base need not be at all strong and can be Et₃N, NaOAc or aqueous Na₂CO₃.



R¹ = Aryl, vinyl, alkyl without beta-H's on an sp³ carbon
X = halide, triflate

Scheme 15: The Heck reaction

The mechanism of the reaction involves oxidative addition of the halide or triflate, insertion of the olefin, and elimination of the product by a β-hydride elimination process. A base then generates the palladium (0) catalyst. The whole process is a catalytic cycle (scheme 16).



Scheme 16: Mechanism of Heck Reaction

$^1\text{H-NMR}$ spectroscopic evidence for the confirmation of the structure of the intermediate alcohol **54** from 2-quinolinecarboxaldehyde **30** was observed by the disappearance of the aldehyde (CHO) proton, which appears in the region 9.89–10.3 ppm. This proton is replaced by methyl (CH_3) protons, which appear as a doublet at 1.58 ppm and hydrogen on the adjacent carbon, which appears as a quartet at 5.04 ppm. The methyl (CH_3) protons and the hydrogen at 1.58 and 5.04 ppm respectively, disappear and were replaced by a singlet for COCH_3 at 2.86 ppm to confirm the structure of the methyl ketone **55**. Similar $^1\text{H-NMR}$ spectroscopic evidence was observed for 8-acetylquinoline **58**, with the COCH_3 protons appearing as a singlet at 2.94 ppm.

All the (thio)semicarbazones were obtained as powders with different colours. The synthetic yields for the products ranged from very low (8 % for **53** and 22 % for **50**, Table 1) to very high (87 % for **39** and **41** and 92 % for **43**, Table 1), however other compounds were obtained in moderate yields. The product yields for the

N1'-substituted thiosemicarbazones **49-53** were low to moderate (Table 1). This could be partly due to the poor solubility of the starting material in methanol which required the use of a co-solvent. Column chromatography was used to purify products **49-53**.

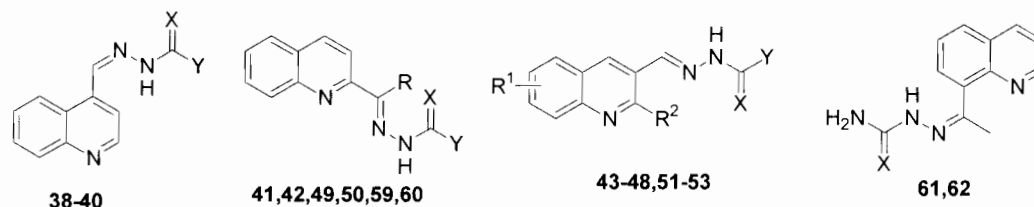


Table 1: First series of (thio)semicarbazones synthesized with their respective yields and melting points.

Cmpd	X	Y	R	R ¹	R ²	%Yield	mp (°C)
38	S	NH ₂	-	-	-	77	241- 242
39	S	SCH ₃	-	-	-	87	183- 185
40	O	NH ₂	-	-	-	78	238- 240
41	S	SCH ₃	H	-	-	87	197- 199
42	S	NH ₂	H	-	-	77	232- 233
43	S	SCH ₃	-	H	H	92	208- 212
44	S	NH ₂	-	6-OMe	Cl	71	253- 255
45	O	NH ₂	-	6-OMe	Cl	76	265- 267
46	S	NH ₂	-	6-Me	Cl	74	270- 272
47	O	NH ₂	-	6-Me	Cl	44	274- 276
48	S	NH ₂	-	8-Me	Cl	56	256- 258

49	S		H	-	-	55	195- 197
50	S		H	-	-	22	209- 212
51	S		-	H	H	57	174- 176
52	S		-	H	H	63	195- 198
53	S		-	H	H	8	209- 212
59	S	NH ₂	CH ₃	-	-	40	160- 162
60	O	NH ₂	CH ₃	-	-	48	228- 232
61	S	-	-	-	-	44	208- 211
62	O	-	-	-	-	48	177- 179

¹H-NMR spectroscopic evidence for the confirmation of the structures of (thio)semicarbazones from aldehydes was observed by the disappearance of the aldehyde (*CHO*) proton which appears as a singlet in the region 9.89-10.3 ppm. This is replaced by the imine (*HC=N*) proton which is observed as a singlet around 8.2-8.9 ppm. The other noticeable observation in the ¹H-NMR spectra is the secondary NH proton of the (thio)semicarbazone moiety in the region around 11.7 ppm. The thiosemicarbazone thioester has a singlet for the SCH₃ protons in the region 2.53-2.73 ppm. The methyl (CH₃) protons of the methyl ketone around 2.86-2.94 ppm, shift slightly upfield to the region 2.35-2.59 ppm when the methyl ketone is incorporated into the thiosemicarbazone scaffold. Further evidence for the confirmation of the products is also observed in the ¹³C-NMR spectra. The thiocarbonyl carbon of the thiosemicarbazone (S=C-NH₂) is observed at about 180 ppm. On the other hand the thiocarbonyl carbon for the thiosemicarbazone thioester (S=C-SCH₃) is observed around 200 ppm, while the carbonyl carbon of

the semicarbazone ($\text{O}=\text{C}-\text{NH}_2$) chemical shift is observed at about 157 ppm. The NH peak observed in the region $3000-3500\text{ cm}^{-1}$ in the IR spectra was also evidence that the (thio)semicarbazones had been formed. The HRMS data of the derived compounds indicated the molecular ion peak corresponding to the molecular formulae of the compounds. The elemental analyses of the (thio)semicarbazones confirmed the molecular formula as well as the purity of the compounds.

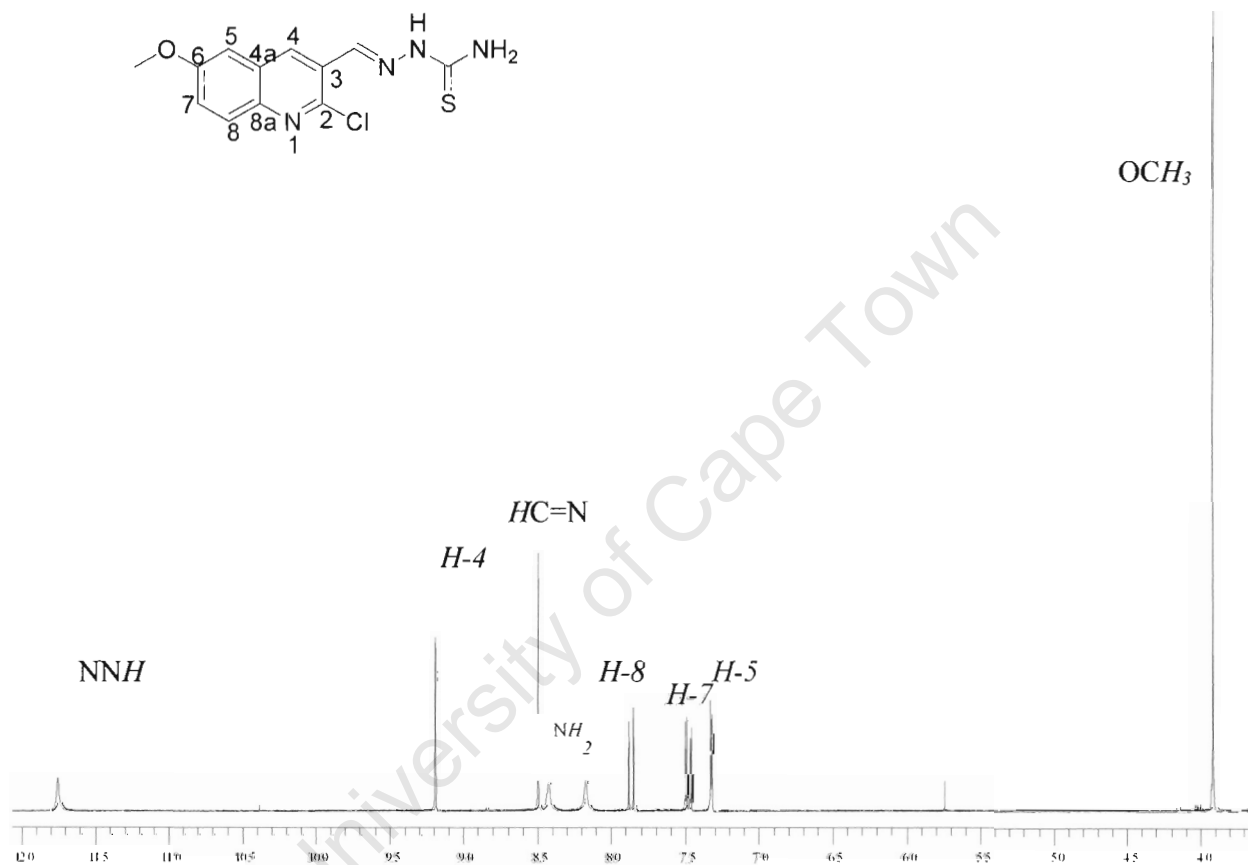


Figure 13: $^1\text{H-NMR}$ spectrum of compound 44 in DMSO-d_6 at 300 MHz

3.2. CHALCONES AND PYRAZOLINES

3.2.1. BACKGROUND AND RATIONALE

3.2.1.1. Background on Chalcone derivatives

Chalcones are α,β -unsaturated ketones (figure 14). Chalcones have demonstrated preferential reactivity towards thiols in contrast to amino and hydroxyl groups. This has implications in terms of eliminating problems of mutagenicity and carcinogenicity which would result from interactions with nucleic acids.⁸² They are a structurally simple group of compounds that have displayed an impressive range of pharmacological activities; among which includes antiprotozoal⁸³⁻⁸⁵, anti-inflammatory⁸⁶, immunomodulatory⁸⁷, nitric oxide inhibition⁸⁸ and anticancer activities⁸⁹. Chalcones that combine a wide range of activities may be advantageous or disadvantageous. A combination of pharmacological activities can enhance the likelihood of undesirable side effects. On the other hand, it may be advantageous for dual-acting drug development, especially if related activities (for example, antiprotozoal) are involved.⁸⁹

Reports on chalcones that combine activities against a number of protozoal infections are rare. However, there are reports about licochalcone A, a natural chalcone derivative isolated from Chinese licorice roots, and other chalcones which possess both antileishmanial and antimalarial activities.^{83,90-92} This shows that the combination of activities is not an unreasonable expectation for this class of compounds. Within the context of antimalarial cysteine protease inhibitors, Li and coworkers⁸³ have reported a series of chalcones that were evaluated against both the sensitive and resistant strains of *P. falciparum*. The observed resistant indexes for the series of chalcones were substantially lower than that of chloroquine, thus suggesting that their mechanism of action is different from that of chloroquine. It was suggested that chalcones inhibit malarial cysteine protease *via* a Michael addition type of reaction, by binding to the nucleophilic side chain of the enzyme.⁸³

Chalcones have similarities with acyl hydrazides. They roughly have the same overall size, and they also have aromatic rings at both ends of the chain linker. It was suggested that both the chalcones and acyl hydrazides (figure 14) could occupy similar positions in the active site of the malarial cysteine protease and

adopt similar binding modes. However, since the inhibition of the malarial cysteine protease occurs inside the acidic food vacuole of the malaria parasite, the relative acid stability of the α,β -unsaturated ketone linker of chalcones offers an advantage over the more potentially labile hydrazide linker in the acyl hydrazides.^{83,93,94} Such an effect could be partly responsible for the observed higher potency of chalcones relative to the corresponding acyl hydrazides. In addition, the α,β -unsaturated ketone linker provides conjugation between aromatic groups at both ends of the linker. Furthermore, the presence of the α,β -unsaturated ketone linker has been shown to be critical for activity, as reduction to the saturated linker resulted in lower potencies against the malaria parasite.⁸³

The DOCK program and modeling studies further suggest that chalcones adopt a more extended conformation, due to the conjugated linker. Therefore, this generates a linear, almost planar structure which fits well into the long cleft of the active site of the malarial cysteine protease. On the other hand the saturated ketone counterpart would be expected to adopt less extended conformation because of the two sp^3 hybridized carbons in the linker, thereby giving rise to less favorable interactions with the protease.⁸³

It has been suggested that chalcones with a quinoline ring attached to the β -carbon of the α,β -unsaturated linker have enhanced antimalarial activity due to the following reasons:⁸³

- (i) Protonation of the nitrogen atom in the quinoline ring under weakly acidic conditions may enhance their interactions with the protease at His67 in the active site, or else protonated His67 may form a hydrogen-bond with the inhibitors.
- (ii) The quinolines may be somewhat concentrated inside the malarial acidic food vacuole (pH \sim 5), i.e. they have enhanced accumulation into the food vacuole.

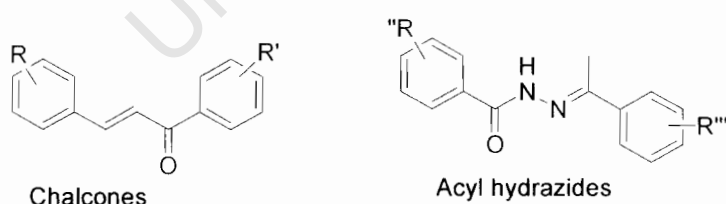


Figure 14: General presentation of chalcone and acyl hydrazide derivatives

3.2.1.2 Background on Pyrazoline derivatives

Pyrazoline derivatives have been reported to have activity against a wide range of therapeutic targets.⁹⁵⁻¹⁰¹ Pyrazoline derivatives of thiosemicarbazones have been studied recently in an effort to investigate the influence of restricted molecular flexibility in the activity of the compounds against cruzain and *T. cruzi*.⁴⁹ Molecular flexibility is a property that has not received much attention in the study of bioactive molecules as therapeutic agents. In addition; oral bioavailability is also an important factor to consider in the development of therapeutic agents. Thus an important goal for therapeutic research, is sufficient knowledge and understanding of the molecular properties that limit oral bioavailability.¹⁰² Factors that have been considered as a primary guide to correlating physical properties with successful drug development by previous researchers are; lipophilicity, molecular weight (MW < 500), and the number of hydrogen bond donors and acceptors.¹⁰²⁻¹⁰⁴ However, one of the properties, namely molecular flexibility has not received much attention, and it is postulated to be important for membrane permeation.^{102,105} Membrane permeation in return is important for oral bioavailability in the absence of active transport, and failure to achieve this usually results in poor oral bioavailability. Studies on molecular flexibility suggest that molecular rigidity plays a pivotal role in blocking out access to the clearance enzymes; while the activity against the target is not compromised.¹⁰²

3.2.1.3 Rationale for Pyrazoline derivatives as Potential Antimalarial agents

We have thus reasoned that pyrazoline derivatives are an interesting class of compounds due to their activity against a wide range of therapeutic targets on the basis of the aforementioned background (3.2.1.2). Thus our rationale is three-fold (partly summarized in figure 15):

- A comparative study of antimalarial activity of chalcones and corresponding pyrazolines has to date not been carried out. We wished to conduct a preliminary study in this regard.
- We reasoned that the presence of the two nitrogen atoms in the pyrazoline ring could lead to increased accumulation of the compounds in the acidic vacuole of the malaria parasite, which could in turn lead to enhanced antiplasmodial activity.

- The NH group in pyrazoline provides a nucleophilic site for introducing further chemical diversity by reacting with a wide range of readily available electrophilic building blocks such as acid chlorides, isocyanates and sulfonyl isocyanates. This would facilitate SAR studies.

In this regard sulfonyl ureas were rationally targeted because they are known to possess some antimalarial activity by blocking permeation pathways.¹⁰⁶

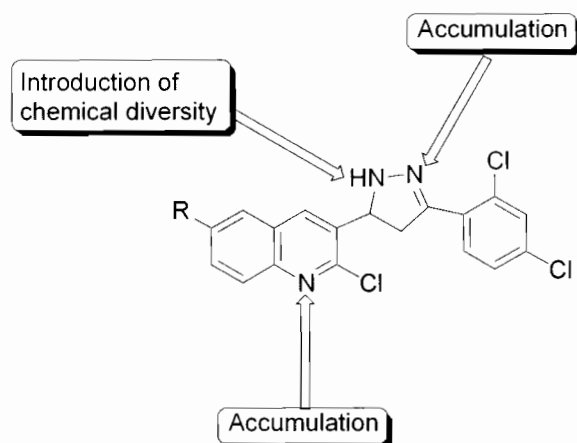
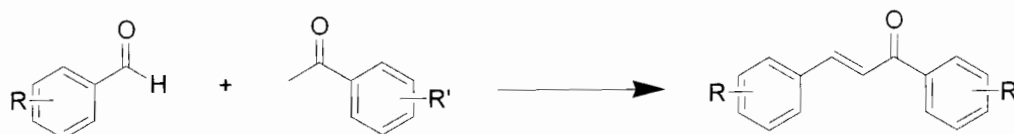


Figure 15: Envisaged structure-activity relationships of Pyrazoline derivatives

3.2.1.4 Synthesis and characterization

General synthesis and characterization of precursor chalcones

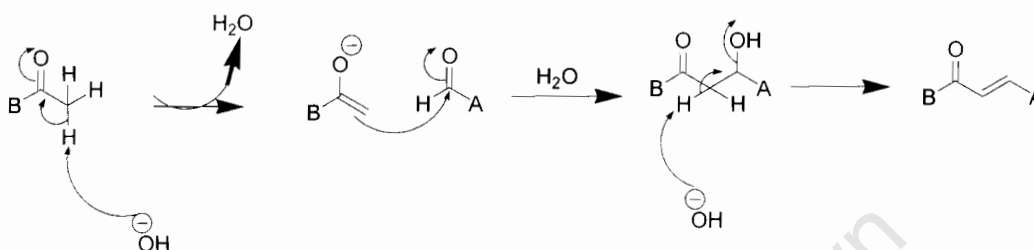
The synthesis of chalcone derivatives is based on a simple procedure, the Claisen-Schmidt condensation reaction. A substituted aldehyde and an aromatic methyl ketone are condensed under base-catalyzed (NaOH or NaOMe) conditions, in methanol or ethanol at room temperature (Scheme 17).^{83,90} Co-solvents were used in cases where the starting materials are insoluble in methanol or ethanol. A minimum amount of methanol or ethanol must be used to ensure formation of a solid product, and the products are usually obtained as yellow precipitates. The corresponding structures of the products were readily characterized with the following spectroscopic techniques; ¹H-NMR, ¹³C-NMR, MS, IR spectroscopy and elemental analysis.



Scheme 17: Reagents and conditions: NaOH or NaOMe; MeOH or EtOH; RT; 24hrs

Mechanism of reaction

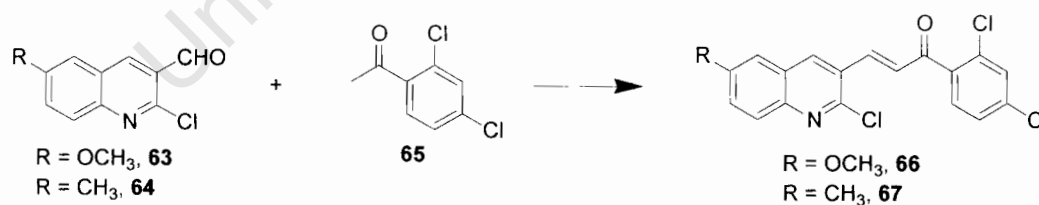
The mechanism involves base-mediated enolization of the methyl ketone followed by reaction of the resulting enolate with the aldehyde in a classical aldol reaction. The resulting β -hydroxy ketone then undergoes a base-mediated elimination to deliver the chalcone. The driving force for this elimination is the formation of a more stable conjugated chalcone system (Scheme 18).



Scheme 18: Mechanism of chalcone synthesis

Synthesis and characterization of target chalcone analogues

The chalcone analogues **66** and **67** were prepared from the Claisen-Schmidt condensation reaction using the quinolinecarboxaldehydes **63** and **64** with 2,4-dichloroacetophenone **65** using sodium methoxide as a base in methanol at room temperature (Scheme 19). The formation of the product was observed immediately after the addition of sodium methoxide to the stirred solution of aldehyde and methyl ketone, however the reactions took 24 hours to go to completion. The chalcone analogues **66** and **67** were obtained in 85 % and 87 % respectively.

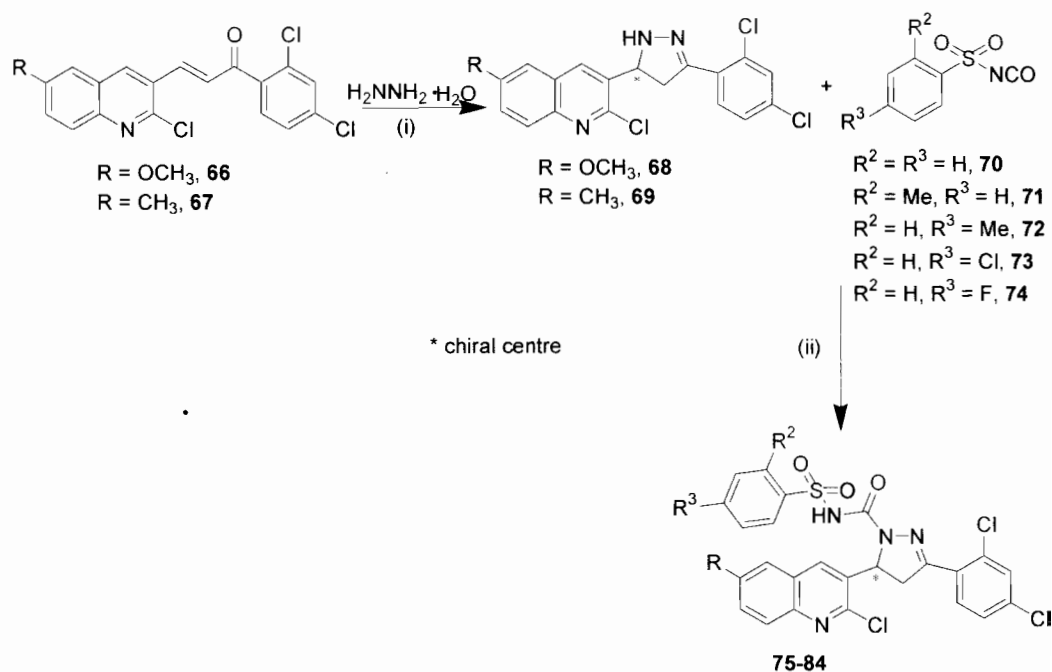


Scheme 19: Reagents and Conditions: NaOMe (1.0 equiv.), MeOH, RT, 24 hrs, 85-87 %.

The $^1\text{H-NMR}$ spectra confirmed the structures of the products. A noticeable change from the starting materials is the disappearance of the aldehyde and methyl ketone chemical shifts, which appear as singlets around 9.89-10.3 ppm and 2.5 ppm respectively. These protons are replaced by the α,β -unsaturated ketone linker protons observed as doublets in the region of 7.2-8.1 ppm with coupling constants of 16 Hz. It is evident from these coupling constants that the products formed are predominantly the *trans*-isomers (*E*-form). The rest of the protons appear in their expected regions with their usual coupling constants. Additional support for the structures of the chalcones comes from the $^{13}\text{C-NMR}$ spectra, where the carbonyl carbon (C=O) of the α,β -unsaturated ketone linker is observed in the region 191 ppm, compared to the aldehyde carbonyl group (C=O) at 195 ppm and methyl ketone carbonyl group (C=O) which appears in the region 179 ppm. Their molecular weights are confirmed by the molecular ions observed in the mass spectra.

Synthesis and characterization of target Pyrazoline derivatives

The chalcone analogues **66** and **67** were treated with hydrazine hydrate in ethanol under reflux, to obtain the pyrazoline products **68** and **69** as white powders in 54 % and 83 % yields respectively. The resulting pyrazolines **68** and **69** were then condensed with substituted aromatic sulfonyl isocyanates **70-74** in THF at room temperature to produce a class of *N*-substituted pyrazole sulfonyl ureas **75-84** as white powders in moderate to good yields as well.⁹⁴⁻⁹⁶ The yields for compounds **75**, **78**, and **83** were very low, and this could be partly due to the partial self-condensation of the aromatic sulfonyl isocyanates. This postulated self-condensation involves decomposition of the corresponding sulfonyl isocyanate to the corresponding sulfonamide via loss of CO_2 in the presence of water, if the conditions are not strictly anhydrous. The resulting primary sulfonamide could then react with another molecule of the sulfonyl isocyanate. The synthesis of the target pyrazolines is illustrated in scheme 20.



Scheme 20: Reagents and conditions: (i) EtOH, reflux, 12 hrs; (ii) THF, RT, 3hrs

Table 5: Chalcones and Pyrazoline derivatives with their respective yields and melting points

Cmpd	R	R ²	R ³	%Yield	mp (°C)
75	OCH ₃	H	H	38	229-230
76	CH ₃	H	H	57	217-220
77	OCH ₃	CH ₃	H	85	213-215
78	CH ₃	CH ₃	H	12	213-214
79	OCH ₃	H	CH ₃	57	219-222
80	CH ₃	H	CH ₃	64	217-219
81	OCH ₃	H	Cl	60	235-236
82	CH ₃	H	Cl	52	233-234
83	OCH ₃	H	F	9	226-227
84	CH ₃	H	F	74	219-221

The ¹H-NMR spectra confirmed the structures of the products. The formation of the pyrazoline analogues **68** and **69** was first recognized by the disappearance of the α,β-unsaturated ketone linker protons, which appear as doublets around 7.2-8.1 ppm with their coupling constants of 15-16 Hz. These were replaced by the pyrazoline ring

protons, with the chiral centre proton (*H-15*) appearing as a ddd (coupling to *NH* and 2x *H-16*) at 5.4 ppm and the other two protons at the adjacent carbon observed at 3.9 and 3.13 ppm. Further evidence from the ^{13}C -NMR spectra was observed from the disappearance of the carbonyl carbon ($\text{C}=\text{O}$) chemical shift in the region 187-192 ppm. The molecular ions observed in the mass spectra confirm the molecular weights of the pyrazoline derivatives formed. Elemental analyses confirmed the molecular formulae and the purity of the compounds.

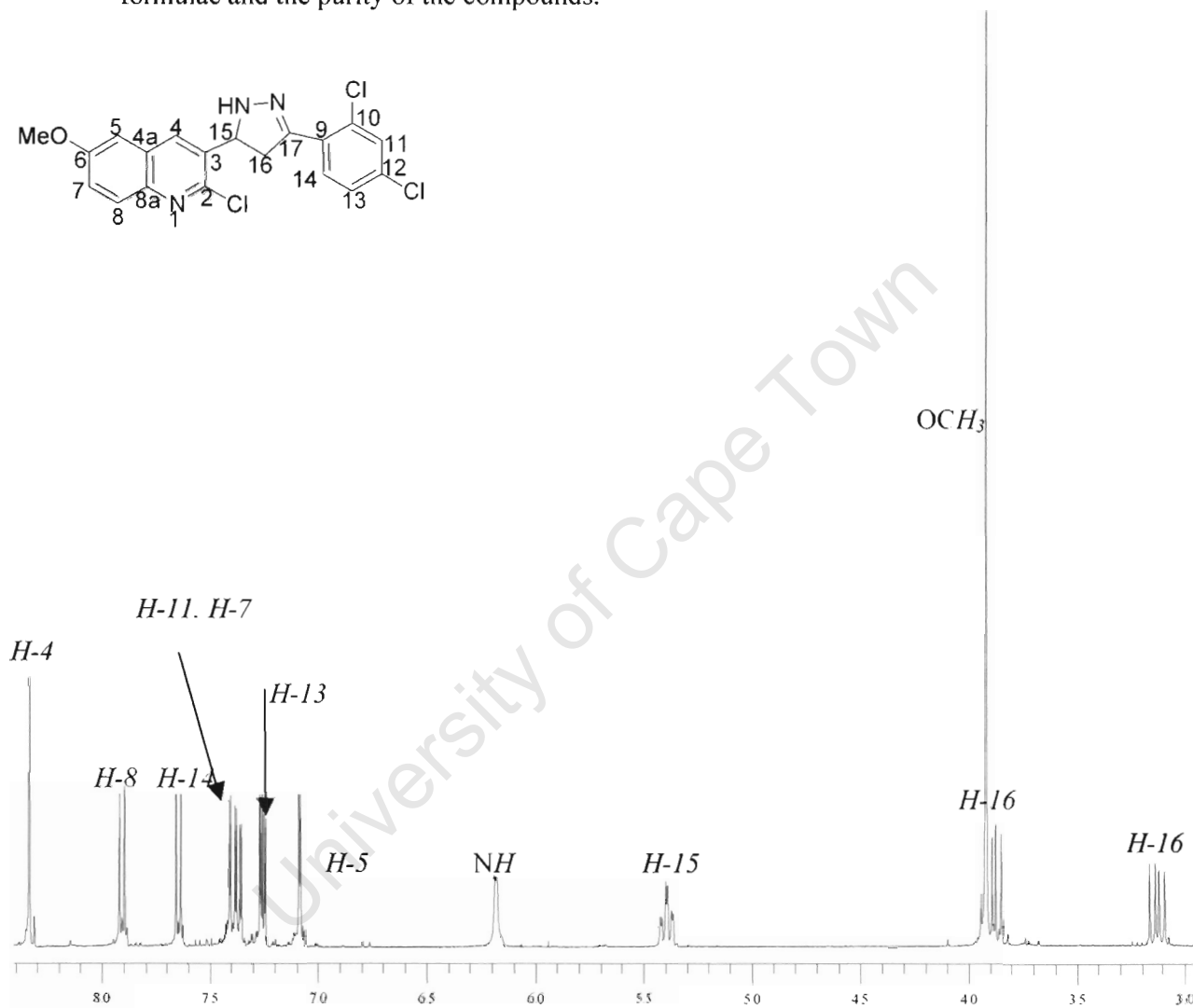


Figure 15: ^1H -NMR spectrum of compound **68** in CDCl_3 at 300 MHz

The structures of *N'*-substituted pyrazoles **75-84** were confirmed by the slight downfield shift of the chiral centre proton (*H-15*) and the protons on the adjacent carbon in the ¹H-NMR spectra. This shift is due to the electron-withdrawing ability of the sulfonyl urea group attached to one of the nitrogen atoms of the pyrazoline ring. The chiral centre proton shifted slightly from 5.4 ppm to 5.82 ppm, while on the other hand the protons on the adjacent carbon shifted slightly from 3.9 and 3.13 ppm to 4.16 and 3.34 ppm respectively. In the sulfonyl ureas *H-15* appears as a dd (coupling to 2x *H-16*) confirming NH substitution.

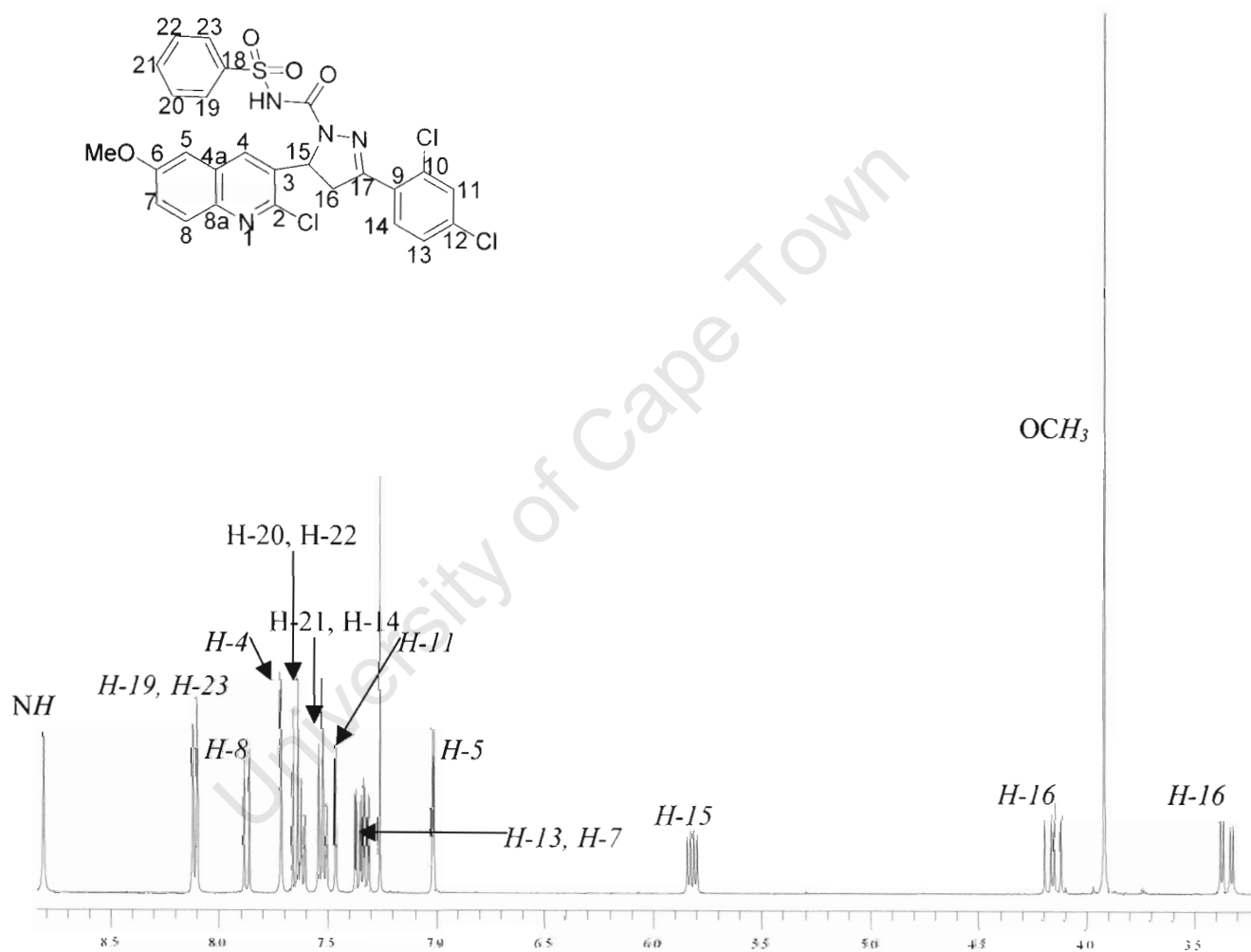
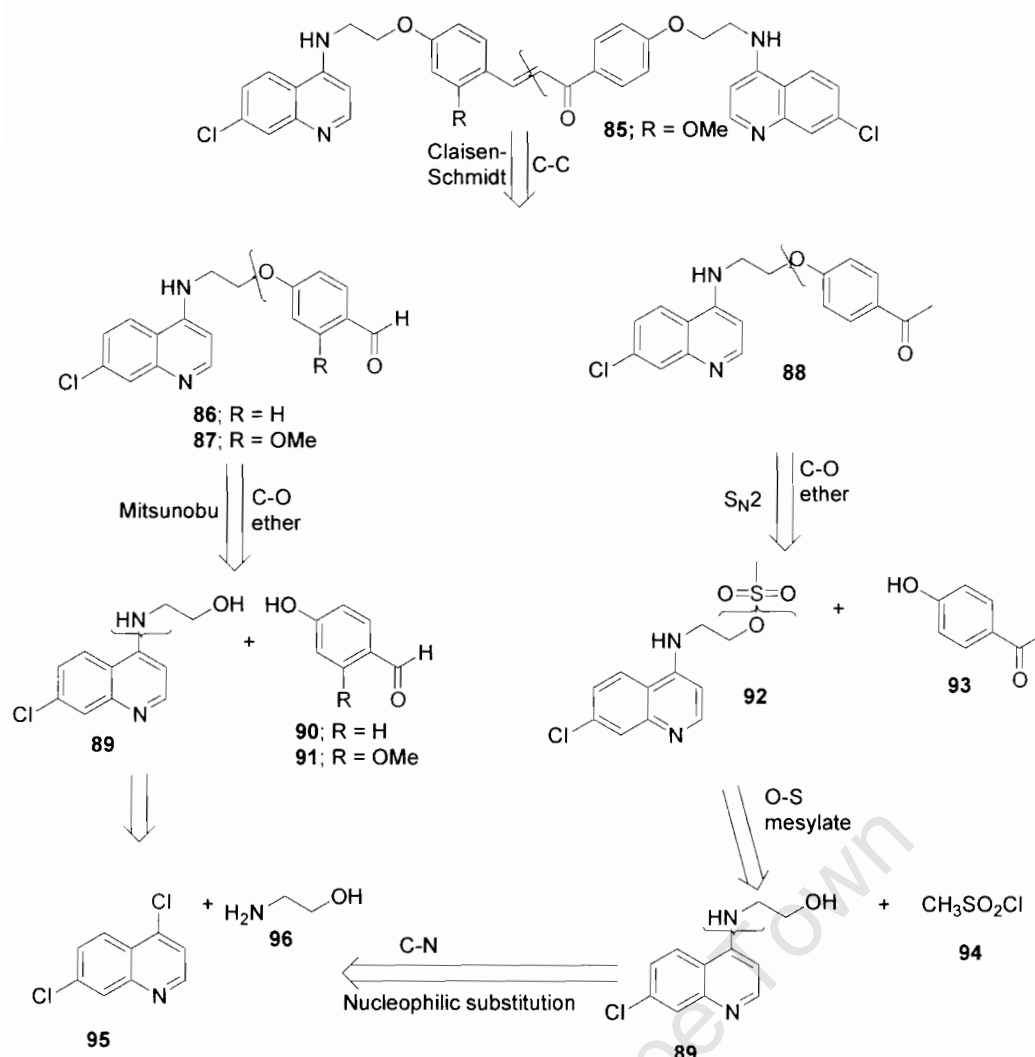


Figure 16: ¹H-NMR spectrum of compound **75** in CDCl₃ at 400 MHz

Alternative synthesis of a potent Bisquinoline chalcone and its analogue

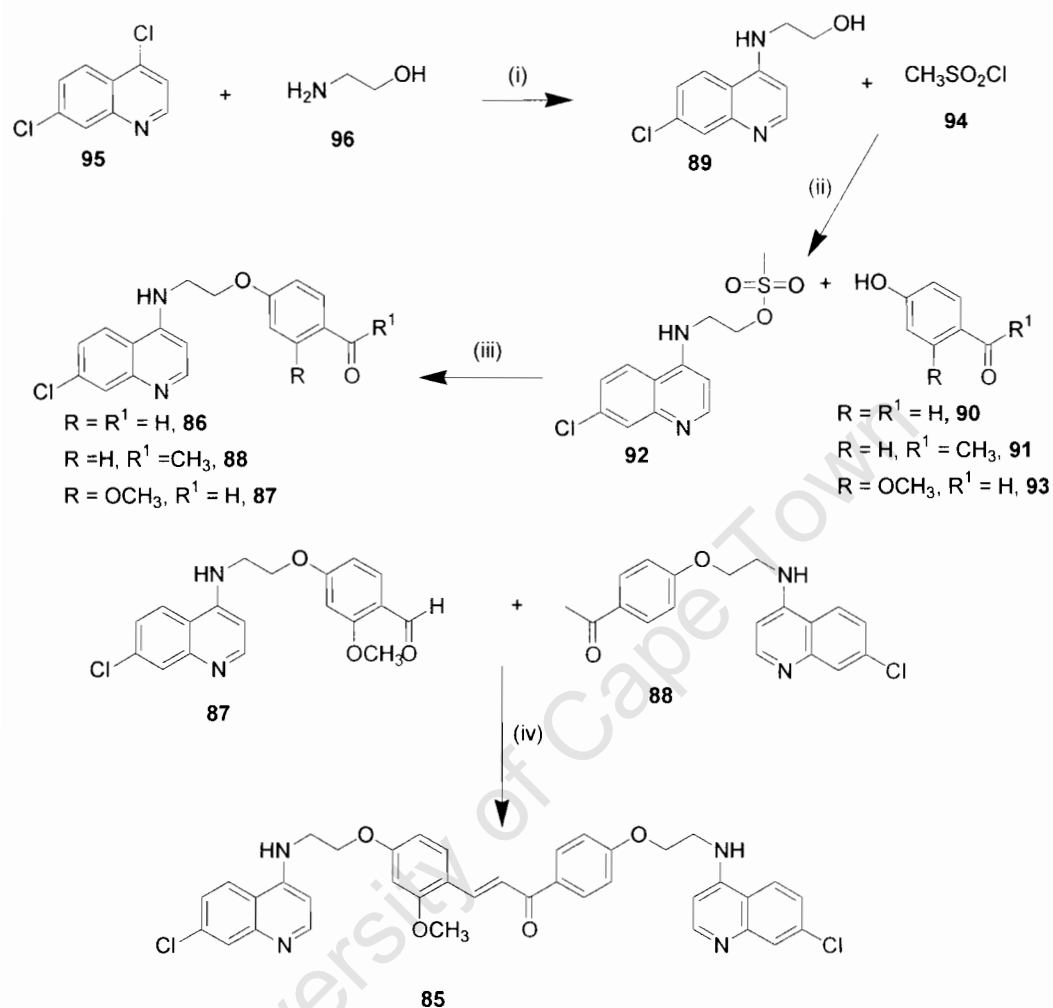
Bisquinoline chalcone **85** was previously designed as part of a bitherapeutic strategy in which a haem-binding (bis)quinoline moiety is attached to an electrophilic linker in such a way that the two quinoline moieties are placed on either side of the electrophilic linker. The electrophilic α,β -unsaturated linker was expected to inhibit falcipain-2 by alkylation of the protease active site thiol. The low nanomolar ($IC_{50} = 7$ nM) potency of chalcone **85** against a chloroquine resistant W2 strain is noteworthy (M. Gamildien; E. Sickle; K. Chibale: unpublished data). This compound also inhibits the *Plasmodium falciparum* cysteine protease falcipain-2 ($IC_{50} = 3.4$ μ M). We decided to explore an alternative synthesis with a view to improving the yield of compound **85**, which would in turn allow preparation of sufficient quantities for *in vivo* (animal) experiments. The previous synthesis utilized Mitsunobu chemistry¹⁰⁷⁻¹¹¹ (Ph_3P , DEAD, Et_3N) as the key step (M. Gamildien; E. Sickle; K. Chibale: unpublished data). Unfortunately this approach resulted in poor yields for the Mitsunobu key step. As an alternative protocol, we decided to use standard S_N2 chemistry to construct the required ether bonds. The retrosynthetic analyses of both the Mitsunobu as well as S_N2 are summarized in scheme 21. Both analyses require key intermediate **89** which was readily prepared from the commercially available 4,7-dichloroquinoline and ethanolamine, *via* the well known nucleophilic substitution reaction.



Scheme 21: Retrosynthetic analysis of compound **85**

The intermediate alcohol **89** was prepared from the neat nucleophilic substitution reaction of 4,7-dichloroquinoline **95** with ethanolamine **96** under reflux for 5 hours.¹¹² Triethylamine (0.3 equiv.) and anhydrous potassium carbonate (0.3 equiv.) were used to facilitate the reaction. The product **89** was obtained in 99 % yield as an off-white powder. The alcohol **89** was then converted into mesylate **92** via another nucleophilic substitution reaction with methanesulfonyl chloride **94** in pyridine at 0 °C.^{113,114} The mesylate **92** was obtained in 71 % yield as a white powder after recrystallization. The resulting mesylate **92** was then reacted with 4'-hydroxybenzaldehyde **90**, 4'-hydroxyacetophenone **91** and 4'-hydroxy-2-methoxybenzaldehyde **93** in DMF under reflux, to yield the compounds **86-88** in 53 %, 84 % and 58 % yields, respectively as white powders. Aldehyde **87** and methyl ketone **88** were then condensed with sodium

hydroxide as a catalyst in methanol (Claisen-Schmidt condensation). A minimum amount of THF (co-solvent) was used because the starting materials were insoluble in methanol. The chalcone **85** was obtained as a bright yellow powder after column chromatography, in 38 % yield (scheme 22). The new synthetic procedure employed was not better than the old one, because it did not improve the yield of the final chalcone analogue **85**. However, the number of purification steps was reduced, because there was no need to purify the intermediate analogues **86-88** by column chromatography as was the case with the old procedure.

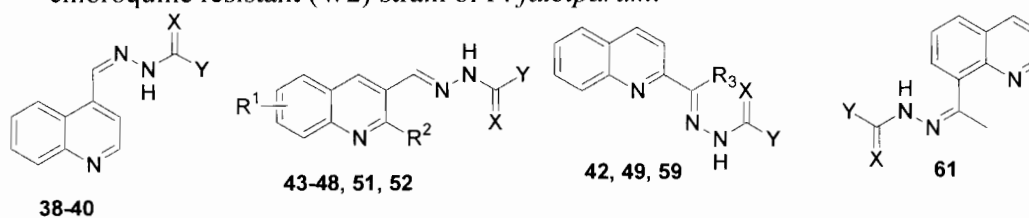



Scheme 22: Reagents and conditions: (i) Et₃N (0.3 equiv.), K₂CO₃ (0.3 equiv.), reflux, 5hrs, 99%; (ii) Pyridine, 0 °C, 5 hrs, 71%; (iii) K₂CO₃ (1.1 equiv.), DMF, reflux, 58%, 53% and 84% respectively; (iv) NaOH (1.0 equiv.), MeOH (1% THF), RT, 24 hrs, 38%

CHAPTER 4
BIOLOGICAL RESULTS AND DISCUSSION

4.1 Data against Cysteine proteases (Falcipain-2 , Cruzain, Rhodesain), and chloroquine resistant W2 strain of *P. falciparum*

Table 6: IC₅₀ values of selected thiosemicarbazones against Falcipain and chloroquine resistant (W2) strain of *P. falciparum*.



Cmpd	X	Y	R ¹	R ²	R ³	Falcipain IC ₅₀ (μM)	W2 IC ₅₀ (μM)
38	S	NH ₂	-	-	-	>20	>10
39	S	SCH ₃	-	-	-	>20	>10
40	O	NH ₂	-	-	-	>20	>10
42	S	NH ₂	-	-	H	>20	>10
43	S	SCH ₃	H	H	-	14.17	>10
44	S	NH ₂	6- OMe	Cl	-	16.70	>10
45	O	NH ₂	6- OMe	Cl	-	>20	>10
46	S	NH ₂	6-Me	Cl	-	>20	>10
47	O	NH ₂	6-Me	Cl	-	>20	>10
48	S	NH ₂	8-Me	Cl	-	18.11	>10
49	S	NH(CH ₂) ₃ NMe ₂	-	-	H	>20	7.752
51	S		H	H	-	>20	>10
52	S	NH(CH ₂) ₃ NMe ₂	H	H	-	>20	3.46
59	S	NH ₂	-	-	CH ₃	16.18	2.46
61	S	NH ₂	-	-	-	>20	>10

4.1.1 Thiosemicarbazones: Falcipain-2 and *P. falciparum* SAR (Table 6)

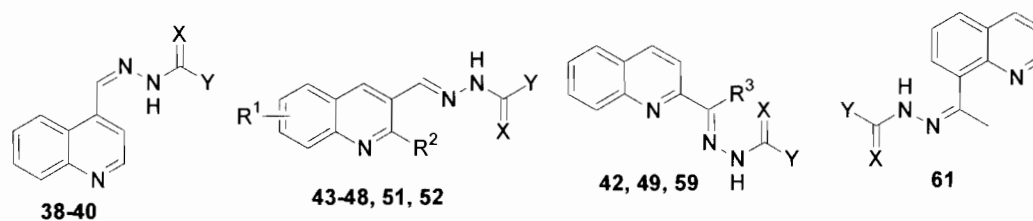
Discussion of results

Most compounds failed to inhibit the target enzyme falcipain-2 at the set concentration limit of 20 μM . The exceptions (**43**, **44**, **48** and **59**) were weak inhibitors. Of these, only **59** showed modest efficacy against the chloroquine resistant W2 strain. Although comparison to chloroquine, a 4-aminoquinoline structurally different from the compounds under study is not appropriate, it is noteworthy that the IC_{50} of chloroquine in the same strain is around 0.3 μM . The other compounds (**49** and **52**) with some weak or modest efficacy against the W2 strain failed to inhibit the target enzyme at 20 μM . There was generally no correlation between the ability of a compound to inhibit falcipain-2 and antiparasmodial activity against W2 *in vitro*. This suggests that falcipain-2 is not the target of these compounds. Compounds (**49**, **52** and **59**) with modest antimalarial activity against W2 maybe acting *via* a protease-independent mechanism. It is also possible that compounds **49**, **52** and **59** may be acting through inhibition of another cysteine protease not contained in our screen. This is because there are many cysteine proteases in *Plasmodium falciparum* some of which have not been fully identified and characterized. Given that these compounds have the ability to chelate a transition metal and it is likely that this mechanism of action is responsible for the observed modest antimalarial activity.

4.1.2 Thiosemicarbazones: Cruzain and Rhodesain SAR (Table 7)

Discussion of Results

The data presented in Table 7 partly indicates activity of the enzyme (cruzain or rhodesain) in the presence of 10 μM of the compounds. If a compound is an effective inhibitor, the enzyme activity should be reduced in the presence of such an inhibitor at a given concentration. Relatively speaking, low % enzyme activity values imply inhibition of the enzyme. Consequently, IC_{50} values were generally only determined in those cases where enzyme activity was reduced to 50 % or less.

Table 7: % Activity of Cruzain and Rhodesain in the presence of 10 μM of selected thiosemicarbazones

Cmpd	X	Y	R ¹	R ²	R ³	Cruzain % Activity (10 μM)	Cruzain IC ₅₀ (μM)	Rhodesain % Activity (10 μM)	Rhodesain IC ₅₀ (μM)
38	S	NH ₂	-	-	-	25	0.906	4	0.4
39	S	SCH ₃	-	-	-	100	ND ^a	89	ND
40	O	NH ₂	-	-	-	95	ND	91	ND
42	S	NH ₂	-	-	H	65	ND	38	5
43	S	SCH ₃	H	H	-	88	ND	87	ND
44	S	NH ₂	6- OMe	Cl	-	89	ND	18	9
45	O	NH ₂	6- OMe	Cl	-	97	ND	94	ND
46	S	NH ₂	6-Me	Cl	-	100	ND	10	-
47	O	NH ₂	6-Me	Cl	-	81	ND	92	ND
48	S	NH ₂	8-Me	Cl	-	40	0.806	4	5
49	S	NH(CH ₂) ₃ NMe ₂	-	-	H	85	ND	81	ND
51	S	ⁿ	H	H	-	99	ND	93	ND
52	S	NH(CH ₂) ₃ NMe ₂	H	H	-	95	ND	83	ND
59	S	NH ₂	-	-	CH ₃	37	4.75	0	0.8
61	S	NH ₂	-	-	-	94	ND	68	ND

^a Not determined

Compared to falcipain-2, the SAR data against cruzain and rhodesain is striking in that a number of compounds were potent in the low micromolar range. In this regard, compounds **38**, **48** and **59** are noteworthy. Against cruzain, compounds **38** and **48** were the most effective. These compounds are structurally different and direct comparison is not quite appropriate. In comparing activity against cruzain, it is necessary to look at compounds from the same structural series. Compounds **38**, **39** and **40** are 4-substituted quinolines. From this series, the most potent compound (**38**) is a thiosemicarbazone. The corresponding thioester **39** was essentially inactive. The poor activity with **40**, a semicarbazone underscores the importance of the thiosemicarbazone moiety compared to the corresponding semicarbazone moiety in the inhibition of cruzain. This result is consistent with earlier findings by Du and coworkers.⁴⁹ Although 3-substituted quinolines are missing from these preliminary SAR studies against cruzain, a comparison between compound **38** (4-substituted quinoline) and compound **42** (2-substituted quinoline) suggests that the 4-position in the quinoline nucleus is a favorable position against cruzain.

Against rhodesain, many more compounds were effective as compared to cruzain although the basis SAR was similar. Compounds **38**, **42**, **44**, **48** and **59** were effective against rhodesain in the low to mid micromolar range. As with cruzain, the most effective compound (**38**) is a 4-substituted quinoline. This once again indicates that a thiosemicarbazone moiety at this position may be important for binding to cysteine proteases within a series of substituted quinolines. Consistent with observations by Du and coworkers⁴⁹, N1 NH₂-substitution was found to lower potency against cruzain for thiosemicarbazones. The same trend was observed for rhodesain in this study. This fact is highlighted by the results obtained from compounds **42** and **49**. Previous studies on unrelated aromatic thiosemicarbazones have highlighted the importance of the C=S double bond of the thiosemicarbazones in the inhibition of cruzain.⁴⁹ In our study, this was indeed also confirmed against both cruzain and rhodesain when comparing thiosemicarbazones and corresponding semicarbazones (**38** vs **40**; **44** vs **45**; **46** vs **47**). The effect of the substituents at position 5 (R³ at the imine carbon (C=N)) was most evident when comparing compounds **42** and **59**. The compound (**59**) with a methyl substituent was more potent than the compound (**42**) without this substituent. Once again this result is consistent with data reported for the unrelated non-quinoline based aromatic thiosemicarbazones.⁴⁹

Within the limited series of compounds, preliminary SAR data against the protease targets gives some insight into the significant differences in the active site topology between all three (falcipain-2, cruzain and rhodesain) related target proteases. On one hand more inhibitors of rhodesain were identified while on the other hand there were no (as) effective inhibitors of falcipain-2. This may suggest that rhodesain is a promiscuous parasite protease which accommodates a broad range of inhibitors. Nevertheless it is clear based on the data against cruzain and rhodesain that quinoline-based thiosemicarbazones can bind to a cysteine protease target. These findings are in agreement with those by Greenbaum et al.⁵⁷ The mode of inhibition maybe by reversible alkylation of the protease active site cysteine thiol requiring nucleophilic addition to either the imine (C=N) carbon or the thiocarbonyl (C=S) group of the thiosemicarbazone moiety. Alternatively the mode of inhibition may be metal-interactive inhibition in which the protease active site thiol (in the thiolate form) coordinates to the metal complex formed between the thiosemicarbazone moiety and endogenous metals such as Fe²⁺/Fe³⁺, Cu²⁺, Zn²⁺ and Cd²⁺.

In this preliminary study of quinoline thiosemicarbazones, we endeavored to expand SAR of thiosemicarbazones against parasite cysteine proteases and hence building on work conducted in our laboratories.^{55,57} Although the focus of the work was on *Plasmodium falciparum* and falcipain-2 from this parasite, we also screened exploratory compounds against two other parasitic cysteine proteases, cruzain and rhodesain. In so doing we were not only able to analyze SAR across three related parasitic cysteine proteases, but we also identified a novel scaffold (compound **59**) that exemplifies a more broadly acting scaffold capable of inhibiting cysteine proteases from multiple protozoan parasites. Incidentally this scaffold is capable of tridentate metal chelation *via* the sulfur, hydrazinic and quinoline nitrogen atoms (figure 17). This compound may be acting as a tridentate metal-interactive cysteine protease inhibitor. As far as inhibiting the growth of *Plasmodium falciparum* is concerned, compound **59** may be acting as an iron chelator in *P. falciparum*. Tridentate chelating thiosemicarbazones have been shown to inhibit ribonucleotide reductase, an enzyme essential for DNA synthesis.^{115,116} Lastly, evidence exists that suggests that copper complexes of thiosemicarbazones produce significant oxidative stress by binding endogenous reducing agents such as glutathione. Ultimately, we think that these biological results should encourage the screening of not only the best

inhibitors of both cruzain and rhodesain against the parasite sources, *T. cruzi* and *T. brucei*, but also the ineffective compounds. This is because the combined data may reveal hidden target complexity as has been recently reported.⁵⁷

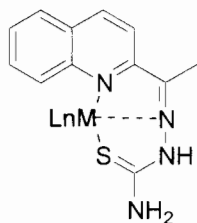


Figure 17: Tridentate metal chelation of compound **59**; M = metal; Ln = ligands

4.1.3 Compliance to Lipinski's Rule

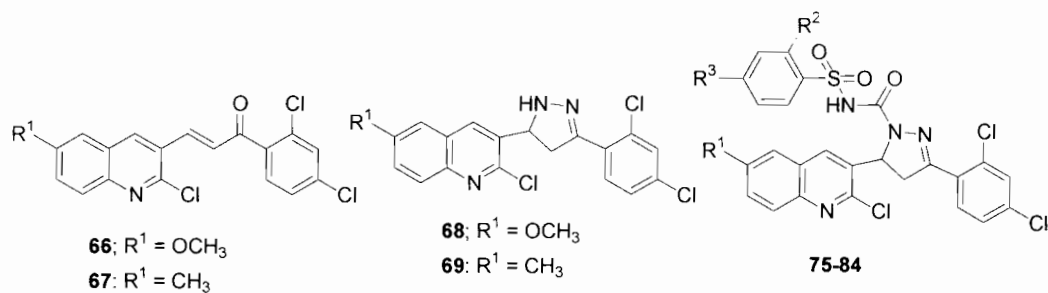
As earlier mentioned factors that have been considered as a primary guide to correlating physical properties with successful drug development by previous researchers are; lipophilicity, molecular weight, and the number of hydrogen bond donors and acceptors.¹⁰²⁻¹⁰⁴ Lipinski's "rule of 5" states that for an oral drug candidate to have good absorption and permeability the following criteria should be met:

- i) Molecular weight < 500
- ii) CLogP < 5
- iii) Number of H-bond acceptors (expressed as the sum of nitrogen atoms and oxygen atoms) ≤ 10 .
- iv) Number of H-bond donors (expressed as the sum of N-H and O-H) ≤ 5 .

Most of these thiosemicarbazones meet these criteria, and any drug-like compound which meets any three of the four criteria set by Lipinski has potential to be developed as an oral drug. However, only the most potent compounds in the series of thiosemicarbazones should be regarded in this case.

4.2 Data against the chloroquine sensitive strain (D10) of *Plasmodium falciparum* Pyrazoline Analogues of Chalcones

Table 8. Percentage parasite viability against *P. falciparum* D10



Cmpd	R ¹	R ²	R ³	1000 ng/mL	500 ng/mL	250 ng/mL
66	OCH ₃	-	-	85.67	78.41	86.09
67	CH ₃	-	-	77.74	80.16	84.88
68	OCH ₃	-	-	84.53	92.77	87.93
69	CH ₃	-	-	62.17	80.49	84.88
75	OCH ₃	H	H	85.74	91.87	85.49
76	CH ₃	H	H	86.99	108.64	55.76
77	OCH ₃	CH ₃	H	80.52	86.05	76.55
78	CH ₃	CH ₃	H	85.21	100.58	63.39
79	OCH ₃	H	CH ₃	90.78	98.35	82.63
80	CH ₃	H	CH ₃	68.18	80.20	78.68
81	OCH ₃	H	Cl	69.62	77.11	82.77
82	CH ₃	H	Cl	88.29	109.67	58.40
83	OCH ₃	H	F	85.13	67.26	74.24
84	CH ₃	H	F	86.61	71.02	81.18

4.2.1 Pyrazoline Analogues of Chalcones

Discussion of Results

Since one of the initial aims of the project was to conduct a preliminary comparative study of chalcones and corresponding unsubstituted and *N*-substituted sulfonyl urea derivatives, the IC₅₀ values for each compound were not determined. Instead the effect of each compound on malarial parasite viability (survival) at three different concentrations was studied. The results are presented in Table 8 above. As can be seen from the table, all compounds were generally not effective at the tested concentrations. At any given concentration, the % parasite viability should be substantially low for an effective antiplasmodial agent. Besides the general lack of potency of all derivatives, interpretation of the data was made much more difficult by the inconsistent data obtained from the biological testing. The expected trend was to be such that the % parasite viability should increase with decrease in the concentration of each compound. This expected trend was observed in only a few cases for compounds **67**, **69** and **81**. The inconsistent biological data casts doubt on the validity of such data. These experiments need to be repeated and more consistent results obtained.

Although the manner in which the compounds may have been handled at the biological testing site is not known, instability and/or precipitation of the compounds under the test conditions cannot be ruled out and need thorough investigation in future. However, suffices to conclude that all compounds were too inactive to permit meaningful comparisons at a given concentration.

4.3 CONCLUSION

Most of the thiosemicarbazones were not potent against falcipain-2 and the W2 strain of *P. falciparum*, but a few compounds (**43**, **44**, **48** and **59**) were weak inhibitors with compound **59** being the most effective against the chloroquine resistant W2 strain. However, various potential inhibitors of cruzain and rhodesain have been identified in this study. Compounds **38** and **48** were the most effective against cruzain while there were more compounds (**38**, **42**, **44**, **48** and **59**) that were effective against rhodesain. Comparison within each series of compounds has shown that the thiosemicarbazones are the most potent against both cruzain

and rhodesain. Though comparison between the three series is inappropriate, the data shows that the 4-position in the quinoline nucleus is a favorable position against both cruzain and rhodesain. Therefore, future work should include evaluation of this 4-substituted quinoline compound against the causative agents, *T. cruzi* and *T. brucei*. Furthermore SAR studies on the 4-substituted quinoline compound should be carried out. These SAR studies should include the variation of the group attached to C-5 (C=N) of the thiosemicarbazone moiety. Substitution of alkyl groups on the C-5 carbon could enhance the activity of this compound against all three cysteine proteases (cruzain, falcipain-2 and rhodesain), the result of which is observed when comparing compounds **42** and **59**. As alluded to earlier, compounds that combine pharmacological activities may be advantageous or disadvantageous. In the case of thiosemicarbazones, the combination of pharmacological activities against the cysteine proteases (cruzain, falcipain-2 and rhodesain) is advantageous.

The pyrazoline analogues of chalcones were generally not effective at the tested concentrations. In addition the data obtained is inconsistent to make any meaningful interpretation.

CHAPTER 5

EXPERIMENTAL

5.1 General

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded at ambient temperature using the following instruments: Varian Mercury (300MHz) or Varian Unity Spectrometer (400MHz) and TMS was used as an internal standard. The chemical shifts (δ) are given in ppm relative to TMS ($\delta = 0.00$).

Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 75MHz or 100MHz with the same internal standard. Diverse solvents were used in the determination of spectra for different compounds. The chemical shifts (δ) are given in ppm relative to TMS ($\delta = 0.00$).

The following abbreviations were used in the ^1H NMR spectra: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; q, quartet; m, multiplet; and J , coupling constant.

Mass spectra were recorded by means of a VG micromass 16 F spectrometer at 70eV with accelerating voltage 4kV. Accurate masses were determined using a VG-70E spectrometer (at Cape Technikon, South Africa) and VG (Micromass) 70-SE magnetic sector mass spectrometer (at Kent, UK). Other mass spectra were recorded by means of micromass 70-70E double focusing magnetic sector mass spectrometer fitted with an Ion Tech B11N saddle field FAB gun (Ion Tech Ltd, Teddington, UK) and a VG11-250J Data system and micromass Autospec-TOF double focusing magnetic sector mass spectrometer (at University of Potchefstroom, South Africa).

Infrared spectra were measured either in solution form using chloroform or as solids (KBr pellets) or Nujol mulls on a satellite FT-IR spectrophotometer.

Micro (elemental) analysis was performed using a Fisons EA 1108 CHNS-O instrument.

Melting points were determined by using a Reicher-Jung Thermovar (temperature range 0-350 °C) on cover slips and are uncorrected.

Column chromatography and preparative layer chromatography (p.l.c), carried out on silica gel (Merck Kieselgel 60) were used in purification of samples.

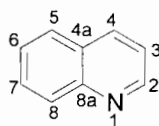
Reactions were monitored by thin-layer chromatography (tlc) using coated silica gel plates, detection by an ultra-violet lamp.

Commonly used solvents were purified and dried accordingly as described in the literature.

Anhydrous sodium sulphate and anhydrous magnesium sulphate were used in the drying of organic solvents after extraction.

Concentration of organic phase (removal of solvents) was achieved by using a Buchi Rotary Evaporator under reduced pressure.

Below is the general numbering system that is used for the quinoline ring system.



5.2 Biology

5.2.1 Thiosemicarbazones

Cruzain and Rhodesain

Recombinant cruzain (from *T. cruzi*) and rhodesain (from *T. brucei rhodesiense*) were recombinantly expressed as previously described by Du and coworkers.⁴⁹ Cruzain (2 nM) and rhodesain (3 nM) were incubated with 0.1-20 μ M inhibitor in 100 mM sodium acetate, pH 5.5 containing 5 mM DTT buffer, for 5 minutes at room temperature. The buffer containing Z-Phe-Arg-AMC (Bachem, $K_m = 1 \mu$ M) was added to the enzyme-inhibitor reaction to give a 20 μ M substrate concentration. The increase in fluorescence (excitation at 355 nM and emission at 460 nM) was followed with an automated microtitre plate spectrofluorimeter (Molecular Devices, spectraMAX Gemini). Inhibitor stock solutions were prepared at 20 mM in DMSO, and serial dilutions were made in DMSO (0.7 % DMSO in assay). Controls were performed using enzyme alone, and enzyme with DMSO. IC_{50} values were determined graphically using inhibitor concentrations in the linear portion of the plot of inhibition versus log [I] (seven concentrations were tested, and at least two were in the linear range).

Percentage activity of the cysteine proteases (cruzain and rhodesain) were determined at 10 μ M concentrations of the inhibitor. IC_{50} values were determined for only the compounds that had less than 50 % activity of the cysteine proteases (cruzain and rhodesain).

IC_{50} values against recombinant falcipain-2 was determined as previously described by Rosenthal and coworkers.¹¹⁷ The enzyme (falcipain-2) was incubated for 30 minutes at room temperature in 100 mM sodium acetate, pH 5.5 containing 10 mM DTT buffer with different concentrations of tested inhibitors. Inhibitor solutions were prepared from stock in DMSO (maximum concentration of DMSO in the assay was 1 %). After 30 minutes of incubation, the substrate Z-Leu-Arg-AMC (benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin) in the same buffer was added to a final concentration of 25 μ M. Fluorescence was monitored for 15 minutes at room temperature in a Fluroskan Ascent spectrofluorometer (Labsystems). IC_{50} values were determined from plots of percentage of activity

over compound concentration using data analysis program Prism (GraphPad software).

***P. falciparum* culture assay**

W2 strain of *P. falciparum* parasites (1 % parasitemia, 2 % hematocrit) were cultured in 0.5 ml of medium in 48-well culture dishes.¹¹⁸ Selected inhibitors from 10 mM stocks in DMSO were added to cultured parasites to a final concentration of 20 μ M. From 48-well plates, 125 μ L of culture was transferred to two 96-well plates (duplicates). Serial dilutions (1:5) of inhibitors were made to final concentrations of 10 μ M, 2 μ M, 400 nM, 80 nM, 16 nM and 3.2 nM. Cultures were maintained at 37 °C for two days. The parasites were washed and fixed with 1 % formaldehyde in PBS. After two days, parasitemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival.

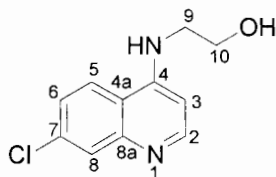
5.2.2 Chalcones and Pyrazoline derivatives

Data against the Chloroquine-sensitive strain of *P. falciparum* (D10)

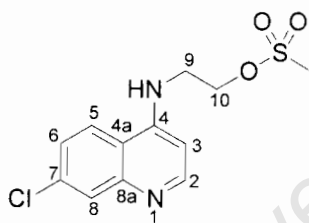
All samples were tested in duplicate on a single occasion against the chloroquine-sensitive strain of *P. falciparum* (D10). Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.¹¹⁸ Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler and coworkers.¹¹⁹ The average percentage parasite viability is determined at three different concentrations.

Samples were stored at -20 °C until use. The pre-weighed samples were first dissolved in 1000 μ L of 100 % DMSO to give a 2 mg/mL concentration each. The samples were then diluted with water to reach the desired concentration of 20 μ g/mL. All samples were further diluted to 2 μ g/mL in complete medium on the day of the experiment. The highest concentration of DMSO to which the parasites were exposed to had no measurable effect on the parasite viability. Chloroquine (CQ) was used as the positive control for all the experiments.

5.3 Chemistry



2-(7-Chloro-quinolin-4-ylamino)-ethanol (89). A solution of 4,7-dichloroquinoline (7.50 g; 37.87 mmol), ethanolamine (46 ml; 757 mmol), triethylamine (1.6 ml; 11.36 mmol) and anhydrous potassium carbonate (1.57 g; 11.36 mmol) was stirred under reflux at 120-140 °C for 5 hrs. The reaction mixture was then cooled to room temperature during which a precipitate formed. It was then filtered, washed with water and recrystallised from methanol to obtain the product **89** (8.34 g; 99 %) as an off-white powder. mp: 216-218 °C (from methanol), R_f (methanol: dichloromethane, 1:9) 0.17; ν_{\max} (KBr)/ cm^{-1} 3618 (O-H), 3305 (N-H), 3029 (C-H; aromatic), 1578 (C=C). δ_H (400 MHz, DMSO- d_6) 8.36 (d, 1H, J 5.4 Hz, H -2), 8.23 (d, 1H, J 8.8 Hz, H -5), 7.76 (d, 1H, J 2.0 Hz, H -8), 7.41 (dd, 1H, J 2.0 and 8.8 Hz, H -6), 7.22 (s, 1H, NH), 6.48 (d, 1H, J 5.4 Hz, H -3), 3.65 (t, 2H, J 5.8 Hz, 2x H -10), 3.34 (t, 2H, J 5.8 Hz, 2x H -9). δ_C (100 MHz, DMSO- d_6) 152.59 (C-2), 150.95 (C-4), 149.82 (C-8a), 134.03 (C-7), 128.19 (C-8), 124.77 (C-6), 124.67 (C-5), 118.18 (C-4a), 99.40 (C-3), 59.48 (C-10), 45.85 (C-9). Anal Found: C, 59.67; H, 4.63; N, 12.58 %. Calc. for $\text{C}_{11}\text{H}_{11}\text{ClN}_2\text{O}$: C, 59.33; H, 4.98; N, 12.58 %. Calc. for $\text{C}_{11}\text{H}_{11}\text{ClN}_2\text{O}$; m/z 222.7. Found MS (FAB) m/z 223.0 (M+1)⁺.

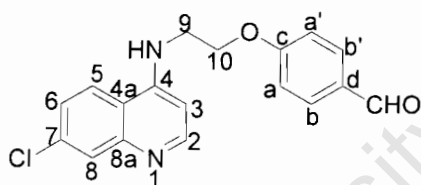


(7-Chloro-quinolin-4-yl)-(3-methanesulfonylpropyl)amine (92). A solution of compound **89** (2.0 g; 9.0 mmol) in 6ml of pyridine was stirred at 0 °C for 30 min. To the above solution, was added a solution of methanesulfonyl chloride (1.74 ml; 22.52 mmol) in 5.40 ml of pyridine and the reaction mixture was stirred at 0 °C for 5 hrs. It was then diluted with 17% ammonium hydroxide, extracted in dichloromethane, dried over anhydrous MgSO_4 , concentrated in *vacuo* and recrystallized from ethyl acetate-hexane to obtain the product **92** [1.93

g; 71 %] as a white powder. mp: 139-142 °C (from ethyl acetate-hexane), R_f (methanol: dichloromethane, 1:9) 0.27; ν_{\max} (KBr)/ cm^{-1} 3218 (N-H), 1579 (C=C). δ_H (300 MHz, CDCl_3) 8.57 (d, 1H, J 5.4 Hz, $H-2$), 7.98 (d, 1H, J 2.4 Hz, $H-8$), 7.73 (d, 1H, J 8.8 Hz, $H-5$), 7.41 (dd, 1H, J 2.4 and 8.8 Hz, $H-6$), 6.42 (d, 1H, J 5.4 Hz, $H-3$), 4.59 (t, 2H, J 5.7 Hz, 2x $H-10$), 3.70 (t, 2H, J 5.7 Hz, 2x $H-9$), 3.08 (s, 3H, SO_2CH_3). δ_C (75 MHz, CDCl_3) 151.94 (C-2), 149.09 (C-8a, C-4), 135.73 (C-7), 128.92 (C-8), 125.92 (C-6), 121.10 (C-5), 118.54 (C-4a), 99.04 (C-3), 66.80 (C-10), 42.49 (C-9), 37.86 (CH_3). Anal Found: C, 48.07; H, 4.39; N, 9.32; S, 10.60 %. Calc. for $\text{C}_{12}\text{H}_{13}\text{ClN}_2\text{OS}$: C, 47.92; H, 4.36; N, 9.31; S, 10.66 %. Calc. for $\text{C}_{12}\text{H}_{13}\text{ClN}_2\text{OS}$; m/z 300.8. Found MS (FAB) m/z 301.0 ($\text{M}+1$)⁺.

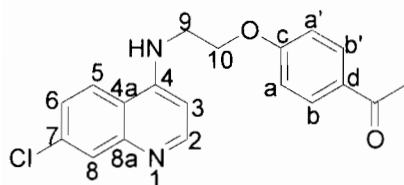
General procedure for the preparation of compounds 86-88

A solution of compound **92** (0.50 g; 1.67 mmol), respective 4-hydroxybenzaldehyde or 4-hydroxyacetophenone (1.67 mmol) and anhydrous potassium carbonate (0.25 g; 1.84 mmol) in 5 ml of DMF was stirred under reflux for 3hrs with TLC monitoring. The reaction mixture was cooled to room temperature, then diluted with distilled water and extracted in ethyl acetate, washed with saturated sodium chloride solution, dried over anhydrous MgSO_4 and concentrated in *vacuo* to afford the desired products.

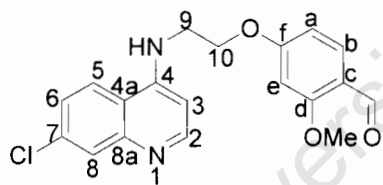


4-[2-(7-Chloro-quinolin-4-ylamino)-ethoxy]-benzaldehyde (86). Yield: 0.32 g; 58 %, obtained as an off-white powder mp: 175-176 °C (from methanol), R_f (methanol: dichloromethane, 1:19) 0.20; ν_{\max} (KBr)/ cm^{-1} 3342 (N-H), 2920 (C-H, aliphatic), 1705 (C=O), 1573 (C=C). δ_H (400 MHz, $\text{DMSO}-d_6$) 9.85 (s, 1H, CHO), 8.41 (d, 1H, J 5.2 Hz, $H-2$), 8.26 (d, 1H, J 9.2 Hz, $H-5$), 7.84 (d, 2H, J 9.0 Hz, H_b, H_b'), 7.78 (d, 1H, J 2.0 Hz, $H-8$), 7.45 (dd, 1H, J 2.0 and 9.2 Hz, $H-6$), 7.14 (d, 2H, J 9.0 Hz, H_a, H_a'), 6.60 (d, 1H, J 5.2 Hz, $H-3$), 4.36 (t, 2H, J 5.6 Hz, 2x $H-10$), 3.72 (t, 2H, J 5.6 Hz, 2x $H-9$). δ_C (100 MHz, $\text{DMSO}-d_6$) 193.98 (CHO), 164.11 (C-c), 152.61 (C-2), 150.74 (C-4), 149.75 (C-8a), 134.16 (C-7), 132.28

(*C-b,b'*), 130.47 (*C-d*), 128.22 (*C-8*), 124.90 (*C-6*), 124.74 (*C-5*), 118.14 (*C-4a*), 115.69 (*C-a,a'*), 99.66 (*C-3*), 66.85 (*C-10*), 42.50 (*C-9*). Anal Found: C, 65.87; H, 4.67; N, 8.05 %. Calc. for $C_{18}H_{15}ClN_2O_2$: C, 66.16; H, 4.63; N, 8.57 %. Calc. for $C_{18}H_{15}ClN_2O_2$; m/z 326.8. Found MS (FAB) m/z 327.1 ($M+1$)⁺.

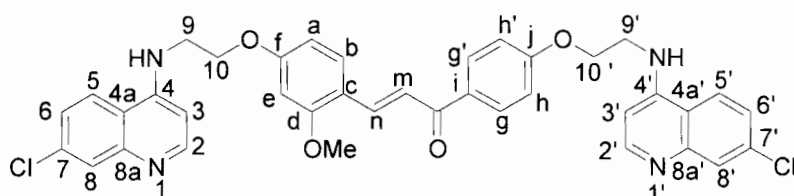


1-[4-[2-(7-Chloro-quinolin-4ylamino)-ethoxy]-phenyl]-ethanone (88). Yield: 0.30 g; 53 %, obtained as off-white powder mp: 173-175 °C (from methanol), R_f (ethyl acetate: hexane, 1:4) 0.18; ν_{max} (KBr)/ cm^{-1} 3382 (N-H), 3200 (C-H, aromatic), 2935 (C-H, aliphatic), 1691 (C=O), 1581 (C=C). δ_H (400 MHz, $CDCl_3$), 8.58 (d, 1H, J 5.2 Hz, $H-2$), 7.99 (d, 1H, J 2.0 Hz, $H-8$), 7.95 (d, 2H, J 9.2 Hz, H_b, H_b'), 7.70 (d, 1H, J 9.2 Hz, $H-5$), 7.39 (dd, 1H, J 2.0 and 9.2 Hz, $H-6$), 6.98 (d, 2H, J 9.2 Hz, H_a, H_a'), 6.51 (d, 1H, J 5.2 Hz, $H-3$), 4.36 (t, 2H, J 5.6 Hz, $2 \times H-10$), 3.78 (t, 2H, J 5.6 Hz, $2 \times H-9$), 2.56 (s, 3H, $ArC=OCH_3$). δ_C (100 MHz, $CDCl_3$) 179.56 (C=O), 162.06 (*C-c*), 151.93 (*C-2*), 149.41 (*C-4*), 149.30 (*C-8a*), 135.17 (*C-7*), 130.68 (*C-b,b'*), 129.71 (*C-d*), 129.67 (*C-8*), 128.92 (*C-6*), 125.71 (*C-5*), 120.84 (*C-4a*), 114.23 (*C-a,a'*), 99.31 (*C-3*), 66.00 (*C-10*), 42.43 (*C-9*), 26.30 (CH_3). Calc. for $C_{19}H_{17}ClN_2O_2$ m/z 340.8. Found MS (FAB) m/z 341.1 ($M+1$)⁺.



4-[2-(7-Chloro-quinolin-4ylamino)-ethoxy]-2-methoxy-benzaldehyde (87). Yield: 0.50 g; 84 %, obtained as an off-white powder. mp: 132-134 °C (from methanol), R_f (methanol: dichloromethane, 1:19) 0.31; ν_{max} (KBr)/ cm^{-1} 3394 (N-H), 3205 (C-H, aromatic), 2945 (C-H, aliphatic), 2884 (C-H, CHO), 1709 (C=O), 1578 (C=C). δ_H (300 MHz, $CDCl_3$) 10.30 (s, 1H, CHO), 8.59 (d, 1H, J 5.4 Hz, $H-2$), 7.99 (d, 1H, J 2.1 Hz, $H-8$), 7.83 (d, 1H, J 9.0 Hz, H_b), 7.70 (d, 1H, J 8.7 Hz, $H-5$), 7.40 (dd, 1H, J 2.1 and 8.7 Hz, $H-6$), 6.58 (d, 1H, J 9.0 Hz,

Ha), 6.50 (m, 2H, *He* and *H-3*), 4.37 (t, 2H, *J* 5.2 Hz, 2x*H-10*), 3.89 (s, 3H, OCH₃), 3.79 (t, 2H, *J* 5.2 Hz, 2x*H-9*). δ_C (75 MHz, CDCl₃) 191.23 (CHO), 164.72 (*C-f*), 163.61 (*C-d*), 152.00 (*C-2*), 151.04 (*C-4*), 149.40 (*C-8a*), 135.14 (*C-7*), 130.86 (*C-b*), 128.96 (*C-8*), 125.70 (*C-6*), 120.94 (*C-5*), 119.68 (*C-4a*), 117.32 (*C-c*), 106.06 (*C-a*), 99.32 (*C-3*), 98.58 (*C-e*), 66.14 (*C-10*), 55.68(OCH₃), 42.44(*C-9*). Anal Found: C, 63.85; H, 5.10; N, 7.43 %. Calc. for C₁₉H₁₇ClN₂O₃: C, 63.96; H, 4.80; N, 7.85 %. Calc for C₁₉H₁₇ClN₂O₃ *m/z* 356.8(M+1)⁺. Found MS (FAB) *m/z* 357.1 (M+1)⁺.



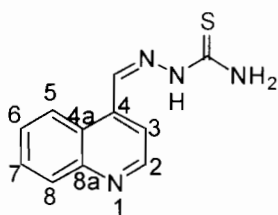
3-{4-[2-(7-Chloro-

quinolin-4-ylamino)-ethoxy]-2-methoxy-phenyl}-1-{4-[2-(7-chloro-quinolin-4-yl)-ethoxy]-phenyl}-propenone (85). A solution of compound **88** (0.1 g; 0.28 mmol) and compound **87** (96 mg; 0.28 mmol) in 3ml of methanol (0.5 ml THF, cosolvent) was stirred at room temperature for 10 minutes to dissolve the starting materials. NaOH (25 mg; 0.56 mmol) was added to the reaction mixture and it was stirred at room temperature for 24 hrs. A yellow precipitate was formed and it was filtered by suction, washed with water. Column chromatography (SiO₂, methanol: dichloromethane, 1:9), yielded the desired product (0.72 g; 38 %) as a yellow powder. mp: 143-145 °C (from ethanol), *R_f* (methanol: dichloromethane, 1:9) 0.39. ν_{\max} (KBr)/cm⁻¹ 3374 (N-H), 3095 (C-H, aromatic), 2976 (C-H, aliphatic), 1669 (C=C, mn), 1579 (C=C, aromatic). δ_H (400 MHz, DMSO-*d*₆) 8.43 (d, 2H, *J* 5.4 Hz, *H-2, H-2'*), 8.28 (d, 2H, *J* 2.0 Hz, *H-8, H-8'*), 8.26 (d, 2H, *J* 9.2 Hz, *Hg, Hg'*), 8.07 (d, 1H, *J* 9.0 Hz, *Hb*), 7.93 (d, 1H, *J* 16.0 Hz, *Hn*), 7.87 (d, 2H, *J* 9.2 Hz, *H-5, H-5'*), 7.72 (d, 1H, *J* 16.0 Hz, *Hm*), 7.45 (dd, 2H, *J* 2.0 and 9.2 Hz, *H-6, H-6'*), 7.08 (d, 2H, *J* 9.2 Hz, *Hh, Hh'*), 6.64 (d, 1H, *J* 9.0 Hz, *Ha*), 6.60 (s, 1H, *He*), 6.51 (d, 2H, *J* 5.4 Hz, *H-3, H-3'*). 4.35 (t, 4H, *J* 5.2 Hz, 2x*H-10, 2xH-10'*), 3.85 (s, 3H, OCH₃), 3.71 (t, 4H, *J* 5.2 Hz, 2x*H-9, 2xH-9'*). δ_C (75 MHz, DMSO-*d*₆) 187.33 (C=O), 162.09 (*Cj*), 162.02 (*Cd*), 159.75 (*Cf*), 151.90 (*C-2, C-2'*), 149.98 (*C-4, C-4'*), 149.96 (*C-8a, C-8a'*), 146.43 (*C-4a, C-4a'*), 137.78 (*Cn*), 133.40 (*C-7, C-7'*), 130.91 (*Cg, Cg'*), 130.57 (*Cl*), 127.50 (*C-8, C-8'*), 127.13 (*Cb*), 124.14 (*C-6, C-6'*), 124.01 (*Cm*), 121.76 (*C-5, C-5'*), 117.40 (*C-*

h,C-h'), 114.38 (*C-c*), 106.77 (*C-a*), 98.90 (*C-3,C-3'*), 98.74 (*Ce*), 65.90 (*C-10,C-10'*), 55.77 (*OCH₃*), 41.86 (*C-9,C-9'*). Calc. for $C_{38}H_{32}Cl_2N_4O_4$, m/z 679.18788 ($M+1$)⁺. Found HRMS (FAB) m/z 679.18725 ($M+1$)⁺.

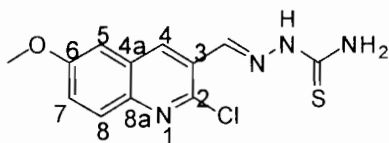
General procedure for preparation of compounds 38, 41, 44, 46, 48, 59, 61

A mixture of the appropriate quinolinecarboxaldehyde (1.0 equiv.) and thiosemicarbazide (1.0 equiv) were added to 5 ml of warm ethanol (50 °C) and the reaction mixture was stirred for 3 hrs at 50 °C. It was then left to stand at room temperature overnight to enhance precipitation and filtered by suction to afford the desired products.

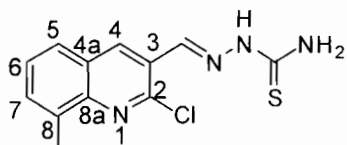


N'-Quinolin-4-yl-methylene-thiosemicarbazone (38).

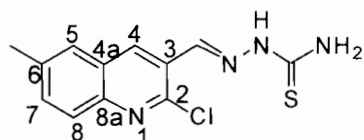
Yield: 0.34 g; 77 %, obtained as a yellowish powder, mp: 241-242 °C (from methanol), R_f (ethyl acetate: hexane, 1:1) 0.18, ν_{max} (KBr)/ cm^{-1} 3433 (N-H, NH_2), 3261 (N-H), 3138 (C-H, aromatic), 1544 (C=C). δ_H (300 MHz, $DMSO-d_6$) 11.74 (s, 1H, NNH), 8.94 (d, 1H, J 4.5 Hz, $H-2$), 8.89 (s, 1H, $HC=N$), 8.41 (s, 1H, NH), 8.33 (s, 1H, NH), 8.31 (dd, 1H, J 1.5 and 8.2 Hz, $H-8$), 8.17 (d, 1H, J 4.5 Hz, $H-3$), 8.08 (dd, 1H, J 1.5 and 8.2 Hz, $H-5$), 7.82 (ddd, 1H, J 1.5, 7.2 and 8.2 Hz, $H-6$), 7.73 (ddd, 1H, J 1.5, 7.2 and 8.2 Hz, $H-7$). δ_C (100 MHz, $DMSO-d_6$) 179.56 (C=S), 150.81 (HC=N), 149.09 (C-2), 138.54 (C-8a), 138.00 (C-4), 130.53 (C-8), 130.23 (C-7), 128.10 (C-4a), 125.75 (C-5), 123.71 (C-6), 118.67 (C-3). Anal Found: C, 56.81; H, 4.25; N, 24.32; S, 13.68 %. Calc for $C_{11}H_{10}N_4S$: C, 57.37; H, 4.38; N, 24.33; S, 13.92 %. Calc for $C_{11}H_{10}N_4S$ m/z 231.07043 ($M+1$)⁺. Found HRMS (FAB) m/z 231.07061 ($M+1$)⁺.



N'-(2-Chloro-6-methoxy)-quinolin-3-yl-methylene-thiosemicarbazone (44). Yield: 0.29 g; 71 %, obtained as a yellowish powder. mp: 253-255 °C (from methanol), R_f (ethyl acetate: hexane; 1:1) 0.17; ν_{\max} (KBr)/ cm^{-1} 3418 (N-H, NH_2), 3257 (N-H), 3140 (C-H, aromatic), 1591 (C=N), 1529 (C=C). δ_{H} (300 MHz, $\text{DMSO-}d_6$) 11.79 (s, 1H, NNH), 9.19 (s, 1H, *H-4*), 8.49 (s, 1H, HC=N), 8.41 (s, 1H, NH), 8.19 (s, 1H, NH), 7.86 (d, 1H, *J* 9.0 Hz, *H-8*), 7.48 (dd, 1H, *J* 2.7 and 9.0 Hz, *H-7*), 7.32 (d, 1H, *J* 2.7 Hz, *H-5*), 3.92 (s, 3H, OCH_3). δ_{C} (100 MHz, $\text{DMSO-}d_6$) 179.18 (C=S), 158.69 (HC=N), 146.56 (C-6), 143.76 (C-2), 137.57 (C-8a), 135.43 (C-4), 129.92 (C-8), 128.91 (C-4a), 126.94 (C-3), 124.71 (C-7), 106.60 (C-5), 56.29 (OCH_3). Anal Found: C, 48.50; H, 3.54; N, 18.82; S, 10.70 %. Calc. for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{OS}$: C, 48.90; H, 3.76; N, 19.01; S, 10.88 %. Calc for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{OS}$, m/z 295.04203 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 295.04155 ($\text{M}+1$)⁺.

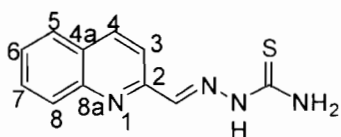


N'-(2-Chloro-8-methyl)-quinolin-3-yl-methylene-thiosemicarbazone (48). Yield: 0.23 g; 56 %, obtained as a yellowish powder. mp: 256-258 °C (from methanol), R_f (ethyl acetate: hexane, 1:1) 0.59; ν_{\max} (KBr)/ cm^{-1} 3430 (N-H, NH_2), 3233 (N-H), 3127 (C-H, aromatic), 1585 (C=N), 1532 (C=C). δ_{H} (300 MHz, $\text{DMSO-}d_6$) 11.75 (s, 1H, NNH), 9.26 (s, 1H, *H-4*), 8.52 (s, 1H, HC=N), 8.41 (s, 1H, NH), 8.22 (s, 1H, NH), 7.83 (dd, 1H, *J* 2.4 and 8.0 Hz, *H-5*), 7.70 (dd, 1H, *J* 2.4 and 8.0 Hz, *H-7*), 7.58 (t, 1H, *J* 8.0 Hz, *H-6*), 2.66 (s, 3H, CH_3). δ_{C} (100 MHz, $\text{DMSO-}d_6$) 179.17 (C=S), 148.24 (C-2), 146.77 (HC=N), 137.58 (C-8a), 137.04 (C-4), 136.28 (C-8), 132.14 (C-7), 128.28 (C-6), 127.76 (C-4a), 127.04 (C-5), 126.63 (C-3), 17.93 (Ar- CH_3). Anal Found: C, 51.67; H, 3.93; N, 20.10; S, 11.67 %. Calc for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{S}$: C, 51.70; H, 3.98; N, 20.10; S, 11.50 %. Calc for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{S}$, m/z 279.04711 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 279.04724 ($\text{M}+1$)⁺.



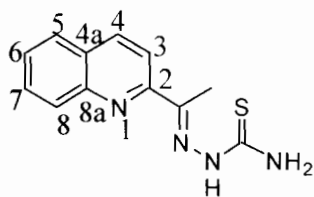
N'-(2-Chloro-6-methyl)-quinolin-3-yl-methylene-

thiosemicarbazone (46). Yield : 0.30 g; 74 %, obtained as a yellowish powder. mp: 270-272 °C (from methanol), R_f (ethyl acetate: hexane, 1:1) 0.48; ν_{\max} (KBr)/ cm^{-1} 3432 (N-H, NH_2), 3246 (N-H), 3135 (C-H, aromatic), 1585 (C=N), 1530 (C=C). δ_{H} (300 MHz, $\text{DMSO-}d_6$) 11.75 (s, 1H, NNH), 9.19 (s, 1H, $H-4$), 8.50 (s, 1H, HC=N), 8.41 (s, 1H, NH), 8.20 (s, 1H, NH), 7.85 (d, 1H, J 8.7 Hz, $H-8$), 7.68 (dd, 1H, J 2.0 and 8.7 Hz, $H-7$), 7.66 (d, 1H, J 2.0 Hz, $H-5$), 2.52 (s, 3H, CH_3). δ_{C} (100 MHz, $\text{DMSO-}d_6$) 179.16 (C=S), 148.26 (C-2), 147.34 (HC=N), 146.30 (C-8a), 138.17 (C-4), 137.61 (C-6), 136.01 (C-7), 134.38 (C-8), 128.17 (C-4a), 127.71 (C-5), 126.78 (C-3), 21.82 (Ar- CH_3) Anal Found: C, 51.30; H, 3.73; N, 20.18; S, 11.56 %. Calc. for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{S}$: C, 51.70; H, 3.98; N, 20.10; S, 11.50 %. Calc for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{S}$, m/z 279.04711 (M+1)⁺. Found HRMS (FAB) m/z 279.04748 (M+1)⁺.



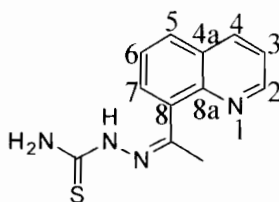
N'-Quinolin-2-yl-methylene-thiosemicarbazone (42).

Yield: 0.34 g, 77 %, obtained as an off-white powder, mp: 232-233 °C (from methanol), R_f (ethyl acetate: hexane, 4:1) 0.41, ν_{\max} (KBr)/ cm^{-1} 3391 (N-H, NH_2), 3142 (N-H), 2994 (C-H, aromatic), 1601 (C=N), 1517 (C=C). δ_{H} (300 MHz, $\text{DMSO-}d_6$) 11.78 (s, 1H, NNH), 8.44 (d, 1H, J 8.4 Hz, $H-4$), 8.42 (s, 1H, NH), 8.38 (s, 1H, NH), 8.35 (dd, 1H, J 1.5 and 8.4 Hz, $H-8$), 8.24 (s, 1H, HC=N), 8.00 (d, 1H, J 8.4 Hz, $H-3$), 7.96 (dd, 1H, J 1.5 and 8.4 Hz, $H-5$), 7.77 (ddd, 1H, J 1.5, 6.9 and 8.4 Hz, $H-6$), 7.61 (ddd, 1H, J 1.5, 6.9 and 8.4 Hz, $H-7$). δ_{C} (100 MHz, $\text{DMSO-}d_6$). 179.26 (C=S), 154.59 (HC=N), 148.04 (C-2), 143.27 (C-8a), 136.94 (C-4), 130.58 (C-4a), 129.47 (C-7, C-8), 128.58 (C-5), 127.80 (C-6), 118.80 (C-3) Anal Found: C, 57.17; H, 4.33; N, 24.24; S, 13.78 %. Calc. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{S}$: C, 57.37; H, 4.38; N, 24.33; S, 13.92 %. Calc. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{S}$, m/z 253.05238 (M+Na). Found HRMS (FAB) m/z 253.06301 (M+Na).



N'-Quinolin-2-yl-ethylene-thiosemicarbazone (59).

Yield: 0.12 g; 40 %, obtained as a brown powder, mp: 160-162 °C (from methanol), R_f (ethyl acetate: hexane, 1:1) 0.51, ν_{\max} (KBr)/ cm^{-1} 3623 (N-H, NH_2), 3163 (N-H), 1602 (C=N). δ_{H} (400 MHz, CDCl_3), 8.98 (s, 1H, NNH), 8.31 (d, 1H, J 8.4 Hz, $H-4$), 8.26 (dd, 1H, J 1.2 and 8.8 Hz, $H-8$), 8.10 (d, 1H, J 8.4 Hz, $H-3$), 7.82 (dd, 1H, J 1.2 and 8.8 Hz, $H-5$), 7.74 (ddd, 1H, J 1.2, 7.2 and 8.8 Hz, $H-6$), 7.57 (ddd, 1H, J 1.2, 7.2 and 8.8 Hz, $H-7$), 6.64 (s, 2H, NH_2) 2.59 (s, 3H, CH_3). δ_{C} (100 MHz, CDCl_3) 179.53 (C=S), 153.80 ($\text{CH}_3\text{C}=\text{N}$), 149.30 (C-2), 147.43 (C-8a), 137.62 (C-4), 136.06 (C-4a), 128.32 (C-8), 128.11 (C-7), 127.40 (C-5), 127.31 (C-6), 120.40 (C-3), 11.98 (CH_3). Calc for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{S}$; m/z 244.07826 (M)⁺. Found HRMS (EI) m/z 244.07832 (M)⁺.

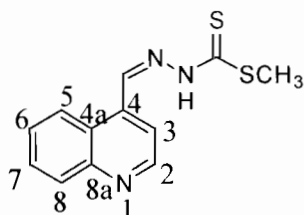


N'-Quinolin-8-yl-ethylene-thiosemicarbazone (61). Yield:

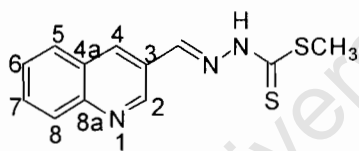
0.19 g; 44 %, obtained as a yellowish powder, mp: 208-211 °C (from methanol), R_f (ethyl acetate: hexane, 1:1) 0.38, ν_{\max} (KBr)/ cm^{-1} 3169 (N-H), 1603 (C=N). δ_{H} (300MHz, CDCl_3) 8.96 (dd, 1H, J 2.1 and 4.2 Hz, $H-2$), 8.36 (s, 1H, NNH), 8.24 (dd, 1H, J 2.1 and 8.4 Hz, $H-4$), 7.95 (dd, 1H, J 1.5 and 8.1 Hz, $H-5$), 7.64 (t, 1H, J 8.1 Hz, $H-6$), 7.56 (dd, 1H, J 1.5 and 8.1 Hz, $H-7$), 7.50 (dd, 1H, J 4.2 and 8.4 Hz, $H-3$), 7.36 (s, 1H, NH), 6.18 (s, 1H, NH), 2.46 (s, 3H, CH_3). δ_{C} (100 MHz, CDCl_3) 179.45 (C=S), 152.74 ($\text{CH}_3\text{C}=\text{N}$), 151.74 (C-2), 151.63 (C-8a), 136.81 (C-4), 136.50 (C-8), 130.36 (C-5), 129.72 (C-7), 129.39 (C-4a), 126.78 (C-6), 122.50 (C-3), 20.79 (CH_3). Anal Found: C, 58.13; H, 4.86; N, 22.83; S, 12.92 %. Calc. for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{S}$; C, 58.99; H, 4.95; N, 22.93; S, 13.12 %. Calc for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{S}$; m/z 244.07826 (M)⁺. Found HRMS (EI) m/z 244.07828 (M)⁺.

General procedure for the preparation of compounds 39, 41 and 43

A solution of the appropriate quinoline-carboxaldehyde (4.01 g; 25.45 mmol) and methylhydrazinecarbodithioate (3.11 g; 25.45 mmol.) in isopropanol (20 ml) was stirred at room temperature for 3 hrs, whereby a yellowish-orange precipitate started forming. This was filtered by suction, washed with cold isopropanol to afford the desired products.

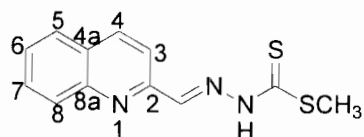


N'-Quinolin-4-yl-methylene-thiosemicarbazone methyl ester (39). Yield: 5.82 g; 87 %, obtained as a yellowish-orange powder. mp: 183-185 °C (from methanol), R_f (ethyl acetate: hexane, 1:1) 0.50; ν_{\max} (KBr)/ cm^{-1} 3088 (C-H, aromatic), 1537 (C=C). δ_{H} (300 MHz, CDCl_3) 10.40 (s, 1H, NNH), 9.00 (d, 1H, J 5.0 Hz, H -2), 8.73 (dd, 1H, J 1.0 and 8.4 Hz, H -8), 8.39 (s, 1H, $\text{HC}=\text{N}$), 8.19 (dd, 1H, J 1.0 and 8.4 Hz, H -5), 7.79 (ddd, 1H, J 1.0, 6.6 and 8.4 Hz, H -6), 7.72 (d, 1H, J 5.0 Hz, H -3), 7.68 (ddd, 1H, J 1.0, 6.6 and 8.4 Hz, H -7), 2.73 (s, 3H, SCH_3). δ_{C} (100 MHz, CDCl_3) 200.20 (C=S), 151.06 ($\text{HC}=\text{N}$), 149.14 (C-2), 144.86 (C-8a), 137.07 (C-4), 130.59 (C-8), 130.43 (C-7), 128.62 (C-4a), 125.13 (C-5), 125.03 (C-6), 121.90 (C-3), 17.70 (SCH_3). Calc for $\text{C}_{12}\text{H}_{11}\text{N}_3\text{S}_2$, m/z 262.04726 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 262.04802 ($\text{M}+1$)⁺.



N'-Quinolin-3-yl-methylene-thiosemicarbazone methyl ester (43). Yield: 6.20 g; 92 %, obtained as a yellow powder. mp: 208-212 °C (from methanol), R_f (methanol: dichloromethane, 1:1) 0.54; ν_{\max} (KBr)/ cm^{-1} 3077 (N-H), 1522 (C=C) δ_{H} (400 MHz, $\text{DMSO}-d_6$) 13.45 (s, 1H, NNH), 9.25 (d, 1H, J 2.0 Hz, H -2), 8.59 (d, 1H, J 2.0 Hz, H -4), 8.43 (s, 1H, $\text{HC}=\text{N}$), 8.07 (dd, 1H, J 1.2 and 8.4 Hz, H -8), 8.03 (dd, 1H, J 1.2 and 8.4 Hz, H -5), 7.81 (ddd, 1H, J 1.2, 7.0 and 8.4 Hz, H -7),

7.65 (ddd, 1H, *J* 1.2, 7.0 and 8.4 Hz, *H*-6), 2.56 (s, 3H, SCH₃). δ_c (100 MHz, DMSO-*d*₆). 189.56 (C=S), 148.67 (C-2), 144.76 (HC=N), 136.56 (C-8a), 131.49 (C-4), 129.60 (C-7), 129.45 (C-8), 128.23 (C-5), 127.93 (C-6), 127.36 (C-4a), 126.73 (C-3), 17.52 (SCH₃) Anal Found: C, 55.05; H, 4.18; N, 15.62; S, 23.34 %. Calc for C₁₂H₁₁N₃S₂: C, 55.14; H, 4.24; N, 16.08; S, 24.54 %. Calc. for C₁₂H₁₁N₃S₂, *m/z* 262.04726 (M+1)⁺. Found HRMS (FAB) *m/z* 262.04730 (M+1)⁺.

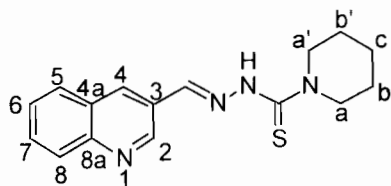


N'-Quinolin-2-yl-methylene-thiosemicarbazone

methyl ester (41). Yield: 5.80 g; 87 %, obtained as a yellow-orange powder, mp: 197-199 °C (from methanol), *R_f* (methanol: dichloromethane, 1:19) 0.8; δ_H (300 MHz, DMSO-*d*₆) 13.59 (s, 1H, NNH), 8.44 (d, 1H, *J* 9.0 Hz, *H*-4), 8.41 (s, 1H, HC=N), 8.07 (dd, 1H, *J* 1.5 and 8.4 Hz, *H*-8), 8.04 (d, 1H, *J* 9.0 Hz, *H*-3), 7.99 (dd, 1H, *J* 1.5 and 8.4 Hz, *H*-5), 7.81 (ddd, 1H, *J* 1.5, 6.6 and 8.4 Hz, *H*-6), 7.66 (ddd, 1H, *J* 1.5, 6.6 and 8.4 Hz, *H*-7), 2.58 (s, 3H, SCH₃). δ_c (75 MHz, DMSO-*d*₆). 188.56 (C=S), 154.67 (HC=N), 146.70 (C-2), 140.67 (C-8a), 137.59 (C-4), 135.60 (C-4a), 134.48 (C-7, C-8), 129.23 (C-5), 127.33 (C-6), 126.73 (C-3), 17.52 (SCH₃). Calc. for C₁₂H₁₁N₃S₂, *m/z* 262.04726 (M+1)⁺. Found HRMS (FAB) *m/z* 262.04730 (M+1)⁺.

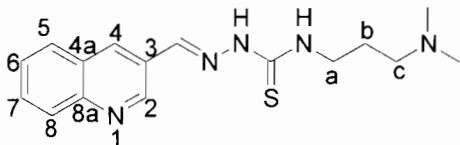
General procedure for the preparation of compounds 49-53

A solution of thiosemicarbazone thioester (**41/43**) (0.20 g; 0.764 mmol) and primary/secondary amine (4.0 equiv) in 5ml methanol and co-solvent (1ml THF) was stirred under reflux for 12 hrs. The solvent was removed in vacuo to afford the product as a powder.



N²-Quinolin-3-yl-methylene-1'-piperidine-

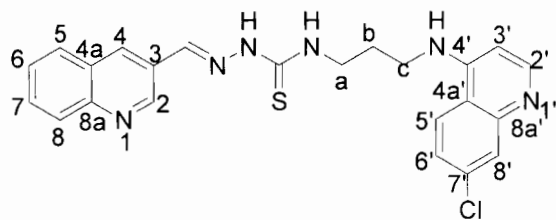
thiosemicarbazone (51). Yield: 0.13 g; 57 %, obtained as a yellow powder mp: 174-176 °C (from ethanol), R_f (ethyl acetate: hexane, 3:7) 0.17; ν_{\max} (KBr)/ cm^{-1} 3426 (N-H), 3127 (C-H, aromatic), 2936 (C-H, aliphatic), 1508 (C=C). δ_{H} (400 MHz, CDCl_3) 9.26 (d, 1H, J 2.0 Hz, $H-2$), 8.82 (s, 1H, NNH), 8.17 (d, 1H, J 2.0 Hz, $H-4$), 8.10 (dd, 1H, J 1.2 and 8.4 Hz, $H-8$), 7.84 (dd, 1H, J 1.2 and 8.4 Hz, $H-5$), 7.82 (s, 1H, $\text{HC}=\text{N}$), 7.74 (ddd, 1H, J 1.2, 7.0 and 8.4 Hz, $H-7$), 7.58 (ddd, 1H, J 1.2, 7.4 and 8.4 Hz, $H-6$), 4.03 (t, 4H, J 4.4 Hz, $2xH_a, 2xH_a'$), 1.80 (m, 6H, $2xH_b, 2xH_b', 2xH_c$). δ_{C} (75 MHz, CDCl_3) 186.34 (C=S), 148.14 (C-2), 145.06 (HC=N), 136.77 (C-8a), 130.41 (C-4), 129.54 (C-7), 129.34 (C-8), 128.12 (C-5), 127.87 (C-6), 127.47 (C-4a), 126.86 (C-3), 53.27 (C-a, -Ca'), 26.18 (C-c), 24.34 (C-b, C-b'). Anal Found: C, 63.61; H, 5.88; N, 18.56; S, 10.28 %. Calc. for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{S}$: C, 64.40; H, 6.08; N, 18.78; S, 10.75 %. Calc. for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{S}$ m/z 299.13303 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 299.13319 ($\text{M}+1$)⁺.



N²-Quinolin-3-yl-methylene-1'-(3-

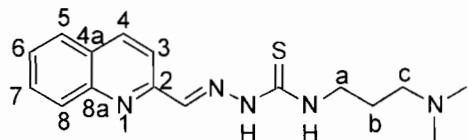
dimethylamino-1-propylamine)-thiosemicarbazone (52). Yield: 0.15 g; 63 %, obtained as a yellow powder after column chromatography (SiO_2 , methanol: dichloromethane, 1:9), mp: 195-198 °C (from ethanol), R_f (methanol: dichloromethane, 1:9) 0.11; ν_{\max} (KBr)/ cm^{-1} 3144 (C-H, aromatic), 2942 (C-H, aliphatic), 1527 (C=C). δ_{H} (400 MHz, CDCl_3) 9.80 (s, 1H, NNH), 9.35 (s, 1H, NH), 9.30 (d, 1H, J 2.0 Hz, $H-2$), 8.26 (d, 1H, J 2.0 Hz, $H-4$), 8.01 (s, 1H, $\text{HC}=\text{N}$), 8.12 (dd, 1H, J 1.2 and 8.4 Hz, $H-8$), 7.84 (dd, 1H, J 1.2 and 8.4 Hz, $H-5$), 7.74 (ddd, 1H, J 1.2, 7.0 and 8.4 Hz, $H-7$), 7.58 (ddd, 1H, J 1.2, 7.0 and 8.4 Hz, $H-6$), 3.87 (t, 2H, J 5.2 Hz, $2xH_a$), 2.56 (t, 2H, J 5.2 Hz, $2xH_c$), 2.37 (s, 6H, $\text{N}(\text{CH}_3)_2$), 1.87 (m, 2H, $2xH_b$). δ_{C} (75 MHz, CDCl_3) 186.98 (C=S), 148.49 (HC=N), 148.19 (C-2, 8a), 139.72 (C-4), 139.21 (C-7), 134.94 (C-8), 130.39 (C-4a), 129.48 (C-5), 128.09 (C-6), 127.57 (C-3),

59.12 (*C-c*), 45.51 (*C-a*), 45.45 (N(CH₃)₂), 36.74 (*C-b*). Calc. for C₁₆H₂₁N₅S, *m/z* 316.15958 (M+1)⁺. Found HRMS (FAB) *m/z* 316.15913 (M+1)⁺.

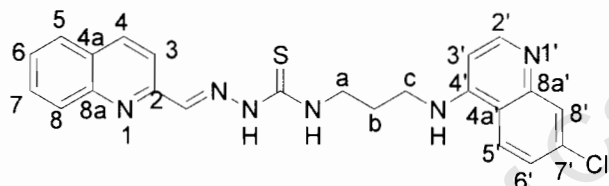


N'-Quinolin-3-yl-methylene-1'-(N'-[7-chloro-quinoline-4-yl]propane-1,3-diamine)-thiosemicarbazone (53).

Yield: 27.5 mg; 8%, obtained as a yellowish-brown powder after column chromatography (SiO₂, methanol: dichloromethane, 1:9), mp: 209-212 °C (from ethanol), R_f (methanol: dichloromethane, 1:9) 0.32; ν_{\max} (KBr)/ cm⁻¹ 3278 (N-H), 2936 (C-H, aliphatic), 1546 (C=C). δ_{H} (400 MHz, DMSO-*d*₆) 11.66 (s, 1H, NNH), 9.52 (d, 1H, *J* 2.2 Hz, *H-2*), 8.81 (s, 1H, NH), 8.53 (d, 1H, *J* 2.2 Hz, *H-4*), 8.40 (d, 1H, *J* 5.6 Hz, *H-2'*), 8.28 (d, 1H, *J* 8.8 Hz, *H-5'*), 8.23 (s, 1H, HC=N), 8.03 (dd, 1H, *J* 1.2 and 8.4 Hz, *H-8*), 7.97 (d, 1H, *J* 1.8 Hz, *H-8'*), 7.76 (dd, 1H, *J* 1.2 and 8.4 Hz, *H-5*), 7.65 (ddd, 1H, *J* 1.2, 7.2 and 8.4 Hz, *H-7*), 7.60 (ddd, 1H, *J* 1.2, 7.2 and 8.4 Hz, *H-6*), 7.41 (dd, 1H, *J* 1.8 and 8.8 Hz, *H-6'*), 6.56 (d, 1H, *J* 5.6 Hz, *H-3'*), 3.77 (t, 2H, *J* 6.8 Hz, 2x *Ha*), 3.02 (t, 2H, *J* 6.8 Hz, 2x *Hc*), 2.04 (m, 2H, 2x *Hb*). δ_{C} (100 MHz, DMSO-*d*₆) 186.75 (C=S), 153.43 (C-2'), 149.56 (C-4'), 148.56 (HC=N), 148.13 (C-8a'), 147.89 (C-2,8a), 140.12 (C-4), 139.34 (C-7), 135.16 (C-8), 133.43 (C-7'), 131.13 (C-4a), 129.54 (C-5), 128.19 (C-8'), 127.76 (C-6), 127.37 (C-6'), 125.44 (C-3), 123.87 (C-5'), 119.12 (C-4a'), 105.23 (C-3'), 48.31 (*C-c*), 46.16 (*C-a*), 34.63 (*C-b*). Calc. for C₂₃H₂₁ClN₆S, *m/z* 449.13151 (M+1)⁺. Found HRMS (FAB) *m/z* 449.13118 (M+1)⁺.



N'-Quinolin-2-yl-methylene-1'-(3-dimethylamino-1-propylamine)-thiosemicarbazone (49). Yield: 0.13 g; 55 %, obtained as a cream fluffy powder, mp: 195-197 °C (from ethanol), R_f (methanol: dichloromethane, 1:9) 0.14; ν_{\max} (KBr)/ cm^{-1} 3134 (N-H), 2942 (C-H, aliphatic), 1523 (C=C). δ_{H} (400 MHz, CDCl_3) 9.32 (s, 1H, NNH), 9.18 (s, 1H, NH), 8.15 (d, 1H, J 8.4 Hz, $H-4$), 8.07 (dd, 1H, J 1.5 and 8.4 Hz, $H-8$), 8.03 (d, 1H, J 8.4 Hz, $H-3$), 7.98 (s, 1H, HC=N), 7.82 (dd, 1H, J 1.5 and 8.4 Hz, $H-5$), 7.74 (ddd, 1H, J 1.5, 7.2 and 8.4 Hz, $H-6$), 7.57 (ddd, 1H, J 1.5, 7.2 and 8.4 Hz, $H-7$), 3.87 (t, 2H, J 5.6 Hz, 2x H_a), 2.52 (t, 2H, J 5.6 Hz, 2x H_c), 2.32 (s, 6H, $\text{N}(\text{CH}_3)_2$), 1.85 (m, 2H, 2x H_b). δ_{C} (100 MHz, CDCl_3) 187.67 (C=S), 155.54 (HC=N), 145.19 (C-2), 141.95 (C-8a), 138.62 (C-4), 137.21 (C-4a) 136.03 (C-7, C-8), 129.95 (C-5), 129.40 (C-6), 127.50 (C-3), 59.29 (C-c), 45.71 (C-a), 45.53 $\text{N}(\text{CH}_3)_2$, 25.40 (C-b). Anal Found: C, 61.55; H, 6.88; N, 21.27; S, 9.52 %. Calc. for $\text{C}_{16}\text{H}_{21}\text{N}_5\text{S}$: C, 60.92; H, 6.71; N, 22.20; S, 10.17 %. Calc. for $\text{C}_{16}\text{H}_{21}\text{N}_5\text{S}$, m/z 316.15958 (M+1)⁺. Found HRMS (FAB) m/z 316.15913 (M+1)⁺.

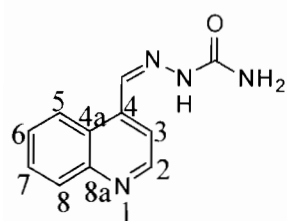


N'-[7-chloro-quinoline-4-yl]propane-1,3-diamine)-thiosemicarbazone (50). Yield: 55 mg; 22 %, obtained as brownish powder after column chromatography (SiO_2 , methanol: dichloromethane, 1:9), mp: 209-212 °C (from ethanol), R_f (methanol: dichloromethane, 1:9) 0.40; ν_{\max} (KBr)/ cm^{-1} 3279 (N-H), 2937 (C-H, aliphatic), 1532 (C=C). δ_{H} (400 MHz, CDCl_3) 8.49 (d, 1H, J 5.6 Hz, $H-2'$), 8.30 (d, 1H, J 8.4 Hz, $H-4$), 8.26 (dd, 1H, J 1.5 & 8.4 Hz, $H-8$), 8.13 (d, 1H, J 8.4 Hz, $H-5'$), 8.07 (d, 1H, J 8.4 Hz, $H-3$), 8.03 (s, 1H, HC=N), 7.94 (d, 1H, J 1.8 Hz, $H-8'$), 7.91 (dd, 1H, J 1.5 and 8.4 Hz, $H-5$), 7.83 (ddd, 1H, J 1.5, 7.2 and 8.4 Hz, $H-6$), 7.66 (ddd, 1H, J 1.5, 7.2 and 8.4 Hz, $H-7$), 7.35 (dd, 1H, J 1.8 and 8.4 Hz, $H-6'$), 6.43 (d, 1H, J 5.6 Hz, $H-3'$), 4.02 (t, 2H, J 6.8 Hz, 2x H_a), 3.54 (t, 2H, J 6.8 Hz, 2x H_c), 2.08 (m,

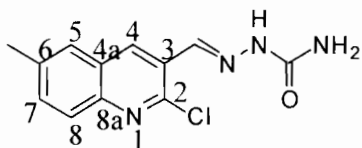
2H, 2x *Hb*). δ_c (100 MHz, CDCl_3).187.32 (C=S), 155.63 (HC=N), 149.68 (C-2), 149.43 (C-2'), 149.26 (C-4'), 148.63 (C-8a'), 142.63 (C-8a), 139.21 (C-4), 136.71 (C-4a) 136.37 (C-7,C-8), 135.33 (C-7'), 130.95 (C-5), 129.65 (C-6), 128.19 (C-8'), 127.63 (C-3), 127.37 (C-6'), 123.87 (C-5'), 119.12 (C-4a'), 105.23 (C-3'), 47.52 (C-c), 46.37 (C-a), 35.23 (C-b). Calc. for $\text{C}_{23}\text{H}_{21}\text{ClN}_6\text{S}$, m/z 449.13151 (M+1)⁺. Found HRMS (FAB) m/z 449.13076 (M+1)⁺.

General procedure for the preparation of compounds 40, 45, 47, 60 and 62

A solution of the appropriate quinolinecarboxaldehyde (1.0 equiv.) semicarbazide hydrochloride (1.0 equiv.) and triethylamine (0.31 ml) in methanol was stirred under reflux until a white precipitate started forming. It was then cooled to room temperature, filtered by suction and washed with cold methanol to afford the desired products.

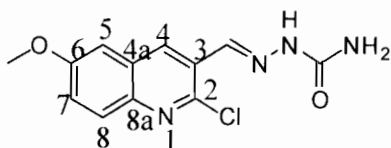


N²-Quinolin-4-yl-methylene-semicarbazone (40). Yield: 0.32 g; 78 %, obtained as a white powder, mp: 238-240 °C (from ethanol), R_f (Ethyl acetate: hexane, 4:1) 0.34, ν_{max} (KBr)/ cm^{-1} 3463 (N-H, NH_2), 3086 (C-H, aromatic), 1700 (C=O), 1575 (C=C and C=N). δ_H (300 MHz, $\text{DMSO}-d_6$) 10.62 (s, 1H, NNH), 8.92 (d, 1H, J 4.6 Hz, $H-2$), 8.63 (s, 1H, HC=N), 8.36 (dd, 1H, J 2.0 and 8.2 Hz, $H-8$), 8.08 (d, 1H, J 4.6 Hz, $H-3$), 8.00 (dd, 1H, J 2.0 and 8.2 Hz, $H-5$), 7.82 (ddd, 1H, J 2.0, 6.6 and 8.2 Hz, $H-6$), 7.71 (ddd, 1H, J 2.0, 6.6 and 8.2 Hz, $H-7$), 6.68 (s, 2H, NH_2). δ_c (100 MHz, $\text{DMSO}-d_6$) 156.96 (C=O), 150.46 (HC=N), 148.57 (C-2), 139.04 (C-8a), 135.55 (C-4), 130.37 (C-8), 130.04 (C-7), 128.02 (C-4a), 125.50 (C-5), 124.01 (C-6), 118.37 (C-3). Anal Found: C, 61.41; H, 4.69; S, 25.77 %. Calc. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}$: C, 61.67; H, 4.71; N, 26.15 %. Calc. for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}$, m/z 214.08546 (M)⁺. Found HRMS (FAB) m/z 214.08549 (M)⁺.



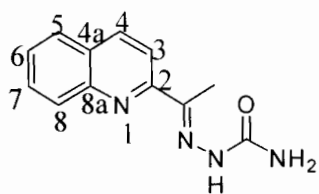
N'-(2-Chloro-6-methyl)-quinolin-3-yl-methylene-

semicarbazone (47). Yield: 0.17 g, 44 %, obtained as a white powder, mp: 274-276 °C (from ethanol), R_f (Ethyl acetate: hexane, 4:1) 0.43, ν_{\max} (KBr)/ cm^{-1} 3462 (N-H, NH_2), 3160 (N-H), 1719 (C=O), 1660 (C=N), 1589 (C=C). δ_{H} (400 MHz, $\text{DMSO-}d_6$) 10.60 (s, 1H, NNH), 9.06 (s, 1H, *H*-4), 8.25 (s, 1H, *HC*=N), 7.83 (d, 1H, *J* 8.6 Hz, *H*-8), 7.75 (d, 1H, *J* 2.0 Hz, *H*-5), 7.64 (dd, 1H, *J* 2.0 and 8.6 Hz, *H*-7), 6.65 (s, 2H, NH_2), 2.50 (s, 3H, 6- CH_3). δ_{C} (100 MHz, $\text{DMSO-}d_6$) 157.12 (C=O), 155.30 (C-2), 153.34 (HC=N), 147.93 (C-8a), 146.02 (C-4), 138.05 (C-6), 135.26 (C-7), 134.88 (C-8), 134.56 (C-5), 134.03 (C-4a), 128.12 (C-3), 21.83 (CH_3) Anal Found: C, 54.27; H, 4.20; N, 20.65 %. Calc. for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{O}$: C, 54.87; H, 4.22; N, 21.33 %. Calc. for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{O}$, m/z 262.06213 (M)⁺. Found HRMS (FAB) m/z 262.06213 (M)⁺.



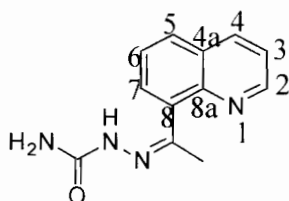
N'-(2-Chloro-6-methoxy)-quinolin-3-yl-

methylene-semicarbazone (45). Yield: 0.31 g, 76 %, obtained as a white powder, mp: 265-267 °C (from ethanol), R_f (Ethyl acetate: hexane, 4:1) 0.38, ν_{\max} (KBr)/ cm^{-1} 3479 (N-H, NH_2), 3179 (N-H), 1713 (C=O), 1588 (C=C). δ_{H} (400 MHz, $\text{DMSO-}d_6$) 10.63 (s, 1H, NNH), 9.06 (s, 1H, *H*-4), 8.24 (s, 1H, *HC*=N), 7.83 (d, 1H, *J* 9.2 Hz, *H*-8), 7.44 (dd, 1H, *J* 2.8 and 9.2 Hz, *H*-7), 7.34 (d, 1H, *J* 2.8 Hz, *H*-5), 6.66 (s, 2H, NH_2), 3.90 (s, 3H, OCH_3). δ_{C} (100 MHz, $\text{DMSO-}d_6$) 162.13 (C=O), 158.66 (C-6), 157.12 (C-2), 156.20 (HC=N), 146.43 (C-8a), 143.45 (C-4), 134.68 (C-8), 134.55 (C-4a), 129.84 (C-3), 124.34 (C-7), 106.55 (C-5), 56.28 (OCH_3). Anal Found: C, 51.19; H, 3.92; N, 19.86 %. Calc. for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{O}_2$: C, 51.72; H, 3.98; N, 20.10 %. Calc. for $\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{O}_2$, m/z 278.05705 (M)⁺. Found HRMS (FAB) m/z 278.05701 (M)⁺.



N'-Quinolin-2-yl-ethylene-semicarbazone (60). Yield:

0.13 g; 48 %, obtained as an off-white powder, mp: 228-232 °C (from ethanol), R_f (ethyl acetate: hexane, 1:1) 0.11, ν_{\max} (KBr)/ cm^{-1} 3473 (N-H, NH_2), 3161 (N-H), 1719 (C=O), 1583 (C=C and C=N) δ_{H} (400 MHz, $\text{DMSO-}d_6$) 9.59 (s, 1H, NNH), 8.48 (d, 1H, J 8.4 Hz, H -4), 8.26 (dd, 1H, J 2.1 and 8.8 Hz, H -8), 8.00 (d, 1H, J 8.4 Hz, H -3), 7.93 (dd, 1H, J 2.1 and 8.8 Hz, H -5), 7.72 (ddd, 1H, J 2.1, 7.0 and 8.8 Hz, H -6), 7.56 (ddd, 1H, J 2.1, 7.0 and 8.8 Hz, H -7), 6.72 (s, 2H, NH_2), 2.40 (s, 3H, CH_3). δ_{C} (100 MHz, DMSO) 157.50 (C=O), 156.08 ($\text{CH}_3\text{C}=\text{N}$), 147.40 (C-2), 145.64 (C-8a), 136.40 (C-4), 130.21 (C-4a), 129.67 (C-8), 128.38 (C-7), 128.20 (C-5), 127.32 (C-6), 119.21 (C-3), 11.88 (CH_3). Calc for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}$; m/z 228.10111 (M)⁺. Found HRMS (EI) m/z 228.10121 (M)⁺.

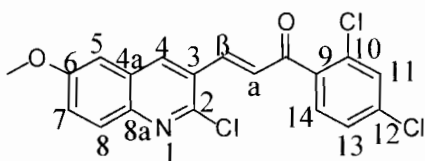


N'-Quinolin-8-yl-ethylene-semicarbazone (62). Yield: 0.19

g, 48 %, obtained as a white powder, mp: 177-179 °C (from ethanol), R_f (Ethyl acetate: Hexane, 1:1) 0.32, ν_{\max} (KBr)/ cm^{-1} 3464 (N-H, NH_2), 3236 (N-H), 1683 (C=O), 1577 (C=C). δ_{H} (400 MHz, $\text{DMSO-}d_6$) 9.34 (s, 1H, NNH), 8.90 (dd, 1H, J 2.0 and 4.4 Hz, H -2), 8.38 (dd, 1H, J 2.0 and 8.4 Hz, H -4), 7.98 (dd, 1H, J 1.6 and 8.4 Hz, H -5), 7.75 (t, 1H, J 8.4 Hz, H -6), 7.59 (dd, 1H, J 1.6 and 8.4 Hz, H -7), 7.54 (dd, 1H, J 4.4 and 8.4 Hz, H -3), 6.27 (s, 2H, NH_2), 2.35 (s, 3H, CH_3). δ_{C} (100 MHz, $\text{DMSO-}d_6$) 157.95 (C=O), 150.69 ($\text{CH}_3\text{C}=\text{N}$), 148.74 (C-2), 146.37 (C-8a), 139.90 (C-4), 137.13 (C-8), 129.81 (C-5), 129.33 (C-7), 128.73 (C-4a), 126.91 (C-6), 122.14 (C-3), 19.84 (CH_3). Calc for $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}$; m/z 228.10111 (M)⁺. Found HRMS (EI) m/z 228.10108 (M)⁺.

General procedure for the preparation of compounds 66 and 67

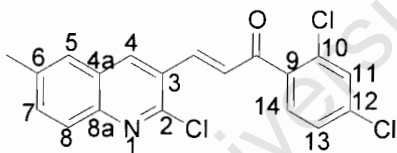
A mixture of the appropriate 3-formylquinoline (1.0 equiv.), 2,4-dichloroacetophenone (1.0 equiv.) and sodium methoxide (1.0 equiv.) in 6ml of ethanol was stirred at room temperature for 24 hrs. It was then filtered by suction and washed with water to afford the desired products.



3-(2-Chloro-6-methoxy-quinolin-3-yl)-1-(2,4-

dichloro-phenyl)-propenone (66). Yield: 1.50 g; 85 %, obtained as a yellow powder.

mp: 176-178 °C (from methanol-water), R_f (ethyl acetate: hexane, 2:8) 0.35, ν_{\max} (KBr)/ cm^{-1} 3086 (C-H), 1666 (C=C), 1621 (C=C). δ_H (400 MHz, CDCl_3) 8.34 (s, 1H, *H*-4), 7.95 (d, 1H, *J* 16.0 Hz, *H* $_{\beta}$), 7.89 (d, 1H, *J* 9.0 Hz, *H*-8), 7.52-7.49 (m, 2H, *H*-11 & *H*-14), 7.43 (dd, 1H, *J* 2.7 and 9.0 Hz, *H*-7), 7.38 (dd, 1H, *J* 1.6 and 8.6 Hz, *H*-13), 7.22 (d, 1H, *J* 16.0 Hz, *H* $_{\alpha}$), 7.11 (d, 1H, *J* 2.7 Hz, *H*-5), 3.94 (s, 3H, OCH_3). δ_C (75 MHz, CDCl_3) 192.43 (C=O), 159.25 (C-6), 155.12 (C-2), 144.23 (C-8a), 141.41 (C $_{\beta}$), 138.45 (C-12), 137.83 (C-10), 136.11 (C-9), 133.83 (C-4), 133.10 (C-14), 130.63 (C-3), 130.53 (C-11), 130.10 (C $_{\alpha}$), 129.34 (C-8), 128.23 (C-4a), 127.78 (C-13), 124.41 (C-7), 106.47 (C-5), 58.53 (OCH_3), Anal Found: C, 58.03; H, 2.70; N, 3.53 %. Calc. for $\text{C}_{19}\text{H}_{12}\text{Cl}_3\text{NO}_2$: C, 58.12; H, 3.08; N, 3.57 %. Calc. for $\text{C}_{19}\text{H}_{12}\text{Cl}_3\text{NO}_2$, m/z 390.99336 (M) $^+$. Found HRMS (EI) m/z 390.99338 (M) $^+$.



3-(2-Chloro-6-methyl-quinolin-3-yl)-1-(2,4-

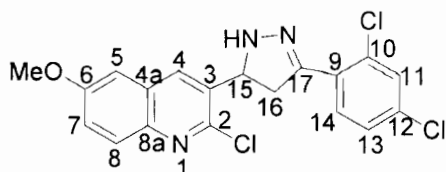
dichloro-phenyl)-propenone (67). Yield: 1.60 g; 87 %, obtained as a yellow powder,

mp: 162-165 °C (from methanol-water), R_f (ethyl acetate: hexane, 2:3) 0.59, ν_{\max} (KBr)/ cm^{-1} 3067 (C-H), 1654 (C=C), 1613 (C=C). δ_H (400 MHz, CDCl_3) 8.35 (s, 1H, *H*-4), 7.95 (d, 1H, *J* 16.0 Hz, *H* $_{\beta}$), 7.88 (d, 1H, *J* 9.6 Hz, *H*-8), 7.62 (d, 1H, *J* 8.4 Hz, *H*-14), 7.59 (d, 1H, *J* 2.4 Hz, *H*-11), 7.52-7.49 (m, 2H, *H*-5 and *H*-7), 7.40 (dd, 1H, *J*

2.4 and 8.4 Hz, *H-13*), 7.24 (d, 1H, *J* 16.0 Hz, *Ha*), 2.53 (s, 3H, 6-*CH*₃). δ_c (100 MHz, CDCl₃) 191.73 (*C=O*), 152.29 (*C-2*), 146.74 (*C-8a*), 140.91 (*C β*), 137.99 (*C-12*), 137.50 (*C-10*), 136.86 (*C-6*), 135.81 (*C-9*), 134.25 (*C-4*), 132.50 (*C-14*), 130.70 (*C-7*), 130.53 (*C-3*), 130.33 (*C-11*), 129.25 (*C α*), 128.13 (*C-13*), 127.47 (*C-8*), 127.15 (*C-5*), 126.94 (*C-4a*), 21.59 (6-*CH*₃). Calc for C₁₉H₁₂C₁₃NO, *m/z* 375.99844 (M)⁺. Found HRMS (EI) *m/z* 375.99895 (M)⁺.

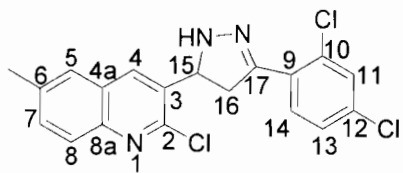
General procedure for the preparation of compounds 68 and 69

A solution of the appropriate chalcone (1.0 equiv.) and hydrazine hydrate (1.05 equiv.) in 20ml of ethanol was stirred under reflux with TLC monitoring for 12 hrs. It was cooled to room temperature, then in ice to enhance precipitation. The crude was filtered and washed with cold methanol to afford the desired products.



2-Chloro-3-[5-(2,4-dichloro-phenyl)-3,4-

dihydro-2H-pyrazol-3-yl]-6-methoxy-quinoline (68). Yield: 0.56 g; 54 %, obtained as an off-white powder. mp: 118-120 °C (from methanol), *R_f* (ethyl acetate: hexane, 2:8) 0.19, ν_{\max} (KBr)/ cm⁻¹ 3347 (N-H), 3054 (C-H, aromatic), 2875 (C-H, aliphatic), 1623 (C=C). δ_H (300 MHz, CDCl₃) 8.34 (s, 1H, *H-4*), 7.91 (d, 1H, *J* 9.6 Hz, *H-8*), 7.65 (d, 1H, *J* 8.4 Hz, *H-14*), 7.41 (d, 1H, *J* 2.1 Hz, *H-11*), 7.36 (dd, 1H, *J* 2.7 and 9.6 Hz, *H-7*), 7.26 (dd, 1H, *J* 2.1 and 8.4 Hz, *H-13*), 7.09 (d, 1H, *J* 2.7 Hz, *H-5*), 6.22 (d, 1H, *J* 3.0 Hz, NH), 5.40 (ddd, 1H, *J* 3.0, 10.2 and 16.5 Hz, *H-15*), 3.92 (s, 3H, OCH₃), 3.88 (dd, 1H, *J* 10.2 and 16.5 Hz, *H-16*), 3.13 (dd, 1H, *J* 10.2 and 16.5 Hz, *H-16*). δ_c (75 MHz, CDCl₃) 158.45 (*C-6*), 153.76 (*C-2*), 149.74 (*C-17*), 146.85 (*C-8a*), 137.51 (*C-12*), 135.13 (*C-10*), 135.03 (*C-9*), 134.53 (*C-4*), 133.62 (*C-14*), 130.88 (*C-3*), 130.41 (*C-11*), 129.63 (*C-8*), 128.52 (*C-13*), 127.25 (*C-4a*), 123.20 (*C-7*), 105.24 (*C-5*), 66.73 (OCH₃), 55.63 (*C-16*), 42.61 (*C-15*). Anal Found: C, 55.95; H, 3.01; N, 10.16 %. Calc. for C₁₉H₁₄Cl₃N₃O: C, 56.11; H, 3.47; N, 10.33 %. Calc. for C₁₉H₁₄Cl₃N₃O, *m/z* 405.02024 (M)⁺. Found HRMS (EI) *m/z* 405.02023 (M)⁺.

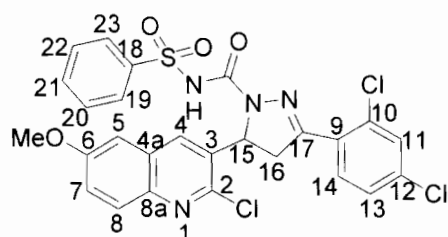


2-Chloro-3-[5-(2,4-dichloro-phenyl)-3,4-dihydro-

2H-pyrazol-3-yl]-6-methyl-quinoline (69). Yield: 0.86 g; 83 %, obtained as a white powder, mp 130-133 °C (from methanol), R_f (ethyl acetate: hexane, 2:3) 0.57. ν_{\max} (KBr)/ cm^{-1} 3317 (N-H), 3034 (C-H, aromatic), 2905 (C-H, aliphatic), 1603 (C=C). δ_H (300 MHz, CDCl_3) 8.35 (s, 1H, *H*-4), 7.91 (d, 1H, *J* 8.4 Hz, *H*-8), 7.65 (d, 1H, *J* 8.4 Hz, *H*-14), 7.59 (d, 1H, *J* 2.0 Hz, *H*-11), 7.54 (dd, 1H, *J* 2.0 and 8.4 Hz, *H*-7), 7.41 (d, 1H, *J* 2.0 Hz, *H*-5), 7.26 (dd, 1H, *J* 2.0 and 8.4 Hz, *H*-13), 6.18 (d, 1H, *J* 3.0 Hz, NH), 5.40 (ddd, 1H, *J* 3.0, 10.2 and 16.2 Hz, *H*-15), 3.90 (dd, 1H, *J* 10.2 and 16.2 Hz, *H*-16), 3.13 (dd, 1H, *J* 10.2 and 16.2 Hz, *H*-16), 2.53 (s, 3H, 6-*CH*₃). δ_C (75 MHz, CDCl_3) 151.72 (*C*-2), 148.60 (*C*-17), 145.69 (*C*-8a), 137.47 (*C*-12), 137.30 (*C*-10), 136.37(*C*-6), 135.10 (*C*-9), 133.27 (*C*-4), 133.03 (*C*-14), 132.80 (*C*-7), 130.87 (*C*-3), 130.38 (*C*-11), 127.90 (*C*-13), 127.39 (*C*-8), 127.24 (*C*-5), 126.53 (*C*-4a), 61.74 (*C*-16), 42.53 (*C*-15), 21.60 (6-*CH*₃). Anal Found: C, 58.12; H, 3.39; N, 10.74 %. Calc for $\text{C}_{19}\text{H}_{14}\text{Cl}_3\text{N}_3$: C, 58.41; H, 3.61; N, 10.76 %. Calc. for $\text{C}_{19}\text{H}_{14}\text{Cl}_3\text{N}_3$, m/z 389.02533 (M^+). Found HRMS (EI) m/z 389.02534 (M^+).

General procedure for the preparation of compounds 75-84

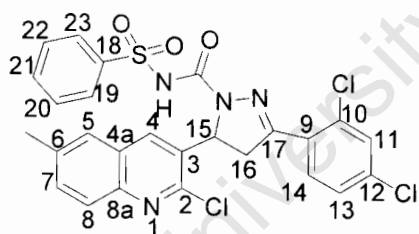
A solution of pyrazoline derivative **68/69** (1.0 equiv.) and the appropriate substituted phenyl sulfonyl urea (2.0 equiv.) in 1 ml of THF was stirred at room temperature with TLC monitoring for 3 hrs. The products precipitated out of solution and were filtered by suction and washed with cold diethyl ether to obtain the desired products as white powders.



N-[5-(2-Chloro-6-methoxyquinolin-3-yl)-3-

(2,4-dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-benzenesulfonamide

(75). Yield: 55 mg; 38 %, obtained as a white flaky powder mp: 229-230 °C (from ethyl acetate-hexane), R_f (Methanol: Dichloromethane, 1:50) 0.44. ν_{\max} (KBr)/ cm^{-1} 3304 (N-H), 3053 (C-H, aromatic), 2922 (C-H, aliphatic), 1692 (C=O), 1627 (C=C). δ_H (400 MHz, CDCl_3) 8.81 (s, 1H, NH), 8.10 (dd, 2H, J 2.0 and 8.4 Hz, H-19 and H-23), 7.87 (d, 1H, J 9.2 Hz, H-8), 7.71 (s, 1H, H-4), 7.64 (t, 2H, J 8.4 Hz, H-20 and H-22), 7.54-7.50 (m, 2H, H-21 and H-14), 7.46 (d, 1H, J 2.0 Hz, H-11), 7.37 (dd, 1H, J 2.0 and 8.4 Hz, H-13), 7.30 (dd, 1H, J 2.8 and 9.2 Hz, H-7), 7.01 (d, 1H, J 2.8 Hz, H-5), 5.82 (dd, 1H, J 12.4 and 18.4 Hz, H-15), 4.16 (dd, 1H, J 12.4 and 18.4 Hz, H-16), 3.92 (s, 3H, OCH_3), 3.34 (dd, 1H, J 12.4 and 18.4 Hz, H-16), δ_C (75 MHz, CDCl_3) 161.21 (C=O), 158.75 (C-6), 154.98 (C-2), 153.57 (C-17), 143.50 (C-8a), 139.17 (C-18), 137.48 (C-12), 135.92 (C-10), 135.03 (C-9), 134.02 (C-4), 131.42 (C-14), 131.25 (C-3), 130.88 (C-21), 129.78 (C-11), 129.14 (C-8), 128.64 (C-20, C-22), 127.82 (C-13), 127.97 (C-4a), 125.33 (C-19, C-23), 123.83 (C-7), 105.67 (C-5), 58.49 (OCH_3), 55.90 (C-16), 45.00 (C-15). Anal Found: C, 52.83; H, 3.14; N, 9.50; 5.02 %. Calc. for $\text{C}_{26}\text{H}_{19}\text{Cl}_3\text{N}_4\text{O}_4\text{S}$: C, 52.94; H, 3.25; N, 9.50; S, 5.44 %. Calc. for $\text{C}_{26}\text{H}_{19}\text{Cl}_3\text{N}_4\text{O}_4\text{S}$, m/z 589.02708 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 589.02754 ($\text{M}+1$)⁺.

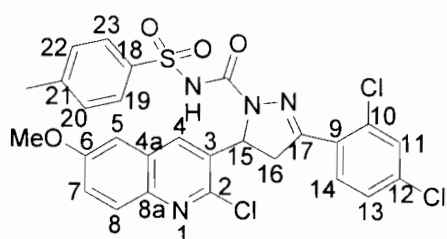


N-[5-(2-Chloro-6-methyl-quinolin-3-yl)-3-(2,4-

dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-benzenesulfonamide (76).

Yield: 84 mg, 57 %, obtained as a white flaky powder, mp: 217-220 °C (from ethyl acetate-hexane), R_f (ethyl acetate: hexane, 1:1) 0.35. ν_{\max} (KBr)/ cm^{-1} 3294 (N-H), 3097 (C-H, aromatic), 2872 (C-H, aliphatic), 1683 (C=O), 1586 (C=C). δ_H (400 MHz, CDCl_3) 8.82 (s, 1H, NH), 8.11 (dd, 2H, J 2.0 and 9.2 Hz, H-19, H-23), 7.87 (d, 1H, J

8.8 Hz, *H*-8), 7.70 (s, 1H, *H*-4), 7.65 (d, 1H, *J* 8.8 Hz, *H*-14), 7.60 (dd, 1H, *J* 2.0 and 8.8 Hz, *H*-7), 7.56 (d, 1H, *J* 2.0 Hz, *H*-5), 7.54-7.49 (m, 3H, *H*-13, *H*-20, *H*-22), 7.46 (d, 1H, *J* 2.0 Hz, and *H*-11), 7.32 (dd, 1H, *J* 2.0 and 8.8 Hz, *H*-21), 5.83 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-15), 4.16 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-16), 3.34 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-16), 2.51 (s, 3H, 6-CH₃). δ_c (100 MHz, CDCl₃) 162.33 (C=O), 152.42 (C-2), 148.53 (C-17), 145.19 (C-8a), 139.32 (C-18), 138.27 (C-12), 137.50 (C-10), 136.53 (C-6), 134.98 (C-9), 133.34 (C-4), 133.12 (C-14), 132.78 (C-7), 131.43 (C-21), 131.17 (C-3), 130.48 (C-11), 128.63 (C-20, C-22), 128.13 (C-13), 127.49 (C-8), 127.13 (C-5), 126.91 (C-19, C-23), 126.64 (C-4a), 58.51 (C-16), 44.97 (C-15), 21.75 (6-CH₃). Anal Found: C, 54.27; H, 3.08; N, 9.54; S, 5.87 %. Calc for C₂₆H₁₉Cl₃N₄O₃S: C, 54.42; H, 3.34; N, 9.76; S, 5.59 %. Calc for C₂₆H₁₉Cl₃N₄O₃S, *m/z* 574.03999 (M+1)⁺. Found HRMS (FAB) *m/z* 574.03964 (M+1)⁺.

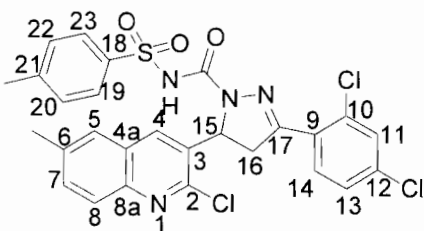


N-[5-(2-Chloro-6-methoxy-quinolin-3-yl)-3-

(2,4-dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-4-methyl-

benzenesulfonamide (79). Yield: 83 mg; 57 %, obtained as white flaky powder, mp: 219-222 °C (from ethyl acetate-hexane), *R_f* (ethyl acetate: hexane, 1:1) 0.48. ν_{\max} (KBr)/ cm⁻¹ 3354 (N-H), 3076 (C-H, aromatic), 2856 (C-H, aliphatic), 1696 (C=O), 1590 (C=C). δ_H (400 MHz, CDCl₃) 8.83 (s, 1H, *NH*), 7.98 (d, 2H, *J* 8.4 Hz, *H*-19 and *H*-23), 7.86 (d, 1H, *J* 9.2 Hz, *H*-8), 7.73 (s, 1H, *H*-4), 7.65 (d, 2H, *J* 8.4 Hz, *H*-20 and *H*-22), 7.45 (d, 1H, *J* 2.0 Hz, *H*-11), 7.37 (dd, 1H, *J* 2.4 and 9.2 Hz, *H*-7), 7.32 (dd, 1H, *J* 2.0 and 8.4 Hz, *H*-13), 7.29 (d, 1H, *J* 8.4 Hz, *H*-14), 7.03 (d, 1H, *J* 2.4 Hz, *H*-5), 5.82 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-15), 4.14 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-16), 3.91 (s, 3H, OCH₃), 3.33 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-16), 2.41 (s, 3H, 21-CH₃). δ_c (100 MHz, CDCl₃) 160.03 (C=O), 158.72 (C-6), 155.08 (C-2), 153.46 (C-17), 143.48 (C-8a), 140.27 (C-21), 137.43 (C-12), 136.18 (C-18), 135.92 (C-10), 133.78 (C-4), 131.44 (C-14), 131.21 (C-3), 129.88 (C-11), 129.77 (C-20, C-22), 129.06 (C-9), 128.68 (C-8), 127.81 (C-13), 127.47 (C-4a), 124.73 (C-19, C-23), 123.73 (C-7), 105.66 (C-5), 58.48 (OCH₃), 55.90 (C-16), 44.97 (C-15), 21.85 (21-CH₃). Anal Found:

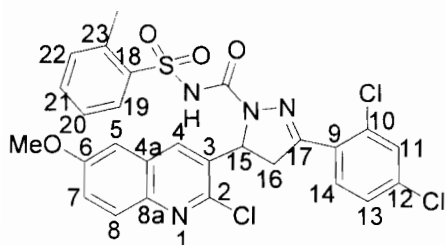
C, 53.71; H, 3.84; N, 8.88; S, 5.28 %. Calc. for $C_{27}H_{21}Cl_3N_4O_4S$: C, 53.70; H, 3.50; N, 9.28; S, 5.31 %. Calc. for $C_{27}H_{21}Cl_3N_4O_4S$, m/z 604.05056 (M+1)⁺. Found HRMS (FAB) m/z 604.05096 (M+1)⁺.



N-[5-(2-Chloro-6-methyl-quinolin-3-yl)-3-(2,4-

dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-4-methyl-

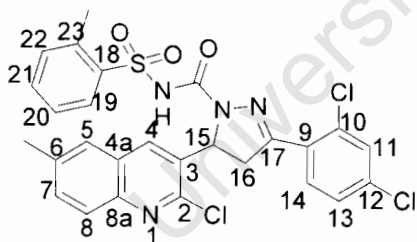
benzenesulfonamide (80). Yield: 96 mg, 64 %, mp: 217-219 °C (from ethyl acetate-hexane) obtained as a white powder. R_f (ethyl acetate: hexane, 1:1) 0.50. ν_{max} (KBr)/ cm^{-1} 3316 (N-H), 3034 (C-H, aromatic), 2986 (C-H, aliphatic), 1682 (C=O), 1602 (C=C). δ_H (400 MHz, $CDCl_3$) 8.85 (s, 1H, NH), 7.97 (d, 2H, J 8.4 Hz, H-19, H-23), 7.86 (d, 1H, J 8.8 Hz, H-8), 7.69 (s, 1H, H-4), 7.65 (d, 1H, J 8.4 Hz, H-14), 7.55 (dd, 1H, J 2.0 and 8.8 Hz, H-7) 7.49 (d, 1H, J 2.0 Hz, H-5), 7.44 (d, 1H, J 2.0 Hz, H-11), 7.30 (dd, 1H, J 2.0 and 8.4 Hz, H-13), 7.28 (d, 2H, J 8.4 Hz, H-20 and H-22), 5.82 (dd, 1H, J 12.0 and 18.0 Hz, H-15), 4.13 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 3.34 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 2.50 (s, 3H, 6- CH_3), 2.40 (s, 3H, 21- CH_3). δ_C (100 MHz, $CDCl_3$) 161.76 (C=O), 154.32 (C-2), 153.44 (C-17), 145.07 (C-8a), 141.06 (C-21), 137.80 (C-18), 137.41 (C-12), 136.22 (C-6), 134.44 (C-10), 133.99 (C-4), 131.46 (C-14), 131.17 (C-3), 130.97 (C-9), 130.34 (C-20, C-22), 130.18 (C-11), 129.76 (C-7), 128.67 (C-13), 127.24 (C-5), 126.87 (C-4a), 125.02 (C-19, C-23), 124.13 (C-8), 58.52 (C-16), 44.98 (C-15), 21.84 (21- CH_3), 21.76 (6- CH_3). Calc. for $C_{27}H_{21}Cl_3N_4O_3S$, m/z 588.05564 (M+1)⁺. Found HRMS (FAB) m/z 588.05544 (M+1)⁺.



N-[5-(2-Chloro-6-methoxy-quinolin-3-yl)-3-

(2,4-dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-2-methyl-

benzenesulfonamide (77). Yield: 82 mg; 85 %, obtained as white flaky powder, mp: 213-215 °C (from ethyl acetate-hexane), R_f (ethyl acetate: hexane, 1:1) 0.45. ν_{\max} (KBr)/ cm^{-1} 3403 (N-H), 3097 (C-H, aromatic), 2882 (C-H, aliphatic), 1685 (C=O), 1622 (C=C). δ_{H} (400 MHz, CDCl_3) 8.87 (s, 1H, NH), 8.16 (d, 1H, J 8.8 Hz, H-8), 7.86 (d, 1H, J 9.6 Hz, H-19), 7.72 (s, 1H, H-4), 7.63 (d, 1H, J 9.6 Hz, H-22), 7.50 (d, 1H, J 1.2 Hz, H-11), 7.48 (t, 1H, J 9.6 Hz, H-20), 7.37-7.31 (m, 4H, H-7, H-13, H-14, H-21), 7.03 (d, 1H, J 2.4 Hz, H-5), 5.80 (dd, 1H, J 12.0 and 18.0 Hz, H-15), 4.15 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 3.92 (s, 3H, OCH_3), 3.35 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 2.74 (s, 3H, 23- CH_3). δ_{C} (100 MHz, CDCl_3) 159.98 (C=O), 156.68 (C-6), 155.08 (C-2), 153.48 (C-17), 143.48 (C-8a), 140.01 (C-18), 137.48 (C-12), 134.09 (C-10), 132.60 (C-4), 131.56 (C-23), 131.39 (C-14), 131.32 (C-21), 129.98 (C-11), 129.77 (C-3), 129.12(C-9), 128.46 (C-22), 128.25 (C-8), 127.84 (C-13), 126.67 (C-4a), 125.60 (C-20), 123.83 (C-19), 121.37 (C-7), 105.27 (C-5), 58.44 (OCH_3), 55.90 (C-16), 44.96 (C-15), 20.69 (23- CH_3). Anal Found: C, 53.82; H, 3.42; N, 9.33; S, 5.51 %. Calc. for $\text{C}_{27}\text{H}_{21}\text{Cl}_3\text{N}_4\text{O}_4\text{S}$: C, 53.70; H, 3.50; N, 9.28; S, 5.31 %. Calc. for $\text{C}_{27}\text{H}_{21}\text{Cl}_3\text{N}_4\text{O}_4\text{S}$, m/z 604.05056 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 604.05014 ($\text{M}+1$)⁺.

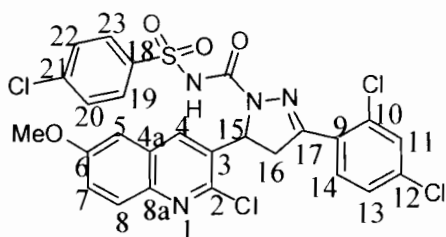


N-[5-(2-Chloro-6-methyl-quinolin-3-yl)-3-(2,4-

dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-methyl-benzenesulfonamide

(78). Yield: 18 mg, 12 %, obtained as white flaky powder, mp: 213-214 °C (from ethyl acetate-hexane), R_f (ethyl acetate: hexane, 1:1) 0.40. ν_{\max} (KBr)/ cm^{-1} 3339 (N-

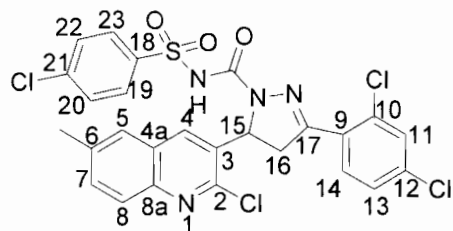
H), 3039 (C-H, aromatic), 2943 (C-H, aliphatic), 1688 (C=O), 1522 (C=C). δ_{H} (400 MHz, CDCl_3) 8.86 (s, 1H, NH), 8.15 (d, 1H, J 8.4 Hz, H-8), 7.86 (d, 1H, J 8.4 Hz, H-19), 7.70 (s, 1H, H-4), 7.63 (d, 1H, J 8.4 Hz, H-22), 7.56 (d, 1H, J 8.8 Hz, H-14), 7.55 (dd, 1H, J 2.0 and 8.4 Hz, H-7), 7.52 (d, 1H, J 2.0 Hz, H-5), 7.48 (d, 1H, J 2.0 Hz, H-11), 7.46 (t, 1H, J 8.4 Hz, H-20), 7.35 (dd, 1H, J 2.0 and 8.8 Hz, H-13), 7.32 (t, 1H, J 8.4 Hz, H-21), 5.81 (dd, 1H, J 12.0 and 18.0 Hz, H-15), 4.15 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 3.36 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 2.75 (s, 3H, 23-Phe- CH_3), 2.52 (s, 3H, 6- CH_3). δ_{C} (100 MHz, CDCl_3) 162.36 (C=O), 153.92 (C-2), 153.54 (C-17), 144.77 (C-8a), 141.43 (C-18), 137.65 (C-12), 135.92 (C-6), 135.43 (C-23), 134.87 (C-10), 134.49 (C-4), 132.36 (C-14), 131.76 (C-21), 131.56 (C-3), 131.34 (C-9), 130.74 (C-22), 130.56 (C-7), 130.28 (C-11), 129.17 (C-13), 127.54 (C-5), 127.07 (C-4a), 126.80 (C-20), 125.54 (C-19), 124.43 (C-8), 58.60 (C-16), 45.48 (C-15), 21.65 (6- CH_3), 20.65 (23- CH_3). Anal Found: C, 54.90; H, 3.17; N, 9.21; S, 5.49 %. Calc for $\text{C}_{27}\text{H}_{21}\text{Cl}_3\text{N}_4\text{O}_3\text{S}$: C, 55.16; H, 3.60; N, 9.53; S, 5.45 %. Calc. for $\text{C}_{27}\text{H}_{21}\text{Cl}_3\text{N}_4\text{O}_3\text{S}$, m/z 588.05564 (M+1)⁺. Found HRMS (FAB) m/z 588.05594 (M+1)⁺.



N-[5-(2-Chloro-6-methoxyquinolin-3-yl)-3-(2,4-dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-4-chloro-

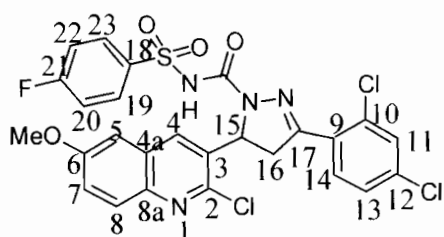
benzenesulfonamide (81). Yield: 92 mg; 60 %, obtained as white flaky powder. mp: 235-236 °C (from ethyl acetate-hexane), R_f (ethyl acetate: hexane, 1:1) 0.51. ν_{max} (KBr)/ cm^{-1} 3348 (N-H), 3005 (C-H, aromatic), 1689 (C=O), 1578 (C=C). δ_{H} (400 MHz, CDCl_3) 8.76 (s, 1H, NH), 8.04 (d, 2H, J 8.8 Hz, H-19, H-23), 7.88 (d, 1H, J 9.2 Hz, H-8), 7.71 (s, 1H, H-4), 7.62 (d, 2H, J 8.8 Hz, H-20, H-22), 7.48 (d, 1H, J 2.0 Hz, H-11), 7.39 (dd, 1H, J 2.4 and 9.2 Hz, H-7), 7.35 (dd, 1H, J 2.0 and 8.4 Hz, H-13), 7.28 (d, 1H, J 8.4 Hz, H-14), 7.02 (d, 1H, J 2.4 Hz, H-5), 5.82 (dd, 1H, J 12.0 and 18.0 Hz, H-15), 4.14 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 3.92 (s, 3H, OCH_3), 3.36 (dd, 1H, J 12.0 and 18.0 Hz, H-16). δ_{C} (100 MHz, CDCl_3) 159.83 (C=O), 158.91 (C-6), 154.98 (C-2), 153.69 (C-17), 143.58 (C-8a), 140.75 (C-21), 137.75 (C-18), 137.57 (C-12), 134.02 (C-10), 133.80 (C-4), 131.34 (C-14), 131.27 (C-3), 130.15 (C-

9), 130.08 (C-11), 129.86 (C-20, C-22), 129.40 (C-8), 128.47 (C-13), 127.80 (C-4a), 127.05 (C-19, C-23), 123.78 (C-7), 105.63 (C-5), 58.61 (OCH₃), 55.85 (C-16), 45.03(C-15). Calc. for C₂₆H₁₈Cl₄N₄O₄S, *m/z* 623.99593 (M+1)⁺. Found HRMS (FAB) *m/z* 623.99564 (M+1)⁺.



4-Chloro-N-[5-(2-chloro-6-methyl-quinolin-3-yl)-3-(2,4-dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-

benzenesulfonamide (82). Yield: 80 mg, 52 %, obtained as white flaky powder, mp: 233-234 °C (from ethyl acetate-hexane), *R_f* (ethyl acetate: hexane, 1:1) 0.27. *v*_{max} (KBr)/ cm⁻¹ 3412 (N-H), 3091 (C-H, aromatic), 2856(C-H, aliphatic), 1681 (C=O), 1532 (C=C). *δ*_H(400 MHz, CDCl₃) 8.79 (s, 1H, NH), 8.04 (d, 2H, *J* 8.4 Hz, *H*-19, *H*-23), 7.88 (d, 1H, *J* 8.8 Hz, *H*-8), 7.67 (s, 1H, *H*-4). 7.64 (d, 1H, *J* 8.4 Hz, *H*-14), 7.56 (dd, 1H, *J* 1.6 and 8.8 Hz, *H*-7), 7.50 (d, 1H, *J* 1.6 Hz, *H*-5), 7.48 (d, 1H, *J* 2.0 Hz, *H*-11), 7.46 (dd, 1H, *J* 2.0 and 8.4 Hz, *H*-13), 7.33 (d, 2H, *J* 8.4 Hz, *H*-20, *H*-22), 5.83 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-15), 4.16 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-16), 3.37 (dd, 1H, *J* 12.0 and 18.0 Hz, *H*-16), 2.53 (s, 3H, 6-CH₃). *δ*_C(100 MHz, CDCl₃) 161.37 (C=O), 155.76 (C-2), 155.64 (C-17), 146.09 (C-8a), 140.78 (C-18), 137.98 (C-12), 137.56 (C-21), 135.35 (C-6), 134.43 (C-10), 134.06 (C-4), 131.40 (C-14), 131.26 (C-3), 130.78 (C-9), 130.54 (C-11), 130.18 (C-7), 129.84 (C-20, C-22), 129.47 (C-13), 128.10 (C-19, C-23), 127.84 (C-5), 127.26 (C-4a), 126.80 (C-8), 58.53 (C-16), 44.99 (C-15), 21.80 (6-CH₃). Anal Found: C, 51.29; H, 2.69; N, 9.19; S, 5.13 %. Calc for C₂₆H₁₈Cl₄N₄O₃S: C, 51.33, H, 2.98; N, 9.21; S, 5.27 %. Calc. for C₂₆H₁₈Cl₄N₄O₃S, *m/z* 609.00884 (M+1)⁺. Found HRMS (FAB) *m/z* 609.00896 (M+1)⁺.



N-[5-(2-Chloro-6-methoxy-quinolin-3-yl)-3-

(2,4-dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-4-fluoro-

benzenesulfonamide (**83**). Yield: 13.33 mg; 9.1 %, obtained as a white flaky powder.

mp: 226-227 °C (from ethyl acetate-hexane), R_f (ethyl acetate: hexane, 1:1) 0.52. ν_{\max}

(KBr)/ cm^{-1} 3376 (N-H), 3078 (C-H, aromatic), 2909 (C-H, aliphatic), 1701 (C=O),

1576 (C=C). δ_{H} (400 MHz, CDCl_3) 8.80 (s, 1H, NH), 8.13 (d, 2H, J 8.8 Hz, H-19, H-

23), 7.88 (d, 1H, J 9.2 Hz, H-8), 7.71 (s, 1H, H-4), 7.64 (d, 2H, J 8.8 Hz, H-20, H-22),

7.47 (d, 1H, J 2.0 Hz, H-11), 7.38 (dd, 1H, J 2.4 and 9.2 Hz, H-7) 7.32 (dd, 1H, J 2.0

and 8.4 Hz, H-13), 7.21 (d, 1H, J 8.4 Hz, H-14), 7.02 (d, 1H, J 2.4 Hz, H-5), 5.82 (dd,

1H, J 12.0 and 18.0 Hz, H-15), 4.16 (dd, 1H, J 12.0 and 18.0 Hz, H-16), 3.92 (s, 3H,

OCH_3), 3.36 (dd, 1H, J 12.0 and 18.0 Hz, H-16). δ_{C} (100 MHz, CDCl_3) 167.33 (C-21),

160.23 (C=O), 158.82 (C-6), 155.06 (C-2), 153.74 (C-17), 143.51 (C-8a), 138.05 (C-

18), 137.54 (C-12), 133.92 (C-10), 133.80 (C-4), 131.72 (C-14), 131.62 (C-3), 131.42

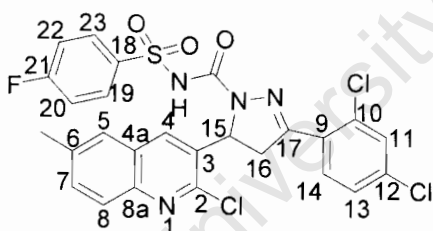
(C-9), 130.36 (C-11), 129.81 (C-8), 128.46 (C-13), 127.83 (C-19, C-23), 127.73 (C-

4a), 123.92 (C-7), 116.54 (C-20, C-22), 105.48 (C-5), 58.52 (OCH_3), 55.87 (C-16),

45.02 (C-15), Anal Found: C, 51.38; H, 2.69; N, 9.19; S, 5.18 %. Calc. for

$\text{C}_{26}\text{H}_{18}\text{Cl}_3\text{FN}_4\text{O}_4\text{S}$: C, 51.37; H, 2.98; N, 9.22; S, 5.28 %. Calc. for $\text{C}_{26}\text{H}_{18}\text{Cl}_3\text{FN}_4\text{O}_4\text{S}$,

m/z 607.12559 ($\text{M}+1$)⁺. Found HRMS (FAB) m/z 607.13447 ($\text{M}+1$)⁺.



N-[5-(2-Chloro-6-methyl-quinolin-3-yl)-3-(2,4-

dichloro-phenyl)-4,5-dihydro-pyrazole-1-carbonyl]-4-fluoro-benzenesulfonamide

(**84**). Yield: 0.112 g, 74 %, obtained as white flaky powder, mp: 219-221 °C (from

ethyl acetate-hexane), R_f (ethyl acetate: hexane, 1:1) 0.35. ν_{\max} (KBr)/ cm^{-1} 3309 (N-

H), 3090 (C-H, aromatic), 2950 (C-H, aliphatic), 1643 (C=O), 1688 (C=C). δ_{H} (400

MHz, CDCl_3) 8.80 (s, 1H, NH), 8.13 (d, 2H, J 8.8 Hz, H-19, H-23), 7.88 (d, 1H, J 8.8

Hz, *H-8*), 7.67 (s, 1H, *H-4*), 7.64 (d, 1H, *J* 8.8 Hz, *H-14*), 7.56 (dd, 1H, *J* 2.0 and 8.8 Hz, *H-7*), 7.48 (d, 1H, *J* 2.0 Hz, *H-5*), 7.46 (d, 1H, *J* 2.0 Hz, *H-11*), 7.33 (dd, 1H, *J* 2.0 and 8.8 Hz, *H-13*), 7.20 (d, 2H, *J* 8.8 Hz, *H-20*, *H-22*), 5.83 (dd, 1H, *J* 12.0 and 18.0 Hz, *H-15*), 4.16 (dd, 1H, *J* 12.0 and 18.0 Hz, *H-16*), 3.36 (dd, 1H, *J* 12.0 and 18.0 Hz, *H-16*), 2.52 (s, 3H, 6-*CH*₃). δ_{C} (100 MHz, CDCl₃) 163.00 (*C-21*), 160.97 (*C=O*), 153.76 (*C-2*), 153.70 (*C-17*), 144.15 (*C-8a*), 137.96 (*C-18*), 137.54 (*C-12*), 135.56 (*C-6*), 134.41 (*C-10*), 133.78 (*C-4*), 131.69 (*C-14*), 131.57 (*C-3*), 131.41 (*C-9*), 130.69 (*C-11*), 129.75 (*C-7*), 128.53 (*C-13*), 127.83 (*C-19*, *C-23*), 127.24 (*C-5*), 126.82 (*C-4a*), 123.92 (*C-8*), 116.54 (*C-20*, *C-22*), 58.52 (*C-16*), 44.98 (*C-15*), 21.73 (6-*CH*₃). Calc. for C₂₆H₁₈Cl₃FN₄O₃S, *m/z* 592.03057 (M+1)⁺. Found HRMS (FAB) *m/z* 592.03085 (M+1)⁺.

University of Cape Town

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