SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTIPARASITIC CYSTEINE PROTEASE INHIBITORS BASED ON THE ISATIN SCAFFOLD

By

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ABSTRACT

Widespread drug resistance, loss of efficacy and toxicity has limited the full utilization of the current available drugs against malaria and other parasitic diseases. This necessitates the development of new drugs. Meanwhile, the cysteine protease family of enzymes has been identified as potential targets for new modes of chemotherapy due to the numerous critical roles they play in the disease-causing agents.

In this project, a non-peptidic and low molecular weight isatin (indole-2, 3-dione) possessing a wide range of pharmacological properties was used as a scaffold to which different moeities were appended. Potential inhibitors of parasitic cysteine proteases and three strains of *P. falciparum* were identified from synthesized libraries of compounds.

Various *N*-substituted isatin derivatives were synthesized by KF/Al_2O_3-mediated reaction of isatins with an alkyl, acyl or sulfonyl halide. A series of isatin-3-thiosemicarbazones were prepared by condensation of isatin / substituted isatins with thiosemicarbazide, and also a series of isatin-based Schiff and Mannich bases were prepared by reacting selected isatin-3-thiosemicarbazones with formaldehyde and appropriate secondary amines. To compare the effects of replacing the Mannich bases, a similar series of aminoquinoline-ethylene isatin-based derivatives were then synthesized. The synthesis was accomplished by condensation of quinoline-ethylene ketone forms with thiosemicarbazide. All synthesized compound were obtained in reasonable to excellent yields and characterized by spectroscopic and analytical techniques.

These synthesized compounds were then subsequently tested for inhibition of cysteine proteases (*falcipain-2*, *cruzain* and *rhodesain*) and also for *in vitro* activity against strains of the parasitic protozoa *P. falciparum* (chloroquine-sensitive D10 and chloroquine-resistant W2 and K1). The structure-activity relationships (SARs) among the classes of compounds for inhibition of *falcipain-2*, *cruzain*, *rhodesain* and *P. falciparum* malaria parasites were studied. Compound N-*biphenyl-2-yl-5-methyl-1H-indole-2, 3*-dione (**57f**) in the class of *N*-substituted isatin derivatives showed good inhibitory activity against *falcipain-2* and *cruzain*. Another compound from the thiosemicarbazones found to be very active was *5-chloro-7-isatin-3-thiosemicarbazone* (**65f**), with IC_{50} values of 10 μM or less against *falcipain-2*, *cruzain* and *rhodesain*.

The quinoline-ethylene isatin thiosemicarbazone derivatives gave optimal *in vitro* antimalarial activity with N^1-*[2-(7-chloro-quinol-4-ylamino)-ethyl]-5-methyl*-1H-*idole-2,3-dione-3-thiosemicarbazone* (**71b**) showing excellent activity against a chloroquine-resistant *P. falciparum* (W2) strain (IC_{50} = 0.051 μM) substantially lower than chloroquine in W2 (IC_{50} = 0.24 μM). Similarily, *5-chloro*-N^1-*[2-(7-chloro-quinol-4-ylamino)-ethyl]-1*H-*indole-2,3-dione* (**71c**) showed high activity against another chloroquine-resistant *P. falciparum* (K1) strain (IC_{50} = 0.054 μM). The IC_{50} of chloroquine in K1 was 0.312 μM. This did not correlate with the inhibitory potency against the *P. falciparum* cysteine protease *falcipain-2*. The lack of correlation between inhibitory activity against *falcipain-2* and the three malaria parasite strains suggests that *falcipain-2* is not the target in quinoline-ethylene isatin thiosemicarbazone inhibitors. Alternatively, these metal-chelating compounds may be metal (Fe^{3+})-interactive inhibitors of *falcipain-2* under the pro-oxidative intra-erythrocytic conditions of oxidative stress in *P. falciparum*.

ABBREVIATIONS

Ac	acetyl
Anal.	analytical
Ar	aryl
Bn	benzyl
bp	boiling point
br	broad (in NMR)
Bu	butyl
Bz	benzoyl
t-Bu	tertiary butyl
^{o}C	degrees Celsius
Calcd.	calculated
CH_2Cl_2	dichloromethane
CH_3CN	acetonitrile
CO_2	carbon dioxide
CP	cysteine protease
CQ	chloroquine
Cys	cysteine
d	doublet (in NMR)
dd	doublet of doublets (in NMR)
ddd	doublet of double of doublet or double double doublet (in NMR)
DMAP	4-dimethylaminopyridine
DMF	*N*, *N*-dimethylformamide
DMSO	dimethylformamide
δ	chemical shift in parts per million downfield from tetramethylsilane (in NMR)
ED_{50}	effective dosage required to inhibit 50 percent of parasite growth
EI	electron impact
Equiv.	equivalents
Et	ethyl
Et_3N	triethylamine

EtOAc	ethyl acetate
EtOH	ethanol
FAB	fast atomic bombardment
FT	Fourier transform
g	gram(s)
h	hour(s)
HAT	Human African trypanosomiasis
HRMS	high-resolution mass spectrometry
Hz	hertz (in NMR)
IC_{50}	inhibitory concentration to inhibit 50 % of enzyme activity
IR	infrared
J	coupling constant (in NMR)
LRMS	low-resolution mass spectrometry
µ	micro (10^{-6})
Ms	Methanesulfonyl
MsCl	Methanesulfonyl chloride
M	moles per cubic decimeter ($mol.dm^{-3}$)
m	multiplet (in NMR)
MDR	multidrug resistance
MeOH	methanol
min	minute
Me	methyl
MHz	megahertz (in NMR)
mL	millilitre
mol	mole(s)
mp	melting point
MS	mass spectrometry
m/z	mass to charge ratio (in mass spectra)
NMP	*N*-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
Nu	nucleophile

p	*para*
P.	*Plasmodium*
Ph	phenyl
Pgp	P-glycoprotein
Phe	phenylalanine
Pro	proline
ppm	parts per million (in NMR)
q	quartet (in NMR)
R_f	retention factor (in chromatography)
rt	room temperature
s	singlet (in NMR)
Ser	serine
t	triplet (in NMR)
tert	tertiary
TBDMS	*tert*-butyldimethylsiloxy
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane (in NMR)
TMSCl	tetramethylsilyl chloride
UV	ultraviolet
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Introduction to Parasitic Diseases

Parasites, a group of microorganisms of the *protista kingdom*, have played a dominant role in the history of human diseases. Not only are parasites common in humans, they are also found among plants and animal groups where they live for survival and completion of their life cycles. The animal-like unicellular parasites also known as *protozoa* are of medical importance because of their ability to cause severe organ dysfunctions in the hosts. Examples of protozoan parasites include *Entamoeba* (amoebiasis/amoebic dysentery), *Trypanosomes* (African trypanosomiasis, Chagas disease), *Balantidium* (colitis, diarrhea) and *Plasmodium* (malaria). [1]

Globally, parasitic diseases such as Trypanosomiasis, Malaria and Leishmaniasiss are major causes of illness today particularly in the developing tropical and subtropical countries, [2] and some sections of the United States. [3] In the developing countries, poverty and climate are the key contributors to the rapid transmission and prevalence of the diseases. In the 1998 World Health Organization report, of a global total of 52.2 million deaths, 17.3 million were due to infectious and parasitic diseases. [4] The burden of parasitic diseases remains a major concern as recent estimates stand at 273 million infections each year for malaria; 1.2 million for African trypanosomiasis and 1.7 million for leishmaniasis. On the other hand, schistosomiasis and filariasis were reported to affect 200 million and 120 million people respectively. [5]

For most of the parasitic diseases, there are adequate drugs available to completely treat these infections. The major drawback faced with these drugs includes loss of efficacy, unacceptable toxicity and widespread drug resistance. Socio-economic factors have further added to the problem of antiparasitic chemotherapy. The diseases are mostly prevalent in poor and highly indebted nations where drug research and development costs are out of reach. [6] On the other hand, the access to available drugs, and more general to health services, is more and more restricted on economical grounds. Coupled with the profit driven trends in the pharmaceutical industries in the western world today, the tropical diseases always remain unattended to. [7]

Within the context of parasitic infections, malaria is the key parasitic disease and was the main focus of this MSc research project.

1.2 Malaria

Malaria, a deadly fever probably among the oldest known to mankind, is caused by *plasmodium* protozoa and transmitted *via* bites by female mosquitoes belonging to the *Anopheles* species. Malaria is now mainly confined to Africa, Asia and Latin America. It kills between 1 and 1.5 million people each year. The four species of *Plasmodium* parasite responsible for causing human malaria are, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, and *Plasmodium malariae*, with the *Plasmodium falciparum* strain being responsible for most fatalities. [8] *P. falciparum* is the prevalent species in sub-Sahara Africa accounting in large part for the extremely high mortality in this region. [9] *P. vivax* is second cause of malaria death, it is also known to cause gradual health deterioration as it remains in the body for a long time. *P. vivax* is the predominant malaria species in most of Asia, Oceania, North Africa, Central and South America.

In malaria areas, women and children are most vulnerable and at the highest risk of morbidity and mortality. Children die in their first or second year of their life before they have adequately developed immunity. As for women, they are at risk during the first pregnancy when their immunity is impaired and so susceptible to *P. falciparum* malaria.

Malaria has 3 prime ways in which it can cause death in individuals.

i). presence of seizures or coma in case of cerebral malaria.

ii). Severe anaemia due to excessive breakdown of red blood cells.

iii). High body temperatures that is accompanied by erythrocytic phase of the ailment.

Malaria is known to cause long-term disabilities in children who frequently get the fever or those who survive cerebral form of malaria. The most notable effects in survivals include learning impairment, mental disabilities due to brain damage, epilepsy and spasticity. [10]

For a better understanding of the pathogenesis and the current chemotherapies, it is necessary to look at the life cycle of the malaria parasite.

1.2.1 Life Cycle of the Malaria Parasite

The life cycle of the malaria parasite involves two stages: a sexual reproductive stage which occurs in the mosquito, and an asexual reproduction stage, which takes place in the human host. [11] The latter can be distinctively categorized as exoerythrocytic (liver) and the erythrocytic (blood) phases. The human part of the cycle is responsible for the disease pathology and target by all antimalarial chemotherapeutic agents.

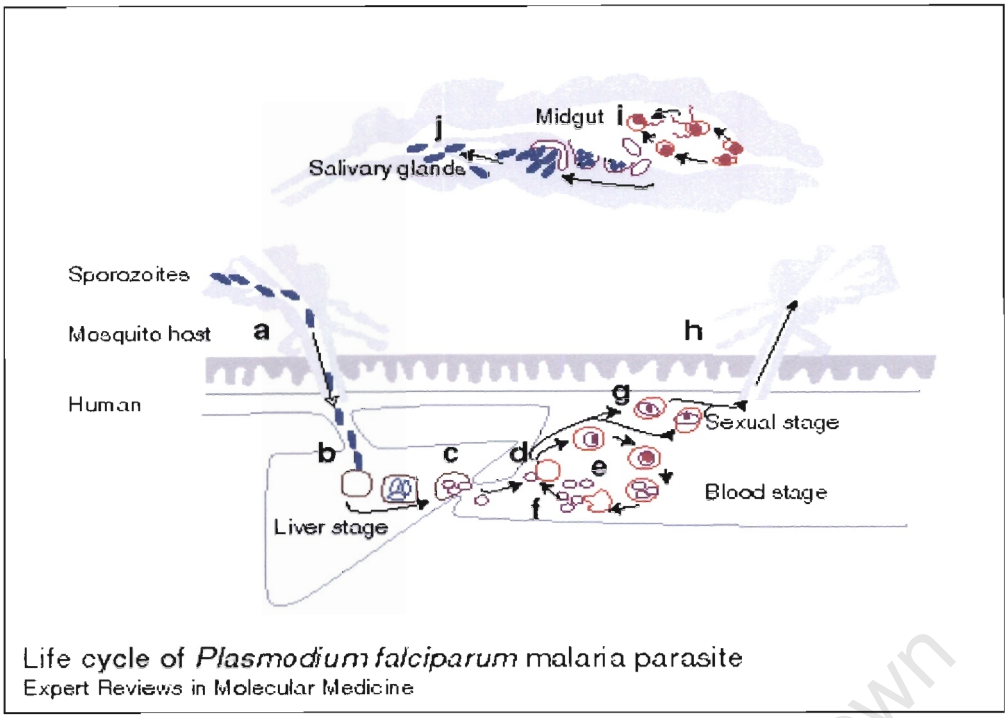


Figure 1. A representation of the *P. falciparum* life cycle. [12]

Figure 1 depicts the life cycle of *P.falciparum* [12] whose basic features are identical to the other *Plasmodium* species. The cycle starts when sporozoites are injected in human peripheral circulation during the blood-meal of an infected *anopheline* mosquito (**a**). Within minutes they migrate to the hepatocytes in the liver (**b**) and undergo asexual multiplication yielding merozoites, a new form of parasites within 1 to 2 weeks. Infected hepatocytes rupture to release the merozoites into the blood stream (**c**) to continue with the cycle. Merozoites then invade the erythrocytes (**d**) where they progressively break down human hemoglobin into amino acids destroying the erythrocytes. Using the amino acid, merozoites then either divide or differentiate into male (microgametocytes) and female (macrogametocyte) which either invades other red blood cells (**f**) to continue the erythrocytic cycle (**e**) or re-enter the blood stream (**g**) eventually taken up by a feeding *Anopheles sp*. mosquito thus completing the cycle (**h**). This erythrocytic stage of the parasitic cycle is thought to be responsible for the periodic clinical manifestations of malaria (*e.g.* fever and chills). [13]

1.2.2 History and Limitation of Current Antimalarials

Malaria has a long-standing history of chemotherapy that goes back to more than 350 years ago when quinine was first used in treating the fever. Several classes of antimalarial drugs with different structural features and targeting different stages of the malaria parasite's life cycle have been developed in the last century (fig. 2).[14] Amongst the most successful antimalarial drugs ever used is the chloroquine, **1** (fig 2) which was discovered in 1934 by a German scientist and consequently launched in 1944.

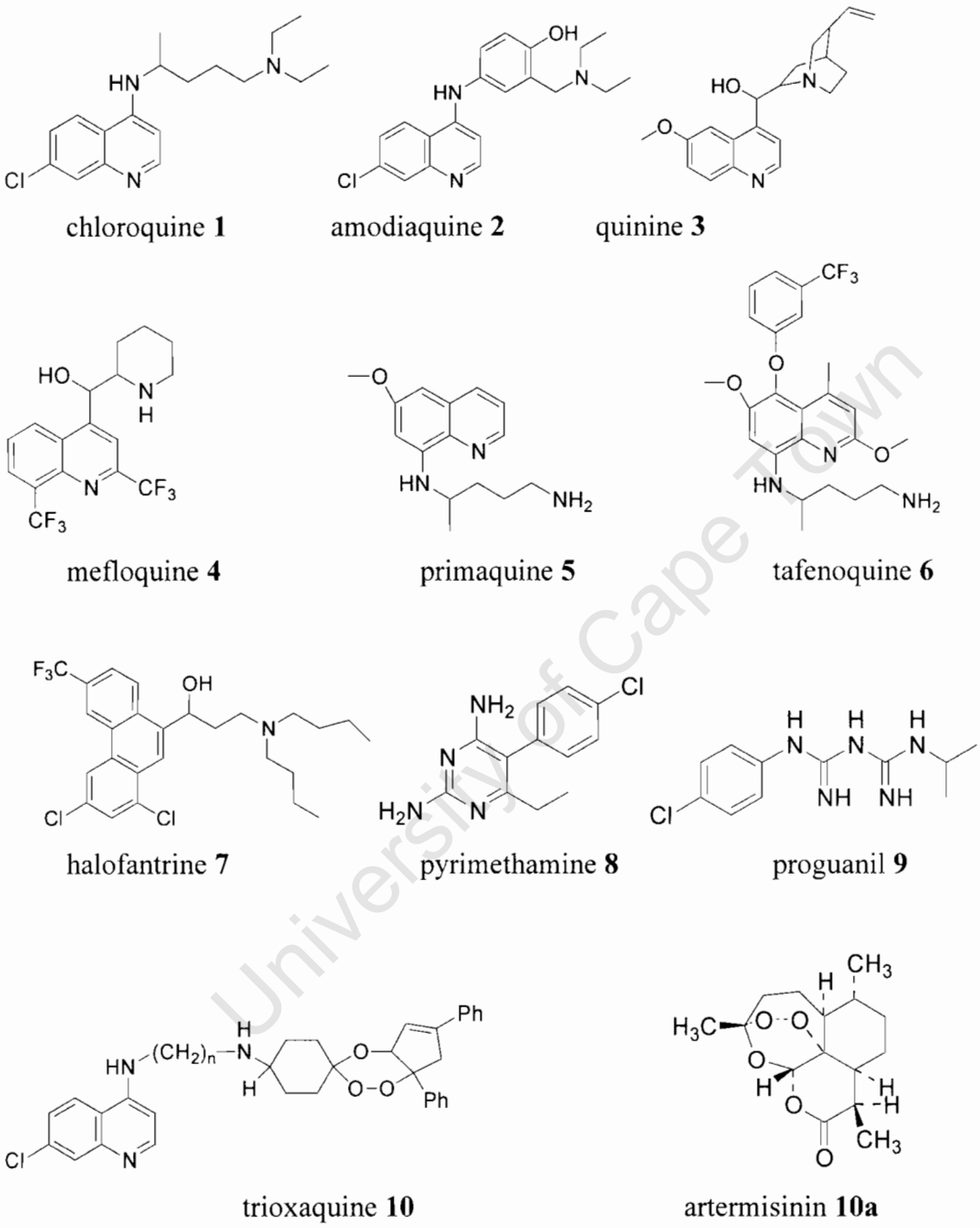


Figure 2. Structures of quinoline and non-quinoline based antimalarial drugs.

For many years chloroquine has proved to be a highly effective, safe [15], and affordable drug for the treatment and prophylaxis of malaria mainly in Southeast Asia, portions of South America, and much of Sub-Sahara Africa. Despite the widespread resistance to chloroquine, the drug is still active against the erythrocytic stage of *P. malariae*, *P. vivax* and even chloroquine-sensitive *P. falciparum* and *P. vivax* species. Chloroquine transverses through the membranes of the parasite and moves against a pH gradient subsequently accumulating in the acidic parasite's food vacuole by a pH trapping mechanism. Accumulation of chloroquine is facilitated by the weak base properties of the drug. At neutral conditions, chloroquine diffuses through the membranes while at acidic pH of the vacuole, it is protonated and trapped. However, the involvement of the drug in inhibitory process is not clear; presumably it interferes with heme polymerization leading to accumulation of toxic heme that becomes fatal to the parasite. [16-18] Serious adverse effects of chloroquine are rare, sometimes headaches, nausea, vomiting and gastrointestinal symptoms may be experienced with its administration.

Amodiaquine (**2**) (fig 2) an analog of chloroquine was used in the treatment and prevention of uncomplicated malaria and some chloroquine-resistant strains. [19] It was introduced about 40 years ago as an alternative to chloroquine. Nowadays, amodiaquine is not recommended due to some toxic side effects such as agranulocytosis and hepatotoxicity.

Quinine (**3**) (fig 2), a natural alkaloid, is the longest serving antimalarial discovered in the 17th century in South America by Augustinian monks. [20, 21] It is available commercially and has excellent activity against all *Plasmodium* malaria cases. The mode of action quinine is similar to other quinolines group of drugs as they all bind strongly to haematin, and form complexes which are toxic to the malarial parasite. [22] However, quinine is associated with toxicity resulting from repeated doses. Symptoms (also known as cinchonisms) include tinnitus, headache, deafness, and occasionally, anaphylactoid shock are characteristic of extensive quinine use. [23]

Mefloquine (**4**) (fig 2), a structural analog of quinine, is a relatively new drug used for treatment and as a prophylactic. It is a potent long-acting schizonticide active against *P. falciparum* resistant to 4-aminoquinoline and folate antangonist drugs. Neuropsychiatric adverse reactions have been reported that include anxiety disorders, hallucinations, sleeping disturbances, convulsions and acute brain syndromes. [24] Sporadic cases of mefloquine resistance have been reported from Thailand [25] and Kenya. [26]

Primaquine (**5**) (fig 2) and tafenoquine (**6**) (fig 2) belong to the 8-aminoquinoline class of antimalarials. Primaquine possess weak antiplasmodial properties and is not normally used for

treating *P. falciparum* malaria. The drug is instead used to eradicate early stages of *P. falciparum* and hypnozoites of *P. vivax* and *P. ovale*. [27] Primaquine acts by interfering with mitochondrial functions of *Plasmodium*. Toxic effects of the drug include mild anemia, cyanosis and haemolysis. [28] Tafenoquine is a long-acting 8-aminoquinoline with a half-life of 2weeks and used by all short-time visitors to malarial areas to prevent the risk of *P. falciparum* malaria infections. [29] Tafenoquine is commonly used for treating multidrug-resistant *P. falciparum* malaria, particularly in combination with artemisinin derivatives.

Folate antagonists are a new group of drugs also known as folate blockers. They act on erythrocytic *P. falciparum*, but not sporozoites or hypnozoites. The drugs (pyrimethamine **8** and proguanil **9**) (fig 2) inhibit preferentially the dihydrofolate reductase (DHFR) necessary for synthesis of tetrahydrofolate, a precursor in the parasite DNA synthesis, whilst (sulfones and sulfonamides) inhibit dihydro-pteroate synthetase (DHPS) enzymes of malaria parasites. Widespread resistance of parasites to the drug has been reported in most malaria-endemic areas. [30] Furthermore, high cost of the drug ($40 per treatment) and reported side effects (severe skin and mucous membrane reactions) further hampers full utilization of this class of drugs. [31 - 33]

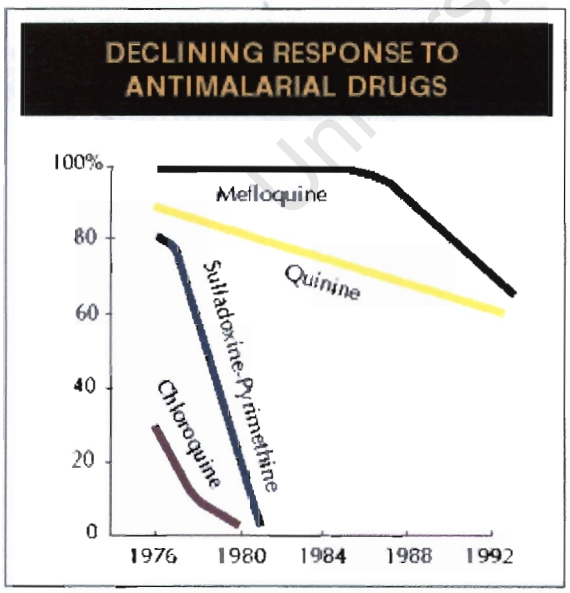
Artemisinin (10a) (fig 2) is an active ingredient of the Chinese herb quinghao and has been used in the cure for fevers and malaria over the past two-three decades. [34 - 38] It is the fastest active antimalarial drug that exists in several forms including artemether, arteether, and artesunate. It has been used in the treatment of severe cases of malaria in combination with other drugs like mefloquine, sulfadoxine-pyrimethamine and benflumetol mainly because of its short half–life (3-5 hours). [39] The drugs are known to generate free radicals which are toxic to the parasites.

Recently a series of trioxaquine (**10**) (fig 2) were reported as potential agents against chloroquine-resistant malaria. Trioxaquine combines an artemisinin and the basic 4-aminoquinoline moiety of chloroquine which presumably is necessary for concentration in the acidic food vacuole and binding to heme. These molecules were designed with an effort to delay or prevent the early development of resistance and increasing the efficacy of the compound. The drug was tested *in vitro* against chloroquine-sensitive and chloroquine-resistant strains of *P.falciparum* all with IC_{50} values below 30nM. Trioxaquine is highly active against Chloroquine-resistant strains (IC_{50} = 21 nM) compared to 116 nM for chloroquine. These encouraging results indicate that the use of bitherapeutic approach may be the best and promising approach to fight the emerging drug resistance.

1.2.3 Drug Resistance

Increasing resistance of malaria parasites to drugs is threatening to undermine efforts to treat the disease in endemic areas and is a major source of concern. Clinically, drug resistance is the ability of the malaria parasite (*P. falciparum* and *P. vivax*) to mutate, thus restraining the power of conventional antimalarial drugs to fight it. This, ultimately, allows the parasite's rapid replication.

The emergence and spread of resistance are not clear though studies indicate several factors, including abuse, underuse or misuse of antimicrobials, poor patient compliance, poor quality of available drugs and spontaneously-occurring gene mutation that affect the structure and activity at the drug target in the malaria parasite *P. falciparum* and *P. vivax*. The latter is more pronounced and affects the accessibility of the drug to that target. Antifolates, for example, act by inhibiting folate biosynthetic pathway in dihydropteroate synthetase (*dhps*) and dihydrofolate reductase (*dhfr*). Resistance of parasites against proguanils (*dhfr* inhibitors) is attributed to a simple single mutations in the *dhfr* enzyme, [40] while resistance to sulfonamides (*dhps* inhibitors) is due to a similar mutations in the corresponding gene. [41] On the other hand, studies on chloroquine resistance is attributed to mutations in the *pfcrt* (*P. falciparum* chloroquine resistance transporter) gene located on the surface of the parasite food vacuole and *Pgh1* (P. glycoprotein homolog). [42] *Pgh1* is actually implicated in the development of resistance in other antimalarials as well.



The Southeast Journal of Tropical Medicine and Public Health, Mekong Malaria. Volume 30, Supplement 4, p 68, 1995

Figure 3. Decline in response to Antimalarial Drugs.

The global picture of reduced susceptibility of *P. falciparum* to various antimalarials is provided in Figure 3 and this underlines the importance of development of new and better drugs that are active against the parasites. A change in the present strategy of antimalarial chemotherapy is needed because with the current trend of declining antimalarial response, by 2010 malaria cases will have doubled.

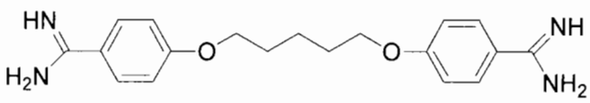
1:3 Other parasitic diseases

While there might be some evidence to suggest that compounds found to be antimalarials can also act against other protozoan parasites, for example, *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania*, it is necessary to briefly look these agents and their associated parasitic diseases although the main focus of the project is on malaria.

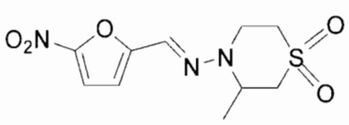
Human African trypanosomiasis, HAT, also known as (sleeping sickness) is a systematic and central nervous system (CNS) infection. Two distinct species of flagellated protozoa (trypanosomes) responsible for the pathological conditions are *Trypanosoma brucei rhodesiense* (occurs in East and Southern Africa) and *Trypanosoma brucei gambiense* (occurs in West and Central Africa). The former is responsible for acute (blood forms), whilst the latter causes chronic forms (tissue forms) of the disease. [43] The only drugs used to treat African Trypanomiasis are, melarsoprol, pentamidine **11** (fig.4) and eflornithine. Melarsoprol is restricted in use due to toxicity and adverse side effects including chest pain, abdominal pain and phlebitis. [44] However, melarsoprol remains the drug of choice for treating the chronic disease stage.

Chagas disease is caused by a parasite of the species *Trypanosoma cruzi* (different from the fellow kinetoplastid species of Leishmania and *Trypanosoma brucei* species families). This is prevalent in Latin and South America where conditions favour the growth of reduviid insects (also called kissing bugs). The protozoan parasite *(T. cruzi*), enters the human body through broken skin from faeces of the bugs. Like HAT, Chagas disease also manifests itself in acute (mild symptoms of fevers and swelling of glands) and chronic forms. The chronic stage is the most fatal as it affects heart muscle, oesophagus and lower intestine causing severe damage. [45] Nifurtimox **12** (fig. 4) and benznidazole **13** (fig.4) are effective against the acute stages of the disease and are inadequate to treat the chronic infections.

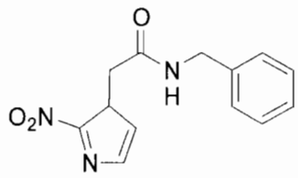
Leishmaniasis is transmitted by the bite of some species of sand flies. The disease most commonly manifests either in a cutaneous (skin) form or in a visceral (internal organ) forms. The 75% of cases that are reported are in Latin America and some areas of South America. Currently, the principal medication for the disease is amphotericin B.



pentamidine **11**



nifurtimox **12**



benznidazole **13**

Figure 4. Structures of some currently used drugs against HAT and Chagas Disease.

CHAPTER 2

BACKGROUND AND RATIONALE: CYSTEINE PROTEASES AND CHEMISTRY OF ISATIN

2.1 CYSTEINE PROTEASES AND THEIR INHIBITORS

2.1.1 Introduction

As mentioned earlier (in Chapter 1), the current chemotherapy for malaria and other parasitic diseases is limited in terms of efficacy, toxicity and resistance. Furthermore, the high cost of current drugs is making it difficult to treat parasitic infections. Hence, there is an urgent need for new treatment approaches that are safe, effective and cheap.

Biochemical pathways found in parasites have provided vital insights in finding alternative routes to current therapies. Enzymes of these pathways are thought to be particularly ideal targets in treatment of parasitic diseases. For example, cysteine proteases, a class of enzymes involved in numerous biological and biochemical processes in parasites, have been reported to be an attractive target in the fight against malaria. [46] In antimalarial chemotherapy, the current focus is particularly on *falcipains*-(2 and 3) cysteine proteases that are located inside the parasitic acidic food vacuole that mediate hemoglobin hydrolysis and are crucial to the survival of the malaria parasite. Previous studies have shown that peptidyl vinyl sulfones effectively inhibit *falcipain-2* or 3 and exhibited strong antimalarial effects *in vitro* and in vivo. [46, 47] In separate studies, peptidyl and non-peptidyl inhibitors of the major cysteine proteases of trypanosomes killed *T. brucei* [48] and *T. cruzi in vitro* and *in vivo*. [49] The results clearly suggest that compounds directed against cysteine proteases may be promising antiparasitic drugs.

2.1.2 Cysteine Proteases

Cysteine proteases are proteolytic enzymes that utilize the cysteine thiol for catalytic activity. These enzymes catalyse the cleavage of amide linkages in proteins and peptides. Generally, the enzymes can be categorized based on occurence as plant proteases, mammalian lysosomal proteases, viral, fungi or bacterial proteases. [50, 51] Proteases clan CA (papain-like) and family C1 are of medical importance and account for more than half of all cysteine proteases. These include the cathepsins B [52] and L [53] (involved in cancer), cathepsin K [54] (involved in bone degradation) as well as parasitic proteases (associated with Chagas disease, malaria and leishmaniasis). Enzymes of family C14 (the caspases) have gained attention because of their involvement in apoptosis. [51] Calpains (belonging to the C2 family) are a unique kind as they

require Ca^{2+} and have been implicated in a number of diseases such as neurological disorders and Alzheimer's disease. [51]

2.1.3 Cysteine Proteases of Parasitic Organisms

Parasitic protozoa contain numerous cysteine proteases all so crucial to the life cycle of the organisms. More recently, a good number of these enzymes have been identified and characterized. Among the most important are cysteine proteases in *P .falciparum*, *Trypanosoma cruzi*, *Trypanosoma brucei rhodesiense* and *Trypanosome brucei gambiense.* These are briefly discussed below:

a) *Falcipains*-2 and 3 are the major cysteine proteases of the protozoan parasite *P. falciparum* and expressed by the erythrocyte trophozoites. Intraerythrocytic trophozoites derive amino acids for protein synthesis from degradation of host cell hemoglobin in the acidic food vacuole. [46, 55] Proteases that hydrolyze hemoglobin include members of aspartic proteases (*plasmepsin* I and II), cysteine proteases (*falcipains* 2 and 3) and *falcilysin* families. *Falcipain-2* and *falcipain-3* appear to be the principal cysteine proteases in this degradation. The most active between the two is *falcipain-2*, however, both have similar specificities of strong preference for the leucine amino acid residue. The role of *falcipain-1* is not clear, but presumed to be active during invasion of erythrocytes. [56]

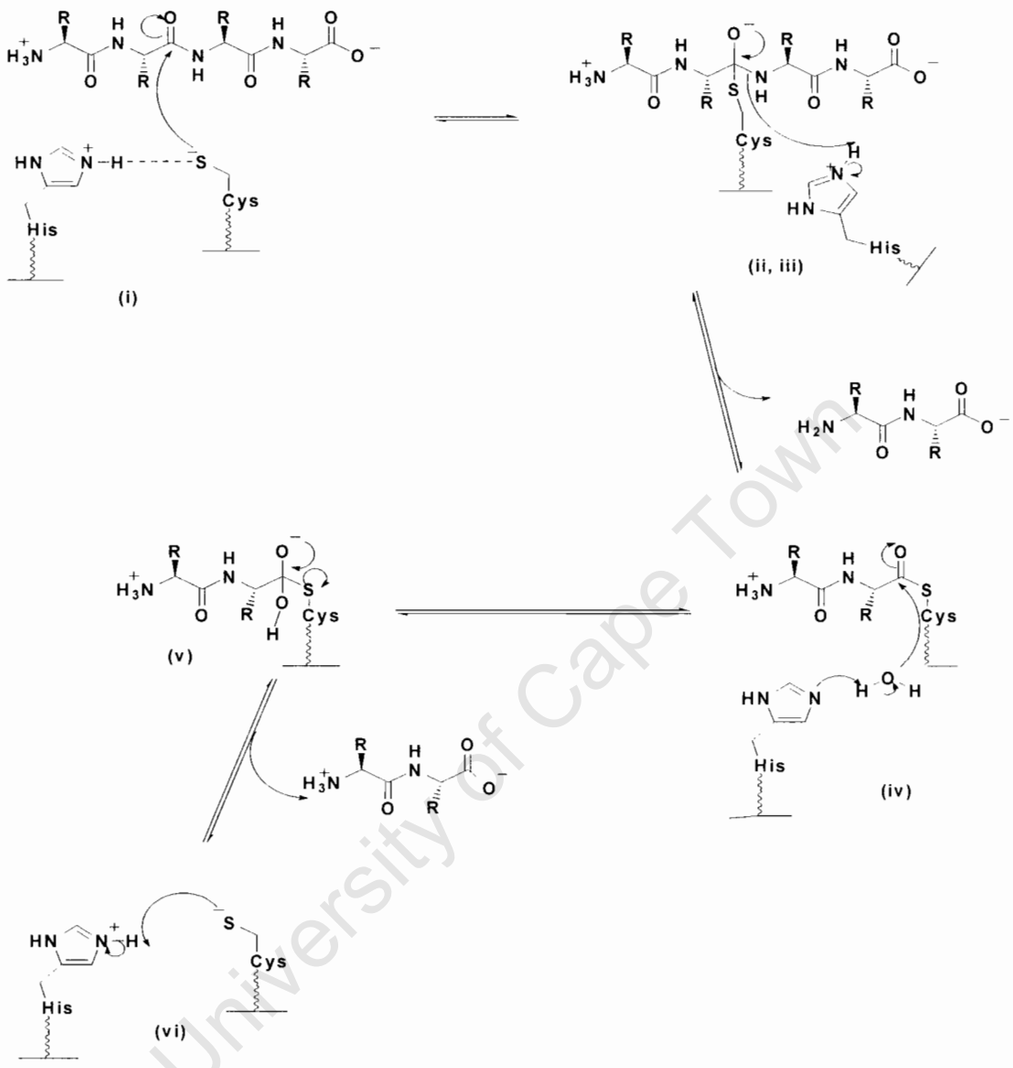
Compounds that inhibit *falcipain-2* or 3 block hemoglobin degradation and prevent parasite development. For example, chalcones and peptidic vinyl sulfones have been shown to cure malaria-infected mice.[46] Correlation between *falcipain-2* or 3 inhibition and parasite development was found, supporting the hypothesis that *falcipain* is the cysteine protease required for hemoglobin degradation.

b) *Cruzain* (also known as *Cruzipain*) is the major cysteine protease in the parasite *Trypanosoma cruzi*, the causative agent for American Trypanosomiasis. This enzyme so much related to cathepsin L, plays an essential role in the life cycle of the parasite such as replication and transformation between stages of *T. cruzi.* [57]

c) *Rhodesain* is a cysteine protease found in *Trypanosoma brucei rhodesiense* a major agent in Human African Trypanomiasis. [58] The enzyme has also been identified to be vital in the life cycle of the parasite.

2.1.4 Postulated Catalytic Mechanism of Cysteine Proteases

The success in development of new potent cysteine protease inhibitors depends on a better understanding of the enzyme's mechanism. Below is a proposed mechanism similar among all cysteine proteases and involves utilization of an active cysteine thiol (as a catalytic nucleophile) for peptide bond cleavage. [59] Histidine and asparagine residues are in close proximity and assist in mechanistic action (Scheme 1).



Scheme 1. Proposed catalytic mechanism for cysteine proteases.

The imidazole group of the histidine polarizes the SH group of the cysteine and enables deprotonation. The thiolate, which is highly nucleophilic, attacks the carbonyl carbon of the peptide bond to be cleaved **(i)** and leads to the formation of a tetrahedral intermediate **(ii)**. Histidine is then used to protonate the tetrahedral intermediate to get the amino group to leave.

The tetrahedral intermediate now breaks down by the departure of protonated amino group (**iii).** Re-protonation of histidine (**iv)** helps to generate a base that attacks the carbonyl carbon of the ester. The tetrahedral adduct collapses (**v)** with the release of a carboxylic end of the peptide followed by protonation of the thiolate anion (**vi)**.

2.2 Mechanistically Distinct Classes of Inhibitors of Cysteine Proteases

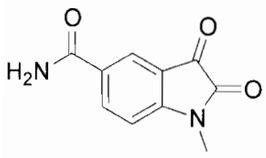
2.2.1 Introduction

Naturally, cysteine proteases are inhibited by cystains and serpins. Cystatins are reversible inhibitors of cysteine proteases and are widely distributed in the human body, while serpins are covalent inhibitors of proteases and exhibit diverse roles, including regulation of blood clotting, fibrin cleavage, and inhibition of apoptosis.

More recently, the development of cysteine protease inhibitors has drastically increased probably driven by advances in knowledge, computer-aided design and use of combinatorial syntheses. The striking features of all cysteine protease inhibitors are; (i) electrophilic head group and (ii) a substrate binding group(s). The most recent cysteine proteases inhibitors reported include reversible, irreversible and slow-turnover inhibitors. [60]

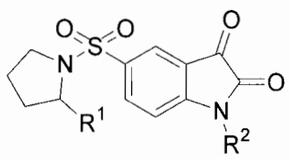
i). Reversible inhibitors

Reversible inhibitors usually involve non-covalent or covalent yet reversible interaction between the active site of the enzyme and the inhibitor. They form weak bonds and do not permanently disable the enzyme. Bonds in the enzyme-inhibitor complex form easily and are easy to break. Methylisatin-5-carboxamide **14** [61], isatin sulfonamide **15** [62], cyclic ketone **16** [63] and 2-benzothiazole **17** [64] and urea **18** [65] are some examples of reversible inhibitors (see fig. 5).



14

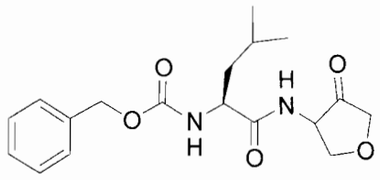
HRV -3C protease: K_i = 51 nM



15

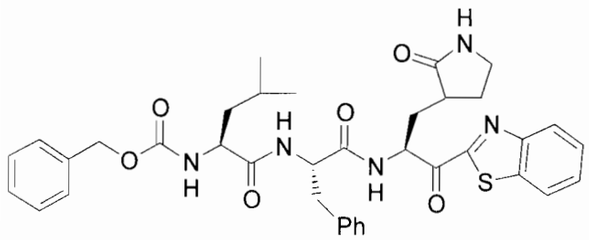
R^1 = CH_2-O-Ph, R2 = CH_2-Ph

caspases-3 and -7: K_i = 1.2 nM



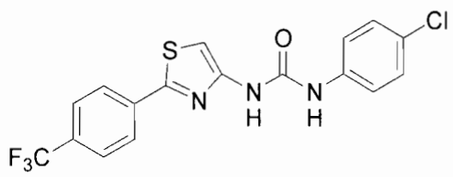
16

cathepsin K: $K_{i\ app}$ = 140 nM



17

HRV -3C protease: K_i = 51 nM



18

cruzain: IC_{50} = 0.8 – 2.9 µM

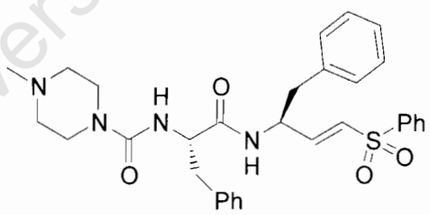
rhodesain: IC_{50} = 2.5 µM

cathepsin B: IC_{50} =10 µM

Figure 5. Reversible inhibitors of cysteine proteases.

ii) Irreversible inhibitors

Irreversible inhibitors act by forming strong bond with the cysteine protease active site whilst the inhibitor inactivating its catalytic activities. They generally stay in the active site for a long time and permanently disable the enzyme. These inhibitors are associated with toxicity *e.g.* vinyl sulfone **19** (fig. 6) is an irreversible inhibitor of cysteine proteases. [66]



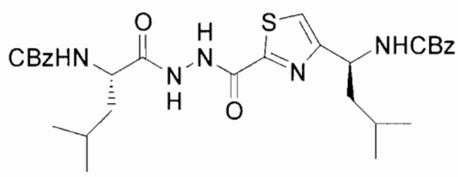
19

cruzipain: k_2/K_i = 6.5 x 10^6 $M^{-1}s^{-1}$

Figure 6. An irreversible inhibitor of cysteine protease.

iii) Slow-turnover Inhibitors

Slow-turnover inhibitors on the other hand, are a series of compounds that form a stable thioacyl-enzyme complex with the active site of the cysteine protease and is very slow to hydrolyze. The best example of this series of inhibitors is the diacyl hydrazine derivative **20** (fig. 7). [67]



20

cathepsin K: K_i = 10 nM

cathepsin B: K_i = 5200 nM

cathepsin L: K_i = 700 nM

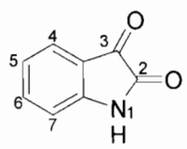
cathepsin S: K_i = 1000 nM

Figure 7. Slow-turnover inhibitor of cysteine protease.

2.3 ISATIN AND ITS CHEMISTRY

2.3.1 Introduction and Occurrences

Isatin **21** (fig. 8), systematically known as indole-2, 3-dione, is a natural product found predominantly in plants of the genus *Isatis*. [68] It is a bright-orange crystalline solid with an unpleasant odour. Its nucleus is basically a nitrogen-containing heterocyclic (pyrrole) fused to a benzene ring forming an indole derivative substituted at positions 2 and 3 with oxygen.

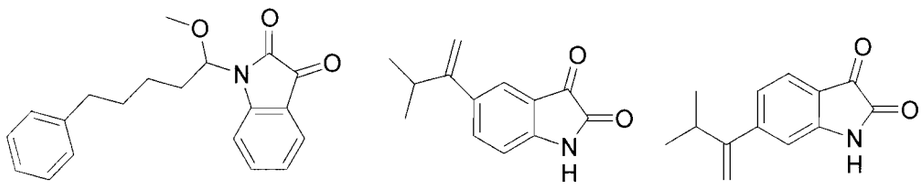


21

Figure 8. Isatin (Indole-2, 3-dione)

Isatin and some of its derivatives are available commercially and all these are produced synthetically; for example, isatin is made by reacting aniline, chloral hydrate and hydroxylamine hydrochloride (Scheme 2).

Historically, Erdman and Laurent discovered isatin in 1841 as a substance obtained from the oxidation of a blue dye known as Indigo. [69] It was later isolated from animal tissue in 1988. [70]



22 **23** **24**

Figure 9. Naturally occurring derivatives of isatin.

Isatin is probably the most versatile and widely distributed heterocyclic compound in nature. For example, in plants isatin is found as a melosatin alkaloid (methoxy phenylpentyl isatin) **22** (fig. 9), [71, 72] in fungi as 5-(3-methylbuten-2-yl) isatin **23** (fig. 9) and 6-(3-methylbuten-2-yl) isatin **24** (fig. 9), both were isolated from *streptomyces albus* [73] and *chaetomium globosum*, [74] respectively.

In animals, it is found as a component of the secretion from parotid glands of a bufo frog, also produced by an *alteromonas* sp., which protects embryos of the shrimp *palaemon macrodaetylus* from the pathogenic fungus *lagenidium callinectes*; whilst in humans as a metabolic derivative of adrenaline and as a component of tribulin in human tissue and body fluid. [75] Isatin has also been found to be a component of coal tar. [76]

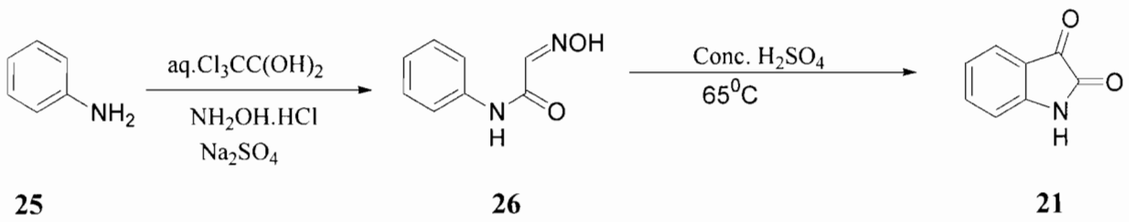
2.3.2 A Review of Isatin Chemistry

2.3.2.1 Synthesis of isatin

A variety of work on synthesis of isatin is well documented since its isolation in 1988. [69] Mostly, the strategy used depends on the availability of reagents, the number of synthetic steps and chemical yields.

a) The Sandmeyer Approach

The commonest and oldest approach is the Sandmeyer synthesis. It involves reacting aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to initially form an intermediate isonitroacetanilide **26.** Cyclization of **26** with concentrated sulfuric acid affords isatin in reasonable yield *ca.* 75%, (Scheme 2). [77]

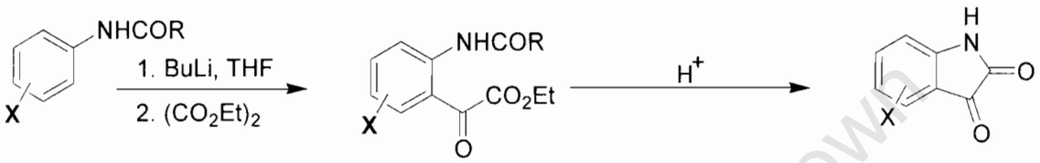


25 **26** **21**

Scheme 2. Synthesis of isatin by Sandmeyer approach.

b) An Alternative Method for the Synthesis of Isatin

Another approach is based on a regiospecific conversion of aniline to isatin. This method makes use of the reaction of an *o*-lithiated and protected aniline derivative **27** with diethyl oxalate to give an α-ketoester **28** which upon deprotection, readily undergoes cyclization to afford isatin in good yield, (Scheme 3). [78]



R = O^{t}Bu, ^{t}Bu

27 **28** **21**

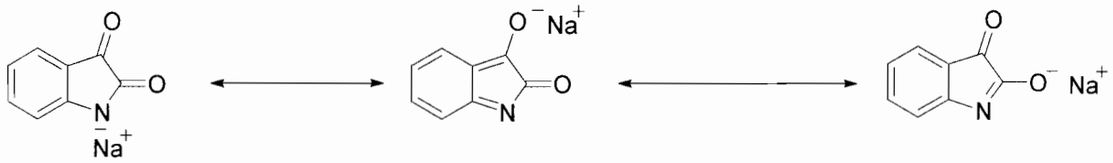
Scheme 3. Synthesis of isatin *via* regiospecific conversion of aniline.

2.3.3 Reactivity of Isatin Derivatives

Isatin and its derivatives have been widely investigated for use as building blocks in synthesis of heterocyclic compounds, natural products, and potentially important pharmaceutical agents. Isatins, for example, may react with nucleophiles, electrophiles and other reagents to give various products. [69]

2.3.3.1 Reactions with Bases

As in pyrrole, the *N*-hydrogen in isatin is acidic and can be deprotonated by a base such as sodium hydride to form sodium isatide **29** (fig. 10), often represented as resonance-stabilized anions **29**, **30** and **31** (fig. 10).



29 **30** **31**

Figure 10. Resonance structures of isatin anion.

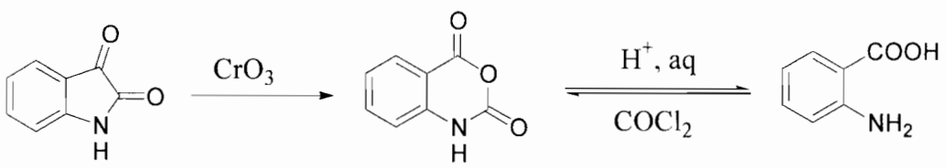
Since the carbonyl group (C-2) is similar to that of normal amides, sodium isatide often hydrolysizes when heated in basic solutions. Hydrolysis of this heterocyclic ring of isatin forms *o*-aminophenylglyoxylic acid **32**, which can be useful in the synthesis of quinoline derivative **33** in the presence of chloroacetone, (fig. 11). This ring opening reaction of isatin has been shown to be a very convenient method for the preparation six-membered quinoline heterocyclic systems.

32 **33**

Figure 11. Hydrolysis and six-membered ring products of isatin.

2.3.3.2 Reactions with Oxidizing and Reducing Agents

Oxidation of isatin with chromic acid or hydrogen peroxide leads to ring expansion giving the corresponding isatoic anhydride **34,** isatoic anhydride is also useful in the synthesis of anthranilic acid **35,** (Scheme 4) [77] and pyrrolo [1, 4] benzodiazepine, a component of antineoplastic antibiotics.



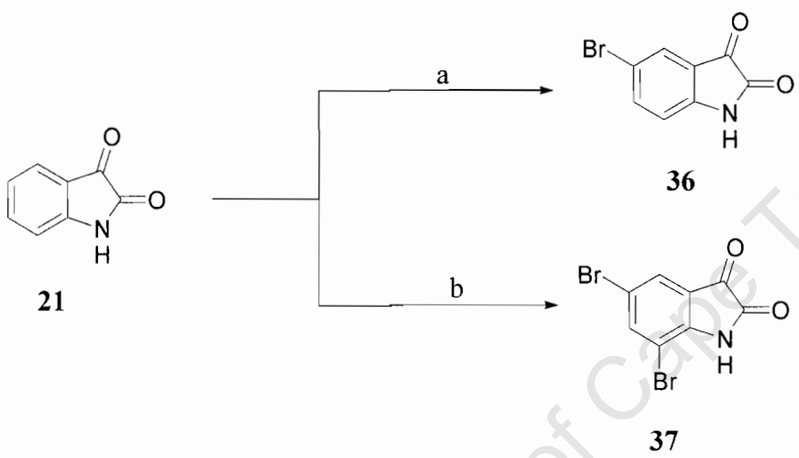
21 **34** **35**

Scheme 4. Oxidation reaction of isatin.

On the other hand, reduction of isatins can lead to either 1 or 3 alkylated indole products depending on the nature of reducing reagent being used. Lithium aluminum hydride and alkyl lithium has been applied to synthesize 3-alkylindoles, whilst sodium hydride, alkyl halide and sodium borohydride in THF reduces isatins to 1-alkylindoles, respectively. [79]

2.3.3.3 Electrophilic Substitution Reactions

Electrophilic substitution of isatins occurs predominantly at positions 5 and/or 7 of the aryl system due to the electronic effect of the nitrogen. The electron-donating nature of the hetero-nitrogen usually directs in coming electrophiles *ortho* and /or *para* positions depending on the solvent. Typical electrophilic reactions such as halogenation, nitration and sulfonation follow the same pattern. For example, bromination of isatin with *N*-bromoacetamide in acetic acid to afford 5-bromoisatin is reported and was achieved in high yield, (Scheme 5**a**). [80]



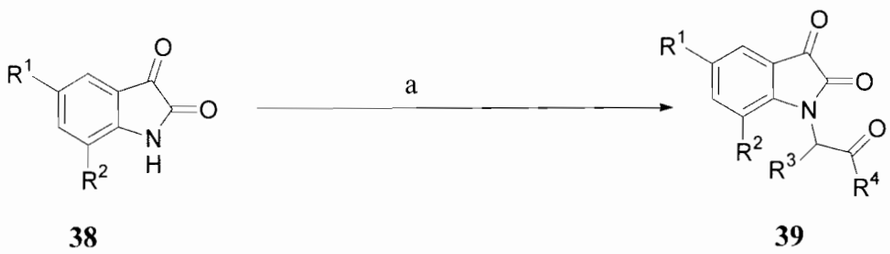
Scheme 5. (a) *N*-bromoacetamide, CH_3OOH, 90 %; (b). Br_2, 95 % warm EtOH, 80 %.

Similarily, Lindwall and co-workers have shown the synthesis of 5, 7-dibromoisatin using bromine in the presence of warm 95 % ethanol to afford the 5, 7-dibromoisatin **37** in 80 % yield, (Scheme 5 b). [81]

2.3.3.4 *N*-substituted Derivatives

Isatin and its derivatives react with alkyl, acyl and sulfonyl halides to give *N*-alkylisatin, *N*-acylisatin and *N*-sulfonylisatin, respectively. For example, *N*-alkylated isatin can be obtained simply by reacting isatin with alkyl electrophiles in the presence of a base under mild reaction conditions. Amongst the most convenient reactions reported is the use of sodium hydride as base. However, Shuttleworth and co-workers used substituted isatins **38** in the synthesis of *N*-functionalized isatin derivatives **39** under mild conditions using 2-*tert*-butylimino-2-

diethylamino-1, 3-dimethylperhydro-1, 3, 2-diazaphosphorine on polystyrene (BEMP) as a base in acetonitrile to give derivatives in high yield >90 %, (Scheme 6). [82]

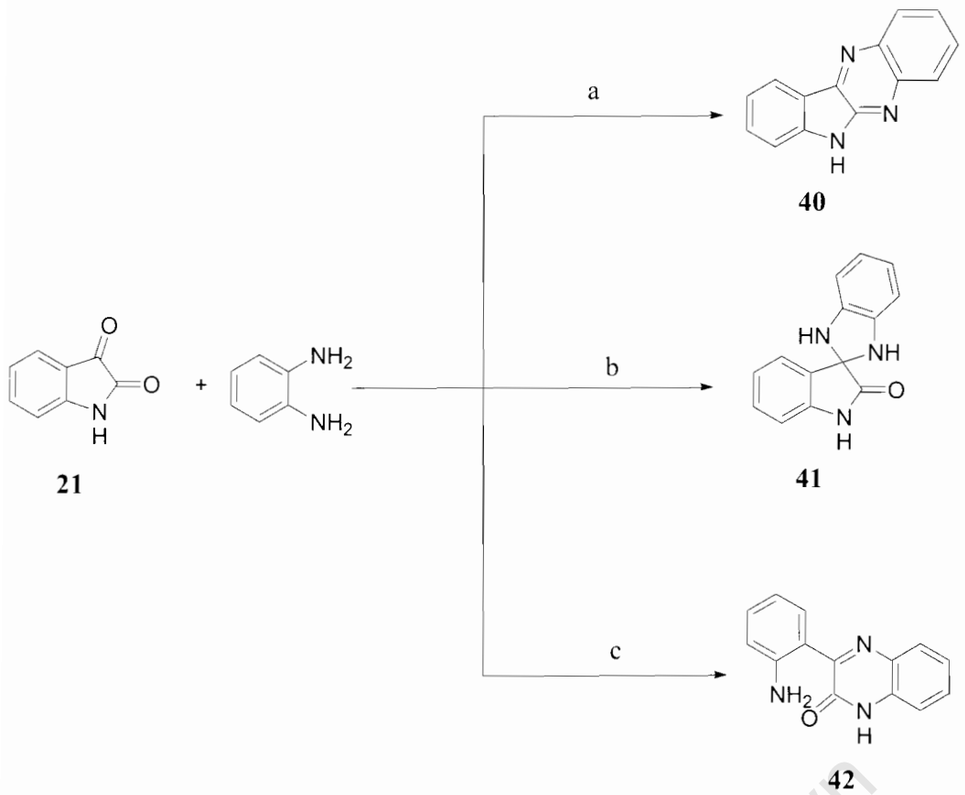


38 **39**

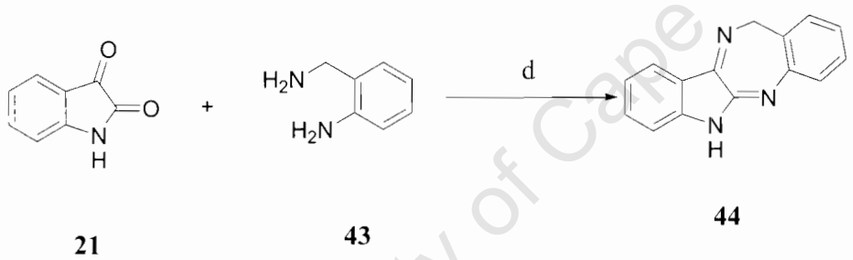
Scheme 6. (a) 1.05 equiv. of BEMP, 1.05 equiv. of α-bromoketone, MeCN, 45 ^{0}C, 16h.

2.3.3.5 Reactions with Nucleophiles

As with ordinary ketones, isatins undergo nucleophilic addition reactions at the ketonic C-3 carbonyl group. Nevertheless, those involving addition at amide C-2 carbonyl have been reported, too. [69] Often the chemoselectivity of these reactions is highly dependent on the nature of nucleophile, solvent and temperature. For instance, *o*-phenylenediamine reacts with isatin and depending on the solvent, gives three different products, (Scheme 7). [83]



Scheme 7. (a) acetic acid (CH_3OOH); (b) *N*-methyl pyrrolidine; (c) THF or Benzene.



Scheme 8. (d) methanol, rt, 48 h, 67%.

As depicted in Scheme 7, acidic solvents such as acetic acid led to the production of a 2, 3-condensation product **40,** while a switch to *N*-methyl-2-pyrrolidine afforded a spiro compound **41** in high yield. On the other hand, a ring-opened quinoxalinone **42** was obtained as the major product when THF or benzene was used as solvent.

In a similar experiment, using a different nucleophile (2-aminobenzylamine) formed sole product **44** and none of 2, 3-condensation products were observed despite varying the solvents, (Scheme 8).

2.4 Other Reactions of Isatins

2.4.1 Baylis-Hillman Reactions of Isatin Derivatives

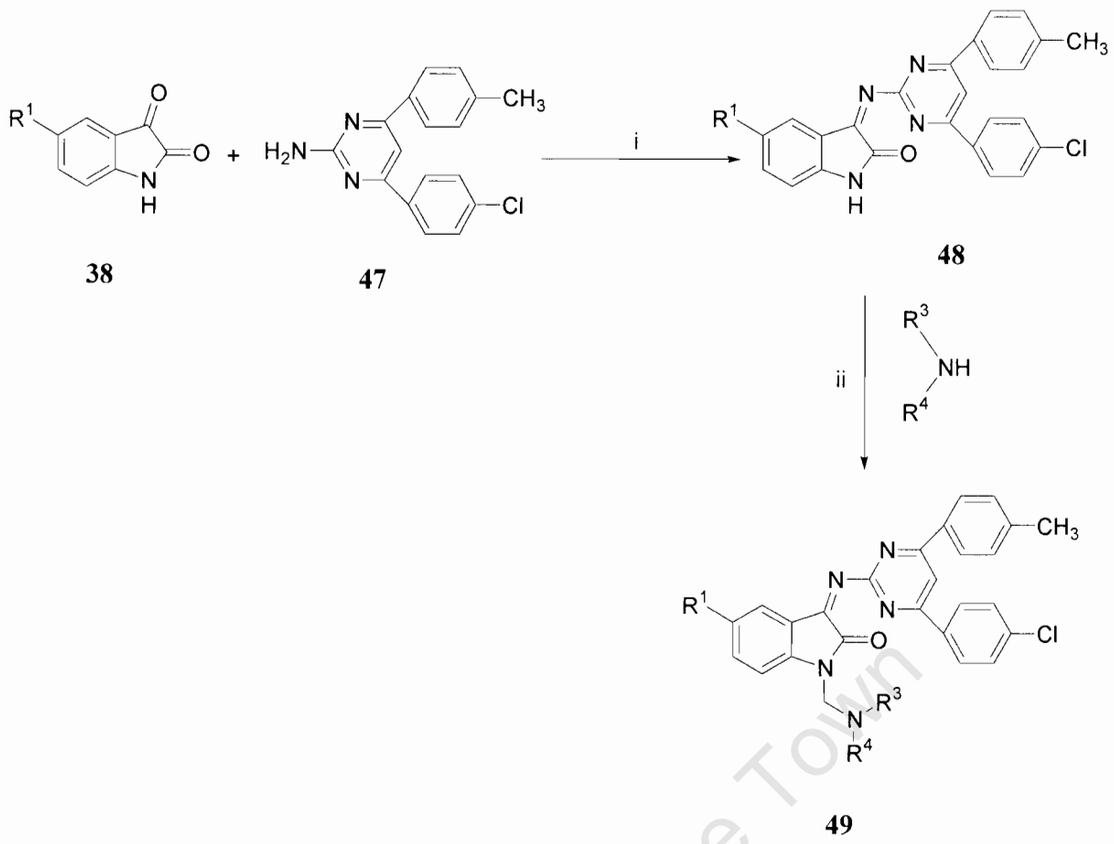
21 **45** **46**

Scheme 9. (i) DABCO (0.15 equiv.), THF/DMF, rt, 20-21 days, 94%.

Since isatins possess a reactive keto-carbonyl (C 3) group, they readily undergo reactions with activated vinyl compounds **45** under mild conditions to afford Baylis-Hillman related products **46**. Chung and co-workers previously reported using THF and DMF to afford desired products in excellent yield, (Scheme 9). [84]

2.4.2 Schiff and Mannich bases of Isatin and its Derivatives

a) Aminopyrimidine Derivatives



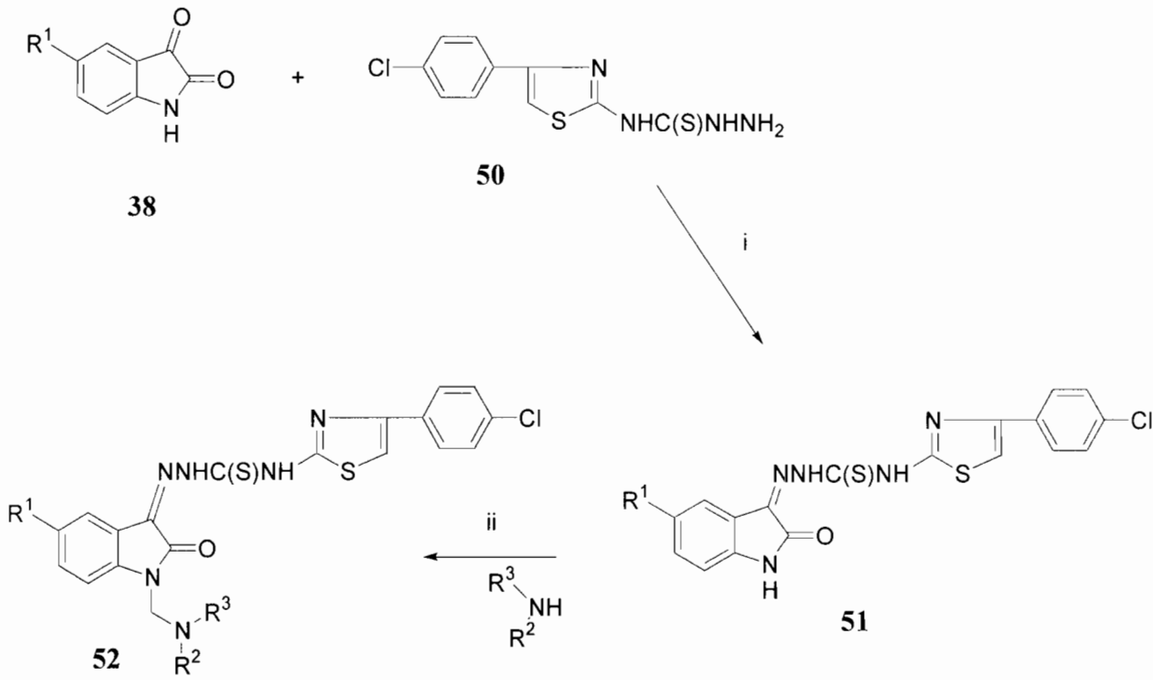
38 **47** **48** **49**

$R^1 = H$, $N(R_1)\,R_2 =$ 

Scheme 10. (i) EtOH, CH_3OOH, 72 %. (ii). CH_2O, THF, 91 %.

Mannich reactions have been applied to isatins. Numerous reactions giving *N*-aminomethylisatin products have been previously reported starting with pyrimidine **47** and intermediate imines **48**. Pandeya and co-workers successfully synthesized a series of pyrimidine Mannich bases **49** in good yield by the condensation of the acidic amino group of the isatin with formaldehyde and secondary amines in THF, (Scheme 10). [85]

b) *N*- [4-(4'-chlorophenyl) thiazol-2-yl] thiosemicarbazone Derivatives



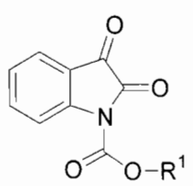
Scheme 11. (i) EtOH; (ii) THF, CH_2O.

Another interesting series of Schiff and Mannich bases of isatin reported by Pandeya *et al* were synthesized from thiosemicarbazide and thiosemicarbazone **50** and **51,** respectively to give (*p*-chlorophenyl) thiazol-2-yl thiosemicarbazones **52**, (Scheme 11). [86] All were achieved using a similar protocol as described above and afforded the desired product in good yield.

2.5 BIOLOGICAL PROPERTIES OF ISATIN AND ITS DERIVATIVES

Isatins are very active in many biological processes. Their biological activity on several important components in both plants and animals are so drastic that they affect the systems in which they are found. For example, it has been found to affect a variety of enzymes, sometimes tissues and receptors. The most notable effects documented are listed below.

i). In 1995, Iyer and co-workers reported that simple *N*-BOC and *N*-CBZ-isatin analogues **53** acted as reversible, slow-binding inhibitors of serine proteases, and exhibited selectivity for α-chymotrypsin.[87]



53

ii) Caspases-3 and 7 are intracellular cysteine proteases involved in apoptosis (programmed cell death). A series of isatin sulfonamide inhibitors have been reported and these have selectivity within the caspase family. As mentioned before, Lee and co-workers have reported isatin sulfonamide 15 as an irreversible inhibitor for caspases 3 and 7 cysteine proteases with inhibitory constant K_i = 1.2 nM and 6.0 nM, respectively. [62]

iii). Recently, Webber and co-workers investigated a series of *N*-aklyl-5-isatin carboxamide derivatives **14, 54, 55** and **56** (fig. 12) as inhibitors of human rhinovirus (HRV) protease. HRVs etiologic agents of the common cold are cysteine protease with a trypsin-like polypeptide structure. [61]

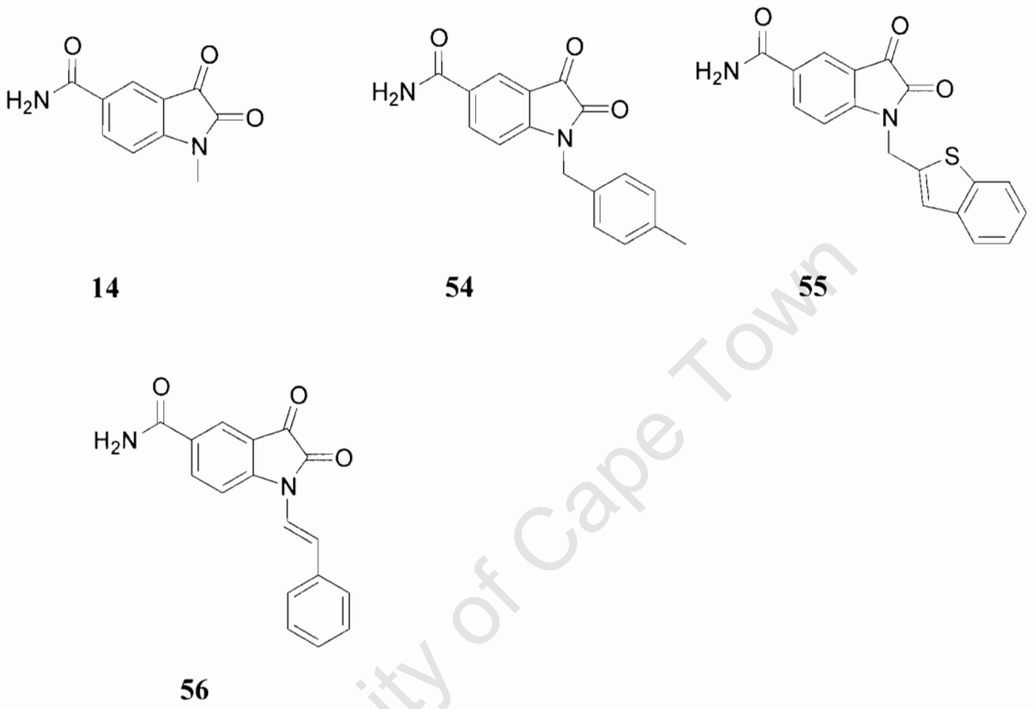


Figure 12. Inhibitors of Human Rhinovirus (HRV) Protease.

iv). Isatin has been shown to inhibit monoamine oxidase MAO in humans. MAO is an enzyme that metabolizes a wide range of mono-amines and is important in the control of the concentration of these neurotransmitters. Several studies have associated stress, anxiety and depression to MAO. [88]

v). Schiff and *N*-Mannich base derivatives of isatin are reported to possess a variety of properties that include; anticonvulsant [89], anti-flammatory [90], antibacterial [91], antifungal [92], antiviral [93], antiprotozoal [94], antihelminthic [95] and anti-HIV. [96]

vi). Isatin also inhibits acetyl cholinesterase (AchE) in the brain and blood. AChE is known to act very rapidly to stop neurotransmission at cholinergic synapses found in the brain and at neuromuscular joints. Inhibition of AchE is important in medicine as a target for drugs for the treatment of Alzheimer's and related diseases. [97]

2.6 RATIONALE FOR CHOICE OF THE ISATIN SCAFFOLD FOR ANTIPARASITIC CYSTEINE PROTEASE INHIBITORS DESIGN

Various derivatives of isatin are known to possess a range of pharmacological properties including antiprotozoal activities [94] as earlier mentioned. Within the context of enzyme inhibitors, isatins have seen recent applications in the inhibition of cysteine and serine proteases. [87] Thus isatin is a biologically validated starting point for the design and synthesis of chemical libraries directed at these targets. Due to the privileged nature of isatin, libraries designed and synthesized around the basic structure of this scaffold should yield medicinally active compounds with high hit rates at significantly reduced library size compared to large classical libraries obtained from combinatorial chemistry efforts based on non-privileged templates.

The aforementioned antiprotozoal and cysteine protease inhibitory activities of isatin derivatives prompted us to investigate this class of compounds as potential ketone inhibitors of parasitic cysteine proteases identified in trypanosomes (*cruzain* and *rhodesain*) and malaria parasites (*falcipain-2*), respectively. [98-101] It is noteworthy that a series of highly potent reversible ketone inhibitors of *cruzain* have previously been disclosed.[102] This has recently culminated in the first crystal structures of these inhibitors being solved.[103] We reasoned that the presence of the ketone functionality adjacent to the aromatic ring on the isatin scaffold provides a diversity point for accessing the corresponding thiosemicarbazones, a class of compounds recently identified as potent antitrypanosomal inhibitors of *cruzain*.[104] Coupled with the molecular simplicity, potentially cost effective synthesis and non-peptidic nature, this makes isatins attractive scaffolds for antiparasitic drug discovery. Cost-effective synthesis should always be a consideration in deciding on potential new antiparasitic agents given the prevalence of major parasitic diseases in poor countries.

2.7 PROJECT AIMS

i). To utilize KF/Al_2O_3 as a convenient supported base in the synthesis of target isatin and 5 and/or 7 *N*-substituted isatin derivatives.

ii). To synthesize isatin-3-thiosemicarbazone derivatives.

iii). To synthesize quinoline and non-quinoline Mannich base isatin derivatives.

iv). To synthesize quinoline-ethylene isatin derivatives for comparison with corresponding Mannich bases.

v). In collaboration with appropriate laboratories, to conduct the biological evaluation of commercially available and synthesized compounds against the three cysteine protease enzymes (*falcipain-2*, *cruzain* and *rhodesain*) and cultured malaria parasites *in vitro*.

vi). To explore structure-activity relationships within each series of compounds in an effort to identify the most promising commercially available and synthesized scaffold for further development and launching of a future medicinal chemistry programme.

CHAPTER 3

ISATIN DERIVATIVES: SYNTHESIS AND CHARACTERIZATION

3.1 *N*-SUBSTITUTED ISATIN DERIVATIVES

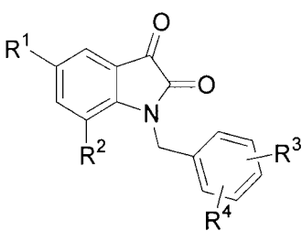
3.1.1 Background and Rationale

A number of biologically active compounds contain aromatic ring systems as part of their structures. Aromatic ring systems are reported to have an excellent binding affinity for proteins. Structure-activity relationship studies have shown that an aromatic moiety is involved in weak, non-covalent π-π interactions with non-polar sites of proteins and also bind to polar substituents such as amides and hydroxyl groups through cation-π interactions. [105, 106] Thus, incorporating aromatic moieties in a drug is vital for enhancing molecular recognition and protein-drug interactions, and hence the desired biological activity. These considerations led us to design an initial exploratory series of potential antiparasitic cysteine protease inhibitors **57a–57i** (*N*-benzyl derivatives), **58a-58b** (*N*-acyl and sulfonyl derivatives) and **59a–59d** (carboxybenzyl derivatives) based on the isatin scaffold.

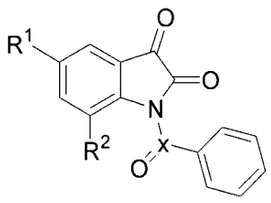
In arriving at exploratory compounds **57a – 59d**, we considered the isatin nucleus as a central scaffold to which suitable substituents could be rationally appended. Previous work on inhibitors of other cysteine proteases pointed to the importance of an *N*-benzyl substituent in compound **57** for effective inhibition. [107] This substituent was thought to bind in the S2 pocket of the enzyme. 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 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.

Since the isatin scaffold had previously not been used for parasitic cysteine protease inhibitor design, we considered the aforementioned previous work on unrelated cysteine protease as a useful starting point. In addition to the more flexible *N*-benzyl system in **57**, we wished to explore the potential of more rigid *N*-acyl and *N*-sulfonyl derivatives **58a–58b** in which rotation about the N-C (amide/sulfonamide) bond is relatively speaking somewhat restricted compared to **57a–57i**. Furthermore we reasoned that the carbonyl oxygen in derivatives **58a–58b** and **59a-59d** could be involved in other (hydrogen bonding) interactions with suitable sites within the protein. The rationale behind derivative **59a–59d** is that a member of this series, *N*-(carbobenzyloxy) isatin, has been investigated as a slow or weak inhibitor of chymotrypsin (chymotrypsin is a serine protease which catalyses the hydrolysis of peptide bonds preferably with aromatic or large hydrophobic side chains).[108]

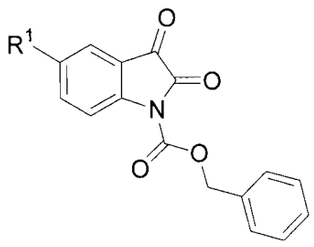
The initial goal was to carry out a limited comparative study of representative compounds from **57a–59d** with respect to inhibition of three parasitic cysteine proteases, *cruzain*, *falcipain* (2 or 3), and *rhodesain*. It was envisaged that preliminary biological data on representative compounds would be used as a basis to launch a more focused future medicinal chemistry programme around the most promising series. Although various substituted isatins can be synthesized, [69] we initially concentrated on the use of commercially available isatins.



57a–57i



58a–58b: X = C, SO

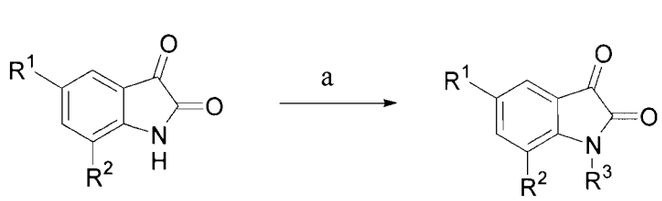


59a–59d

3.1.2 Synthesis and Characterization

3.1.2.1 Synthesis of derivatives 57a–57i and 58a–58b

Compounds in the series **57a–57i** and **58a–58b** were prepared as depicted in Scheme 12. For the synthesis of compounds **57a –57i,** a series of commercially available isatins (1.0 equiv) were treated with benzyl halides (1.2 equiv) in CH_2Cl_2 as solvent in the presence of 4.0 equivalents of potassium fluoride on alumina (KF/Al_2O_3). The reaction mixtures were stirred at room temperature for 16 hours, after which the resultant mixtures were simply diluted with diethyl ether and the crude isolated. Purification of the crude reaction mixtures by column chromatography over silica gel (3:7; EtOAc: Hex) generally gave high yields of the desired products (Table 1). During our method development, we observed that 4 equivalents of the base were necessary to drive reactions to completion and that prior to purification by column chromatography, the reactions had proceeded cleanly with only the excess benzyl halide present in addition to the product.



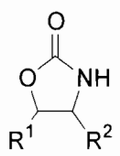
38 **57a-58b**

Scheme 12.(a) 4.0 equiv. of KF/Al_2O_3, 1.2 equiv. of benzyl or benzoyl or benzene sulfonyl halide CH_2Cl_2, rt, 16-24h, 48-95%.

KF/Al_2O_3 was preferred to other bases like sodium hydride (NaH) due to ease and convenience of reaction work-up and product isolation, which made it suitable in parallel synthesis. This immobilized reagent has been used by numerous researchers in similar contexts as ours. Potassium fluoride on alumina was first used for the functionalization of amides in 1981 by Yamawaki [109] but has now recently been utilized in a variety of synthetic transformations including arylation, esterification, alkylation, heterocycle-formation and other important solid phase syntheses. [109] To lay some emphasis on the appreciable work done by employing KF/Al_2O_3;

i). Diaryl ether derivatives previously that could not be formed by the characteristic Ullman-coupling reactions (due to electronically unfavorable substituents) can now be accomplished by using KF/Al_2O_3 at elevated temperatures. [110]

ii). KF/Al_2O_3 has been demonstrated to enhance selectively *N*-alkylation of 2-oxazolidinone and carboxylic acid methyl ester **60** and **61** respectively, completely suppressing the competing *O*-alkylation. In a similar context, mono-functionalization of benzodiazepin-2, 5-diones **62** was accomplished using acetonitrile or dimethoxyethane in the presence of KF/Al_2O. [111-113]



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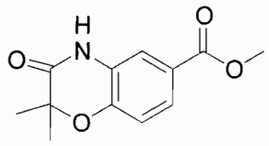

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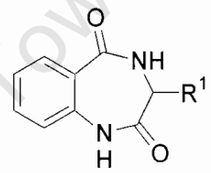


62

iii). Investigations into the possible utilization of KF/Al_2O_3 in palladium-catalyzed cross-coupling reaction has demonstrated that the base is effective over a wide range of both aryl bromides/iodines and boronic acids. However, vinyl halides did not favour KF/Al_2O_3 as base. [114]

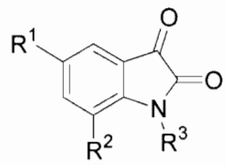
iv). Deprotection of silyl ethers typically requiring various conditions such use of TFA, formic acid and fluoride reagents, can be achieved by using KF/Al_2O_3 in a solvent dependent manner. Interestingly, various silyl protecting groups (TMS, TBDMS and TBDPS) can now be effectively, selectively and cleanly removed. [115]

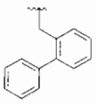
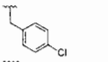
In our work, the use of KF/Al_2O_3 was also extended to the parallel synthesis *N*-acyl and *N*-sulfonyl isatin derivatives. Reaction of isatin with benzoyl or sulfonyl chloride in CH_2Cl_2 in the presence of KF/Al_2O_3 gave the corresponding *N*-acylisatin and *N*-sulfonylisatin in 84 and 48%

yield respectively. Although the yields are unoptimised, the reactions proceeded without any complications. Overall results are presented in Table 1.

The reaction of isatin with benzoyl chloride is particularly noteworthy. The synthesis of *N*-acylisatin derivatives under various conditions have been described, for instance using triethylamine in benzene, pyridine in benzene or triethylamine in chloroform. However, these reactions are associated with a number of drawbacks such as formation of complex products. [69]

Table 1. *N*-alkylated, *N*-acylated and *N*-sulfonylated isatin derivatives prepared by parallel synthesis using KF/Al_2O_3.



Compound	R^1	R^2	R^3	Time (hrs)	%Yield
57a	H	H		16	95
57b	I	H		16	65
57c	Me	H		16	54
57d	Me	H		16	76
57e	Me	H		16	60
57f	Me	H		16	72
57g	Cl	H		16	63
57h	Cl	H		16	70
57i	Cl	H		16	70
58a	H	H		24	84
58b	H	H		20	48

The synthesized compounds were confirmed from their high-resolution mass spectra and spectroscopic data together with their elemental analyses. The infrared spectra in solid state using KBr for the products showed the absence of a broad N-H peak (3400-3150cm^{-1}) confirming that it was not the starting material.

The ^{1}H NMR spectra for the benzyl series of compounds **57a – 57i** show essentially two sets of signals for aromatic and methylene protons. This is exemplified in Figure 13 below, which is the proton NMR spectrum of compound **57a**. The methylene (H-8) protons in the molecule are characteristic of the compound and were observed as a singlet at δ 4.93 ppm, integrating for two protons. The appearance of a multiplet in aromatic region at δ 7.30 ppm, integrating to five protons confirmed the presence of the phenyl moiety of the unsubstituted benzyl (Bn) group.

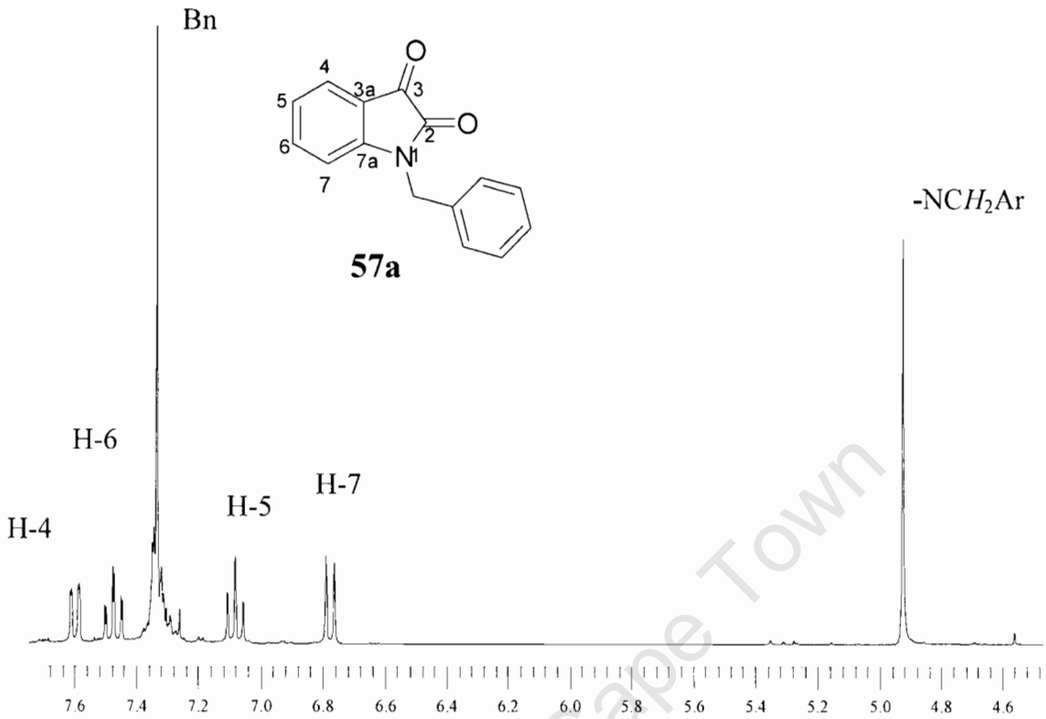


Figure 13. ^{1}H NMR spectrum of compound **57a** in $CDCl_3$

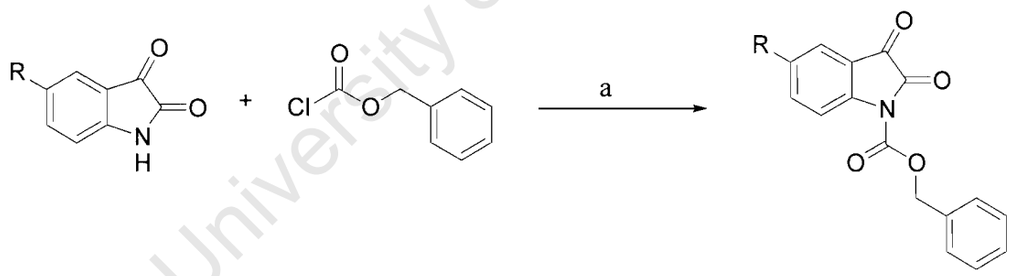
In the aromatic region, H-4 which appears at δ 7.61 ppm as a double doublet (dd) is the most deshielded. This is due to the neighboring electron withdrawing dicarbonyl effect. On expansion of NMR spectrum of **57a**, H-4, H-5, and H-6 were clearly a double doublet, double double doublet and double double doublet, respectively. The expected double doublet for H-7 was not evident even on expansion and the multiplicity observed was a broadened doublet at δ 6.79 ppm. The doublet actually observed for H-7 is probably due to low resolution and /or splitting of H-7 at the NMR field (300 MHz) used. There may be something unusual about the magnetic environment for H-7 in this and related isatins. Since **57a** is a known compound, a literature search revealed the same observation of H-7 as a doublet by other authors. [116] Nevertheless, more evidence for compound **57a** was given by disappearance of N-H signal in the ^{1}H NMR spectrum of the starting isatin.

No significant variation in the chemical shift values with change in substituents was observed across the series of compounds **57** for the common hydrogen and carbon atoms. The ^{13}C NMR spectra of the compounds showed C=O signals at δ 183.0 ppm (C-3) and δ 158.2 ppm (C-2). An additional C=O peak at δ 166.7 ppm was observed for the exocyclic C=O of **58a**.

The high-resolution mass spectrometry (HRMS) was used to confirm the molecular masses of the compounds and in each case found to be very close to the calculated values. Furthermore, attempts to study the fragmentation pattern was made and it was found that the peak corresponding to *m/z* 91.0 $(C_6H_4N)^+$ was observed in compound **57a**. This peak was attributed to the six-membered ring fragment (arene aziridine) and this might arise from the loss of the two carbonyl groups and the *N*-substituent.

3.1.2.2 Synthesis of Isatin Chloroformate Derivatives 59a–59d

In view of the previously mentioned (apparent) potential of *N*- (carbobenzyloxy) isatin as a biologically active molecule, we set out to make a preliminary array of 5-isatin substituted derivatives. The synthesis of derivative **59a** has been previously reported by Ramaswarny A *et al* and was prepared following the literature procedure in 76% yield. [107] For other analogs **59b-59d**, the same procedure was used. Accordingly, isatin / 5-substituted isatins **63** (1.0 equiv) was dissolved and stirred in CH_3CN at 0 ^0C in an ice bath. Benzyl chloroformate (1.1 equiv) was then added slowly followed by 0.1 equiv. of 4-dimethylaminopyridine (DMAP) and triethylamine (1.1 equiv). After stirring for 3 hours at 0 ^0C the desired products were obtained by filtration from the reaction mixture, washed with cold ethanol, dried and recrystallized from ethanol to give varying yields of the products, Table 2.



23 **63** **59**

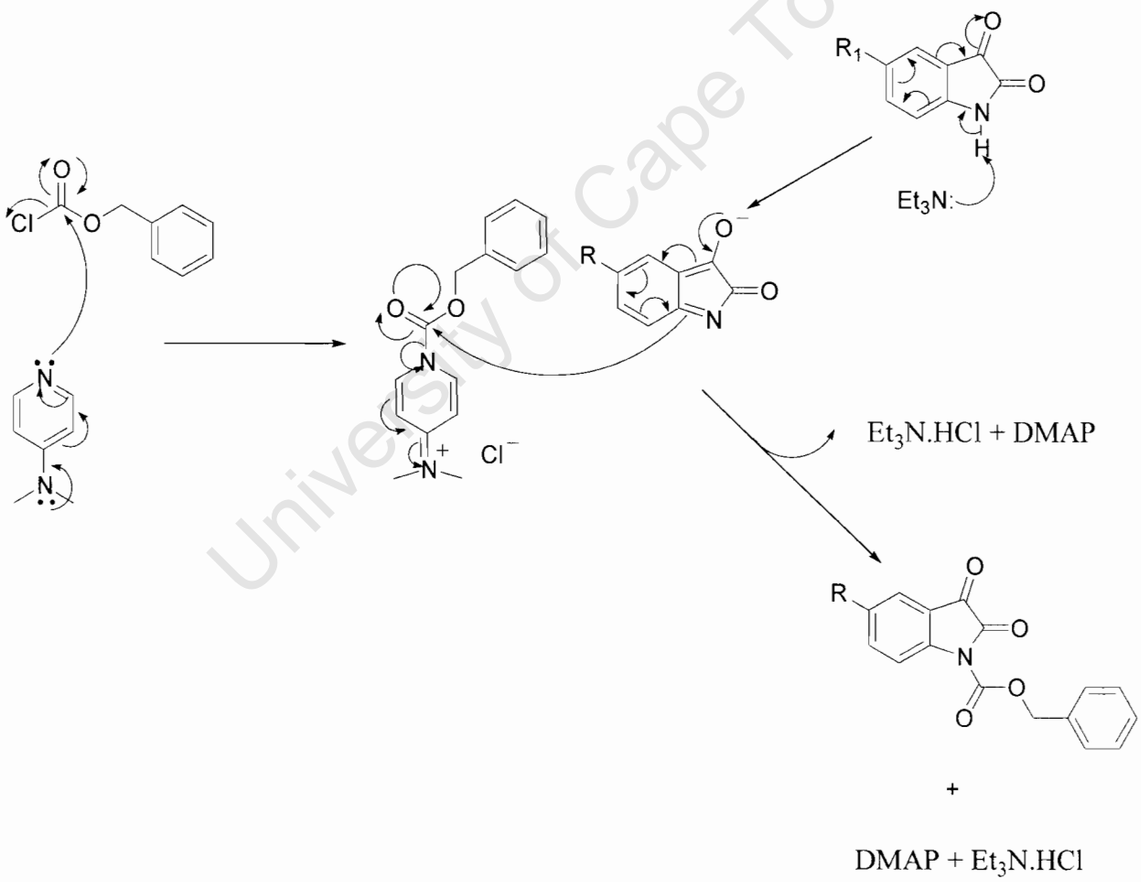
Scheme 13. (a) 0.1 equiv. of DMAP, 1.1 equiv. of Et_3N, CH_3CN, 0 ^0C, 3h, 19-76%.

Table 2. *N*- (carbobenzyloxy) isatin derivatives synthesized and their percentage yields.

Compound	R	% Yield	mp (^{0}C)
59a	H	97	152 - 154
59b	Cl	19	166
59c	Me	24	144-150
59d	F	35	>350

3.1.2.2 Mechanism

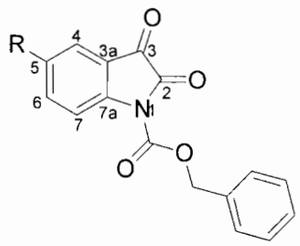
The supposed reaction mechanism of acylation of isatin, in which DMAP acts as an acyl transfer catalyst, is schematically represented in Scheme 14 below. Nucleophilic attack of DMAP on the carbonyl group of benzyl chloroformate results in a more reactive intermediate which is rapidly attacked by the nucleophilic deprotonated isatin to give the final product **59**. Regeneration of DMAP accompanies product formation along with triethylammonium hydrochloride.



Scheme 14. Mechanism of DMAP-catalyzed acylation of isatin.

The key spectroscopic indicators in the ^{1}H NMR spectrum of the compounds includes the presence of additional aromatic protons between δ 8.15-7.32 ppm, a singlet for the benzylic (H-9) protons around δ 5.48 ppm. Pertinent data is summarized in Table 6.

Table 3. ^{1}H NMR Chemical shifts of *N*-chloroformate isatin derivatives



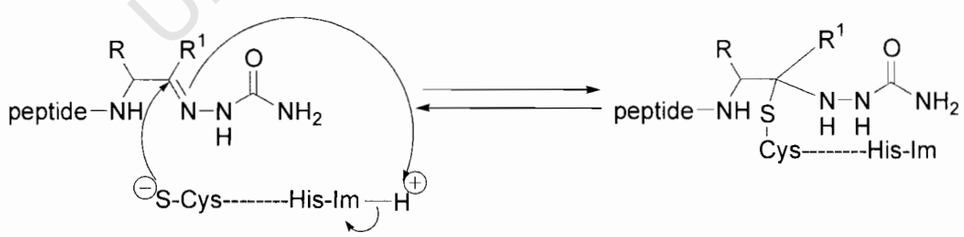
Compound	H-4	H-5	H-6	H-7	-OCH_2Ar	Ph
59a	7.78	7.32	7.74	8.15	5.48	7.54-7.35
59b	7.72	~	7.68	8.13	5.47	7.49-7.38
59c	7.55	~	7.54	8.01	5.46	7.42-7.35
59d	7.50	~	7.54	8.18	5.36	7.39-7.32

3.2 ISATIN-3-THIOSEMICARBAZONES

3.2.1 Background and Rationale

Within the context of cysteine protease inhibitors, the rationale for pursuing thiosemicarbazone derivatives of isatin is at least five-fold.

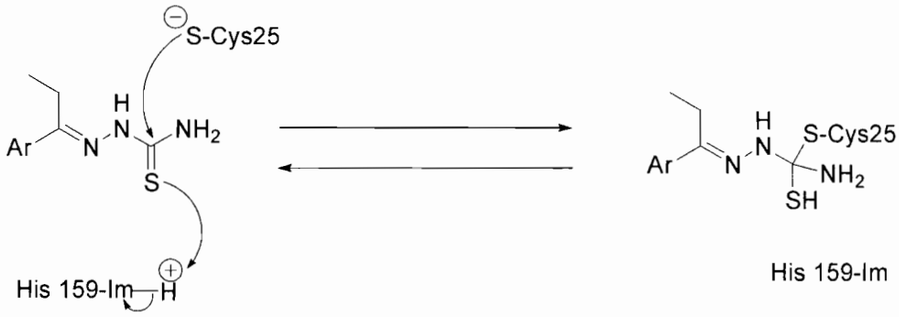
i) First, the corresponding semicarbazones, as a class, are known general cysteine protease inhibitor scaffolds. Peptidyl semicarbazones have been shown to be inhibitors of cysteine proteases (albeit weaker than the corresponding aldehydes). Inhibition of cysteine proteases by peptidyl semicarbazones is due to the formation of a tetrahedral adduct by attack of the active site thiolate on the imine carbon of the semicarbazone, [117] (Scheme 15).



Scheme 15. Inhibition of cysteine protease by peptidyl semicarbazones.

A histidine group which is in close proximity polarizes the enzyme-cysteine thiol group allowing deprotonation even under neutral to weakly acidic pH conditions. The resulting nucleophilic thiolate/imidazolium ion pair allows the thiolate anion attack the imine as mentioned before.

ii) Second, non-peptidic thiosemicarbazones have been recently identified as potent anti-trypanosomal inhibitors of *cruzain*. [104]



Scheme 16: Inhibition of cysteine protease by thiosemicarbazones.

The mechanism of inhibition of *cruzain* by thiosemicarbazones has been proposed *via* attack of the enzyme-thiolate anion on the thiol carbonyl resulting in formation of tetrahedral adduct. The attack is driven by the assisted protonation of thiosemicarbazone sulfur by His 159, (Scheme 16). Experimental and computer modeling results supports this mechanism. [104]

iii) Third, irrespective of the mechanism of inhibition described in (i) and (ii) above, the reactions are reversible. This has implications for (low) host toxicity and purification of target cysteine proteases by affinity chromatography.

iv). Compared to 3-imine derivatives, (thio) semicarbazone derivatives of isatin are more stable and less prone to hydrolysis. This has implications for bioavailability *in vivo*. The presumed stability of (thio) semicarbazone derivatives (X = O, S) relative to simple imines is based on stabilization of the imine double bond by the electronegative nitrogen substituent. This makes the imine double bond (C=N) of (thio) semicarbazones less prone to nucleophilic attack compared to simple imines, (Scheme 17).

Scheme 17. Stabilization of the imine double bond.

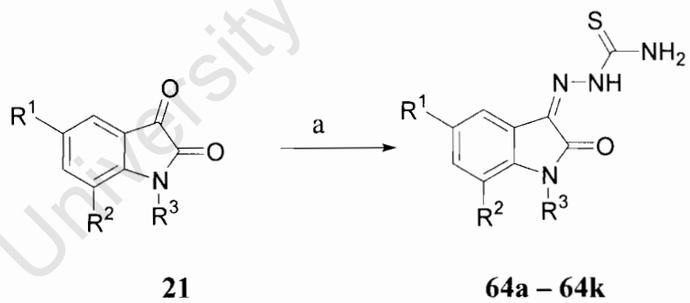
v). Like biguanides, thiosemicarbazones are metal chelators (*via* the sulfur and hydrazinic nitrogen atoms) which may act as metal-interactive inhibitors of cysteine proteases. [118]

Initially we aimed to only study commercially available isatins with respect to substituents R^1, R^2 and R^3 (Scheme 18). The ultimate aim is to identify the most promising commercially available isatin scaffold on which to base inhibitor design and synthesis. Coupled with previous studies on *N*-substituted isatins described before, it was envisaged that this would lead to preliminary structure-activity relationship (cysteine protease inhibitor) data to be generated and used in further future rounds of design and synthesis.

3.2.2 Synthesis and Characterization

3.2.2.1 Synthesis

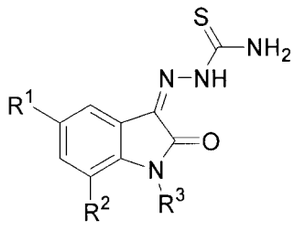
The synthesis of thiosemicarbazones **64a – 64k** was carried out according to a literature procedure by straightforward condensation of a number of commercially available isatins with thiosemicarbazide. In each case, equimolar quantities of isatin and thiosemicarbazide were initially dissolved in 10 mL of warm 95% ethanol and heated with continuous stirring at 45 °C for 3-6 hours. The resultant mixtures were cooled to room temperature and allowed to stand for 24 hours. Crystalline products **64a–64k** were formed which were filtered, washed with ethanol, dried and recrystallized (from ethanol). The overall yields were in the range 39-98%. The yields were dependant on the rate of crystallization of each individual compound from solution. The low yield of **64a** may be due the fact that part of the product had remained in the mother liquor. Scheme 18 below depicts the synthesis of isatin-3-thiosemicarbazone and its derivatives. The overall results are presented in Table 4.



21 **64a – 64k**

Scheme 18. (a) 1.2 equiv. of thiosemicarbazone, EtOH, 3h, 45 °C, 39-98%.

Table 4. Isolated yields and data for synthesized isatin-3-thiosemicarbazones

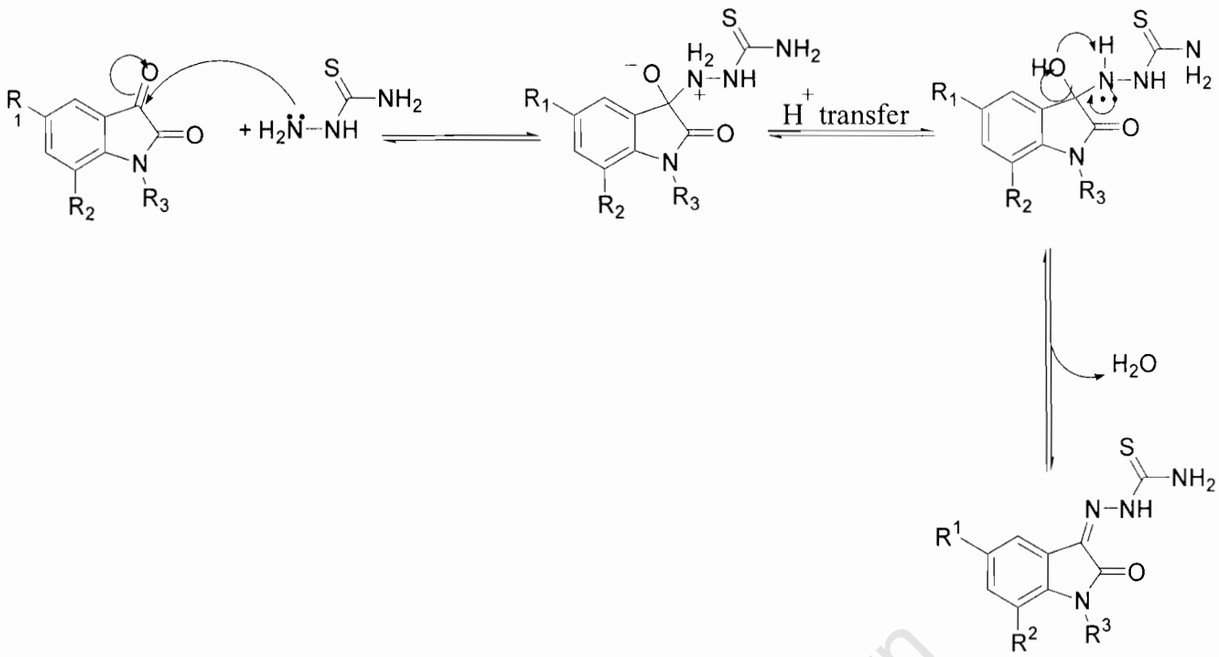


Compound	R^1	R^2	R^3	Time (Hours)	% Yield	mp (^{0}C)
64a	H	H	H	4	43	249-253
64b	F	H	H	4	82	287-289
64c	Cl	H	H	4	73	283-285
64d	Br	H	H	4	43	281-284
64e	I	H	H	4	67	265
64f	Cl	Me	H	4	98	>350
64g	Me	H	H	4	74	281-283
64h	Me	Me	H	4	90	286-287
64i	NO_2	H	H	4	88	>350
64j	H	H	Ph	6	76	245-249
64k	H	H	Me	6	97	253

3.2.2.1 Mechanism

A possible mechanism for the formation of compounds **64a–64k** is presented in Scheme 19 below. Nucleophilic addition of the more nucleophilic terminal amino group to the ketone carbonyl of the isatin results in an intermediate from which proton transfer from the nitrogen to the negatively charged oxygen follows to give a neutral molecule. Subsequently, elimination of water gives the required products **64**. Nucleophilic attack occurs preferentially at the ketone carbonyl group (C-3) since this carbonyl group is more electrophilic than the amide carbonyl. However, it is noteworthy that the reaction of 1-aryl or 1-arylsulfonylisatin with hydrazine or thiosemicarbazine is reported to (sometimes) lead to products of nucleophilic attack at amide carbonyl and /or ketonic carbonyl in aq. PrOH/AcOH. When EtOH as a solvent is used as in our

work, only one (predominant) C-3 product is obtained. It may be assumed that solvents play a role in reactions too.



Scheme 19. Mechanism for the formation of isatin-3-thiosemicarbazones.

The synthesized compounds **64a-64k** were fully characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectra as well as elemental analysis. The infrared spectra of showed characteristic peaks in respect of four major functional groups, secondary N-H (3414 cm^{-1}), amide C=O (1684 cm^{-1}), C=N (1699 cm^{-1}) and C=S (1137 cm^{-1}). The absence of a ketone (C=O) band at 1730 cm^{-1} provided further proof of product formation. The ^{1}H NMR spectrum (DMSO-d$_6$) for compound **64a** is shown (Figure 14). Key signals in the spectra of all the compounds are three sets of chemical shifts at δ 12.46 ppm (NNH), δ 11.15 ppm (H-1) and δ 8.98-8.62 ppm (N*H*H and NH*H*). Aromatic ring protons appear in the region δ 7.65 – 6.92 ppm, where a double doublet is observed at δ 7.64 ppm for H-4 with J = 1.2 and 7.5 Hz, a double double doublet at δ 7.34 ppm with J = 1.2, 6.9 and 7.6 Hz for H-6, another double double doublet at δ 7.09 ppm with J = 0.9, 6.9 and 7.5 Hz for H-5 and a doublet at δ 6.92 ppm attributed to H-7. The expansion (not shown in Figure 14) clarified these assignments and a literature search revealed the same observation. [119]

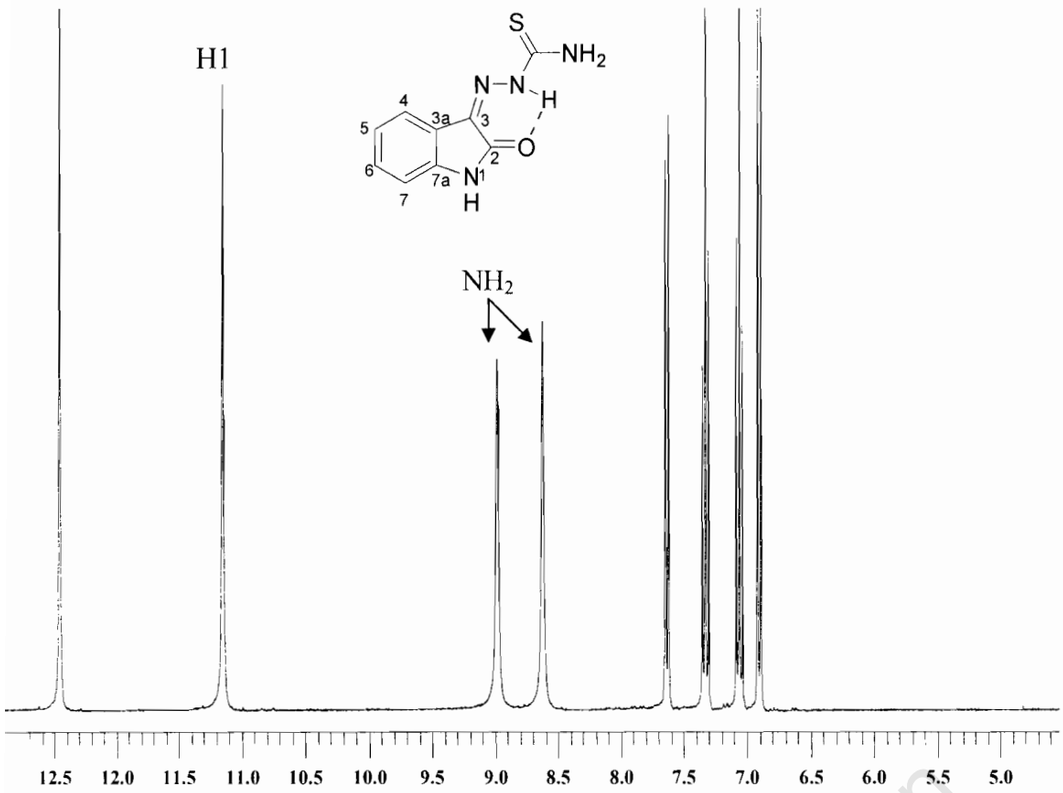


Figure 14. ^{1}H NMR spectrum of compound **64a** in DMSO-d_6.

The ^{1}H NMR for the 5 and 7-substituted isatin derivatives are similar to **64a** with no significant changes in chemical shift values for common protons. The stereochemistry (*E*, *Z*) of isatin-3-thiosemicarbazone about the double bond was not investigated. However, from the NMR spectra, one predominant isomer was obtained. The possibility of intra-molecular H-bonding involving the thiosemicarbazone NH and the C-2 carbonyl oxygen would favour the *Z* isomer as shown for **64a** in Figure 14. This intra-molecular H-bonding partly accounts for the deshielding of the NNH proton which appears at 12.5 ppm. The ^{13}C NMR spectra revealed further evidence on the structures of the products. For instance, the spectrum of compound **64a**, shows the C=S carbon at δ 178.7 ppm, resonance for the amide carbonyl (C-2) at δ 162.6 ppm, the C=N at δ 142.3 ppm and disappearance of the ketonic carbonyl (C-3) peak at 183.2 ppm in the starting material. The aromatic carbons appear in the region δ 131.9-110.9 ppm.

Further support for the structures was provided by the mass spectra, which indicated the (found) molecular ion close to the calculated masses. For example, the molecular ion observed for compound **64a** was *m/z* 220.04245. A fragmentation pattern was observed for the molecule, which shows the following peaks that correspond to $[C_8H_{10}N_4S]$,$^{+}$ *m/z* 194.04647 (98%);

$[C_3H_8N_4S]$,$^+$ *m/z* 132.04598 (30%) and $[C_7H_6N]$,$^+$ *m/z* 104.0507 (35%). A similar fragmentation pattern was observed in the other derivatives.

3.3 QUINOLINE *N*-MANNICH ISATIN DERIVATIVES

3.3.1 Background and Rationale

Quinolines are naturally occurring heteroaromatic molecular structures found in coal tar and other products of fossil fuel. [120] Quinoline and its derivatives have interesting chemistry and have been used widely in the treatment malaria for more than 350 years. Quinine was the first quinoline antimalarial natural product, which led to the discovery of synthetic derivatives such as chloroquine, mefloquine, and amodiaquine to mention a few. It is postulated that quinoline antimalarials accumulate in the parasite's acidic food vacuole and inhibit β-haematin formation. Toxic heme thus builds up in the vacuole subsequently killing the parasites. [121, 122] Though the actual mode of action of these agents is still unclear, they still remain potential sources of today's and future antimalarial therapy. This is partly because *Plasmodium falciparum* has had difficulty developing rapid resistance to this class of compounds. For example simple modification of the lateral side chain of chloroquine has resulted in derivatives with activity against chloroquine-resistant strains. [123]

Mannich bases, on the other hand, have received attention in organic chemistry as building blocks for nitrogenous molecules [124] and have recently been found to possess marked and improved antimalarial properties against multi-drug resistant strains of *P. falciparum*. [125] Besides, isatin-based Mannich bases too have been reported to possess antibacterial, [91] antifungal,[92] antiviral,[93] and anti-HIV,[96] antiprotozoal [94] and antihelminthic activities[95]. In continuation of our work on isatin derivatives, we designed quinoline-Mannich based precursors **65** (X=O) and the corresponding thiosemicarbazones **66** (X=NNHC(S)NH_2). We reasoned that a bi-therapeutic strategy, in which a haem-binding 4-aminoquinoline moiety is conjugated to a cysteine protease inhibiting isatin moiety, could be advantageous with respect to slowing down the emergence of drug resistance. Other non-quinoline Mannich bases were also designed with a view to establishing preliminary structure-activity relationships with respect to the role of the 4-aminoquinoline moiety in the activity against the parasite enzymes and parasites themselves. The design principle for quinoline *N*-Mannich base derivatives against *P. falciparum* is depicted in Figure 15.

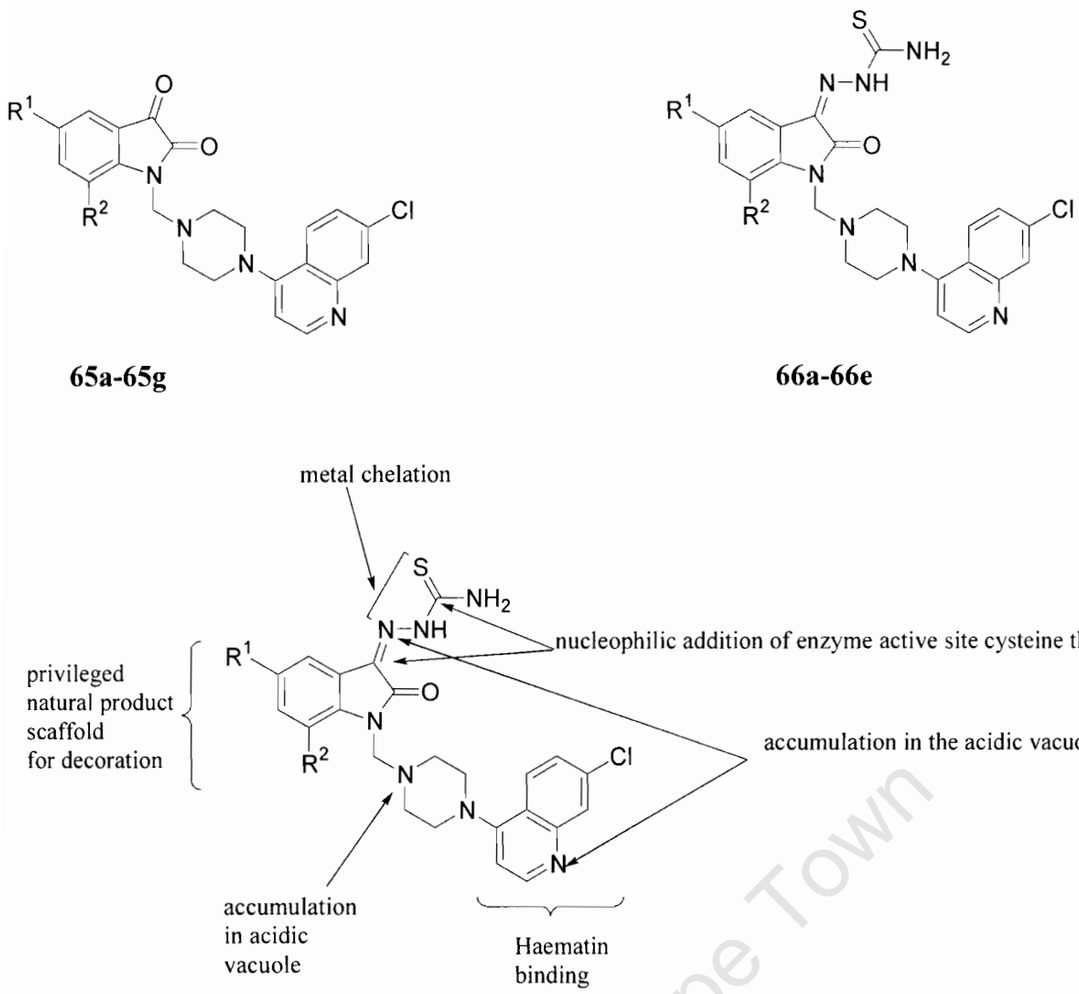


Figure 15. Chemical structures of **65** and **66** and design principle for quinoline *N*-Mannich base derivatives **66** against *P. falciparum*.

The design principle depicted in Figure 15 is summarised as:

i). The isatin provides a hydrophobic aromatic ring template potential for binding to the hydrophobic sites of the target.

ii). The thiosemicarbazone moiety has been selected to provide reactive sites (the imine and thiol carbonyl) for alkylation of the enzyme cysteine thiolate. This moiety is likely a metal chelator in which the hydrazinic nitrogen and sulfur atoms could be involved in binding to endogenous iron. This has implications for metabolism within the parasite by inhibiting iron-dependent enzymes as well as metal-interactive inhibition of *falcipain-2*. The hydrazinic nitrogen could also assist in the accumulation within the acidic food vacuole of *P. falciparum*.

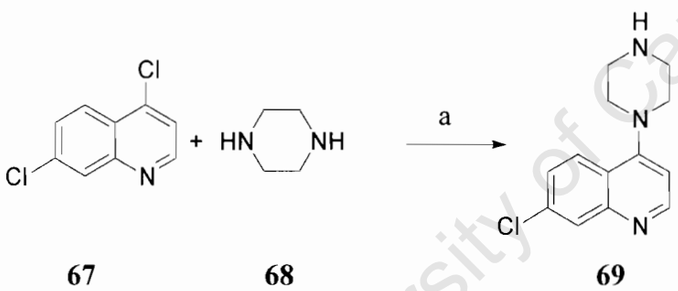
iii). To increase accumulation of the molecule, a piperazine substituent bearing a protonatable nitrogen has been introduced between the isatin and 4-aminoquinoline.

iv). The 7-chloroquinoline moiety has been proposed from earlier findings that it binds to the haematin in the parasite's acidic vacuole thus inhibiting haematin formation and increased in accumulation due to the protonable nitrogen. [126]

3.3.2. Synthesis and Characterization

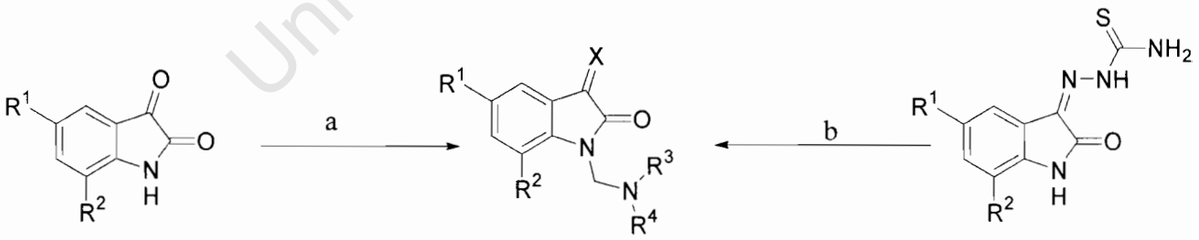
3.3.2.1 Synthesis

Chloro-4-piperazin-1-yl-quinoline, the precursor compound was firstly synthesized in good yield (66%) from condensation of 4, 7-dichloroquinoline **67** and piperazine **68** (excess) in *N*-methyl-2-pyrrolidine (NMP), to give the target compounds **69**, (Scheme 20). Upon the preparation of the required secondary amine precursor, we proceeded with the Mannich reaction. Mannich reactions can be performed *via* a one pot direct multicomponent protocol requiring isatin, an aldehyde and an amine or *via* a preformed iminium ion. The latter approach was used in this project to prepare **65** and **66**. Accordingly, paraformaldehyde (1.0 equiv) and chloro-4-piperazin-1-yl-quinoline **69** (1.0 equiv) were dissolved in ethanol and stirred for 30 mins. The iminiun ion formed *in situ* was then reacted with selected isatins (Scheme 21a) and isatin-3-thiosemicarbazones (Scheme 21b) in ethanol for 3 hours at room temperature to furnish the desired *N*-Mannich isatin derivatives accomplished in low to excellent yields 26-93%, (Table 5). The above mentioned approach was used in the preparation of non-quinoline based Mannich derivatives **70**.



67 **68** **69**

Scheme 20. (a) K_2CO_3, Et_3N, NMP, 135^{0}C, 4h, 66.4%.

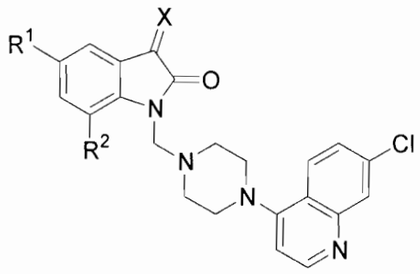


38 **70** **64**

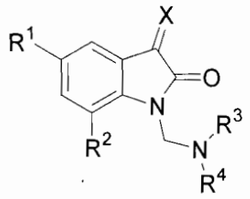
65; X=O;(R^3)R^4=piperazinyl quinoline. **66**; X=NNHC(S)NH_2; (R^3)R^4= piperazinyl quinoline. **70**; X= NNHC(S)NH_2, (R^3)R^4= Et, pyrrodyl, piperidyl, morpholinyl, *N*-benzyl piperazinyl and *N*-phenyl piperazinyl.

Scheme 21. (a) CH_2O, amine, EtOH, rt, 3h, 26-93%; (b) CH_2O, amine, EtOH, 45^{0}C, 3h, 26-93%.

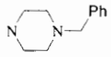
Table 5. Data on a series of quinoline and non-quinoline Mannich based isatin derivatives.



65a–66d

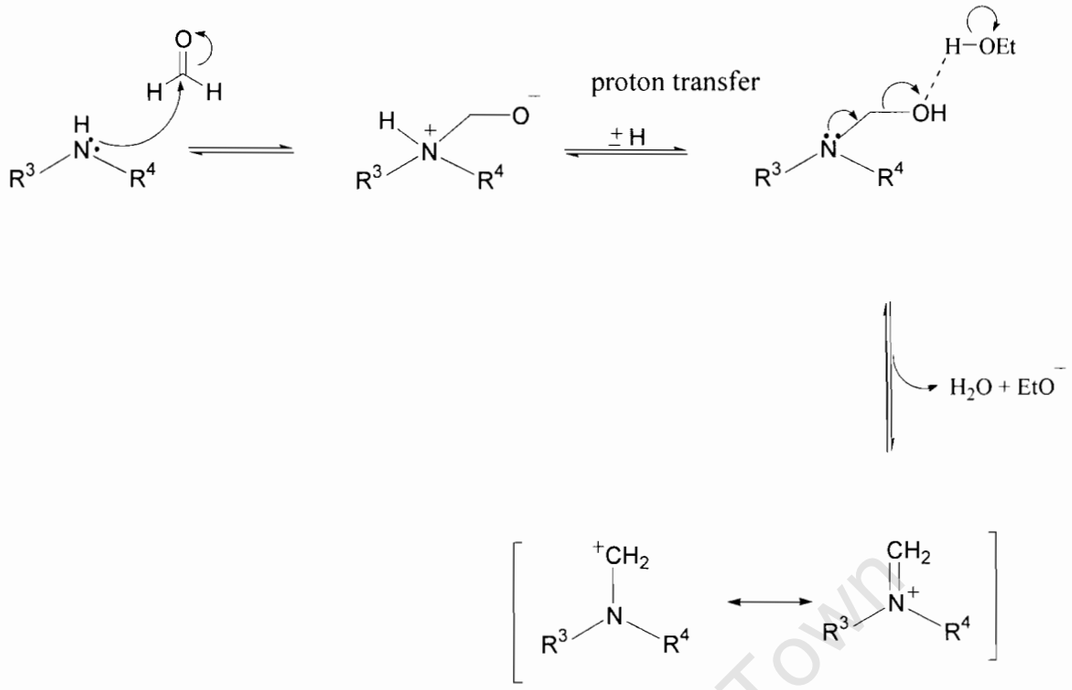


70a–70f

Compound	R^1	R^2	$(R^3)R^4$	X	Time(hrs)	% Yield	m.p (^oC)
65a	H	H	~	O	4	93	203-206
65b	Me	Me	~	O	4	85	209-213
65c	Cl	H	~	O	4	24	153-157
65d	F	H	~	O	4	94	110-117
65e	Me	H	~	O	4	55	202-203
65f	I	H	~	O	4	59	151-153
65g	Br	H	~	O	4	48	210-212
66a	H	H	~	$NNHC(S)NH_2$	3	78	216-217
66b	F	H	~	$NNHC(S)NH_2$	3	51	233-234
66c	Cl	H	~	$NNHC(S)NH_2$	3	86	234-235
66d	Br	H	~	$NNHC(S)NH_2$	3	73	236-237
66e	Me	H	~	$NNHC(S)NH_2$	3	78	232-234
70a	H	H	NEt_2	$NNHC(S)NH_2$	3	54	134-135
70b	H	H		$NNHC(S)NH_2$	3	95	179-181
70c	H	H		$NNHC(S)NH_2$	3	90	181-184
70d	H	H		$NNHC(S)NH_2$	3	92	208-210
70e	H	H		$NNHC(S)NH_2$	3	62	187-188
70f	H	H		$NNHC(S)NH_2$	3	76	188-191

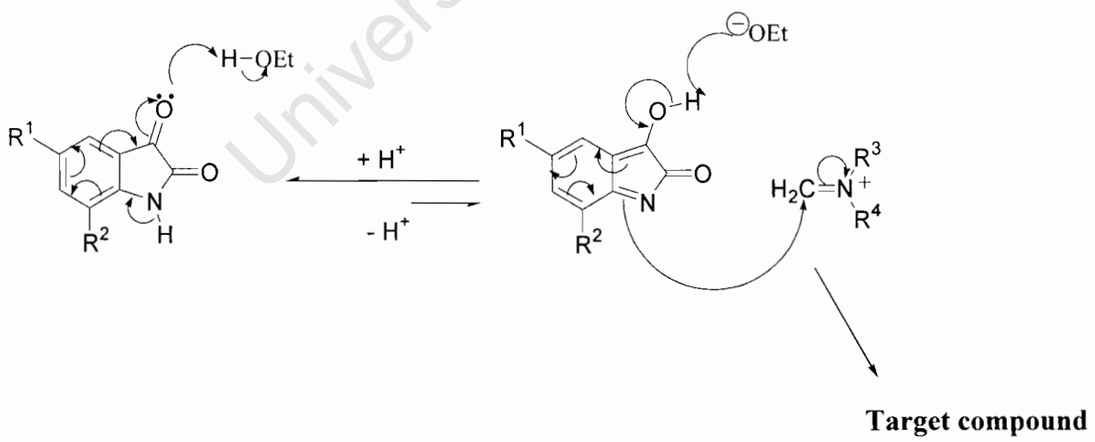
3.3.2.1 Mechanism

The proposed mechanism involves initial amine addition to the carbonyl group of formaldehyde to form an intermediate followed by acid-catalyzed dehydration gives the iminium ion. Since no acid was used in our case, ethanol was likely the proton donor during the dehydration.



Scheme 22. Mechanism for the formation of iminium ion.

The Mannich reaction of ketones with acidic α-protons usually proceed *via* nucleophlic enolate anions or enol equivalents which attack the electrophilic iminium ion. In case of isatin, the acidic NH proton is involved in a kind of keto-enol tautomerism presumably catalyzed by the solvent ethanol. Since the enol is the nucleophilic form, the reaction proceeds *via* this form. Re-aromatization provides an additional driving force.



Scheme 23. Mechanism for the Mannich reaction involving isatin.

The structures of compounds were assigned on the basis of spectra data, including ^{1}H NMR spectra which demonstrated characteristic broad signals at δ 3.25 ppm and δ 2.96 ppm for the piperazinyl ethylene protons, a singlet at δ 4.60 ppm for the methylene (-NCH_2N-) proton while the aromatic protons appeared in the region δ 8.71-6.80 ppm. Further proof of the correct structural assignment of the *N*-Mannich bases was provided by ^{13}C NMR. Aromatic carbons were observed in the region between δ 156.0 -109.0 ppm whilst the sp^3 carbons were observed at 62.0, 52.0 and 51.0 ppm. Below is the ^{1}H NMR spectrum of compound **66a**.

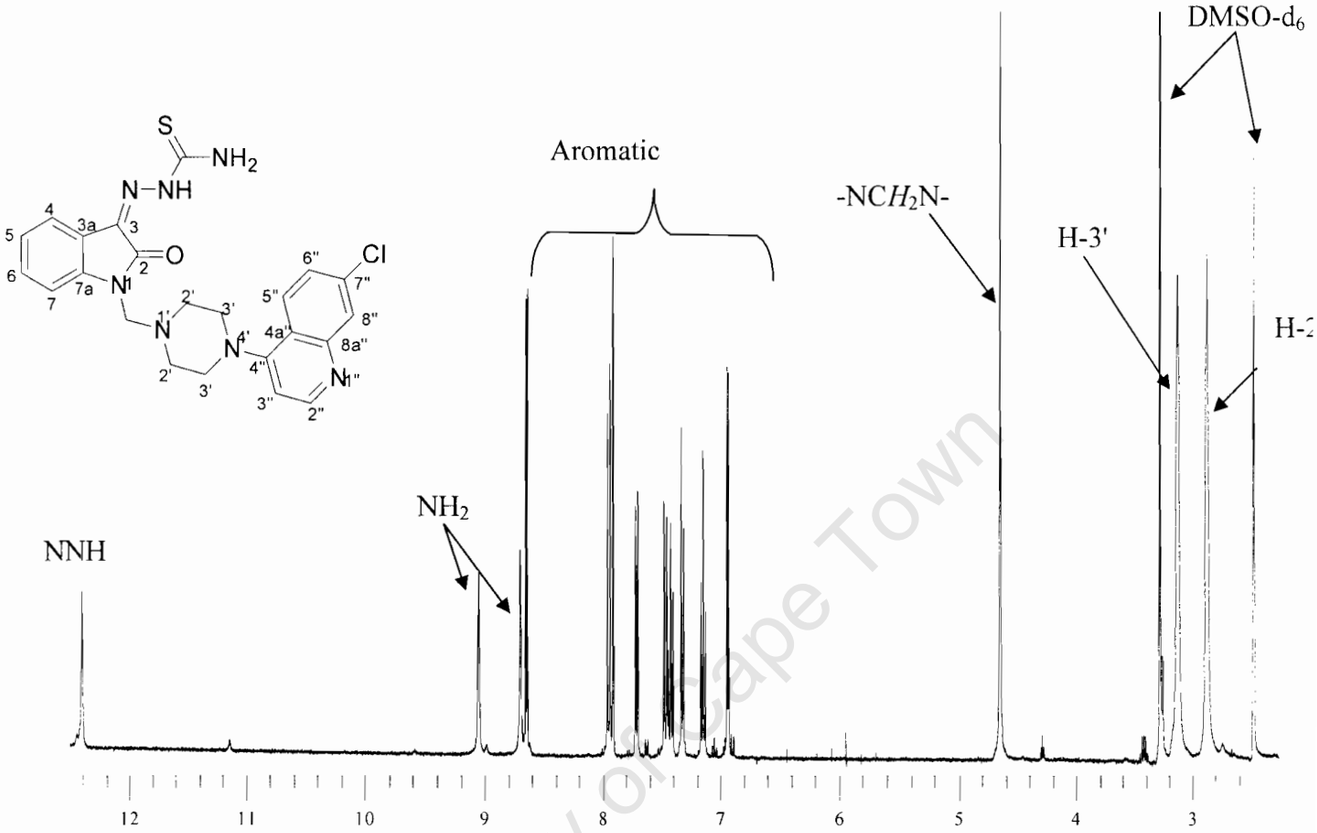


Figure 15: ^{1}H NMR spectrum for **66a** in DMSO-d_6.

From the spectrum, the appearance of singlets at δ 12.40, 9.04, 8.69 and 4.60 ppm representing NNH, NH_2 and –NCH_2N- respectively in the molecule and other derivatives provided evidence for product formation. In the ^{13}C NMR spectra, the appearance of the C=N at δ 162.6 ppm and disappearance of the ketonic C=O at δ 183.0 ppm provided further proof. Infrared spectra exhibited C=N stretching vibrations at 1590cm^{-1} and there was complete disappearance of ketonic C=O at 1730cm^{-1}.

2.4 QUINOLINE ETHYLENE ISATIN DERIVATIVES

2.4.1. Background and Rationale

As with the Mannich base quinolines derivatives **66a-66c**, quinoline ethylene isatin derivatives **71** were essentially aimed at the malaria parasite in a bi-therapeutic strategy similar to the quinoline-Mannich based derivatives. The rationale for this class is two-fold and is depicted in Figure 16.

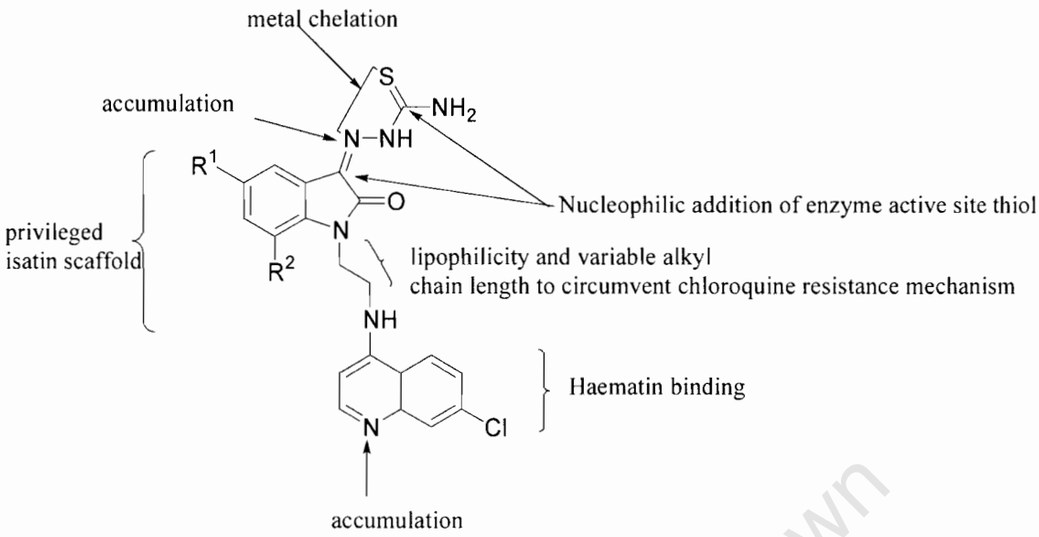


Figure 16. Design principle for quinoline ethylene derivatives **71**.

In addition to the common structural features already discussed for Mannich base quinoline **66,** the additional variable alkyl chain in **71** is important for lipophilicity and avoidance of the chloroquine resistance mechanism:

i). Lipophilicity is an important parameter in the effectiveness of aminoquinoline antimalarial agents such as chloroquine. Sufficient lipophilicity is required for these agents to cross the parasite membrane and enter the acidic vacuole where they accumulate *via* pH trapping. [127] Thus introducing a variable alkyl spacer between the isatin and 4-aminoquinoline moiety would permit studies of the effects of lipophilicity and antimalarial activity within a homologous series. Moreover, for metal (iron) chelators, lipophilicity is an important property in effective antimalarial activity. [128]

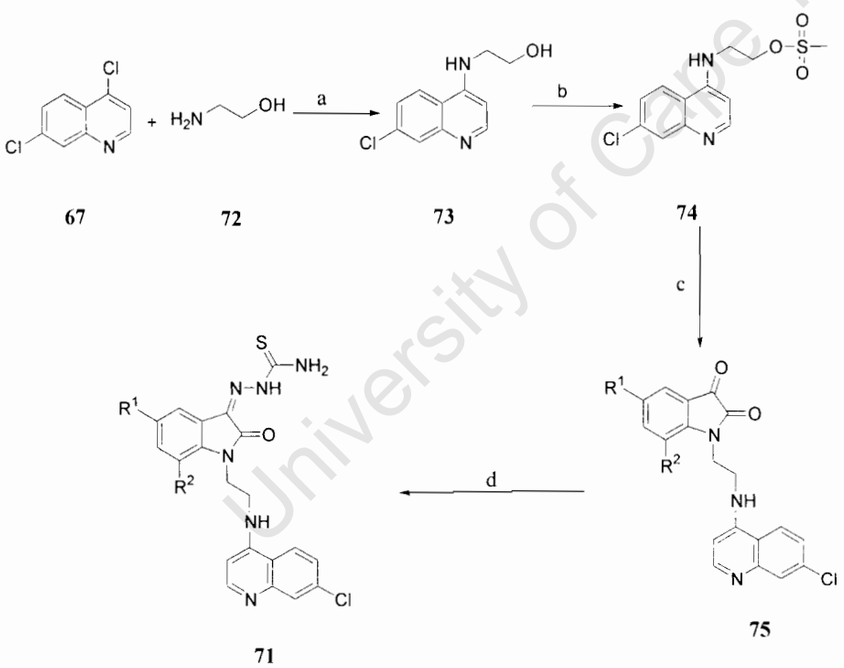
ii). As mentioned earlier, modification of the length of the alkyl side chain of chloroquine has previously led to new aminoquinoline derivatives which circumvent aminoquinoline drug resistance. [123]

For the purpose of the MSc programme, preliminary studies focused only on identifying the optimum commercially available scaffold while keeping the ethylene linker constant.

3.4.2.1 Synthesis of Derivatives 75a–75e and 71a–71c

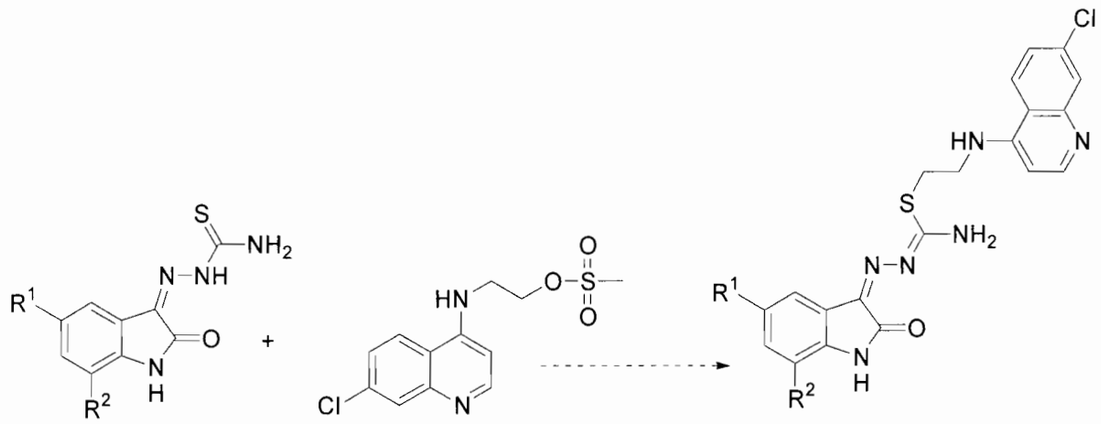
The quinoline-ethylene isatin derivatives listed in Table 9 were prepared according to Scheme 24. Reaction of excess 2-amino ethanol **72** and 4, 7-dichloroquinoline **67** as reported previously [126] gave **73** in a good yield of 92%. This was followed by *o*-mesylation [129] in pyridine at 0 °C for 5 hours to furnish **74** in a yield of 82%. Selectivity in the mesylation of preformed alcohol **73** was not a problem in spite of the presence of the 4-amino (NH) group. This is due to the fact that the lone pair of electrons on the nitrogen is in conjugation with the quinoline nitrogen and is not readily available for reactions. Thus *O*-mesylation proceeded smoothly at low temperature. The reaction could also have proceeded *via* initial attack of the quinoline nitrogen (DMAP effect) on the sulfonyl group of MsCl followed by sulfonyl transfer from the resulting intermediate to the primary hydroxyl group.

The synthesis of quinoline-ethylene isatin derivatives from **74** (as in Scheme 24) employed sodium hydride as the base and dimethylformamide (DMF) as the solvent resulted in good yields (68-88%) of the products, (Table 6). Selected precursors were used in formation of thiosemicarbazones, (Scheme 24).



Scheme 24. (a) 0.3 equiv. of K_2CO_3, 0.3 equiv. of Et_3N, reflux, 8h, 92 %; (b) 2.5 equiv. of methanesulfonyl chloride, pyridine, 0 °C, 5h, 83%;(c) NaH, isatin/5-substituted isatin, DMF, 60 °C, 24h, 68-89%; (d) $H_2NNHC(S)NH_2$, ethanol, 3h, rt, 68-89%.

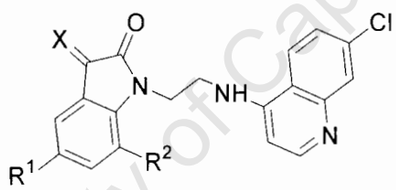
It is noteworthy that compounds **71a–71c** were prepared from precursors **75a–75e** by condensation with thiosemicarbazide as shown in Scheme 24 (d). It was necessary that the target compounds were prepared using this procedure. The alternative procedure of starting with thiosemicarbazone derivatives **64** of isatin followed by alkylation would have led to S-alkylation at the thiosemicarbazone moiety as a competing reaction to give unwanted product **77,** (Scheme 25).



64 **74** **77**

Scheme 25. By product formation via S-alkylation.

Table 6. Data on a series of ethylene-quinoline isatin derivatives



74a – 75c

Compound	R^1	R^2	X	Time(hrs)	%Yield	mp (^{0}C)
75a	H	H	O	16	88	240-243
75b	Me	H	O	16	68	240-243
75c	Cl	H	O	16	69	221-223
75d	Me	Me	O	16	89	219
75e	I	H	O	16	70	200-201
71a	H	H	$NNHC(S)NH_2$	3	70	167-171
71b	Me	H	$NNHC(S)NH_2$	3	89	227-230
71c	Cl	H	$NNHC(S)NH_2$	3	68	221-227

The structures of the respective analogues were confirmed by ^{1}H NMR spectra, below is the ^{1}H NMR spectrum of compound **71a.**

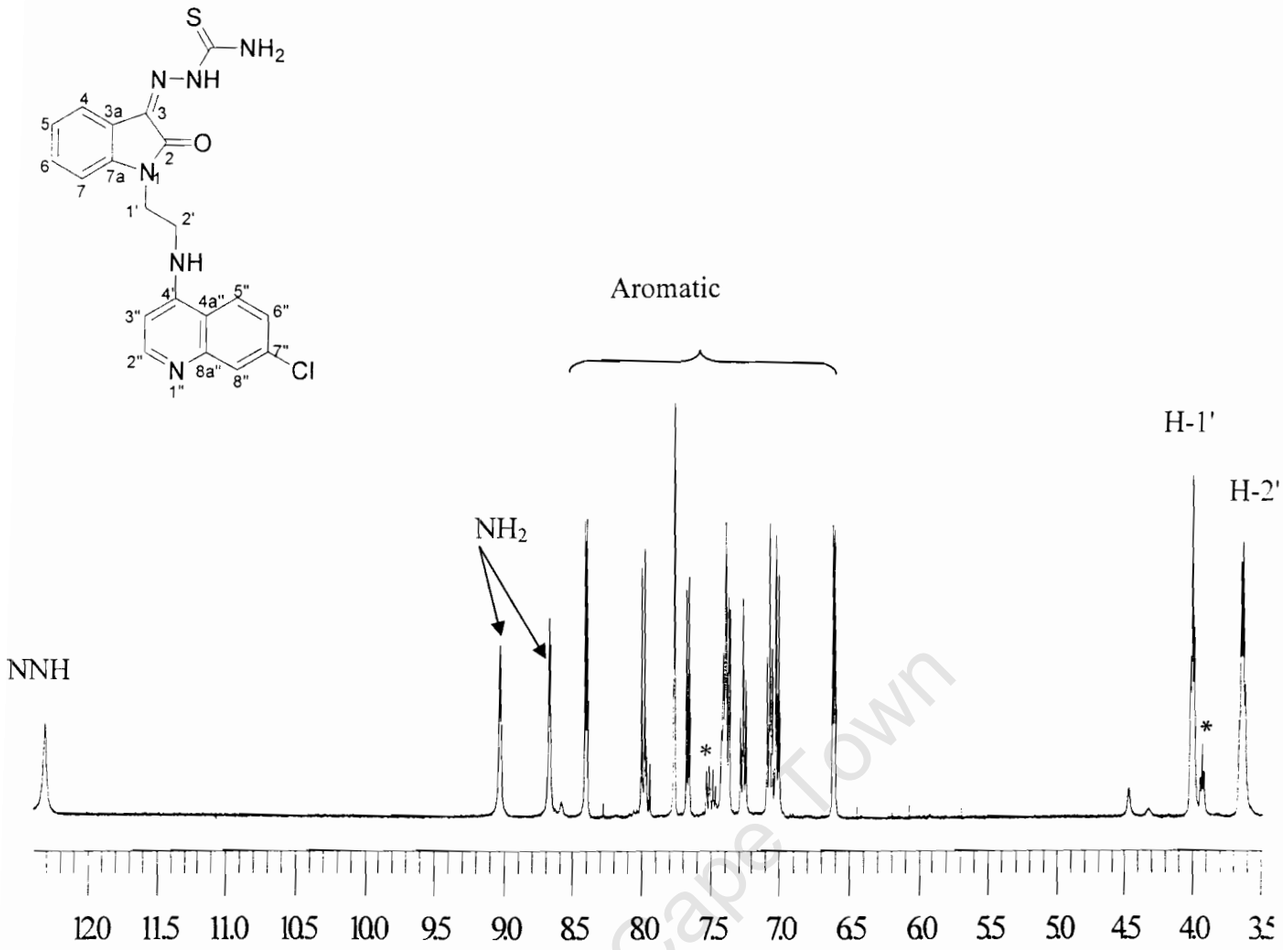


Figure 17. ^{1}H NMR spectrum for **71a** in DMSO-d_6.

The ^{1}H NMR spectra as shown in Figure 17 above explains the following;

i) Presence of NNH, NH_2

ii) Characteristic triplets for H-1′ and H-2′ respectively.

iii) Presence of aromatic protons of isatin and quinoline from δ 8.40 - 6.63 ppm.

iv) Absence of methyl signal of the mesylate at δ 3.07 ppm and NH from the isatin at δ 11.20 ppm.

The small peaks found at δ 3.90 and 7.50 ppm and marked with an asterisk (*) may be due to some unreacted starting material or probably due to *E, Z* isomerism.

The other spectroscopic data was satisfactorily and consistent with the structure. The purity for most derivatives was confirmed by elemental analysis data which was within the acceptable ±0.4 %. However, a few compounds did not show satisfactory elemental analysis data in respect of some atoms.

3.5 Conclusion

The chemical synthesis of series of derivatives based on isatin scaffold have been investigated. *N*-functionalized isatin derivatives **57a–57i** and **58a–58b** were synthesized mainly in one-step by parallel synthesis using immobilized reagents. The success in the use of KF/Al_2O_3 as a support bound base augur well for future parallel synthesis of libraries of these derivatives. The *N*-(carbobenzyloxy) isatins **59a-59d** were successfully synthesis despite obtaining relatively low yields. The reactions involving isatin-3-thiosemicarbazones proceeded accordingly to afford all desired products in excellent yields. Bi-functionalized isatin derivatives (quinoline-Mannich base and quinoline-ethylene thiosemicarbazones) were synthesized in two or more steps involving preformed precursors and all were also obtained in reasonable to excellent yields.

In view of the prevalence of parasitic diseases in poor third world countries, there is a need to consider simple and cost effective synthetic chemistry in order to make potential drugs affordable. These considerations should always guide the selection and prioritization of target compounds. The importance of simple, cost-effective synthesis cannot be over emphasized for tropical diseases. The syntheses just described herein potentially fulfils this criteria.

CHAPTER 4

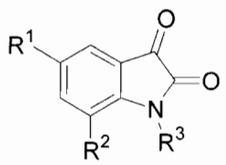
BIOLOGICAL EVALUATION OF SYNTHESIZED COMPOUNDS

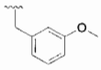
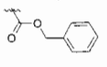
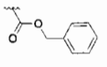
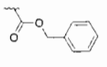
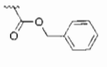
4.1 *N*-SUBSTITUTED ISATIN DERIVATIVES

4.1.1.*In vitro* Activity of *N*-substituted isatins against *Cruzain, Falcipain-2, Rhodesain* and a chloroquine-sensitive (3D7) strain of *P. falciparum.*

IC_{50} values against recombinant proteins (*cruzain* and *falcipain-2*) were determined essentially as described previously [104]. Accordingly, equal amounts (~1 nM) each of recombinant protein was incubated with different concentrations of inhibitors (added from 100x stocks in DMSO in 100 mM sodium acetate (pH 5.5)-10 mM dithiothreitol for 30 min at room temperature before addition of the substrate benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin (final concentration, 25 μM). Fluorescence was continuously monitored for 30 min at room temperature in a Labsystems Fluoroskan II spectrofluorometer. IC_{50} values were determined from plots of activity over inhibitor concentration with GraphPad Prism software. IC_{50} values for *rhodesain* were similarly determined at 3nM. Selected compounds from the series were also tested against a chloroquine-sensitive 3D7 strain of *P.falciparum*. Results of the aforementioned assays are shown in Table 7.

Table 7. Inhibition of *cruzain*, *falcipain-2*, *rhodesain* and chloroquine-sensitive (3D7) strain by *N*-substituted isatin and commercially available derivatives.



Compound	R^1	R^2	R^3	IC_{50} [μM]			ED_{50}[μM]
				Cruzain	*Falcipain-2*	*Rhodesain*	3D7[d]
methylisatin	H	H	Me	>10[a]	NE[b]	ND[c]	ND
phenylisatin	H	H	Ph	>10	NE	ND	ND
57a	H	H		>10	NE	>10	ND
57b	I	H		90	ND	>10	ND
57c	Me	H		80	NE	>10	ND
57d	Me	H		>10	NE	>10	69.14
57e	Me	H		**2**	21.9	>10	5.30
57f	Me	H		**2.8**	**9.2**	>10	9.89
57g	Cl	H		**6**	31.2	>10	ND
57h	Cl	H		>10	34.6	>10	ND
57i	Cl	H		90	46.9	>10	7.40
58a	H	H		>10	NE	100	ND
58b	H	H		>10	NE	15	ND
59a	H	H		>10	NE	30	ND
59b	Cl	H		80	NE	>10	ND
59c	Me	H		>10	NE	>10	ND
59d	F	H		>10	NE	>10	6.17

[a]No inhibition at 10 μM [b]No effect [c]Not determined [d]Chloroquine-sensitive strain of *P .falciparum*

From the *in vitro* results presented in Table 7 [130], several trends were observed as follows:

i). In general compounds without a substituent at position 5 (R^1) of isatin (*N*-methylisatin, *N*-phenylisatin, **57a**, **58a**, **58b** and **59a**) did not generally show good activity (IC_{50} > 10 μM).

ii). A combination of *N*-benzylation and substitution at position 5 seemed favourable and exhibited modest to good activity with **57e**, **57f** and **57g**, showing good activity against both *cruzain* and *falcipain-2*. The best compound in this series was **57f** which showed activity against two cysteine proteases (*falcipain-2* and *cruzain*) with IC_{50} values 2.8 μM and 9.2 μM ,respectively. This compound contains a privileged biaryl substructural unit probably essential for activity.

iii). Alteration of substituents on the isatin and benzyl rings decreased activity against *falcipain-2*, but increased activity against *cruzain* (**57e** and **57g**).

iv). Exchanging the *N*-benzyl for *N*-acyl and *N*-sulfonylisatin derivatives had no beneficial effect regardless of the substituent (R^1, R^2, R^3).

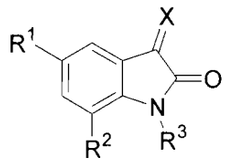
v). Against the chloroquine-sensitive 3D7 strain, compounds **57d**, **57e**, **57f**, **57i** and **59d** demonstrate very weak (**57d**) to moderate (**57e**, **57f**, **57i** and **59d**) inhibitory activity, while the rest were not determined because they were inactive at the initial cut off concentration of 250 mg/ml chosen for % parasite survival.

Against the cysteine protease enzymes, the preliminary study revealed that *N*-benzylated scaffolds are favoured to bind the cysteine proteases. As mentioned previously, the benzyl moiety was presumed to bind tightly in the S2 pocket of the enzyme and this accounts for the increased potency of this class of inhibitors. Apparently, compound **57f** (biaryl containing), is superior to other derivatives against *cruzain* and *falcipain-2*. The biaryl motif is regarded as a privileged substructural unit which binds to a wide range of proteins. [131] From this limited structure-activity relationship (SAR) data, it is tempting to speculate that isatins containing a biaryl moiety might provide ligands for a diverse set of cysteine proteases. However, more biaryl compounds will need to be synthesized and tested against multiple cysteine proteases in order that meaningful SAR maybe delineated.

4.2 ISATIN-3-THIOSEMICARBAZONE DERIVATIVES

4.2.1 Inhibition of *Cruzain*, *Falcipain-2* and *Rhodesain*

Table 8. Inhibition of *cruzain*, *falcipain-2* and *rhodesain* by commercial isatins and thiosemicarbazone derivatives.



Compound	R^1	R^2	R^3	X	IC_{50} [µM]		
					Cruzain	*Falcipain-2*	*Rhodesain*
Isatin	H	H	H	O	>10[a]	NE[b]	>10
5-methylisatin	Me	H	H	O	>10	NE	ND[c]
5-fluoroisatin	F	H	H	O	>10	NE	ND
5-chloroisatin	Cl	H	H	O	>10	NE	ND
5-bromoisatin	Br	H	H	O	>10	NE	ND
5-iodoisatin	I	H	H	O	>10	NE	ND
5-nitroisatin	NO_2	H	H	O	>10	42.6	>10
5-(CF_3O) isatin	CF_3	H	H	O	>10	NE	>10
5,7dimethylisatin	Me	Me	H	O	>10	NE	ND
64a	H	H	H	N-NHC(S)NH_2	**8**	38.8	**3.5**
64b	F	H	H	N-NHC(S)NH_2	30	38.6	15
64c	Cl	H	H	N-NHC(S)NH_2	21	32.8	**6**
64d	Br	H	H	N-NHC(S)NH_2	20	29.6	**7**
64e	I	H	H	N-NHC(S)NH_2	**9**	28.7	**1**
64f	Cl	Me	H	N-NHC(S)NH_2	10.5	**9.4**	**3**
64g	Me	H	H	N-NHC(S)NH_2	20-50	43.9	15
64h	Me	Me	H	N-NHC(S)NH_2	16	13.2	15
64i	NO_2	H	H	N-NHC(S)NH_2	30	**4.4**	17
64j	H	H	Ph	N-NHC(S)NH_2	16	29.7	40
64k	H	H	Me	N-NHC(S)NH_2	58	63	50

[a]No inhibition at 10 µM [b] No effect [c] Not determined

All inhibitors were screened for effectiveness against the three proteases using procedures as described above (4.1.1). The results are presented in Table 8 as IC_{50} values together with those of commercially available isatins which are included for comparison purposes.

On the account of the biological data presented above in Table 8, the following SAR observations were made. [130]

i). All the commercially available derivatives were practically inactive.

ii). The synthesized thiosemicarbazones had better activity ranging from moderate to high in most cases. Compound **64f** exhibited good activity (10μM or less) against all the three enzyme targets with IC_{50} values of 10.5 μM, 9.4 μM and 3 μM against *cruzain*, *falcipain-2* and *rhodesain*, respectively.

iii). Besides **64f**, compound **64i** displayed moderate potency against *falcipain-2*, while **64a** and **64e** are the most promising scaffolds against *cruzain* and *rhodesain*.

iv). The order of activity against the targets (especially for *cruzain* and *rhodesain*) for the 5 halo substituted analogues is I > Br > Cl > F, implying that within this class, a more hydrophobic atom at position 5 is favored for activity (compare **64b**, **64c**, **64d** and **64e**).

Since the initial aim of this work was to identify the promising scaffold for future inhibitors design, these compounds were not tested further against the parasite sources of the proteases. Suffices to note that in terms of future development of parasitic cysteine protease inhibitors based on the isatin scaffold, combination of the thiosemicarbazone moiety with *N*-benzylation might lead to superior inhibitors. Moreover, privileged biphenyl-based *N*-benzylated derivatives might provide diverse ligands for multiple parasitic cysteine proteases. The commercial availability of a diverse range of boronic acids for construction of the biphenyl (biaryl) moiety *via* the palladium (0)-catalyzed Suzuki cross coupling reaction should facilitate SAR studies in this regard. The generally superior activity of thiosemicarbazone derivatives, compared to corresponding commercially available ketone precursors, might suggest the involvement of the thiocarbonyl (C=S) group in the mechanism of inhibition of the parasitic cysteine proteases understudy in this project. This is in line with the mechanism of inhibition of *cruzain* by simple aromatic thiosemicarbazones suggested by Du and co-workers [104] (also described in Scheme 16 on page 36). The differences in activity across the proteases gives some indication in differences in the active site topology amongst the three proteases.

4.3 MANNICH AND ETHYLENE BASED ISATIN DERIVATIVES

4.3.1 Inhibition activity of derivatives 68a–75c

Compounds in the series **65a–71c** were evaluated against *cruzain* and *rhodesain*. Percent activity which gives a measure of the inhibitory activity of each compound in place of IC_{50} values was determined. The lower the % enzyme activity, the more potent a compound is as an inhibitor at a given concentration. The results are presented in Table 9.

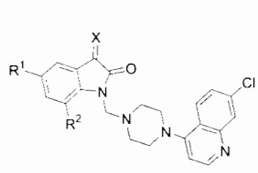
As can be seen from the Table 9,

i). Generally thiosemicarbazones (**66a**, **66b**, **66c**, **66e**, **71a** and **71b**) were most effective at reducing enzyme activity compared to the corresponding ketone derivatives especially in the case of *rhodesain*. Compound **71b** was found to be very potent against both *cruzain* and *rhodesain* by reducing the % activity of the enzyme to 23 and 6% respectively. Similarly, compounds **66b**, **66c**, **66e** and **71a** demonstrated an increased potency and apparently more potent against *rhodesain* (2-4% enzyme activity for the best compounds **66b**, **66c** and **66e**).

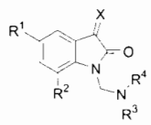
ii). The non-quinoline based derivatives (**70a-70f**) showed lack of activity against both *cruzain* and *rhodesain* as compared to their corresponding quinoline based derivatives (**66b**, **66c**, **66e**, **71a** and **71b**).

iii). Against *cruzain*, the quinoline-Mannich base derivatives (**66b**, **66c** and **66e**) were less superior to the quinoline-ethylene derivatives (**71a** and **71b**) when other moieties were kept constant.

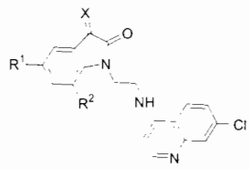
Table 9. Percentage Activity of the enzyme in the presence of 10 μM of compound.



65a-66c



70a-70f



71a-71c

Compound	R^1	R^2	$(R^3)R^4$	X	% Activity	
					Cruzain	Rhodesain
65a	H	H	~	O	55	92
65b	Me	Me	~	O	ND	ND
65c	Cl	H	~	O	ND	ND
65d	F	H	~	O	89	91
65e	Me	H	~	O	ND	ND
65f	I	H	~	O	86	91
65g	Br	H	~	O	ND	ND
66a	H	H	~	N-NHC(S)NH_2	86	46
66b	Me	H	~	N-NHC(S)NH_2	71	**3**
66c	F	H	~	N-NHC(S)NH_2	76	**2**
66d	Cl	H	~	N-NHC(S)NH_2	ND	ND
66e	Br	H	~	N-NHC(S)NH_2	83	**4**
70a	H	H	NEt_2	N-NHC(S)NH_2	71	67
70b	H	H		N-NHC(S)NH_2	82	71
70c	H	H		N-NHC(S)NH_2	84	65
70d	H	H		N-NHC(S)NH_2	81	78
70e	H	H		N-NHC(S)NH_2	85	74
70f	H	H		N-NHC(S)NH_2	92	74
75a	H	H	~	O	93	83
75b	Me	H	~	O	92	72
75c	Cl	H	~	O	ND	ND
75d	Me	Me	~	O	92	71
75e	I	H	~	O	ND	ND
71a	H	H	~	N-NHC(S)NH_2	57	10
71b	Me	H	~	N-NHC(S)NH_2	23	6
71c	Cl	H	~	N-NHC(S)NH_2	ND	ND

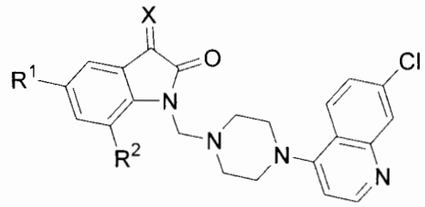
[a] Not determined

4.3.2 *In vitro* Activity of derivatives 65-71 against *P. falciparum* D10, K1, and W2 strains

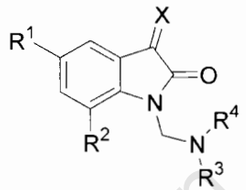
Since *P. falciparum* malaria was the main focus of the MSc project several compounds were selected for *in vitro* testing against *P. falciparum* strains. The detailed procedure for the parasites culture (D10 and K1) were conducted according to the methods described by Trager and Jensen. [132] Initial 1mg/ml stocks of the compounds were made up in DMSO, using the pre-weighed samples provided, and were diluted in water and finally complete medium on the day of the experiment. The highest concentration of DMSO that the parasites were exposed to was 0.05 %, which had no measurable effect on parasite viability. No attempt was made to determine IC_{50} values greater than 1000 $ngml^{-1}$. All experiments were performed in duplicate on a single occasion using a chloroquine-sensitive strain of *P. falciparum* (D10). The more active compounds were also tested against a chloroquine-resistant strain of *P. falciparum* (K1). The parasites were continuously cultured and the parasite lactate dehydrogenase (pLDH) activity was used to measure parasite viability as described by Makler et al. [133] The 50% inhibitory concentration (IC_{50}) values for the compounds were obtained from the dose-response curves, using non-linear dose-response curve fitting analyses with GraphPad Prism v.3.00 software.
W2-strain P. falciparum parasites (1 % parasitemia, 2 % hematocrit) were cultured in 0.5 mL of medium in 48-well cultured dishes. Appropriate inhibitors from 10 mM stocks in DMSO were added to cultured parasites to a final concentration of 20 µM. From 48-well plates, 125 µM of culture was transferred to two 96-well plates (duplicates). Serial dilutions (1:5) of inhibitors were made to final concentrations of 10 µM, 2000 nM, 400 nM, 80 nM, 16 nM, 3.2 nM. Cultured were maintained at 37 ^{o}C for 2 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. [134]

A dose-response curve is a plot of log of concentration of a drug against the plots of response (enzyme activity) expressed in percentage. The IC_{50} values (see Table 10) are simply the concentration of drug required to cause a response halfway between the Y-axis. Depending on which test, dose-response curves can have almost any shape.

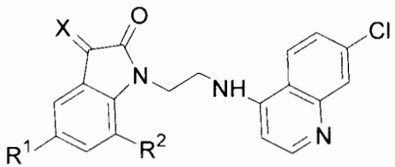
Table 10. IC_{50} values of compounds against chloroquine sensitive (D10), chloroquine resistant (K1 and W2) strains and *Falcipain-2*. (**Table 10 continues on page 61**).



65a-66e



70a-70f



75a-71c

Compound	R^1	R^2	$(R^3)R^4$	X	IC_{50} [µM]				RI[d] (K1)	RI (W2)
					P. falciparum D10[a]	*P. falciparum* K1[b]	*P. falciparum* W2[c]	*Falcipain-2*		
65a	H	H	~	O	0.949	ND[e]	0.961	>20	~	1.013
65b	Me	Me	~	O	ND	ND	ND	ND	~	~
65c	Cl	H	~	O	1.069	ND	ND	ND	~	~
65d	F	H	~	O	0.719	0.720	0.908	>20	1.001	1.263
65e	Me	H	~	O	ND	ND	ND	ND	~	~
65f	I	H	~	O	0.915	ND	0.794	~20	~	0.868
65g	Br	H	~	O	ND	ND	ND	ND	~	~
66a	H	H	~	$NNHC(S)NH_2$	1.370	ND	0.957	**9.265**	~	0.698
66b	Me	H	~	$NNHC(S)NH_2$	ND	ND	ND	ND	~	~

66c	F	H	~	$NNHC(S)NH_2$	1.185	ND	1.193	**7.987**		1.007
66d	Cl	H	~	$NNHC(S)NH_2$	1.370	ND	1.809	ND	~	1.320
66e	Br	H	~	$NNHC(S)NH_2$	0.640	0.521	1.226	**6.069**	0.814	1.916
70a	H	H	NEt_2	$NNHC(S)NH_2$	ND	ND	>>10	>20	~	~
70b	H	H		$NNHC(S)NH_2$	ND	ND	>>10	>20	~	~
70c	H	H		$NNHC(S)NH_2$	ND	ND	>>10	>20	~	~
70d	H	H		$NNHC(S)NH_2$	ND	ND	>>10	>20	~	~
70e	H	H		$NNHC(S)NH_2$	ND	ND	>>10	>20	~	~
70f	H	H		$NNHC(S)NH_2$	ND	ND	>>10	>20	~	~
75a	H	H	~	O	0.905	0.990	0.125	>20	1.094	0.138
75b	Me	H	~	O	0.547	0.727	0.489	>20	1.329	0.894
75c	Cl	H	~	O	0.375	1.507	ND	ND	4.019	~
75d	Me	Me	~	O	0.330	0.454	0.227	~20	1.376	0.688
75e	I	H	~	O	ND	ND	ND	ND	~	~
71a	H	H	~	$NNHC(S)NH_2$	0.320	0.712	0.242	14.650	2.225	0.756
71b	Me	H	~	$NNHC(S)NH_2$	0.079	0.101	**0.051**	11.640	1.278	0.646
71c	Cl	H	~	$NNHC(S)NH_2$	0.095	**0.054**	ND	ND	0.568	~
CQ					**0.033**	**0.312**	**0.240**		9.454	7.273

[a]Chloroquine-sensitive strain; [b]Chloroquine-resistant strain; [c]Chloroquine-resistant strain; [d]Resistance Index IC_{50} (K1or W2)/ IC_{50}(D10) [e] Not determined

4.3.2.1 Inhibition of *falcipain-2* by compounds 65a – 71c

i). SAR studies show that quinoline thiosemicarbazone derivatives **66** and **71** that were assayed generally showed better inhibition activity against *falcipain-2* compared to the corresponding ketones **65** and **75,** respectively. It is noteworthy that all non-quinoline Mannich base thiosemicarbazones **70** were less active (IC_{50} > 20 μM) compared to the corresponding quinoline Mannich bases **66**. This may suggest preference for larger hydrophobic groups in the S2 enzyme binding pocket.

ii). Within the thiosemicarbazones, quinoline-Mannich based derivatives **66a**, **66c** and **66e** more active against the enzyme, with IC_{50} values mostly less than 10 μM, compared to the ethylene quinolines **71a-71b** albeit there is no direct correspondence in some respective structures with regard to substituents.

iii). Considering compounds that were similar (**66a - 66e**) with respect to position 5 substituents, the general order of activity against *falcipain-2* was Br > F > H. This trend is consistent with the trend observed for isatin-3-thiosemicarbazones (Table 8, page 56) in which a large (iodo) substituent at position 5 resulted in high potency against the enzyme.

4.3.2.2 Effects of compounds 65a – 71c on Malaria Parasites D10, K1 and W2

In the study of antimalarial effects of compounds **65a– 71c**, the following observations were made.

i). Thiosemicarbazones **71a**, **71b,** and **71c** were generally superior compared to the corresponding ketone derivatives **75a**, **75b**, and **75c**. This trend was not observed for the Mannich bases **65** and **66**. Compounds **71b** and **71c** were particularly active against all three *P. falciparum* strains D10, K1 and W2. These two compounds were even superior to chloroquine in the resistant W2 and K1 strains, with IC_{50} values of 0.051 μM and 0.054 μM respectively while still retaining respectable activity against D10.

ii). Within the thiosemicarbazones, the ethylene-based derivatives were comparatively more potent than the Mannich bases. For example, compounds **71b** and **71c** had better IC_{50} values, roughly all less than 1 μM.

iii). Removal of the quinoline ring system generally resulted in a significant loss of activity against all the three parasite strains to a point were it was deeded unnecessary to determine IC_{50} values in D10 and K1 strains for compounds **70a – 70f**. Although the IC_{50} values were measured against W2, they were much greater than 10 μM.

iv). Among the more active ethylene-quinoline thiosemicarbazones, methyl substitution at position 5 on the isatin ring resulted in better inhibitors of parasite growth against W2. Compound **71b,** with IC_{50} = 0.051 μM, was 5-fold better compared to chloroquine in the same W2 strain. Chloro substitution at position 5, as in **71c**, also showed excellent inhibitory activity against K1 with an IC_{50} value of 0.054 μM, which was 6-fold better than chloroquine. Considering compounds of series **71** only, the order of antimalarial activity was generally Cl > Me >H. The SAR study within this class of compounds clearly shows no correlation between inhibition of *falcipain-2* and antimalarial activity.

We have shown that thiosemicarbazone derivatives of isatin are potent inhibitors of *falcipain-2* and *P .falciparum* strains D10, K1 and W2. Clearly, quinoline-Mannich base isatin derivatives (**66a**, **66c** and **66e**) block enzyme activity at IC_{50} values of less than 10 μM. In a similar manner, compounds **71a**, **71b** and **71c** (quinoline-ethylene isatin derivatives) effectively blocked parasite development, but had little or no activity against *falcipain-2*. There was no correlation between the potencies of compounds against *falcipain-2* and parasites. This lack of correlation strongly suggests that the antimalarial activity of inhibitors **71a**, **71b** and **71c** were entirely not due to inhibition of *falcipain-2*. The biological assays showed no food vacuole abnormalities that usually accompany *falcipain-2* inhibition. Inhibition of *falcipain-2* is usually associated with food vacuole swelling due to accumulation of undigested hemoglobin that results from blockage of the hydrolysis process. [135] Although *falcipain-2* appears not to be the principle target for these compounds, it is possible that the compounds may be acting through the inhibition of another cysteine protease not contained in our (*falcipain-2*) screen. There are many cysteine proteases in *P. falciparum* (including *falcipain-1*) as already alluded to. The possibility that these compounds may be exerting their antimalarial effects through inhibition of another cysteine protease in *P. falciparum* is supported by the fact that compounds **71b** and **71c** can bind to and inhibit a cysteine protease. The inhibitory activity (albeit weak to modest in some cases) as demonstrated against *falcipain-2* (weak), *cruzain* (modest) and rhodesain (strong) in Tables 9 and 10 is a case in point. Alternatively compounds **71a**, **71b**, **71c** may be acting *via* a protease-independent mechanism. Given that these compounds have the ability

to chelate a transition metal like iron (+2 or +3 oxidation state), it is likely that this mechanism of action is responsible for the observed antimalarial activity. Similar conclusions have recently been made for tridentate chelating 2-acetylpyridine thiosemicarbazones. [134]

4.3.2.3 Effects of Different Moieties on Antimalarial Activities

The excellent inhibitory results against parasite development by compounds **71a**, **71b** and **71c** led to our speculating that the improved potency could have been due to the contribution from specific moieties which are discussed below:

a) Effect of the Thiosemicarbazone Group

i). Thiosemicarbazones are potential iron-chelators in *P. falciparum*, which may exhibit biological activity either by withholding iron from plasmodia or by formation of complexes toxic to the microorganism. This has implications for iron-dependent enzymes. Withholding iron from such enzymes as δ-aminolevulinate synthase (enzymes in the heme biosynthetic pathway) results in reduction in heme synthesis. [136] This may also lead to the malfunctioning of other iron-dependent enzymes such as ribonucleotide reductase which is essential for DNA synthesis. [137, 138] This would result in the disruption of vital metabolism and lead to the death of the parasite. Iron chelators such as desferrioxamine (DFO), the only agent for clinical use as an iron chelator, have been shown to inhibit ribonucleotide reductase activity by binding to the iron. [139, 140] Similarily, the thiosemicarbazones may be exerting their antiplasmodial effects through this mechanism.

ii). Secondly, metal chelators are known to form complexes that subsequently enter the erythrocytes to produce a lethal free radical-mediated reaction. One study gives evidence of copper complexes of thiosemicarbazones producing oxidative stress upon binding to glutathione, a known reducing agent. [141]

iii). Thirdly, the thiosemicarbazone moiety contains a protonatable hydrazinic nitrogen atom (see Figure 16) which possibly increases accumulation in the acidic food vacuole of the parasite *via* the pH trapping principle. [142]

iv). Lastly, the thiosemicarbazone possess an imine (C=N) and a thiol carbonyl (C=S) groups as potential sites for interaction with a cysteine thiolate in the active site of the cysteine proteases (indicated in Scheme 16).

b) Effect of the Ethylene Linker

First, introduction of the ethylene linker in the molecule was more favored compared to Mannich bases with a piperazinyl linker. The ethylene linker presumably allows the molecule to adopt a less restricted conformation due to free rotation, thereby, giving rise to favorable interactions with the active site of the target. On the other hand, the piperazinyl spacer may suffer a loss of rotational freedom.

Secondly, the ethylene linker increases the lipophilicity of compounds **71a**, **71b**, **71c** compared to **65** and **66**. Table 11 summaries the *C* logP (partition coefficient) values of selected Mannich and ethylene-based derivatives. *C* logP measures the lipophilicity characteristic of a molecule that determines whether the molecule can penetrate and transverse many cell membranes to reach the site of action. It is clear that compounds **71a**, **71b** and **71c** have higher *C* logP values which probably aids their passage through parasites membranes on the way to their presumed site of action, the acidic food vacuole. If these compounds are also exerting their antimalarial effects *via* chelation of iron in *P. falciparum*, then the high lipophilicity is a major contributor. Lipophilicity is an important physical property of iron chelators since the iron withheld by chelators from *P. falciparum* most likely resides within the parasitic compartment of the malaria infected red blood cell. As such an effective antimalarial iron chelator should have the ability to cross lipid membranes and display high selective affinity for iron (II) or iron (III) compared to other endogenous transition metals. A correlation between the degree of lipophilicity of an iron chelator and its antiplasmodial activity has been demonstrated. [128]

Table 11. *C* logP values of selected compounds.

Compounds	*C* logP
65 a	2.88
65 b	2.57
65c	3.13
66 a	4.49
66 b	4.98
66d	5.20
71 a	5.15
71 b	5.65
71c	5.86

c) Effect of Quinoline Moiety in the Molecule

The quinoline moiety may be enhancing the antiplasmodial activity in two ways;

i). *Via* increased lipophilicity (high *C* logP values) (Table 11). Since the molecules are able transverse the membranes of the parasitized erythrocytes to reach the acidic food vacuole and accumulate through protonatable nitrogens, which is the basis of the pH-trapping principle.

ii). Through the inhibition of β-haematin formation by the 7-chloro aminoquinoline moiety. [126]

d) Effect of substituents at position 5 in the isatin moiety

Chloro and methyl substitution at position 5 on the aromatic ring of the isatin is favored presumably for binding to the target.

4.3.3 Resistance Index

Resistance index is defined as the ratios of the IC_{50} against resistant strains (W2 or K1 in this case) to the IC_{50} of the same compound against sensitive strains (e.g. D10). The ratio measures the potential of any new compound against chloroquine-resistant strains relative to choloroquine (CQ). Mathematically the Resistance Index (RI) is expressed as,

$$RI = \frac{IC_{50} \text{ of compound in resistant strain}}{IC_{50} \text{ of compound in a sensitive strain}}$$

Relative to CQ, RI greater than CQ indicates a weaker activity against the chloroquine-resistant strains (K1 or W2), while RI less than that of CQ indicates a better activity of the compound against the chloroquine-resistant strains (K1 or W2). Most compounds showed indices far less than CQ (Table 10), signifying that most isatin compounds **65a–71c** are promising agents against K1 and W2 strains Moreover the results suggest that the mechanism of action of these compounds may be different from that of chloroquine.

4.4 Compliance to Lipinki's Rule

In vitro screens for antimicrobial activity provide the capacity of inhibitors against emerging infectious, not necessarily how the drugs will work in humans. For the most potent compounds in the tested series (**57f**, **64f**, **71b** and **71c**) to succeed in animal and human clinical trials as oral drugs depends on absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. Lipinki's "rule of 5" is used to classify which compounds are likely to have good intestinal absorption (membrane absorption or permeability) as a measure of a drug-like compound. [143] The rule states that good absorption and permeability is likely when:

a) Molecular weight < 500

b) *C* logP < 5

c) Number of H-bond acceptors (expressed as the sum of Ns and Os) ≤10.

d) Number of H-bond donors (expressed as the sum of N-H and O-H) ≤ 5

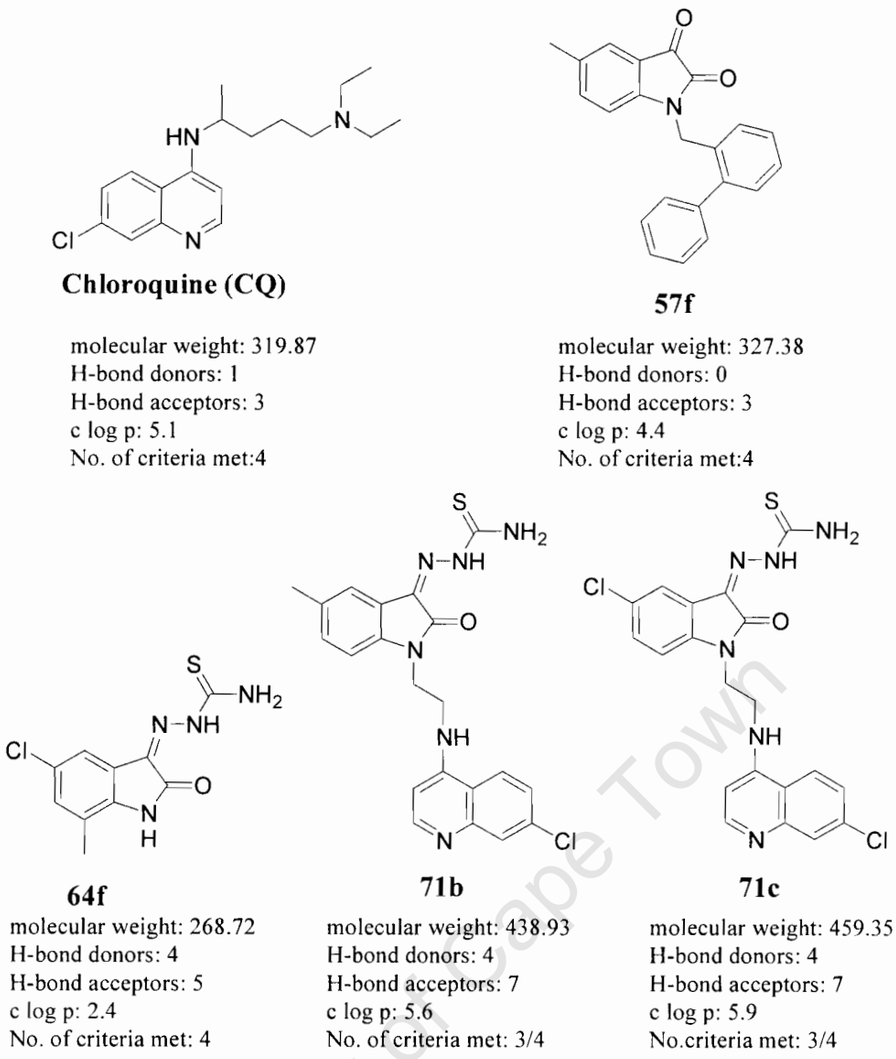


Figure 18. Compliance of chloroquine and the most active synthesized compounds to Lipinski's rule of 5.

A compound that meets at least 3 out of the 4 criteria set by Lipinki's rule of 5 is likely to be developed as an oral drug. All potent compounds described above fall in this category.

4.5 Conclusion

We have identified various novel molecules that can serve as potential inhibitors against cysteine proteases and appropriate parasites. Inhibitor *N-biphenyl-2-yl-5-methyl-1H-indole-2, 3-dione* **57f** from the class of *N*-substituted isatin derivatives has shown potency against *falcipain-2* and *cruzain*. The 5, 7-substituted isatin-3-thiosemicarbazone **64f** exhibited promising inhibitory activities against the 3 enzymes *falcipain-2*, *cruzain* and *rhodesain*. In light of these inhibitors, it is actually advantageous from a therapeutical viewpoint and can be extremely useful in treating numerous parasitic infections, to have compounds acting against targets from multiple protozoan parasites, (see Chapter 1.3). Two quinoline-ethylene thiosemicarbazone derivatives N^1-*[2-(chloro-quinolin-4-ylamino) ethyl]-5-methyl*-1H-*indole-2,3-dione-3-thiosemicarbazone* **71b** and N^1-*[2-(7-chloro-quinolin-4-ylamino) ethyl]-5-chloro*-1H-*indole-2,3-dione-3-thiosemicarbazone* **71c** showed improved efficacy compared to chloroquine against the chloroquine-resistant *P. falciparum* strains K1 and W2. Further comparisons with chloroquine should be done *in vivo* even for the not so potent compounds. However, it is not clear what the structural features controlling the *in vitro* antimalarial activities are, since there was no correlation with inhibition of *falcipain*-2, the presumed target. Investigations into this class of compounds is therefore further warranted as they may prove to be beneficial and will further direct efforts to find even more antimalarial chemotherapies in future. A chemical proteomic approach will particularly be useful in identifying targets for thiosemicarbazones in *P. falciparum*. In this regard the synthesis of biotinylated affinity probes for use in the affinity purification of protein targets should facilitate the understanding of true targets of the most active compounds. This would then be followed by the synthesis of new derivative libraries against the new targets identified. Finally it is worth noting that the potentially low cost and simple synthesis of inhibitor scaffolds augurs well for tropical diseases.[144]

CHAPTER 5
EXPERIMENTAL

5.1 GENERAL

Proton nuclear magnetic resonance (^{1}H 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 (δ = 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 (δ = 0.00).

The following abbreviations were used in the ^{1}H NMR spectra: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; t, triplet; q, quartet; m, multiplet; qd, quartet of doublets; dt, doublet of triplet; td, triplet of doublets; ddt, doublet of doublet of triplets; br, broad and *J*, coupling constant. **The format used for recording ^{13}C NMR data is that accepted by most international journals (including American chemical society journals). In this format chemical shift values are simply listed without specific assignment to carbon atoms.**

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) and VG (Micromass) 70-SE magnetic sector mass spectrometer (at Kent).

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 ^{O}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.

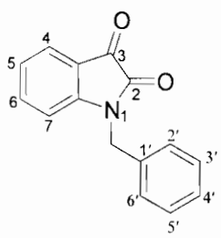
Anhydrous sodium sulphate and anhydrous magnesium sulphate were used in drying of organic solvents after extraction.

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

ChemDraw Ultra 6.0 was used to calculate the logarithm of the partition coefficients (*C* logP).

A. General procedure for preparation of compounds 57a - 58b

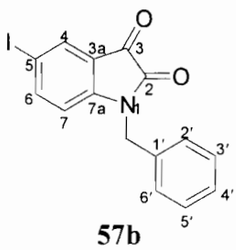
Isatin /5-substituted isatin (0.2 g, 1.35 mmol) and KF/Al_2O_3 (0.8 g, 5.44 mmol) were mixed in 10 mL of anhydrous dichloromethane (DCM). To the resultant mixture was added benzyl, sulfonyl or benzoyl halide (0.16 mL, 1.35 mmol) and the reaction mixture was vigorously stirred at 25 °C for 12 hours, diluted with diethyl ether and filtered. The solvents were removed under reduced pressure. Column chromatography (SiO_2, EtOAc: Hex, 2:8) yielded the desired product. [145]



57a

***N*-Benzyl-1*H*- indole-2, 3-dione**

(0.30g, 95%) obtained as reddish crystals; m.p. 127 °C (from MeOH) (lit 124-125 °C) [146]; R_f 0.45 (EtOAc: Hex 2:8); δ_H (300MHz, $CDCl_3$), 7.61 (1H, dd, *J* 1.2 and 7.2, H-4), 7.50 (1H, ddd, *J* 1.2, 7.5 and 7.8, H-6), 7.34-7.25 (5H, m, H-2′, 3′, 4′, 5′ and 6′), 7.11 (1H, ddd, *J* 0.6, 7.2 and 7.8, H-5), 6.79 (1H, d, *J* 7.5, H-7), 4.93 (2H, s, -NC*H*$_2$Ar).

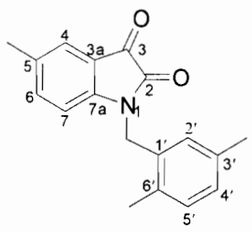


57b

N-*Benzyl-5-iodo*-1H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 0.73 mmol of isatin and halide were used.

(0.17g, 65 %) obtained as reddish crystals, m.p.140-141°C; R_f 0.43 (EtOAc: Hex, 3:7); IR (KBr) ν_{max}/cm^{-1} 1739 (keto C=O), 1601 (amide C=O); δ_H (400MHz, $CDCl_3$), 7.43 (1H, d, *J* 1.2, H-4), 7.38-7.24 (5H, m, H-2′, H-3′, H-4′, H-5′ and H-6′), 7.13 (1H, dd, *J* 1.2 and 7.6, H-6), 6.75 (1H, d, *J* 7.6, H-7), 4.91 (2H,s, -NC*H*$_2$Ar); δ_C ($CDCl_3$, 75 MHz), 183.1, 158.4, 149.9, 140.4, 138.6, 133.5, 129.5 (2 x C), 128.3 (2 x C), 127.1, 125.8, 117.3, 111.2 and 44.2; HRMS (EI) Found *m/z* 362.97519, $[M]^+$ $C_{15}H_{10}INO_2$ requires 362.97563.

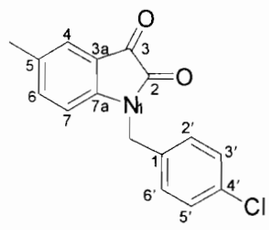


57c

N-(2, 5-Dimethyl-benzyl)-5-methyl-1H-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.24 mmol of isatin and halide were used.

(0.19g, 54%) obtained as orange crystals; m.p. 159-160 °C; R_f 0.40; (EtOAc / Hex, 3:7); IR (KBr) ν_{max}/cm^{-1} 2927 (C-H), 1728 (keto C=O), 1609 (amide C=O); δ_H (400MHz, $CDCl_3$), 7.44 (1H, d, *J* 1.2, H-4), 7.27 (1H, dd, *J* 1.2 and 8.0, H-6), 7.09 (1H, d, *J* 7.6, H-5′), 7.01 (1H, d, *J* 7.6, H-4′), 6.91 (1H, s, H-2′), 6.56 (1H, d, *J* 8.0, H-7), 4.86 (2H, s, -NCH_2Ar); 2.33 (3H, s, CH_3-Ar), 2.30 (3H, s, CH_3-Ar), 2.27 (3H, s, CH_3-Ar); δ_C ($CDCl_3$, 100 MHz), 183.5, 158.4, 148.9, 138.7, 135.9, 133.6, 132.5, 131.8, 130.7, 128.6, 127.2, 125.6, 117.7, 111.0, 42.2, 20.9, 20.5 and 18.8; HRMS (EI) Found *m/z* 279.12570 $[M]^+$ $C_{18}H_{17}NO_2$, requires 279.12593.

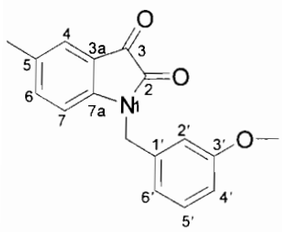


57d

N-(4-chloro-benzyl)-5-methyl-1H-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.24 mmol of isatin and halide were used.

(0.27g, 76%) obtained as orange crystals, m.p. 163-165 °C (ethanol); R_f 0.30; (EtOAc: Hex, 3:7); IR (KBr) ν_{max}/cm^{-1} 1730 (keto C=O), 1613 (amide C=O); δ_H (400MHz, $CDCl_3$), 7.42 (1H, d, *J* 0.8, H-4), 7.34 (1H, dd, *J* 0.8 and 8.0, H-6), 7.30 (2H, d, *J* 7.6, H-3′ and H-5′), 7.26 (2H, d, *J* 7.6, H-2′ and H-6′), 6.96 (1H, d, *J* 8.0, H-7), 4.87 (2H, s, -NCH_2Ar); 2.30 (3H, s, CH_3-Ar); δ_C ($CDCl_3$, 75 MHz), 183.2, 158.3, 148.2, 138.7, 134.1, 133.9, 133.2 (2 x C), 128.8 (2 x C), 127.6, 125.8, 117.8, 110.5, 43.4 and 20.6 ; HRMS (EI) Found *m/z* 285.05587 $[M]^+$, $C_{16}H_{12}ClNO_2$ requires 285.05566.

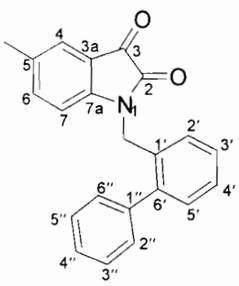


57e

N-*(3-Methoxy-benzyl)-5-methyl*-1H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.24 mmol of isatin and halide were used.

(0.21g, 60%) obtained as orange crystals; m.p. 135-136 °C (from ethanol); R_f 0.46 (EtOAc: Hex, 3:7);IR (KBr) ν_{max}/cm^{-1} 1739 (keto C=O), 1612 (amide C=O); δ_H (400MHz, $CDCl_3$), 7.42 (1H, d, *J* 1.2, H-4), 7.28 (1H, dd, *J* 1.2 and 8.0, H-6), 6.98 (1H, d, *J* 8.0, H-4′), 6.95 (1H, t, *J* 8.0, H-5′), 6.91 (1H, d, *J* 8.0, H-6′), 6.85 (1H, s, H-2′), 6.68 (1H, d, *J* 8.0, H-7), 4.87 (2H, s, -NC*H*$_2$Ar); 3.77 (3H, s, -O-C*H*$_3$), 2.31 (3H, s, C*H*$_3$-Ar); δ_C ($CDCl_3$, 75 MHz), 183.1, 160.1, 158.0, 148.6, 138.6, 135.6, 130.0, 129.7, 125.6, 119.6, 118.4, 113.3, 113.2, 112.8, 55.3, 43.9 and 20.5; HRMS (EI) Found *m/z* 281.10567 $[M]^+$ $C_{17}H_{15}NO_3$ requires 281.10519.

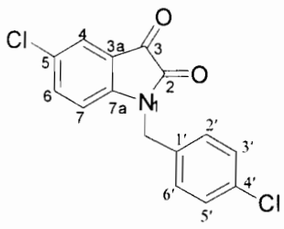


57f

N-*Biphenyl-2-yl-5-methyl*-1H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.24 mmol of isatin and halide were used.

(0.29g, 72%) obtained as red-orange crystals; m.p 159-163 °C (from ethanol); R_f0.42 (EtOAc: Hex, 3:7);IR (KBr) ν_{max}/cm^{-1}, 1733 (keto C=O), 1606 (amide C=O); δ_H (400MHz, $CDCl_3$), 7.47 (1H, d, *J* 2.0, H-4), 7.45 (1H, dd, *J* 2.0 and 8.0, H-6), 7.38-7.28 (9H, m, H-2′, H-3′, H-4′, H-5′, H-2″, H-3″, H-4″, H-5″ and H-6″), 7.15 (1H, d, *J* 8.0, H-7), 4.90 (2H, s, -NC*H*$_2$Ar), 2.25 (3H, s, CH_3-Ar); δ_C ($CDCl_3$, 75 MHz), 183.4, 160.2, 148.9, 148.0, 147.6, 147.2, 147.0, 137.6, 135.6, 130.2, 129.7, 128.2, 128.1, 127.9, 127.7, 127.2, 125.4, 123.8, 117.7, 111.1, 44.0 and 20.6; HRMS (EI) Found *m/z* 327.12535 $[M]^+$ $C_{22}H_{17}NO_2$ requires 327.12590.

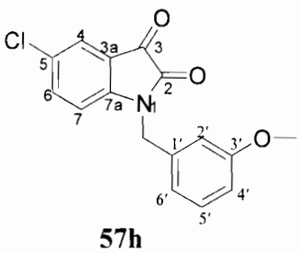


57g

5-Chloro-N-(4-chloro benzyl)-1H-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.10 mmol of isatin and halide were used.

(0.21g, 63%) obtained as orange crystals; m.p. 123-126 °C (from ethanol); R_f 0.46 (EtOAc: Hex, 2:8); IR (KBr) ν_{max}/cm^{-1} 1730 (keto C=O), 1603 (amide C=O); δ_H (300MHz, $CDCl_3$), 7.56 (1H, d, *J* 2.1, H-4), 7.47 (1H, dd, *J* 2.1 and 8.4, H-6), 7.35 (2H, d, *J* 8.4, H-3′ and 5′), 7.26 (2H, d, *J* 8.4, H-2′ and 6′), 6.71 (1H, d, *J* 8.4, H-7), 4.89 (2H, s, -NCH_2Ar); δ_C ($CDCl_3$, 75 MHz), 183.4, 158.1, 148.3, 138.7, 135.1, 133.3, 130.3, 128.7 (2 x C), 126.5 (2 x C), 125.8, 117.9, 111.5 and 43.4; HRMS (EI) Found *m/z* 305.00157 $[M]^+$ $C_{15}H_9Cl_2NO_2$ requires 305.00103.

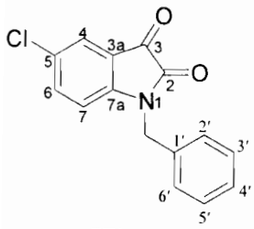


57h

5-Chloro-N-(3-methoxy-benzyl)-1H-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.10 mmol of isatin and halide were used.

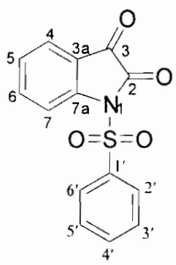
(0.23g, 70%) obtained as yellow crystals; m.p. 94-96 °C (from ethanol); R_f 0.64 (EtOAc: Hex, 3:7);IR (KBr) ν_{max}/cm^{-1} 1733 (keto C=O), 1603 (amide C=O); δ_H (300MHz, $CDCl_3$), 7.56 (1H, d, *J* 1.8, H-4), 7.45 (1H, dd, *J* 1.8 and 8.4, H-6), 7.23 (1H, t, *J* 7.8, H-5′), 6.89 (1H, d, *J* 7.8, H-6′), 6.85 (1H, d, *J* 7.8, H-4′), 6.82 (1H, s, H-2′), 6.74 (1H, d, *J* 8.4, H-7), 4.88 (2H, s, -NCH_2Ar), 3.77 (3H, s, Ar -O-CH_3); δ_C ($CDCl_3$, 75 MHz), 183.6, 160.2, 158.5, 148.9, 137.6, 135.6, 130.2, 129.7, 125.3, 119.4, 118.5, 113.4, 113.2, 112.3, 55.3 and 44.0; HRMS (EI) Found *m/z* 301.05170 $[M]^+$ $C_{16}H_{12}O_3NCl$ requires 301.05057.



57i

N-*Benzyl-5-chloro*-1H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.3953 mmol of isatin and halide were used.(0.21g, 70%) obtained as orange crystals; m.p.139-141 °C (lit 140 °C) [147]; R_f 0.5 (EtOAc: Hex, 3:7); δ_H (300MHz, $CDCl_3$), 7.58 (1H, d, *J* 2.1, H-4), 7.44 (1H, dd, *J* 2.1 and 8.4, H-6), 7.38-7.26 (5H, m, H-2′, H-3′, H-4′, H-5′ and H-6′), 6.73 (1H, d, *J* 8.4, H-7), 4.92 (2H, s, -NCH_2Ar).

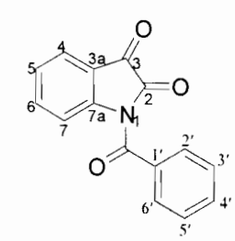


58a

***N*-Phenylsulfonyl-1*H*- indole-2, 3-dione**

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.3953 mmol of isatin and halide were used.

(0.33g, 84%) obtained as orange crystals; mp 185-187 °C (lit 186.5-187 °C) [148]; R_f 0.35 (EtOAc: Hex, 3:7); δ_H (300MHz, $CDCl_3$), 8.12 (1H, d, *J* 7.8, H-7), 8.04 (1H, dd, *J* 0.9 and 7.4, H-4), 7.92 (1H, ddd, *J* 0.9, 6.9 and 7.8, H-6), 7.65-7.55 (5H, m, H-2′, 3′, 4′, 5′ and 6′), 7.32 (1H, ddd, *J* 1.2, 6.9 and 7.4, H-5).



58b

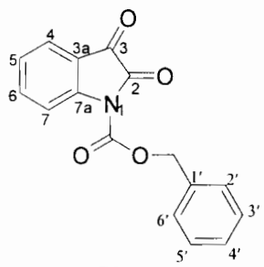
***N*-Benzoyl-1*H*-indole-2, 3-dione**

The conditions employed for the preparation of this compound were those described in general procedure **A.** However, 1.3953 mmol of isatin and halide were used.

(0.16g, 48%) obtained as orange crystals, mp 210-214 °C (lit 209-211 °C) [149, 150]; R_f 0.47 (EtOAc/ Hex, 3:7); δ_H (300MHz, $CDCl_3$), 8.15 (1H, d, *J* 7.2, H-7), 7.78 (1H, dd, *J* 0.6 and 7.8, H-4), 7.69 (1H, ddd, *J* 0.6, 7.2 and 7.8, H-6), 7.56-7.51 (5H, m, H-2′, 3′, 4′, 5′ and 6′), 7.36 (1H, ddd, *J* 1.2, 7.2 and 7.8, H-5).

B. General procedure for the preparation of compounds 59a–59d

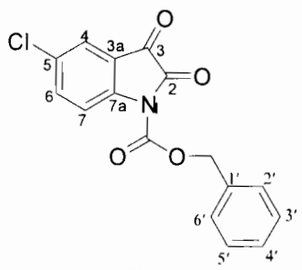
Isatin or 5-substituted isatin (0.2 g, 1.35mmol) was dissolved and stirred in CH_3CN (10mL) at 0 °C. Benzyl chloroformate (0.21 mL, 1.49mmol) was added, followed by 4-dimethylaminopyridine (0.012 mL, 0.135mmol) and triethylamine (0.20 mL, 1.49mmol). After 3 hours, the resulting mixture was removed from ice-bath and 20mL of water was added and a precipitate formed which was filtered, dried under high vacuum pressure and recrystallized from ethanol.



59a

2, 3-Dioxo-2, 3-dihydro-indole-1-carboxylic acid benzyl ester

(0.37g, 97%), obtained as yellow crystals; mp 152-154 °C (from ethanol) (lit 154-156 °C) [151]; R_f 0.5 (MeOH: EtOAc, 2:8); δ_H (300MHz, $CDCl_3$); 8.15 (1H, d, *J* 8.4, H-7), 7.78 (1H, dd, *J* 0.6 and 7.8, H-4), 7.74 (1H, ddd, *J* 0.6, 7.5 and 8.4, H-6), 7.54-7.35 (5H, m, H-2′, H-3′, H-4′, H-5′ and 6′), 7.32 (1H, ddd, *J* 0.9, 7.5 and 7.8, H-5), 5.48 (2H, s, ArCH_2O-).

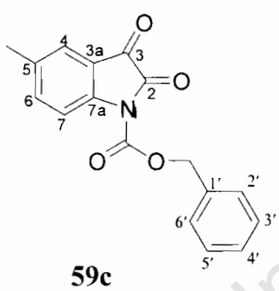


59b

5-Chloro-2, 3-dioxo-2, 3-dihydro-indole-1-carboxylic acid benzyl ester

The conditions employed for the preparation of this compound were those described in general procedure **B.** However, 1.10 mmol of isatin and benzyl chloroformate were used.

(0.07g, 19%) obtained as orange crystals, mp 166 °C (from ethanol); R_f 0.48 IR ($CHCl_3$) ν_{max}/cm^{-1} 1780 (ester C=O), 1745 (keto C=O), 1601 (amide C=O); δ_H (300MHz, $CDCl_3$); 8.13 (1H, d, *J* 8.7, H-7), 7.72 (1H, d, *J* 2.4, H-4), 7.68(1H, dd, *J* 2.4, 8.7, H-6), 7.49-7.38 (5H, m, H-2′, H-3′, H-4′, H-5′ and H-6′), 5.47 (2H, s, ArCH_2O-); δ_C (75 MHz); 183.8, 166.7, 156.2, 144.0, 138.8, 129.1, 128.7 (2 x C), 128.4, 128.1 (2 x C), 127.3, 123.6, 118.6, 117.3 and 68.9; HRMS (EI) Found *m/z* [M^+], 315.02633 $C_{16}H_{10}ClNO_4$ requires 315.02984.

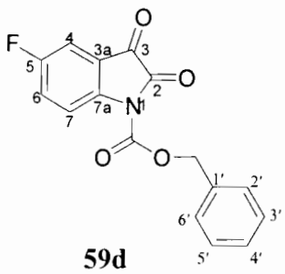


59c

5-Methyl-2, 3-dioxo-2, 3-dihydro-indole-1-carboxylic acid ester

The conditions employed for the preparation of this compound were those described in general procedure **B.** However, 1.24 mmol of isatin and benzyl chloroformate were used.

(0.09g, 24%) obtained as yellow crystals; mp 144-150 °C (ethanol); R_f 0.43; IR ($CHCl_3$) ν_{max}/cm^{-1} 1780 (ester C=O), 1745 (keto C=O), 1602 (amide C=O); δ_H (400MHz, $CDCl_3$); 8.01 (1H, d, *J* 8.8, H-7), 7.55 (1H, d, *J* 2.2, H-4), 7.54 (1H, dd, *J* 2.2 and 8.8, H-6), 7.42-7.35 (5H, m, H-2′, H-3′, H-4′, H-5′ and H-6′), 5.46 (2H,s, ArCH_2O-), 2.38 (3H,s, CH_3-Ar); δ_C (75 MHz); 183.0, 166.4, 156.0, 143.8, 138.4, 129.2, 128.7 (2 x C), 128.3, 127.3 (2 x C), 126.8, 125.6, 117.8, 117.1, 68.4 and 20.9; HRMS (EI) Found *m/z* 295.08463 $[M]^+$ $C_{17}H_{13}O_4N$ requires 295.08446.



59d

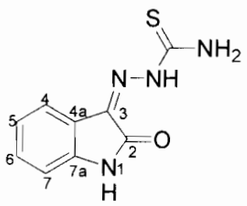
5-Fluoro-2, 3-dioxo-2, 3-dihydro-indole-1-carboxylic acid benzyl ester

The conditions employed for the preparation of this compound were those described in general procedure **B.** However, 1.21 mmol of isatin and chloroformate were used.

(0.12g, 35%) obtained as orange-yellow crystals; mp > 350 ^{0}C (from ethanol); R_f 0.47;IR ($CHCl_3$) ν_{max}/cm^{-1} 1780 (ester C=O), 1745 (keto C=O), 1602 (amide C=O); δ_H (400MHz, $CDCl_3$); 8.18 (1H,d, *J* 8.4, H-7), 7.50 (1H, d, *J* 2.1, H-4), 7.54 (1H, dd, *J* 2.1 and 8.4, H-6), 7.39-7.32 (5H, m, H-2′, H-3′, H-4′ and H-5′), 5.36 (2H,s, ArCH_2O-); δ_C (75 MHz); 183.8, 167.0, 156.1, 142.9, 139.5, 128.2, 127.4 (2 x C), 127.1, 127.0 (2 x C), 125.6, 124.8, 119.9, 117.1 and 69.6; HRMS (EI) Found *m/z* 299.05963 $[M]^+$, $C_{16}H_{10}FNO_4$ requires 299.05939.

C. General procedure for preparation of compounds 64a–64k

Equimolar quantities of isatin or substituted isatin (0.2 g, 1.36 mmol) and thiosemicarbazide (0.12 g, 1.36 mmol) in 10 mL of warm 95% ethanol were stirred at 45 ^{o}C for 3-4 hours. After standing for approximately 24 hours at room temperature, the crystalline products were separated by filtration, washed with ethanol, dried and recrystallised from ethanol.

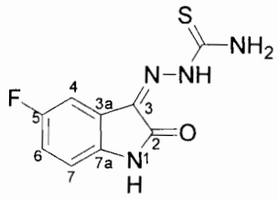


64a

1-*H*-Indole-2, 3-dione-3-thiosemicarbazone

(0.13g, 43%) obtained as orange crystals; m.p. 249-253 ^{o}C (from ethanol) (lit 247-249 ^{o}C) [152]; δ_H (400MHz, DMSO-d_6), 12.46 (1H, s, NNH), 11.15 (1H, s, H-1), 8.98 (1H, s, N*H*H), 8.62 (1H, s, NH*H*), 7.64 (1H, dd, *J* 1.2 and 7.5, H-4), 7.34 (1H, ddd, *J* 1.2, 6.9 and 7.8, H-6), 7.09 (1H, ddd, *J* 0.9, 6.9 and 7.5, H-5), 6.92 (1H, d, *J* 7.8, H-7).

5-Fluoro-1*H- *Indole-2, 3-dione-3-thiosemicarbazone

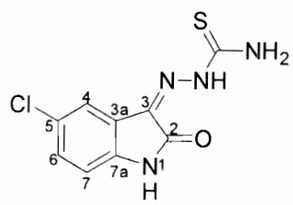


64b

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.12 mmol of isatin and thiosemicarbazide were used.

(0.24g, 82%) obtained as yellow crystals; mp 287-289 ^{o}C (from ethanol); IR (KBr) ν_{max}/cm^{-1} 3417 (N-H), 3272 (N-H), 1677 (C=O), 1611 (C=N), 1137(C=S); δ_H (300MHz;DMSO-d_6); 12.37 (1H, s, NNH), 11.17 (1H, s, H-1), 9.07 (1H, s, N*H*H), 8.72 (1H, s, NH*H*), 7.50 (1H, d, *J* 2.7, H-4), 7.17 (1H, dd, *J* 2.7 and 8.4, H-6), 6.90 (1H, d, *J* 8.4, H-7); δ_C (75 MHz); 179.5, 163.4, 160.1, 157.7, 139.3, 132.1, 122.2, 119.2, 112.8; LRMS (EI) *m/z* 238 [60 %, M^+], 210 [100 %, M-$C_8H_7FN_4S$] and 122 [25 %, M-C_7H_5FN]; Anal. Calcd. for $C_9H_7FN_4OS$ C 45.4%, H 2.9%, N 23.5%, S 13.5%. Found C 45.4%, H 2.8%, N 23.5%, S 13.2%.

5-Chloro-1*H*- indole-2, 3-dione-3-thiosemicarbazone

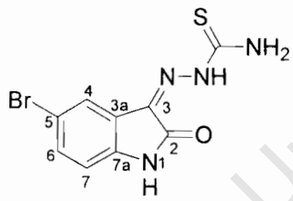


64c

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.10 mmol of isatin and thiosemicarbazide were used.

(0.20g, 73%) obtained as orange crystals; mp 283-285 ^{o}C (from ethanol) (lit. 266 ^{0}C) [153]; δ_H (300MHz; DMSO-d_6); 12.62 (1H, s, NNH), 11.22 (1H, s, H-1), 8.54 (1H, s, N*H*H), 8.17 (1H, s, NH*H*), 7.72 (1H, d, *J* 2.4, H-4), 7.39 (1H, dd, *J* 2.4 and 8.4, H-6), 7.08 (1H, d, *J* 8.4, H-7).

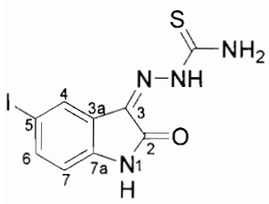
5-Bromo-1*H*-indole-2, 3-dione-3-thiosemicarbazone



64d

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 0.88 mmol of isatin and thiosemicarbazide were used.

(0.11g, 43%) obtained as orange crystals; mp 281-284 ^{o}C (from ethanol) (lit 270 ^{0}C) [154]; δ_H (300MHz; DMSO-d_6); 12.29 (1H, s, NNH), 11.26 (1H, s, H-1), 9.07 (1H, s, N*H*H), 8.78 (1H, s, NH*H*), 7.87 (1H, d, *J* 2.1, H-4), 7.51 (1H, dd, *J* 2.1 and 8.1, H-6), 6.89 (1H, d, *J* 8.1, H-7).

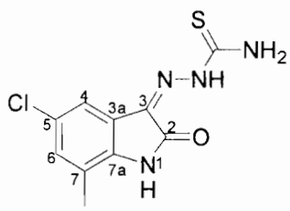


64e

5-Iodo-1*H*-indole-2, 3-dione-3-thiosemicarbazone

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 0.73 mmol of isatin and thiosemicarbazide were used.

(0.13g, 67%) obtained as orange crystals; mp 265 °C (from ethanol) (lit 266-268 °C) [155]; δ_H (300MHz;DMSO-d_6); 12.30 (1H, s, NNH), 11.20 (1H, s, H-1), 8.99 (1H, s, N*H*H), 8.74 (1H, s, NH*H*), 8.03 (1H, d, *J* 1.8, H-4), 7.67 (1H, dd, *J* 1.8 and 8.4, H-6), 6.76 (1H, d, *J* 8.4, H-7).

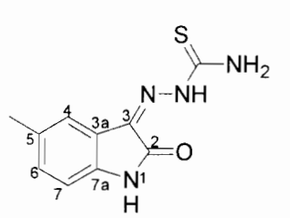


64f

5-*Chloro-7-methyl*-1H- *Indole-2, 3-dione-3-thiosemicarbazone*

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.02 mmol of isatin and thiosemicarbazide was used.

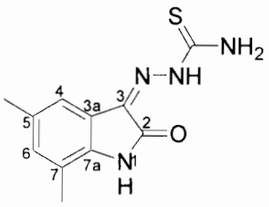
(0.27g, 98%) obtained as yellow crystals; mp > 350 °C (from ethanol); IR (KBr) ν_{max}/cm^{-1} 3412 (N-H), 3258 (N-H), 1677 (C=O), 1612 (C=N), 1130 (C=S); δ_H (300MHz; DMSO-d_6); 12.11 (1H, s, NNH), 11.02 (1H, s, H-1), 9.57 (1H, s, N*H*H), 8.66 (1H, s, NH*H*), 7.58 (1H, d, *J* 1.5, H-4), 7.23 (1H, d, *J* 1.5, H-6), 2.21 (3H, s, C*H*$_3$-Ar); δ_C (75 MHz); 178.7, 162.7, 139.7, 131.3, 126.4, 122.3, 121.3, 117.9, 112.6 and 20.7; LRMS (EI) *m/z* 268 [64 %,M^+], 240 [100 %, M-$C_9H_9ClN_4S$] and 165 [29 %, M-$C_8H_6ClN_2$]; Anal. Calcd. for $C_{10}H_9ClN_4OS$ C 44.7%, H 3.4%, N 20.9% S 11.9%. Found C 44.7% H 4.3% N 17.1% S 9.7%.



64g

5-Methyl-1H- indole-2, 3-dione-3-thiosemicarbazone

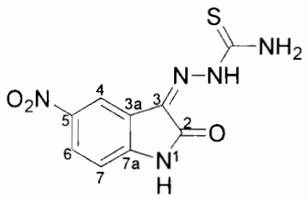
The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.24 mmol of isatin and thiosemicarbazide were used. (0.22g, 74%) obtained as red crystals; mp 281-283 °C (from ethanol); IR (KBr) ν_{max}/cm^{-1} 3418 (N-H), 3286 (N-H), 1696 (C=O), 1606 (C=N), 1127 (C=S); δ_H (400MHz; DMSO-d_6); 12.44 (1H, s, NNH), 11.00 (1H, s, H-1), 8.90 (1H, s, N*H*H), 8.55 (1H, s, NH*H*), 7.47 (1H, d, *J* 1.2, H-4), 7.15 (1H, dd, *J* 1.2 and 8.0, H-6), 6.80 (1H, d, *J* 8.0, H-7), 2.27 (3H, s, C*H*$_3$-Ar); δ_C (75 MHz); 179.4, 163.3, 140.8, 132.8, 132.3, 132.0, 122.0, 120.6, 111.4 and 21.2; LRMS (EI) *m/z* 234 [54 %, M^+], 206 [100 %, M-$C_9H_{10}N_4S$] and 146 [34 %, M-$C_8H_8N_3$]; Anal. Calcd. for $C_{10}H_{10}N_4OS$ C 51.3%, H 4.3%, N 23.9%, S 13.8%. Found C 50.6%, H 4.2%, N 24.4%, S 13.8%.



64h

5, 7-Dimethyl-1H- indole-2, 3-dione-3-thiosemicarbazone

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.14 mmol of isatin and thiosemicarbazide were used. (0.25g, 90%) obtained as orange crystals; mp 286-287 °C (from ethanol); IR (KBr) ν_{max}/cm^{-1} 3463 (N-H), 3267 (N-H), 1688 (C=O), 1605 (C=N), 1124 (C=S); δ_H (300MHz; DMSO-d_6); 12.49 (1H, s, NNH), 11.12 (1H, s, H-1), 8.95 (1H, s, N*H*H), 8.60 (1H, s, NH*H*), 7.32 (1H, d, *J* 1.2, H-4), 6.98 (1H, d, *J* 1.2, H-6), 2.25 (3H, s, CH_3-Ar), 2.17 (3H, s, CH_3-Ar); δ_C (75 MHz); 179.3, 163.8, 139.4, 133.8, 133.2, 131.9, 120.8, 120.4, 119.5, 21.2 and 16.5; LRMS (EI) *m/z* 248 [57 %, M^+], 220 [100 %, M-$C_9H_{12}N_4S$] and 160 [30 %, M-$C_9H_{10}N_3$]; Anal. Calcd. for $C_{11}H_{12}N_4OS$ C 53.2%, H 4.9%, N 22.6%, S 12.9%. Found C 51.4%, H 3.1%, N 21.8%, S 13.3%.

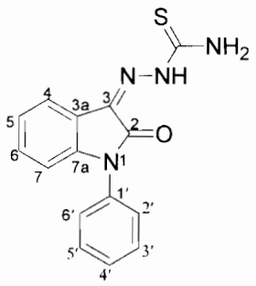


64i

5-Nitro-1H- indole-2, 3-dione-3-thiosemicarbazone

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.04 mmol of isatin and thiosemicarbazide were used.

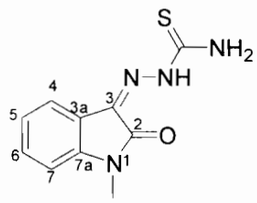
(0.24g, 88 %) obtained as orange crystals; mp >350 °C (from ethanol), IR (KBr) ν_{max}/cm^{-1} 3358 (N-H), 3286 (N-H), 1696 (C=O), 1606 (C=N), 1127 (C=S); δ_H (300MHz; DMSO-d_6); 12.22 (1H, s, NNH), 11.76 (1H, s, H-1), 9.16 (1H, s, N*H*H), 9.01 (1H, s, NH*H*), 8.59 (1H, d, *J* 1.2, H-4), 8.27 (1H, dd, *J* 1.2 and 8.7, H-6), 7.12 (1H, d, *J* 8.7, H-7); δ_C (75 MHz); 178.8, 162.8, 147.3, 142.3, 132.6, 129.9, 120.9, 116.3 and 111.1; LRMS (EI) *m/z* 265 [50 %, M^+], 237 [100 %, M-$C_8H_7N_5O_2S$]; Anal. Calcd. for $C_9H_7N_5O_3S$ C 40.7%, H 2.6%, N 26.4%, S 12.1%. Found C 41.4%, H 2.7%, N 25.6% S 11.8%.



64j

1-Phenylindole-2, 3-dione-3-thiosemicarbazone

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 0.89 mmol of isatin and thiosemicarbazide were used. (0.20g, 76%) obtained as yellow crystals; mp 245-249 °C (from ethanol) (lit 250-256 ^{0}C) [156]; δ_H (400MHz;DMSO-d_6); 12.36 (1H, s, NNH), 9.03 (1H, s, N*H*H), 8.70 (1H, s, NH*H*), 7.82 (1H, dd, *J* 1.2 and 7.6, H-4), 7.59-7.49 (5H,m, H-2′, H-3′, H-4′, H-5′ and H-6′), 7.37 (1H, ddd, *J* 1.2, 6.8 and 7.6, H-6), 7.21 (1H, ddd, *J* 0.8, 6.8 and 7.6, H-5), 6.83 (1H, d, *J* 7.6, H-7).

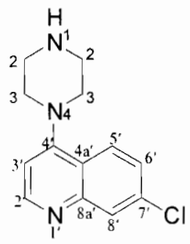


64k

1-Methylindole-2, 3-dione-3-thiosemicarbazone

The conditions employed for the preparation of this compound were those described in general procedure **C.** However, 1.24 mmol of isatin and thiosemicarbazide was used.

(0.28g, 97%) obtained as yellow crystals; mp 253 °C (from ethanol) (lit 244 ^{0}C)[157, 158]; δ_H (300MHz;DMSO-d_6); 12.40 (1H, s, NNH), 9.03 (1H, s, N*H*H), 8.67 (1H, s, NH*H*), 7.70 (1H, dd, *J* 1.2 and 7.8, H-4), 7.44 (1H, ddd, *J* 1.2, 6.9 and 7.8, H-6), 7.17 (1H, ddd, *J* 0.6, 6.9 and 7.8, H-5), 7.13 (1H, d, *J* 7.8, H-7), 3.21 (3H, s, -NC*H*$_3$).



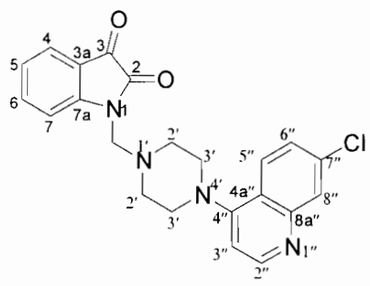
69

7-Chloro-4-piperazin-1-yl-quinoline

A mixture of 4,7-dichloroquinoline (2.0g, 10.10 mmol), piperazine (4.35g, 50.49 mmol), K_2CO_3 (0.419g, 3.03 mmol), triethylamine (0.423 mL, 3.02 mmol) in *N*-methyl-2-pyrrolidinone (10mL) was stirred under nitrogen at 135 ^{0}C for 4 hours. After cooling to room temperature, the mixture was diluted with CH_2Cl_2 (50 mL) and washed with brine (10 × 50 mL). The organic layer was separated, dried over sodium sulfate, filtered and concentrated. Column chromatography (SiO_2; MeOH: EtOAc, 2:80) yielded the amine **33** (1.87g, 67 %) as a white solid; mp 113-114 °C (from methanol) (lit. 113-115 °C) [159]; R_f 0.34 (MeOH: EtOAc, 2:8); δ_H (300MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.1, H-2′), 8.02 (1H, d, *J* 2.1, H-8′), 7.93 (1H, d, *J* 9.0, H-5′), 7.40 (1H, dd, *J* 2.1 and 9.0, H-6′), 6.81 (1H, d, *J* 5.1, H-3′), 3.17-3.12 (8H, br s, H-2 and H-3); δ_C (75 MHz); 157.3, 151.9, 150.2, 134.8, 128.9, 126.1, 125.2, 121.9, 108.9, 53.5 (2 x C) and 46.1 (2 x C); LRMS (EI) *m/z* 247 [66 %, M^+] and 205 [100%, M-$C_{11}H_{10}N_2Cl$]; Anal Calcd. for $C_{13}H_{14}N_3Cl$ C 63.0%,H 5.7%, N 16.9% Found C 62.8%, H 5.6%, N 16.6%.

D. General procedure for preparation compounds 65a–65g

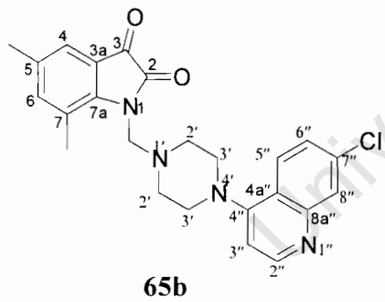
To a solution of isatin or substituted isatin (0.2g, 1.35 mmol) in 5mL of 99.9 % ethanol was added to a mixture of compound **69** (0.27g, 1.35 mmol) and aqueous formaldehyde 37% (0.03 mL, 1.35 mmol) also dissolved in 10ml of 99.9 % ethanol. The reaction mixture was stirred for 3 hours at room temperature, refrigerated for 48 hours to form crystals. The crystalline products were separated by filtration, washed and vacuum dried. Recrystallization from ethanol rendered desired products in pure form.



65a

N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]*-1H-*indole-2, 3-dione*

(0.51g, 93%) obtained as reddish-orange crystals; mp 203-206 °C (from ethanol); IR (KBr) ν_{max}/cm^{-1} 1730 (keto C=O), 1617 (amide C=O), 1572 (C=N); δ_H (300MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.1, H-2″), 8.02 (1H, d, *J* 2.1, H-8″), 7.92 (1H, d, *J* 9.0, H-5″), 7.66 (1H, dd, *J*, 0.6 and 8.0, H-4), 7.62 (1H, ddd, *J* 0.6, 6.8 and 7.8, H-6), 7.43 (1H, dd, *J* 2.1 and 9.0, H-6″), 7.19 (1H, ddd, *J* 0.9, 6.8 and 8.0 and H-5), 7.10 (1H, d, *J* 7.8, H-7), 6.82 (1H, d, *J* 5.1, H-3″), 4.60 (2H, s, -NC*H*$_2$N-), 3.24 (4H, br s, -N(CH$_2$C*H*$_2$)$_2$NAr), 2.95 (4H, br s, -N(C*H*$_2$CH$_2$)$_2$NAr); δ_C (75 MHz); 182.1, 159.0, 156.6, 151.8, 151.5, 150.1, 138.4, 128.9, 128.4, 126.3, 125.4, 125.2, 124.0, 121.8, 117.4, 111.5, 109.1, 62.3, 51.8 (2 x C) and 50.6 (2 x C); HRMS (FAB) Found *m/z* 407.12661 $[M+1]^+$, $C_{22}H_{19}O_2N_4Cl$ requires 407.12747; Anal Calcd for $C_{22}H_{19}O_2N_4Cl$ C 64.4%, H 4.7%, N 13.3%. Found C 64.6%, H 4.5%, N 13.3%.



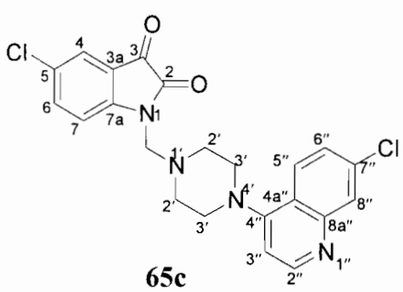
65b

N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5, 7-dimethyl*-1H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **D.** However, 1.14 mmol of isatin and compound **69** were used.

(0.42g, 85%) obtained as red crystals; mp 209-213 °C (from ethanol); IR ($CHCl_3$) ν_{max} /cm^{-1} 1739 (keto C=O), 1635 (amide C=O), 1542 (C=N); δ_H (400MHz; $CDCl_3$); 8.71 (1H, d, *J* 5.2, H-2″), 8.02 (1H, d, *J* 2.0, H-8″), 7.93 (1H, d, *J* 9.0, H-5″), 7.43 (1H, dd, *J*, 2.0 and 9.0, H-6″), 7.31 (1H, d, *J* 2.0, H-4), 7.20 (1H, d, *J* 2.0, H-6), 6.80 (1H, d, *J* 5.2, H-3″), 4.51 (2H, s, -NC*H*$_2$N-), 3.23 (4H, br s, -N-CH$_2$C*H*$_2$NAr), 2.86 (4H, br s, -NC*H*$_2$CH$_2$NAr), 2.58 (3H, s, Ar-

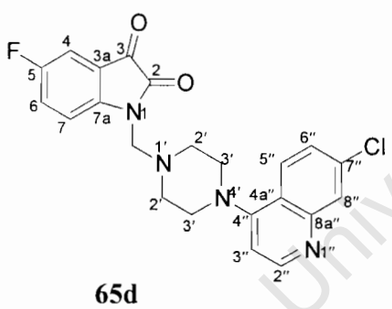
CH_3), 2.29 (3H, s, Ar-CH_3); δ_C (75 MHz); 182.7, 159.0, 157.7, 151.1, 150.1, 138.9, 134.9, 128.8, 128.7, 126.8, 125.9, 125.6, 125.0, 122.2, 118.4, 111.8, 109.8, 62.4, 52.8 (2 x C), 50.6 (2 x C), 20.1 and 19.1; LRMS (EI) *m/z* 434 [58 %, M^+], 119 [100 %, M-C_8H_9N]; Anal. Calcd. for $C_{24}H_{23}O_2N_4Cl$ C 66.3%, H 5.3%, N 12.9%. Found C 67.3%, H 5.5%, N 12.3%.



65c

N^1-*[4-(7-chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-chloro-1*H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **D.** However, 1.10 mmol of isatin and compound **69** were used. (0.13g, 24%) obtained as orange crystals; mp 153-157 °C (from ethanol); IR ν_{max} /cm^{-1} 1736 (keto C=O), 1617 (amide C=O), 1511 (C=N); δ_H (300MHz; $CDCl_3$); 8.69 (1H, d, *J* 5.0, H-2″), 8.00 (1H, d, *J* 2.0, H-8″), 7.89 (1H, d, *J* 8.8, H-5″), 7.61 (1H, d, *J* 2.4, H-4), 7.57 (1H, dd, *J* 2.4 and 8.4, H-6), 7.42 (1H, dd, *J* 2.0 and 8.8, H-6″), 7.09 (1H, d, *J* 8.4, H-7), 6.80 (1H, d, *J* 5.0, H-3″), 4.57 (2H, s, -NCH_2N-), 3.21 (4H, br s, -NCH_2CH_2NAr), 2.91 (4H, br s,-NCH_2CH_2NAr); δ_C (75 MHz); 181.9, 158.3 , 156.6, 151.7, 149.9, 149.5, 138.8, 128.9, 128.4, 126.3, 125.2, 124.9, 123.7, 121.7, 118.4, 112.9, 109.1, 62.4, 51.8 (2 x C) and 50.6 (2 x C); LRMS (EI) *m/z* 440 [3 %, M^+], 260 [100 %, M-$C_{14}H_{15}ClN_3$] ; Anal. Calcd. for $C_{22}H_{18}O_2N_4Cl_2{\cdot}H_2O$ C 57.6%, H 4.1%, N 12.2%. Found C 57.6%, H 4.1%, N 11.9%.

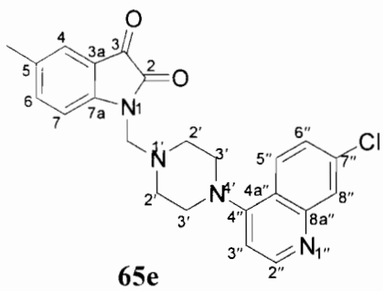


65d

N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-fluro-1H-indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **D.** However, 1.21 mmol of isatin and compound **69** were used. (0.49g, 94%) obtained as orange crystals; mp 110-117 °C (from ethanol); IR ($CHCl_3$) ν_{max} /cm^{-1}, 1744 (keto C=O), 1615 (amide C=O), 1562 (C=N); δ_H (400MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.2, H-2″), 8.03 (1H, d, *J* 2.0, H-8″), 7.90 (1H, d, *J* 9.0, H-5″), 7.42 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.35 (1H, d, *J* 2.1, H-4), 7.31 (1H, dd, *J* 2.1 and 8.2, H-6), 7.11 (1H, d, *J* 8.2, H-7), 6.82 (1H, d, *J* 5.2, H-3″), 4.58 (2H, s, -NCH_2N-), 3.25 (4H, br s, -NCH_2CH_2NAr), 2.93 (4H, br s, -NCH_2CH_2NAr); δ_C (75 MHz); 181.9, 159.6, 156.7, 151.8, 150.1, 150.0, 136.7, 136.1, 128.8, 126.7, 125.3, 125.0, 123.6 , 121.9, 119.3 , 112.4, 109.2, 62.7, 52.1 (2 x C),

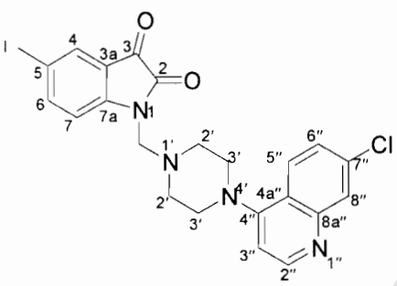
50.8 (2 x C); HRMS (FAB) *m/z* 425.11703 $[M+1]^+$ $C_{22}H_{18}ClFN_4O_2$ requires 425.11805; Anal. Calcd. for $C_{22}H_{18}ClFN_4O_2 \cdot H_2O$ C 60.1%, H 4.3%, N 11.8%. Found C 60.4%, H 4.3%, N 11.5%.



65e

N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-methyl-1H-indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **D.** However, 1.24 mmol of isatin and compound **69** were used. (0.29g, 55 %) obtained as reddish-orange crystals; mp 202-203 °C (from ethanol); IR ($CHCl_3$) ν_{max} /cm^{-1}, 1729 (ketone C=O), 1609 (amide C=O), 1518 (C=N); δ_H (300MHz; $CDCl_3$); 8.69 (1H, d, *J* 5.1, H-2″), 8.02 (1H, d, *J* 2.1, H-8″), 7.90 (1H, d, *J* 8.7, H-5″), 7.45 (1H, d, *J* 2.4, H-4), 7.43 (1H, dd, *J* 2.4 and 7.8, H-6), 7.38 (1H, dd, *J* 2.1 and 8.7, H-6″), 7.02 (1H, d, *J* 7.8, H-7), 6.81 (1H, d, *J* 5.1,H-3″), 4.58 (2H, s, -NC*H*$_2$N-), 3.24 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.94 (4H, br s, -NC*H*$_2$CH$_2$NAr), 2.35 (3H, s, Ar-C*H*$_3$); δ_C (75 MHz); 182.4, 159.1, 156.6, 151.5, 150.7, 150.2, 138.4 , 128.8, 128.4, 126.4, 125.7, 125.6 , 124.4, 121.8, 118.2, 110.5, 109.0, 62.3, 51.6 (2 x C), 50.6 (2 x C) and 21.2; HRMS (FAB) Found *m/z* 421.14346 $[M+1]^+$ $C_{23}H_{21}ClN_4O_2$ requires 421.14312; Anal. Calcd for $C_{23}H_{21}ClN_4O_2 \cdot H_2O$ C 62.8%, H 4.8%, N 12.8%. Found C 63.0%, H 4.9%, N 12.9%.

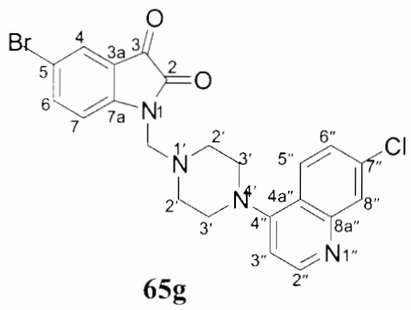


65f

N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-iodo-1H-indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **D.** However, 0.73 mmol of isatin and compound **69** was used. (0.23g, 59%) obtained as red crystals; mp 151-153 °C (from ethanol); IR ($CHCl_3$) ν_{max} /cm^{-1} 1745 (keto C=O), 1602 (amide C=O), 1536 (C=N); δ_H (400MHz; $CDCl_3$); 8.68 (1H, d, *J* 5.2, H-2″), 8.02 (1H, d, *J* 2.0, H-8″), 7.94 (1H, d, *J* 9.0, H-5″), 7.93 (1H, d, *J* 0.3, H-4), 7.90 (1H, dd, *J* 0.3 and 8.0, H-6), 7.43 (1H, dd, *J* 2.0 and 9.0, H-6″), 6.94 (1H, d, *J* 8.0, H-7), 6.81 (1H, d, *J* 5.2, H-3″), 4.58 (2H, s, -NC*H*$_2$N-), 3.22 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.92 (4H, br s, -NC*H*$_2$CH$_2$NAr); δ_C (75 MHz); 181.6, 158.6, 156.9, 151.8, 150.7, 150.0, 136.7, 135.8, 128.8, 126.4, 124.9, 124.5, 123.9, 121.8, 119.2, 113.7, 109.1, 62.4, 51.8 (2 x C) and 50.2 (2 x C); HRMS (FAB) Found *m/z* 534.03212 $[M+1]^+$ $C_{22}H_{18}ClIN_4O_2$ requires 534.03195;

Anal. Calcd. for $C_{22}H_{18}ClIN_4O_2 \cdot 0.25H_2O$ C 49.2%, H 3.4%, N 10.4%. Found C 49.2%, H 3.1%, N10.3 %.



65g

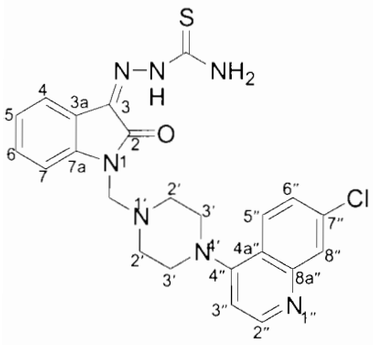
N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl]-5-bromo-*1H-*indole-2, 3-dione*

The conditions employed for the preparation of this compound were those described in general procedure **D.** However, 0.88 mmol of isatin and compound **69** were used.

(0.21g, 48%) obtained as orange crystals; mp 210-212 °C (from ethanol); IR ($CHCl_3$) ν_{max} /cm^{-1} 1736 (keto C=O), 1618 (amide C=O), 1502 (C=N); δ_H (300MHz; $CDCl_3$); 8.70 (1H, d, *J* 5.1, H-2″), 8.02 (1H, d, *J* 2.1, H-8″), 7.90 (1H, d, *J* 9.0, H-5″), 7.72 (1H, d, *J* 2.1, H-4), 7.55 (1H, dd, *J* 2.1 and 8.4, H-6), 7.42 (1H, dd, *J* 2.1 and 9.0, H-6″), 7.05 (1H, d, *J* 8.4, H-7), 6.82 (1H, d, *J* 5.2, H-3″), 4.58 (2H, s, -NC*H*$_2$N-), 3.24 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.93 (4H, br s, -NC*H*$_2$CH$_2$NAr); δ_C (75 MHz); 182.2, 161.1, 156.6, 151.8, 150.8, 150.0, 136.1, 128.8, 128.4, 126.4, 126.0, 124.9, 124.0, 121.8, 118.1, 112.5, 109.0, 62.4, 51.8 (2 x C) and 50.6 (2 x C); LRMS (EI) *m/z* 486 [3 %, M^+], 260 [100%, M-$C_{14}H_{15}ClN_3$]; Anal. Calcd. for $C_{22}H_{18}O_2BrN_4Cl \cdot H_2O$ C 52.4%, H 3.6%, N 11.5%. Found C 52.7%, H 3.5%, N 11.6%.

E. General procedure for the preparation of compounds 66a-66e

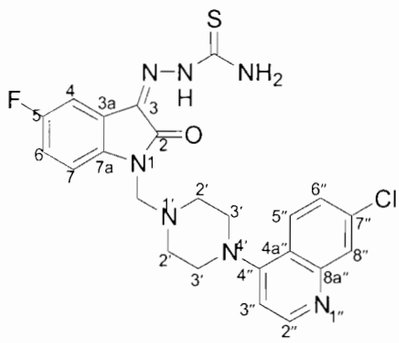
To a solution of isatin or substituted isatin-3-thiosemicarbazone derivative (0.2g, 1.36 mmol) in 5mL of 99.9 % ethanol was added a mixture of compound **69** (0.22g, 1.36mmol) and aqueous formaldehyde 37% (0.027 mL, 1.35mmol) also dissolved in 10mL ethanol (99.9%). The reaction mixture was stirred for 3 hours at room temperature, refrigerated for 48 hours to form crystals. The crystalline product was separated by filtration, vacuum dried and recrystallized from ethanol.



66a

N^1-*[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl*-1H-*indole-2,3-dione 3-thiosemicarbazone*

(0.29g, 78 %) obtained as orange crystals; mp 216-217 ^{o}C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3686 (N-H), 3036 (Ar-H), 1675 (C=O), 1511 (C=N), 1122 (C=S); δ_H (300 MHz; DMSO-d$_6$); 12.40 (1H, s, NNH), 9.04 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 8.65 (1H, d, *J* 5.2, H-2″), 7.96 (1H, d, *J* 9.0, H-5″), 7.91 (1H, d, *J* 2.4, H-8″), 7.66 (1H, dd, *J* 1.2 and 7.6, H-4), 7.48 (1H, dd, *J* 2.4 and 9.0, H-6″), 7.42 (1H, ddd, *J* 1.2, 7.2 and 8.0, H-6), 7.32 (1H, d, *J* 7.2, H-7), 7.15 (1H, ddd, *J* 0.4, 7.6 and 8.0, H-5), 6.61 (1H, d, *J* 5.2, H-3″), 4.60 (2H, s, -NC*H*$_2$N-), 3.13 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.87 (4H, br s, -NC*H*$_2$CH$_2$NAr); δ_C (75 MHz); 179.4, 162.6, 156.9, 152.8, 150.3, 144.5, 134.2, 131.8, 131.7, 128.9, 126.8, 126.4, 123.1, 122.1, 121.3, 120.1, 111.7, 110.2, 61.7, 52.3 (2 x C) and 50.7 (2 x C); HRMS (FAB) *m/z* 480.13677 [M+1]$^+$, $C_{23}H_{22}ClN_7OS$ requires 480.13732; Anal. Calcd. for $C_{23}H_{22}ClN_7OS$ C 57.6%, H 4.6%, N 20.4%, S 6.7%. Found C 57.6%, H 4.7%, N 20.4%, S 6.8%.

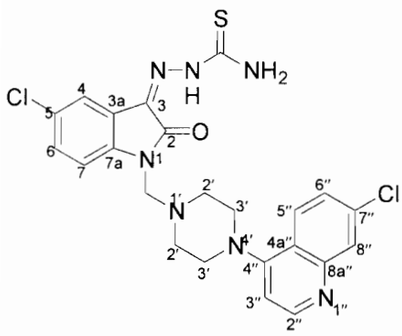


66b

***N^1*-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-5-fluoro-*1H*-indole-2,3-dione-3-thiosemicarbazone**

The conditions employed for the preparation of this compound were those described in general procedure **E.** However, 0.84 mmol of compound **65d** and thiosemicarbazide were used.

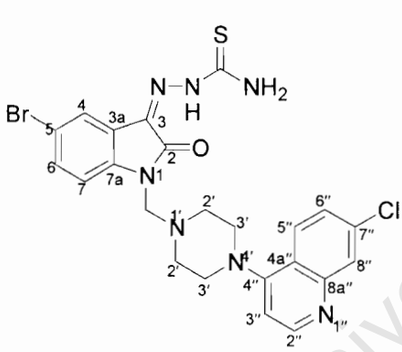
(0.21g, 51 %) obtained as yellowish-orange crystals; mp 233-234 ^{o}C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3686 (N-H), 1630(C=O), 1511(C=N), 1121 (C=S); δ_H (400MHz;DMSO-d$_6$); 12.31 (1H, s, NNH), 9.15 (1H, s, N*H*H), 8.81 (1H, s, NH*H*), 8.67 (1H, d, *J* 5.4, H-2″), 7.98 (1H, d, *J* 8.7, H-5″), 7.93 (1H, d, *J* 2.4, H-8″), 7.57 (1H, d, *J* 2.7, H-4), 7.48 (1H, dd, *J* 2.4 and 8.7, H-6″), 7.38 (1H, dd, *J* 2.7 and 7.6, H-6), 7.28 (1H, d, *J* 7.6, H-7), 6.61 (1H, d, *J* 5.4, H-3″), 4.60 (2H, s, -NC*H*$_2$N-), 3.13 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.88 (4H, br s, -NC*H*2CH$_2$NAr); δ_C (75 MHz); 178.7, 161.8, 156.6, 152.1, 149.5, 139.8, 138.5, 133.5, 131.3, 130.5, 127.9, 126.1, 125.7, 122.0, 121.5, 120.2, 111.9, 109.4, 61.1, 51.6 (2 x C) and 49.9 (2 x C); Anal. Calcd for $C_{23}H_{21}ClFN_7OS$ C 55.5%, H 4.3%, N 19.5%, S 6.4%. Found C 55.9%, H 4.2%, N 19.5%, S 5.8%; HRMS (FAB) *m/z* 498.12924 [M+1]$^+$ $C_{23}H_{21}ClFN_7OS$ requires 498.12790.



66c

***N*^{1}-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-5-chloro-*1H*-indole-2, 3-dione 3-thiosemicarbazone**

The conditions employed for the preparation of this compound were those described in general procedure **E**. However, 0.37 mmol of compound **65c** and thiosemicarbazide were used. (0.35g, 86 %) obtained as orange crystals; mp 234-235 ^{o}C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3687 (N-H), 1675 (C=O), 1518 (C=N), 1120 (C=S); δ_H (300 MHz; $CDCl_3$); 12.3 (1H, s, NNH), 9.10 (1H, s, N*H*H), 8.71 (1H, s, NH*H*), 8.68 (1H, d, *J* 5.4, H-2″), 8.01 (1H, d, *J* 2.0, H-8″), 7.88 (1H, d, *J* 8.8, H-5″), 7.61 (1H, d, *J* 2.1, H-4), 7.59 (1H, dd, *J* 2.1 and 8.4, H-6), 7.42 (1H, dd, *J* 2.0 and 8.8, H-6″), 7.11 (1H, d, *J* 8.4, H-7), 6.81 (1H, d, *J* 5.4, H-3″), 4.58 (2H, s, -NC*H*$_2$N-), 3.21 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.91 (4H, br s, -NC*H*$_2$CH$_2$NAr); δ_C (75 MHz); 178.7, 162.6, 158.3, 156.7, 151.7, 150.1, 149.5, 138.9, 128.9, 128.4, 126.4, 125.2,123.9, 123.7, 121.7, 119.1, 111.9, 110.0, 62.4, 51.9 (2 x C), 51.2 (2 x C); Anal. Calcd. for $C_{23}H_{21}Cl_2N_7OS$ C 53.8%, H 4.1%, N 18.6% S 5.8% Found C 53.7%, H 4.1%, N 19.0%, S 6.2%; HRMS (FAB) *m/z* 514.09889 $[M+1]^+$ $C_{23}H_{21}Cl_2N_7OS$ requires 514.09836.

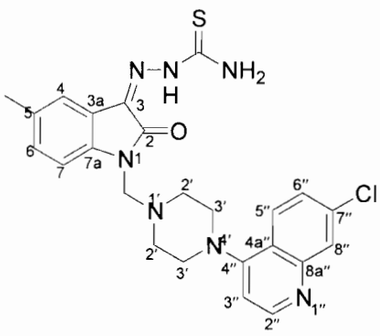


66d

***N*^{1}-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-5-bromo-*1H*-indole-2,3-dione3-thiosemicarbazone**

The conditions employed for the preparation of this compound were those described in general procedure **E.** However, 0.67 mmol of compound **65g** and thiosemicarbazide were used. (0.27g, 73 %) obtained as orange crystals; mp 236-237 ^{o}C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3688 (N-H), 1628 (C=O), 1515 (C=N), 1122 (C=S); δ_H (300 MHz; DMSO-d_6); 12.24 (1H, s, NNH), 9.14 (1H, s, N*H*H), 8.87 (1H, s, NH*H*), 8.69 (1H, d, *J* 5.4, H-2″), 7.97 (1H, d, *J* 9.0, H-5″), 7.91 (1H, d, *J* 2.4, H-8″), 7.62 (1H, d, *J*, 2.1, H-4), 7.50 (1H, dd, *J* 2.1 and 7.8, H-6), 7.39 (1H, dd, *J* 2.4 and 9.0, H-6″), 7.35 (1H, d, *J* 7.8, H-7), 6.69 (1H, d, *J* 5.4, H-3″), 4.67 (2H, s, -NC*H*$_2$N-), 3.15 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.89 (4H, br s, -NC*H*$_2$CH$_2$N-); δ_C (75 MHz); 179.5, 161.8, 156.9, 152.1, 149.5, 143.7 , 133.5,131.6, 131.0, 130.9, 127.9, 126.1, 125.7, 122.9, 121.3, 119.3, 111.0, 109.4, 60.9, 52.5 (2 x C) and 49.9 (2 x C); HRMS (FAB) *m/z* 558.04865

$[M+1]^+$ $C_{23}H_{21}BrClN_6OS$ requires 558.04784; Anal. Calcd. for $C_{23}H_{21}BrClN_7OS$ C 49.4%, H 3.8%, N 17.0%, S 5.7% Found C 50.2%, H 3.9%, N 17.2%, S 5.1%.



66e

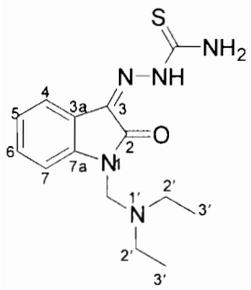
***N*^{1}-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-ylmethyl-methyl-*1H*-indole-2,3-dione-3-thiosemicarbazone**

The conditions employed for the preparation of this compound were those described in general procedure **E.** However, 0.85 mmol compound **65e** and thiosemicarbazide were used.

(0.33g, 78%) obtained as yellowish-orange crystals; mp 232-234 °C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3687 (N-H), 1675 (C=O), 1518 (C=N), 1122 (C=S); δ_H (300 MHz; DMSO-d_6); 12.37 (1H, s, NNH), 9.17 (1H, s, N*H*H), 8.91 (1H, s, NH*H*), 8.68 (1H, d, *J* 5.4, H-2″), 7.99 (1H, d, *J* 9.0, H-5″), 7.94 (1H, d, *J* 2.1, H-8″), 7.78 (1H, d, *J* 2.1, H-4), 7.55 (1H, dd, *J* 2.1 and 8.7, H-6), 7.48 (1H, d, *J* 2.1 and 9.0, H-6″), 7.34 (1H, d, *J* 8.7, H-7), 6.69 (1H, d, *J* 5.4, H-3″), 4.81 (2H, s, -NC*H*$_2$N-), 3.16 (4H, br s, -NCH$_2$C*H*$_2$NAr), 2.90 (4H, br s, -NC*H*$_2$CH$_2$NAr), 2.27 (3H, s, Ar-C*H*$_3$); δ_C (75 MHz); 179.4, 161.7, 156.2, 152.8, 149.5, 144.0, 142.4, 134.6, 132.0, 130.8, 127.9, 126.1, 125.0, 123.6, 121.3, 120.0, 112.3, 109.4, 63.1, 51.6 (2 x C), 49.5 (2 x C) and 21.3; HRMS (FAB) *m/z* 494.15187 $[M+1]^+$ $C_{24}H_{24}ClN_7OS$ requires 494.15297; Anal. for Calcd. $C_{24}H_{24}ClN_7OS$ C 58.4%, H 4.9%, N 19.8%, S 6.5%. Found C 58.4 %, H4.9 %, N19.7 %, S 6.2%.

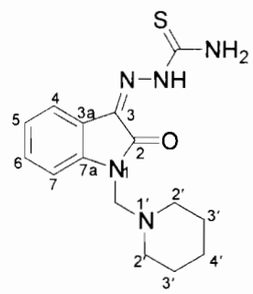
F. General procedure for preparation of compounds 70a–70f

Equimolar quantities of isatin-3-thiosemicarbazone (0.20g, 0.91 mmol), amine (0.09 mL, 0.91 mmol) and paraformaldehyde (0.08g, 0.90mmol) were dissolved in warm 99.9 % ethanol. The mixture was continuously stirred at 25 °C for 6 hours. After standing for approximately 24 hours in the fridge, a crystalline product was formed. The product was then separated by filtration, vacuum dried and recrystallized from ethanol.



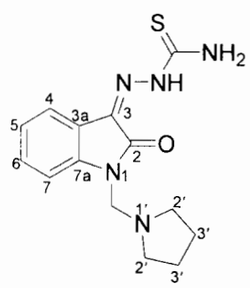
70a

***N*[1]-Diethylaminomethyl-indole-2, 3-dione-3-thiosemicarbazone**
(0.15g, 54%) obtained as yellow crystals; mp 134-135 °C (ethanol) (lit 135 °C) [160]; δH (300MHz; DMSO-d_6); 12.10 (1H, s, NNH), 9.05 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 7.71 (1H, dd, *J* 0.9 and 7.5, H-4), 7.44 (1H, ddd, *J* 0.9, 6.6 and 8.0, H-6), 7.17 (1H, ddd, *J* 0.9, 6.6 and 7.5, H-5), 7.19 (1H, d, *J* 8.0, H-7), 4.52 (2H, s, -NCH_2N-), 2.65 (4H, q, *J* 6.9, -NCH_2CH$_3$), 1.06 (6H, t, *J* 6.9, -NCH$_2$CH_3).



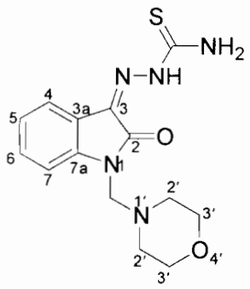
70b

***N*[1]-Piperidin-1-ylmethyl-indole-2, 3-dione-3-thiosemicarbazone**
(0.27g, 95%); obtained as yellow solids; mp 179-180 °C (from ethanol) (lit 174 °C) [160]; δ_H (300 MHz, DMSO-d_6); 12.38 (1H, s, NNH), 9.05 (1H, s, N*H*H), 8.69 (1H,s, NH*H*), 7.71 (1H, dd, *J* 0.9 and 7.5, H-4), 7.41 (1H, ddd, *J* 0.9, 7.8 and 8.1, H-6), 7.26 (1H, d, *J* 7.8, H-7), 7.17(1H, ddd, *J* 1.2, 7.5 and 8.1, H-5), 4.47 (2H, s, -NCH_2N-), 2.54 (4H, t, *J* 4.8, H-2′), 1.46 (4H, m, H-3′ 3′), 1.33 (2H, m, H-4′ 4′);



70c

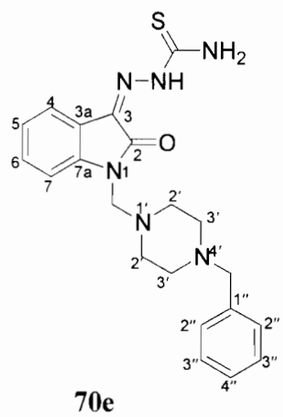
***N*[1]-Pyrrolidin-1-ylmethyl-indole-2, 3-dione-3-thiosemicarbazone**
(0.25g, 90%) obtained as yellow crystals; mp 181-184 °C (from ethanol) (lit 177-178 °C) [160]; δ_H (400 MHz, DMSO-d_6); 12.29 (1H, s, NNH), 9.03 (1H, s, N*H*H), 8.68 (1H, s, NH*H*), 7.68 (1H, dd, *J* 1.2 and 7.6, H-4), 7.42 (1H, ddd, *J* 1.2, 7.2 and 8.0, H-6), 7.23 (1H, d, *J* 8.0, H-7), 7.12 (1H, ddd, *J* 0.9, 7.2 and 7.6, H-5), 4.61 (2H, s, -NCH_2N-), 2.60 (4H, br t, *J* 3.2, H-2′), 1.65 (4H, m, H-3′).



70d

***N*[1]-Morpholin-4-ylmethyl-indole-2, 3-dione-3-thiosemicarbazone**

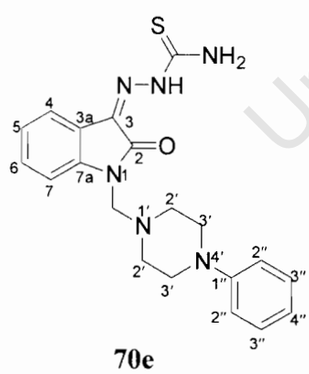
(0.27g, 92 %); as yellow crystals; mp 208-210 °C (from ethanol) (lit 215-216 °C) [160]; δ_H (300MHz; DMSO-d_6); 12.37 (1H, s, NNH), 9.05 (1H, s, N*H*H), 8.70 (1H, s, NH*H*), 7.72 (1H, dd, *J* 0.6 and 7.2, H-4), 7.42 (1H, ddd, *J* 0.9, 7.5 and 8.0, H-6), 7.27 (1H, d, *J* 8.0, H-7), 7.15 (1H, ddd, *J* 0.6, 7.2 and 7.5, H-5), 4.49 (2H, s, -NC*H*$_2$N-), 3.54 (4H, br s, H-3′), 2.57 (4H, br s, H-2').



70e

N[1]-*(4-Benzyl-piperazin-1-ylmethyl-indole-2,3-dione-3-thiosemicarbazone*

(0.23g, 63%); obtained as yellow crystals; mp 187-188 °C; IR (KBr): ν_{max}/cm^{-1} 3680 (N-H), 3428 (N-H), 1637 (C=O), 1505 (C=N), 1123 (C=S); δ_H (300 MHz, DMSO-d_6); 12.38 (1H, s, NNH), 9.06 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 7.71 (1H, dd, *J* 0.9 and 7.5, H-4), 7.43 (1H, ddd, *J* 0.9, 7.2 and 7.8, H-6), 7.25-7.23 (5H, m, H-2″, H-3″, H-4″, H-5″ and H-6″), 7.19 (1H, d, *J* 7.8, H-7), 7.17 (1H, ddd, *J* 0.6, 7.2 and 7.5, H-5), 4.52 (2H, s, -NC*H*$_2$N-), 3.43 (2H, s, -NC*H*$_2$Ar), 2.59 (4H, br s, -NC*H*$_2$CH$_2$NCH$_2$Ar), 2.34 (4H, br s, -NCH$_2$C*H*$_2$NCH$_2$Ar); δ_C (75 MHz); 178.7, 161.6, 143.7, 138.2, 131.0, 130.9, 128.6 (2 x C), 128.0, 126.8 (2 x C), 122.8, 120.5, 119.2, 111.1, 61.8, 52.3 (2 x C), 50.1 (2 x C) and 49.9; Anal. Calcd. for $C_{21}H_{24}N_6OS$; C 62.1%, H 6.6%, N 20.1%, S 6.6 Found C 61.7%, H 5.9%, N 20.6%, S 6.6%; HRMS (FAB) *m/z* 409.18109 $[M+1]^+$, $C_{21}H_{24}N_6OS$ requires 409.18106.



70e

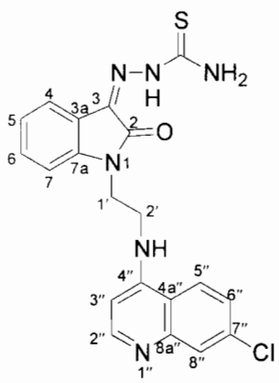
N[1]-*(4-phenyl-piperazin-1-ylmethyl-indole-2,3-dione-3-thiosemicarbazone*

(0.27g, 76%) obtained yellowish crystals; 188-191 °C; IR (KBr): ν_{max}/cm^{-1} 3686 (N-H), 3575 (N-H), 1694 (C=O), 1502 (C=N), 1127 (C=S); δ_H (300 MHz, DMSO-d_6); 12.39 (1H, s, NNH), 9.04 (1H, s, N*H*H), 8.69 (1H, s, NH*H*), 7.79 (1H, dd, *J* 0.9 and 7.6, H-4), 7.45 (1H, ddd, *J* 0.9, 6.6 and 8.0, H-6), 7.18 (1H, d, *J* 8.0, H-7), 7.21-7.13 (5H, m, H-2″, H-3″ and 4″), 7.10 (1H, ddd, *J* 0.6, 6.6 and 7.6, H-5), 4.59

(2H, s, -NCH_2N-), 3.09 (4H, br s, -NCH$_2$CH_2NAr), 2.76 (4H, br s, -NCH_2CH$_2$NAr); δ_C (75 MHz); 178.7, 161.7, 151.0, 143.7, 131.1, 130.9, 128.8, 122.0, 120.6 (2 x C), 120.5, 119.3 (2 x C), 119.2, 111.1, 60.9, 50.1 (2 x C) and 48.1 (2 x C); Anal. Calcd. for $C_{20}H_{22}N_6OS$ C 60.9%, H 5.6%, N 21.3%, S 8.1% Found C 61.3%, H 6.3%, N 18.7%, S 5.7%; HRMS (FAB) *m/z* 395.16544 $[M+1]^+$, $C_{20}H_{22}N_6OS$ requires 395.16541

G. General procedure for the preparation of compounds 71

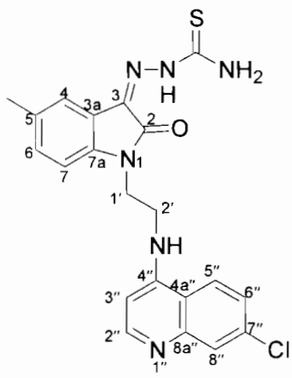
Equimolar quantities of ethyl-quinoline isatin derivative (0.1g, 0.284 mmol) and thiosemicarbazide (0.038g, 0.284mmol) were dissolved in warm 99.9 % ethanol. The mixture was then continuously stirred at 45 °C for 5-6 hours. After standing for approximately 24 hours at room temperature, a crystalline product was formed. The product was then separated by filtration, vacuum dried and recrystallized from acetonitrile.



71a

N^1-*[2-(7-Chloro-quinolin-4-ylamino)-ethyl]*-1H-*indole-2,3-dione-3-thiosemicarbazone*

(0.08g, 70%) obtained as orange crystals; mp 167-171 °C (from acetonitrile); IR (KBr): ν_{max}/cm^{-1} 3680 (N-H), 3404 (N-H), 3012 (Ar-H), 1635 (C=O), 1516 (C=N), 1140 (C=S); δ_H (400MHz, DMSO-d_6); 12.31 (1H, s, NNH), 9.02 (1H, s, N*H*H), 8.67 (1H, s, NH*H*), 8.40 (1H, d, *J* 5.4, H-2″), 7.98 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.0, H-8″), 7.67(1H, dd, *J* 0.6 and 7.4, H-4), 7.38 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.28 (1H, ddd, *J* 0.6, 7.6 and 7.8, H-6), 7.10 (1H, ddd, *J* 0.9, 7.4 and 7.6, H-5), 7.02 (1H, d, *J* 7.8, H-7), 6.63 (1H, d, *J* 5.4, H-3″), 4.01 (2H, t, *J* 5.6, -CH_2CH$_2$NHAr), 3.65 (2H, t, *J* 5.6, -CH$_2$CH_2NHAr); δ_C (75 MHz); 179.4 , 161.8, 152.6, 150.5, 149.7, 149.1, 143.7, 138.6, 134.1, 128.2, 124.9, 124.5, 123.7, 123.1, 118.2, 117.3, 110.3, 104.5, 74.9 and 42.4; HRMS (FAB) Found *m/z* 425.09419 $[M+1]^+$, $C_{20}H_{17}ClN_6OS$] requires 425.09513; Anal. Calcd. for $C_{20}H_{17}ClN_6OS \cdot 0.5H_2O$; C 55.4%, H 3.9%, N 18.8%, S 6.9% Found C 55.3%, H 4.0%, N 18.8%, S 6.9%.

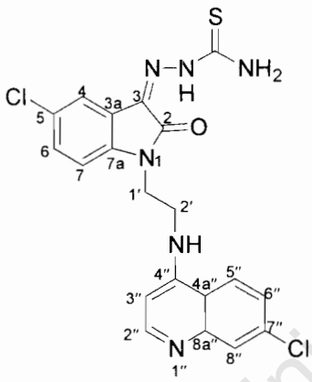


71b

N^1-*[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5-methyl*-1H-*indole-2,3-dione-3-thiosemicarbazone*

The conditions employed for the preparation of this compound were those described in general procedure **G.** However, 0.27 mmol of compound **75b** and thiosemicarbazide were used.

(0.11g, 89 %) obtained as yellow-orange crystals; mp 227-230 °C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3632 (N-H), 3378 (N-H), 1665 (C=O), 1506 (C=N), 1127 (C=S); δ_H (400MHz; DMSO-d_6); 12.29 (1H, s, NNH), 8.98 (1H, s, N*H*H), 8.63 (1H, s, NH*H*), 8.40 (1H, d, *J* 5.6, H-2″), 8.01 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.0, H-8″), 7.49 (1H, d, *J* 2.0, H-4), 7.39 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.08 (1H, dd, *J* 2.0 and 7.4, H-6), 7.00 (1H, d, *J* 7.4, H-7), 6.60 (1H, d, *J* 5.6, H-3″), 3.98 (2H, t, *J* 5.4, -NC*H*$_2$CH$_2$NHAr), 3.62 (2H, t, *J* 5.4, -NCH$_2$C*H*$_2$NHAr), 2.31 (3H, s, Ar-C*H*$_3$); δ_C (75 MHz); 179.4, 161.8, 152.5, 150.6, 149.7 149.0, 141.6, 138.9, 134.2, 128.1, 125.3, 124.9, 124.5, 121.9, 118.2, 118.1, 110.9, 110.6, 74.9, 41.8 and 21.2; LRMS (FAB) *m/z* 439 [5 %, $(M+1)^+$], and 307 [30 %, M-$C_{18}H_{14}ClN_3$]; Anal. Calcd. for $C_{21}H_{19}ClN_6OS$; C 57.5%, H 4.4%, N 19.3%, S 7.3% Found C 57.1%, H 4.3%, N 18.8%, S 7.3%.



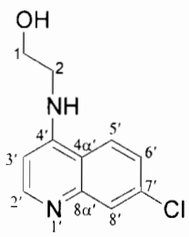
71c

N^1-*[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5-chloro*-1H-*indole-2, 3-dione 3-thiosemicarbazone*

The conditions employed for the preparation of this compound were those described in general procedure **G.** However, 0.26 mmol of compound **75c** and thiosemicarbazide were used.

(0.16g, 68 %) obtained as orange crystals; mp 221-227 °C (from ethanol); IR (KBr): ν_{max}/cm^{-1} 3688 (N-H), 3478 (N-H), 1665 (C=O), 1510 (C=N), 1121 (C=S); δ_H (400MHz, DMSO-d_6); 12.29 (1H, s, NNH), 8.98 (1H, s, N*H*H), 8.64 (1H, s, NH*H*), 8.40 (1H, d, *J* 5.0, H-2″), 8.01 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.1, H-8″), 7.49 (1H, d, *J* 2.0, H-4), 7.39 (1H, dd, *J* 2.0 and 9.0, H-6″), 7.09 (1H, dd, *J* 2.0 and 7.4, H-6), 7.01 (1H, d, *J* 7.4, H-7), 6.60 (1H, d, *J* 5.0, H-3″), 3.98 (2H, t, *J* 6.4, -NC*H*$_2$CH$_2$NHAr), 3.62 (2H, t, *J* 6.4, -NCH$_2$C*H*$_2$NHAr); δ_C (75 MHz); 178.3, 158.2, 151.9, 150.8, 149.8, 149.0, 142.1, 136.7, 127.5, 127.3, 124.8, 124.2, 123.5, 121.9, 117.6, 117.0, 109.7, 104.7, 74.7 and 42.3; HRMS (FAB) Found *m/z* 459.05659 $[M+1]^+$, $C_{20}H_{16}Cl_2N_6OS$

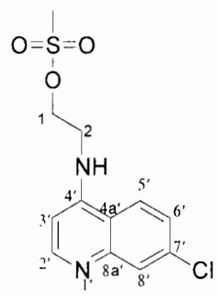
required 459.05615; Anal. Calcd. for $C_{20}H_{16}Cl_2N_6OS$, C 57.5%, H 4.4%, N 19.3%, S 7.3%. Found C 57.1%, H 4.3%, N 19.0%, S 7.2%.



73

2-(7-Chloro-quinolin-4-ylamino)-ethanol

A mixture of 4, 7-dichloroquinoline (4.0g, 20.19 mmol), ethanolamine (24.42mL, 403.9mmol) and triethylamine (0.84mL, 6.06 mmol) was stirred under reflux for 8 hours. The reaction mixture was then cooled to room temperature during which a white precipitate formed. The precipitate was filtered, washed with water (3 x 50mL) and recrystallized from methanol to obtain (4.10g, 92 %) as cream-white crystals, mp 213-215 °C (from methanol) (lit 214 °C) [161]; R_f 0.42 (MeOH/DCM, 2:8); δ_H (300MHz; CD_3OD); 8.35 (1H, d, *J* 6.0, H-2′), 8.09 (1H, d, *J* 9.0, H-5′), 7.78 (1H, d, *J* 2.1, H-8′), 7.40 (1H, dd, *J* 2.1 and 9.0, H-6′), 6.57 (1H, d, *J* 6.0, H-3′), 3.86 (2H, t, *J* 5.7, ArNHCH_2CH_2-), 3.52 (2H, t, *J* 5.7, ArNHCH_2-); δ_C (75 MHz); 151.8, 150.1, 149.0, 133.3, 127.4, 123.9, 117.4, 108.6, 58.7 and 45.0 ; LRMS (EI) m/z 223.1 [M^+,]; Anal. Calcd C 59.3%, H 4.9%, N 12.6% Found C 59.0%, H 4.9%, N 12.6%.



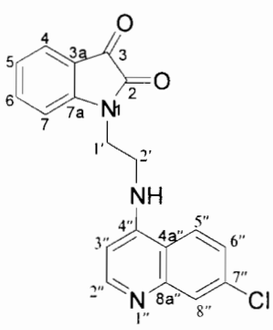
74

Methanesulfonic acid 2-(7-chloro-quinolin-4-ylamino)-ethyl ester

A solution of compound **73** (8.0g, 36 mmol) in 24 mL of pyridine was stirred at 0 °C for 30 minutes. Whilst at 0 °C methanesulfonyl chloride (6.97mL, 90.0 mmol) in 22mL of pyridine was added slowly to the above solution, and the resultant mixture stirred for 5 hours. The reaction mixture was then diluted with 17% aqueous ammonia solution (25 mL), extracted with CH_2Cl_2 (3 x 50 mL), dried over anhydrous $MgSO_4$, concentrated and recrystallized from methanol/ water to obtain yield (8.96g, 83%) obtained as white crystals, mp 138-140 °C (from MeOH / H_2O), R_f 0.76 (MeOH/DCM, 2:8); IR (KBr) ν_{max}/cm^{-1}; 3218 (N-H), 1579 (C=N), 1442(C=C); δ_H (300MHz; CD_3OD); 8.39 (1H, d, *J* 5.7, H-2′), 8.08 (1H, d, *J* 9.0, H-5′), 7.79 (1H, d, *J* 2.1, H-8′), 7.41 (1H, dd, *J* 2.1 and 9.0, H-6′), 6.61 (1H, d, *J* 5.7, H-3′), 4.48 (2H, t, *J* 5.4, ArNHCH_2CH_2-), 3.74 (2H, t, *J*, 5.4, ArNHCH_2-) 3.07 (3H, s, -OSO_2-CH_3); δ_C (100 MHz); 151.9, 150.2, 149.1, 133.2, 127.8, 124.1, 123.9, 117.9, 108.0, 66.8, 42.4 and 37.8; HRMS (EI) Found *m/z* 300.03487 $[M]^+$, $C_{12}H_{13}O_3ClN_2S$ requires 300.03354; Anal. Calcd. for $C_{12}H_{13}O_3N_2SCl$ C 47.9%, H 4.4%, N 9.3%, 10.7%. Found C 48.1%, H 4.4%, N 9.2%, S 10.3%.

H. General procedure for preparation compounds 75a–75e

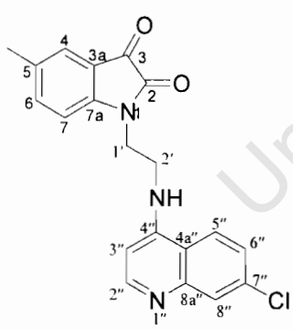
To a mixture of isatin or substituted isatin (0.1g, 0.68 mmol) in 10 mL of anhydrous DMF at 0 °C was added NaH (0.02g, 0.815 mmol) was then added to this mixture followed by Compound **74** (0.27g, 0.88 mmol) warming to room temperature with stirring over 1 hour. The temperature was then raised to 60 °C and stirring continued for a further 12 hours. Ice-cold water was added to the reaction and a coloured precipitate formed. The solid was filtered, washed with water (2 x 25 mL), petroleum ether (1 x 10mL) and dried under vacuum to give the desired product.



75a

N*^{1}*-[2-(7-Chloro-quinolin-4'-ylamino)-ethyl]-1*H*-indole-2, 3-dione

(0.21g, 88%) obtained as orange crystals; mp 240-243 °C (from methanol); R_f 0.43 (MeOH: EtOAc, 2:8); IR ($CHCl_3$) ν_{max}/cm^{-1} 3236 (N-H), 1734 (keto C=O), 1600 (amide C=O), 1514 (C=N); δ_H (300MHz; DMSO-d_6); 8.41 (1H, d, *J* 5.4, H-2″), 7.97 (1H, d, *J* 9.0, H-5″), 7.76 (1H, d, *J* 2.4, H-8″), 7.52 (1H, dd, *J* 0.6 and 7.2, H-4), 7.48 (1H, ddd, *J* 0.6, 6.6 and 7.4, H-6), 7.39 (1H, dd, *J* 2.4, 9.0, H-6″), 7.07 (1H, ddd, *J* 0.9, 6.6 and 7.2, H-5), 7.03 (1H, dd, *J* 0.9 and 7.4, H-7), 6.62 (1H, d, *J* 5.4, H-3″), 3.93 (2H, t, *J* 5.1, ArNHCH$_2$C*H*$_2$-), 3.60 (2H, t, *J* 5.1, ArNHC*H*$_2$-); δ_C (75 MHz); 183.3, 158.3, 151.9, 150.6, 149.7, 149.0, 137.8, 133.3, 127.4, 124.3, 124.0, 123.7, 122.9, 117.4, 117.0, 110.2, 104.6, 74.9 and 42.0; HRMS (FAB) Found *m/z* 352.08494 $[M+1]^+$, $C_{19}H_{14}O_2N_3Cl$] requires 352.08527; Anal. Calcd. for $C_{19}H_{14}O_2N_3Cl$ C 64.8%, H 4.0%, N 11.9%. Found C 64.4%, H 3.9%, N 11.2%.



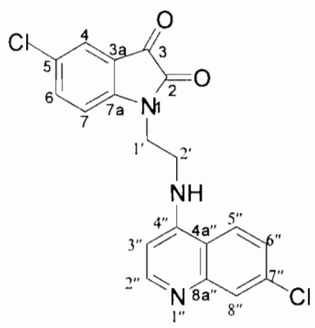
75b

N*^{1}*-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5-methyl-1*H*-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **H.** However, 0.62 mmol of isatin and 0.81 mmol of compound **74** were used.

0.62 mmol, (0.16g, 68 %) obtained as red crystals; mp 240-243 °C (from methanol); R_f 0.28 (MeOH: EtOAc; 2:8); IR (KBr) ν_{max}/cm^{-1} 3236 (N-H), 1737 (keto C=O), 1600 (amide C=O), 1514 (C=N); δ_H (300MHz; $CDCl_3$); 8.53 (1H, d, *J* 5.4, H-2″), 7.93 (1H, d, *J* 2.4, H-8″), 7.68 (1H, d, *J* 9.0, H-5″), 7.43 (1H, d, *J* 2.0, H-4), 7.39 (1H, dd, *J* 2.0 and 7.2, H-6), 7.37 (1H, dd, *J*

2.4 and 9.0, H-6″), 6.81 (1H, d, *J* 7.2, H-7), 6.62 (1H, d, *J* 5.4, H-3″), 4.15 (2H, t, *J* 5.4 , ArNHCH$_2$C*H*$_2$-), 3.67 (2H, t, *J* 5.4, ArNHC*H*$_2$-), 2.32 (3H, s, Ar-C*H*$_3$); δ_C (75 MHz), 183.6, 158.2, 152.0, 150.5, 149.7, 149.1, 137.7, 133.4, 126.8, 124.6 , 124.2, 123.4, 123.1, 117.5, 116.9, 111.2, 104.6, 74.6, 42.1 and 20.3; LRMS (EI) *m/z* 365.0 [16 %, M^+], 191 [100 %, M-$C_{10}H_8ClN_2$]; Anal. Calcd. for $C_{20}H_{16}O_2N_3Cl$ C 65.7%, H 4.4%, N 10.5%. Found C 65.3%, H 4.3%, N 10.9%.

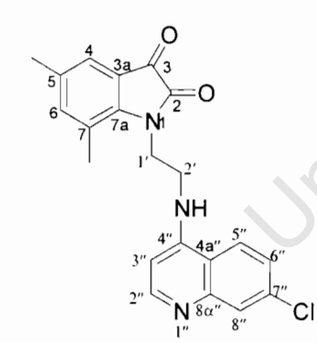


75c

5-Chloro-N*^{1}*- [2 -(7-chloro-quinolin-4'-ylamino)-ethyl]-1H-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **H.** However, 0.55 mmol of isatin and 0.72 mmol of compound **74** were used.

(0.15g, 69%) obtained as orange crystals; mp 221-223 °C (from ethanol); R_f 0.46 (MeOH; EtOAc, 2:8); IR ($CHCl_3$) ν_{max}/cm^{-1}, 3237 (N-H), 1734 (C=O), 1606 (amide C=O), 1519 (C=N); δ_H (300MHz; DMSO-d_6); 8.43 (1H, d, *J* 5.4, H-2″), 7.94 (1H, d, *J* 8.4, H-5″), 7.78 (1H, d, *J* 2.1, H-8″), 7.59 (1H, d, *J* 2.4, H-4), 7.40 (1H, dd, *J* 2.4 and 8.7, H-6), 7.31 (1H, dd, *J* 2.1 and 8.4, H-6″), 7.07 (1H, d, *J* 8.7, H-7), 6.64 (1H, d, *J* 5.4, H-3″), 3.96 (2H, t, *J* 5.4, ArNHCH$_2$C*H*$_2$-), 3.64 (2H, t, *J* 5.4, ArNHC*H*$_2$-); δ_C (75 MHz); 182.3, 158.2, 151.9, 150.7, 149.8, 149.0, 136.7, 127.4, 127.2, 124.8, 124.2, 123.4, 121.9, 117.5, 117.0, 109.7, 104.7, 74.6 and 42.3; HRMS (FAB) Found *m/z* 386.04642 $[M+1]^+$ $C_{19}H_{13}O_2N_3Cl_2$ requires 386.04630; Anal. Calcd. for $C_{19}H_{13}O_2N_3Cl_2$ C 59.1 %, H 3.4 %, N 10.9 %. Found C 59.0 %, H 3.4 %, N 10.8 %.



75d

N*^{1}*-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-5, 7-dimethyl-1H-indole-2, 3-dione

The conditions employed for the preparation of this compound were those described in general procedure **H.** However, 0.57 mmol of isatin and 0.74 mmol of compound **74** were used.

(0.19g, 89%) obtained as orange crystals; mp 219 °C (from ethanol); R_f 0.53; IR ($CHCl_3$) ν_{max} /cm^{-1} 3236 (N-H), 1736 (keto C=O), 1607 (amide C=O), 1519 (C=N); δ_H (300MHz; DMSO-d_6); 8.40 (1H, d, *J* 5.4, H-2″), 8.03 (1H, d, *J* 9.0, H-5″), 7.78 (1H, d, *J* 2.1, H-8″), 7.44 (1H, d, *J* 2.4, H-4), 7.40 (1H, d, *J* 2.4, H-6), 7.39 (1H, dd, *J* 2.1 and 9.0, H-6″), 6.62 (1H, d, *J* 5.4, H-3″), 4.12 (2H, t, *J* 6.3,

ArNHCH$_2$CH_2-), 3.64 (2H, t, *J* 6.3, ArNHCH_2-), 2.40 (3H, s, Ar-CH_3), 2.22 (3H, s, Ar-CH_3); δ_C (75 MHz); 182.2, 158.6, 151.8, 150.4, 149.8, 149.0, 136.3, 127.5, 127.4, 124.1, 123.7, 122.7, 121.2, 117.8, 117.4, 110.6, 104.6, 74.3, 42.7, 19.8 and 18.2; HRMS (FAB) Found *m/z* 380.11717 $[M+1]^+$ $C_{21}H_{18}O_2N_3Cl$ requires 380.11657; Anal. Calcd. for $C_{21}H_{18}O_2N_3Cl$ C 66.4%, H 4.7%, N 11.1%. Found C 66.3%, H 4.6%, N 10.9%.

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