SMALL MOLECULE INHIBITORS OF PARASITIC ENZYMES

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SMALL MOLECULE INHIBITORS OF PARASITIC ENZYMES

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By

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Abstract

The work presented in this thesis is threefold:-(i) development of potential novel inhibitors of cysteine proteases from the causative agents of Malaria, African sleeping sickness and Chagas disease, (ii) development of potential new and novel anti-trypanosomal inhibitors of trypanosome alternative oxidase, and (iii) synthetic and methodological studies.

(i) The emergence of the widespread resistance of *Plasmodium falciparum* to the available drugs has necessitated the search for new lead compounds with high structural variation. Among potential novel targets for antimalarial chemotherapy are enzymes such as cysteine proteases that mediate hemoglobin hydrolysis. The design, synthesis and biological evaluation of four different classes of Mannich bases as potential new inhibitors of a cysteine protease (falcipain-2) from *P.falciparum* was undertaken. Biological screens were also extended to two other important homologous parasitic trypanosomal cysteine proteases, cruzain and rhodesain. From the assays two compounds **83b** and **83c** from the series of 4-aminoquinoline semicarbazones were identified as promising antiparasitic agents against all the three cysteine proteases, and one compound **91a** from the series of Mannich bases of semicabazones of isatin derivatives against chloroquine sensitive (D10) and chloroquine resistant (K1) strains of the malaria parasite.

(ii) Trypanosome alternative oxidase (TAO) is a terminal oxidase present in the bloodstream form of African trypanosomes which are responsible for human African sleeping sickness and nagana in cattle. TAO is present in the mitochondria of the trypanosomes and is an additional (in addition to cytochrome *c* oxidase) protein in the respiratory chain. TAO acts as the terminal electron acceptor and its substrates are ubiquinol and dioxygen which are converted to ubiquinone and water respectively. Based on the hypothesis that salicylhydroxamic acid (SHAM) and related compounds inhibit TAO by interfering with ubiquinone, a series of prenylated amides, which closely resemble ubiquinone, were designed, synthesized and tested against the enzyme. Biological data showed that these compounds have comparable inhibitory activity to SHAM at high concentrations and that they act by interfering with ubiquinone/ubiquinol-

mediated electron transport, more specifically by binding to the terminal ubiquinol receptor on the oxidase component of the TAO.

(iii) Investigations into the copper (I)-catalyzed homologation of alkynes to give allenes *via* Mannich bases revealed that product (allene vs homocoupling product) and yield distribution are dependent upon the nature of the anionic component of the copper catalyst and the electronic nature of a substituent on the aryl ring of an alkyne substrate. When a copper catalyst with a non-linear anionic component was employed, no allene products were obtained. On the other hand, the yields of homocoupling products decreased as the strength of the electron withdrawing group on the aryl ring increased.

Operationally simplified and high yielding methods for the preparation of *N, N'-bis*(benzyloxycarbonyl)-1-L-cysteinyl-glycyl-3-dimethylaminopropylamide, an alternative substrate for trypanothione reductase, and structural analogues, using polymer-supported reagents are described. Solid phase synthesis of a biaryl chalcone is also described.

ABBREVIATIONS

Ac	Acetyl
ADP	Adenosine diphosphate
Aryl	aromatic ring
Asp	Aspartic acid
ATP	Adenosine triphosphate
CAN	Cerium (VI) ammonium nitrate
Cbz	Benzyloxycarbonyl
CNS	Central nervous system
CQ	Chloroquine
Cys	Cysteine
DCC	*N, N'*-Dicycohexylcarbodiimide
DHAP	dihydroxyacetone phosphate
DMAP	4-*N, N*-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dppp	1,1'-bis(diphenylphosphino)propane
EDC	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide
Et	Ethyl
equiv.	Equivalent
Glu	Glutamine
GLUT-1	Glucose transporter-1
G3P	Glycerol-3-phosphate
3GPA	3-phosphoglyceric acid
GR	Glutathione reductase
GSH	Glutathione
GSSH	Glutathione disulfide
h	Hour
His	Histidine

HOBt	1-Hydroxy benzotriazole
HRMS	High Resolution Mass Spectrometry
IC_{50}	Inhibitory concentration to inhibit 50 % of enzyme activity or parasite growth
IR	Infrared
mol	Mole
Me	Methyl
MS	Mass Spectrometry
$NADP^+$	Oxidized nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide
ND	Not determined
NMP	1-methyl-2-pyrrolidinone
NMR	Nuclear Magnetic Resonance
P	*Plasmodium*
PPh_3	Triphenylphosphine
PS	Polymer-supported
3GPA	3-phosphoglyceric acid
RNA	Ribonucleic acid
rt	Room temperature
SAR	Structure-Activity Relationship
S_N2	Nucleophilic bimolecular substitution
SHAM	Salicylhydroxamic acid
T.	*Trypanosoma*
TAO	Trypanosome alternative oxidase
T. b	*Trypanosoma brucei*
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMS	Trimethylsilane
TryR	Trypanothione reductase
Trx	Oxidised thioredoxin (thioredoxin disulfide)
TrxR	Thioredoxin reductase

$T[S]_2$	Trypanothione disulfide
$T[SH]_2$	Trypanothione

The following abbreviations are used in the experimental section

s	singlet
br	broad
br s	broad singlet
d	doublet
dd	double of doublets
ddd	double double doublet or doublet of doublet of doublets
t	triplet
q	quartet
quint	quintet
sep	septet
m	multiplet
δ	chemical shift
Anal.	Analytical
cm^{-1}	wavenumbers
EI	Electron impact
mp	Melting point
m/z	mass to charge ratio
R_f	Retention factor

CONTENTS

CHAPTER 1

INTRODUCTION I

PARASITIC DISEASES

1.1.0 Protozoa

Protozoa are the most abundant animals in the world in terms of numbers and biomass. Their principal importance is as consumers of bacteria (prokaryotes). Bacteria play a vital role in maintaining the earth as a suitable place for inhabitation by other forms of life and protozoa play a vital role in controlling their numbers and biomass. Protozoa are also important as parasites and symbionts of multicellular animals.

Protozoa are defined as single-celled eukaryotic organisms, mostly too small to be seen with the naked eye but visible in the average microscope. Among the unique features in protozoa are the mega- and micronucleus found in ciliates and *kinetoplast*, a deoxyribonucleic acid (DNA) containing structure in the mitochondrion of kinetoplastid flagellates.[1] They feed heterotrophically and exhibit diverse motility mechanisms. Although they are the simplest eukaryotes, they are still far more complex than bacteria. Protozoa are a very diverse group, not closely related to one another. However, it is impossible to draw a line between unicellular animals (consumers), plants (producers) and fungi (decomposers) and it is customary to speak of the Protista as a separate kingdom of unicellular eukaryotes that embraces *both* heterotrophic protozoa and autotrophic algae.

1.1.1 Protozoan classification

The protozoa are subkingdom of the kingdom - Protista.[1] Depending on the taxonomic scheme followed, the protista consist of 7 phyla:

1. Sarcomastigophora,
2. Labyrinthomorpha,
3. Apicomplexa,
4. Microspora,
5. Ascetospora,
6. Myxozoa and
7. Ciliophora.

Almost all of these phyla have parasitic representatives, however, what might be considered as the most important parasitic protozoan species are found in the following two phyla.

Sarcomastigophora - *Order.* Amoebida - amoeba - simple direct life cycles. *Order.* Kinetoplastida - Flagellates- many species have complex life cycles often requiring an insect vector. Chief amongst the flagellates are trypanosomes.

Apicomplexa - Coccidial parasites (including malaria). The life cycles are complex and contain both asexual and sexual reproductive phases.

1.2.0 Parasitic Protozoa

There are more than 65,000 known species of protozoa, of which a fifth are parasitic. Parasitic protozoa are an extremely diverse group of unicellular organisms of the kingdom Protista. They are, in general, small, have short generation times, high rates of reproduction and a tendency to induce immunity to reinfection in those hosts that survive. A small number of these have evolved to parasitize humans and have had a profound effect on shaping the course of human history and evolution.[2] Parasitic protozoa are in no way simple or degenerate and adaptions to parasitism frequently include complex life cycles and specialized ways of entering and maintaining themselves in their hosts. Despite the great advances in combating of infectious diseases over the past century, these parasites continue to inflict a tremendous social and economic burden on human societies, particularly in tropical and subtropical regions of the world. In addition to being fascinating in its own right, the study of protozoan parasite biology may lead to important practical developments such as novel chemotherapeutic agents and vaccines.

1.3.0 Parasitic Diseases

It has long been recognized that various specific pathological conditions are due to the presence and action of parasites in the human body but the part played in the causation of the so-called infective diseases by various members of protozoan have an overwhelming impact on the public health in developing countries. The World Health Report of 2000 lists 55 million deaths in 1999 due to all causes. Tragically, specific protozoal diseases

occupy a prominent place in this list. 1,086,000 people died from malaria, 66,000 from African trypanosomiasis, 21,000 from Chagas' disease, 14,000 from schistosomiasis and 57,000 from leishmaniasis.[3] These sobering statistics are further supplemented with the burden of disease data expressed in DALYs, disability-adjusted life years robbed by disease: 45 million by malaria, 2 million by trypanosomiasis; 0.7 million by Chagas' disease; 2 million by schistosomiasis; and 2 million by leishmaniasis. The geographical distribution of these diseases is mainly centered around the tropics, where the climate favors persistence of the vectors for transmission. Apart from drug treatment of infected patients the disease is generally fought via its vector, the tsetse fly. Vector control is possible, but eradication is probably not.[4] The flies are controlled with traps and with aerial insecticide spraying. The latter measure is quite costly and therefore a renewed interest in trapping to control tsetse fly populations is emerging. It is, however, not an easy task to simultaneously cover a large population and properly monitor for a sufficient period of time. Vaccine development has not been successful thus far, mainly because parasites are experts at evading or dysregulating the human immune system. Chemotherapy to fight the aforementioned diseases exists, but is plagued by high toxicity and increasing resistance. Adding insult to injury, pharmaceutical companies steer away from vaccine and drug development to fight tropical diseases because such programs do not fit in their profit-driven business model.

1.3.1.0 Malaria

Malaria is the world's most important parasitic disease and ranks among the major health and developmental challenges facing large parts of the world, including some of the poorest countries.

Malaria is caused by blood parasites of the genus *Plasmodium*. There are approximately 156 named species of plasmodium which infect species of vertebrates. Four are known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. *P. falciparum* and *P. vivax* cause the majority of infections, whereas the prevalence of *P. ovale* and *P.malariae* infection is low. *P. falciparum* is distributed globally but is especially common in Africa. It is by far the most aggressive species as it can progress to severe or complicated malaria, which are characterized by vital organ dysfunction. This manifests as coma,

acidosis, hypoglycemia, renal failure, pulmonary edema, or severe anemia. Nearly all malaria deaths result from *P. falciparum* infections. *P. vivax* can cause recurring and debilitating infections but rarely kills. It does, however, cause low birthweight, a major determinant of infant mortality.

1.3.1.1 Geographical Distribution:

Malaria is endemic in 91 countries, affecting 40% of the world's population (Figure 1).[5]

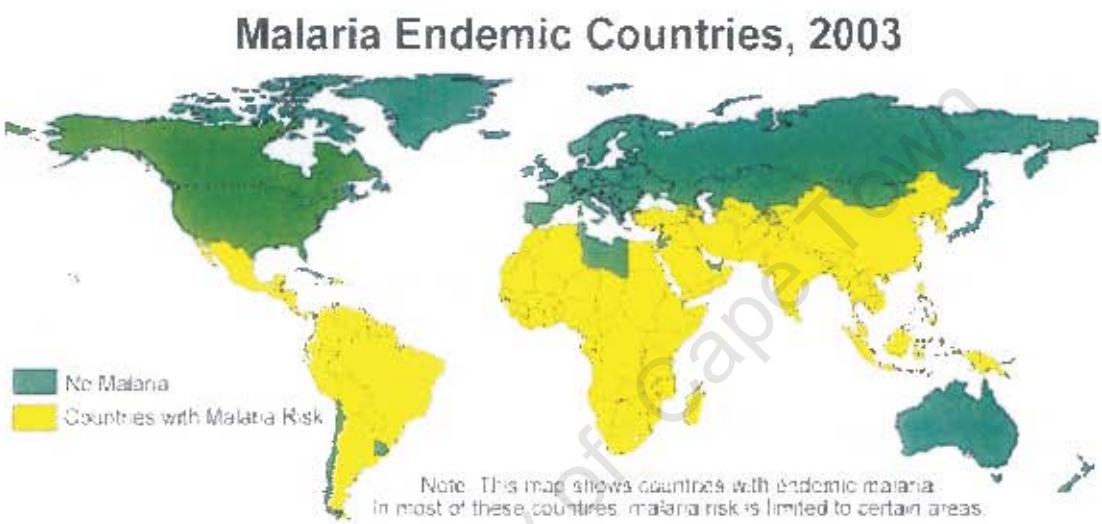


Figure 1. Malaria endemic countries[5]

It is generally prevalent in areas where environmental conditions allow parasite multiplication in the vector. Thus, malaria is usually restricted to tropical and subtropical areas (Figure 1). More than 90% of the world's malaria occurs in sub-sahara Africa. However, this distribution might be affected by climatic changes, especially global warming, and population movements.[6]

1.3.1.2 Life cycle

Knowledge of the life cycle of the malaria parasite is foundational to understanding the methods of prevention, treatment, and research endeavors. Interrupting the life cycle will prevent malaria, but this has proven more difficult than it would appear.

The life cycle of all species of human malaria parasites is essentially the same. It exhibits a complex life cycle that comprises an exogenous sexual phase (sporogony) with

multiplication in certain Anopheles mosquites sexual phase and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host (human) (Figure 2). The major phases of the life cycle are: liver stage, blood stage, sexual stage and sporogony. Malaria infection in the human host starts when the sporozoites are injected in the blood stream during a blood meal by an infectious female anopheles mosquito. The sporozoites enter the liver cells and multiply to form about 30,000 merozoites each. After 5 days, the merozoites are released into the blood stream. They enter red blood cells and develop through the so called ring, trophozoite, and schizont stages (Figure 2).

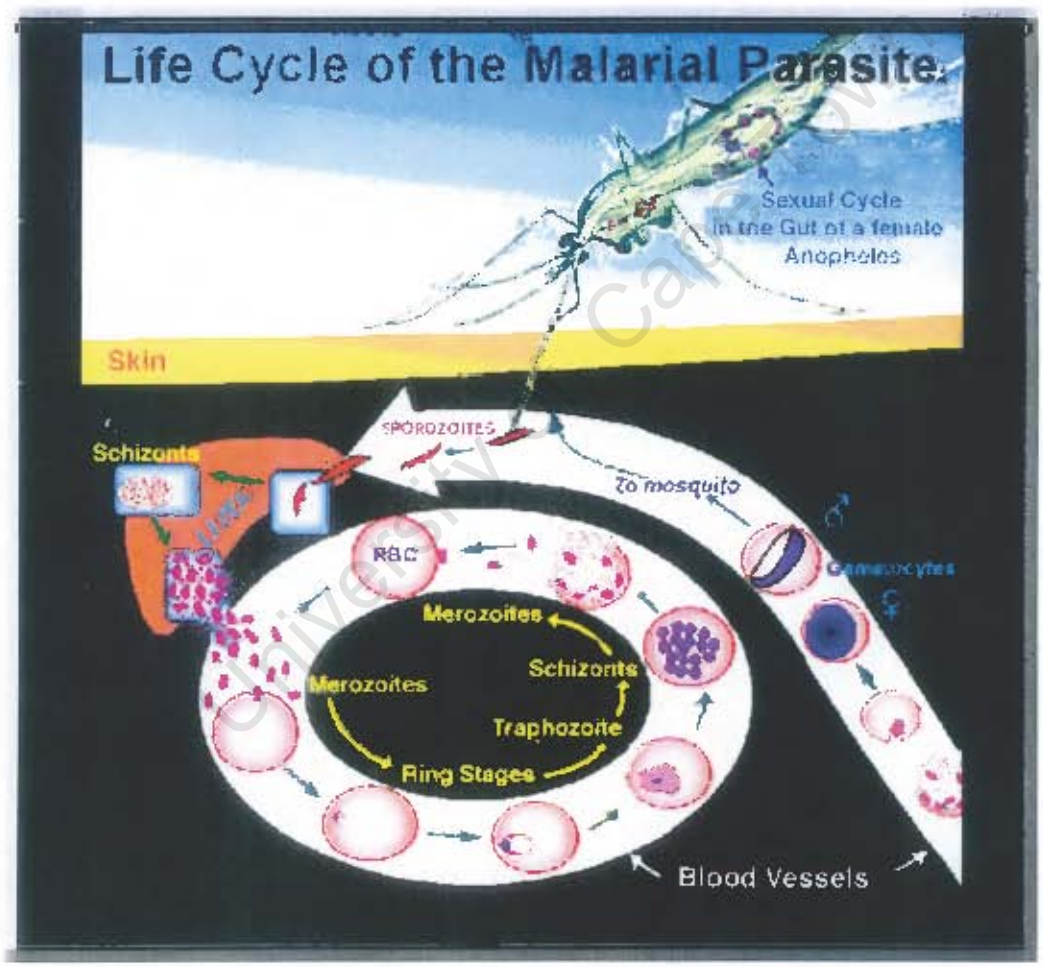


Figure 2. Life cycle of the malaria parasite[7]

The erythrocyte provides the parasite with a safe haven from the host's immune system, but presents certain logistical problems with regard to access to nutrients and disposal of

waste products. Parasite growth is supported by ingestion of host hemoglobin. During a 48 h (or 72 hr for *P. malariae*) cycle the parasite divides to produce 16-20 schizonts, releasing cell debris, which causes a febrile episode in the host. With minutes, the merozoites invade new red blood cells and the life cycle continues. After several cycles, some of the intraerythrocytic parasites develop into sexual gametocytes. The gamates are ingested when the mosquito bites an infected individual. Inside the mosquito, female and male gametes come together to form a zygote- oocyte (sporocyst). Division and multiplication of the sporocyst takes place to produce many sporozoites. These then migrate to the salivary gland, waiting to re-infect again. This completes the life cycle.

1.3.1.3 Treatment and prevention of malaria

Chemotherapy of malaria targets the parasites. It has been shown that the liver is the ideal target for drugs and vaccines to treat and prevent malaria because the liver stage is the longest single stage in the parasite's life cycle.[8] The parasite takes at least five and a half days to develop from entry into the liver stages that are released into the blood. And during those five and a half days the patient is not ill. So it's a tremendously important window to attack the parasite. However, it is extremely difficult to study the liver stages.

1.3.1.4.0 Anti-malarial drugs

Anti-malarial drugs can either be classified according to structure or anti-malarial activity. The system of classification used in this report is according to the structure.[9]

Classification according to the structure:

1. 4-Aminoquinolines: chloroquine **1** and amodiaquine **2**.
2. Aryl amino alcohols: quinine **3**, mefloquine **4** and halofantrine **5**.
3. Folate synthesis inhibitors: Type 1 - competitive inhibitors of dihydropteroate synthase-sulphone (dapsone) **6** and sulphonamide (sulfadiazine) **7**; Type 2 - inhibit dihydrofolate reductase - biguanides like proguanil **8** and chloroproguanil **9**; diaminopyrimidine like pyrimethamine **10**.
4. 8-Aminoquinolines: primaquine **11** and quinocide **12**.
5. Antibiotics: tetracycline **13** and doxycycline **14**.

6. Peroxides: artemisinin or qinghaosu **15** and its derivatives such as artemether **16** and artesunate **17**.
7. Naphthoquinones: atovaquone **18** and
8. Iron chelating agents: desferrioxamine **19**.

The chemical structures of the above listed drugs are depicted in Figure 3 on page 8.

1.3.1.4.1.0 4-Aminoquinolines

The most important of these drugs, chloroquine (CQ) **1** (Figure 3, p.8), has been the mainstay of antimalarial chemotherapy for the last 50 years. It is a very potent schizonticidal drug against the erythrocytic stage of all the 4 Plasmodium species. The compound eradicates parasites rapidly, has minimal toxicity, is a cheap and well tolerated drug for the treatment and prophylaxis of malaria. However, resistance to chloroquine has been steadily increasing since the drug's initial use in South America and Southeast Asia in the late 1950s. Chloroquine resistance is now widespread in most *P. falciparum*-endemic areas of the world (see map on page 4).[10] Thus, the use of chloroquine for presumptive treatment of falciparum malaria or for chemoprophylaxis is usually no longer appropriate.[11] Moreover, resistance to chloroquine of *P. vivax*, the second most lethal human malaria parasite, is increasing in South Asia.[12] Amodiaquine **2** (Figure 3, p.8), is used to treat chloroquine-resistant malaria in developing countries but it is quite toxic, and resistance to it is also common.

Despite years of use, the mechanism of action of chloroquine remains uncertain. Chloroquine and other quinoline drugs target a unique, essential metabolic pathway of the parasite, the ingestion and degradation of host cell hemoglobin (Hb) within the parasite's food vacuole. While globin-derived amino acids are used by the parasite, the remaining free heme moiety must be got rid of: iron(II)protoporphyrin IX (Fe[II]PPIX) is oxidized to iron(III) protoporphyrin IX (Fe[III]PPIX) and, for the most part, converted into a crystalline pigment called haemozoin.[13] It is generally accepted that chloroquine prevents heme disposal through the formation of complexes with Fe[III]PPIX. Nuclear magnetic resonance, UV-visible and Mössbauer spectroscopy data concur to show that π-π interaction between the drug and the electronic system of hematin governs the

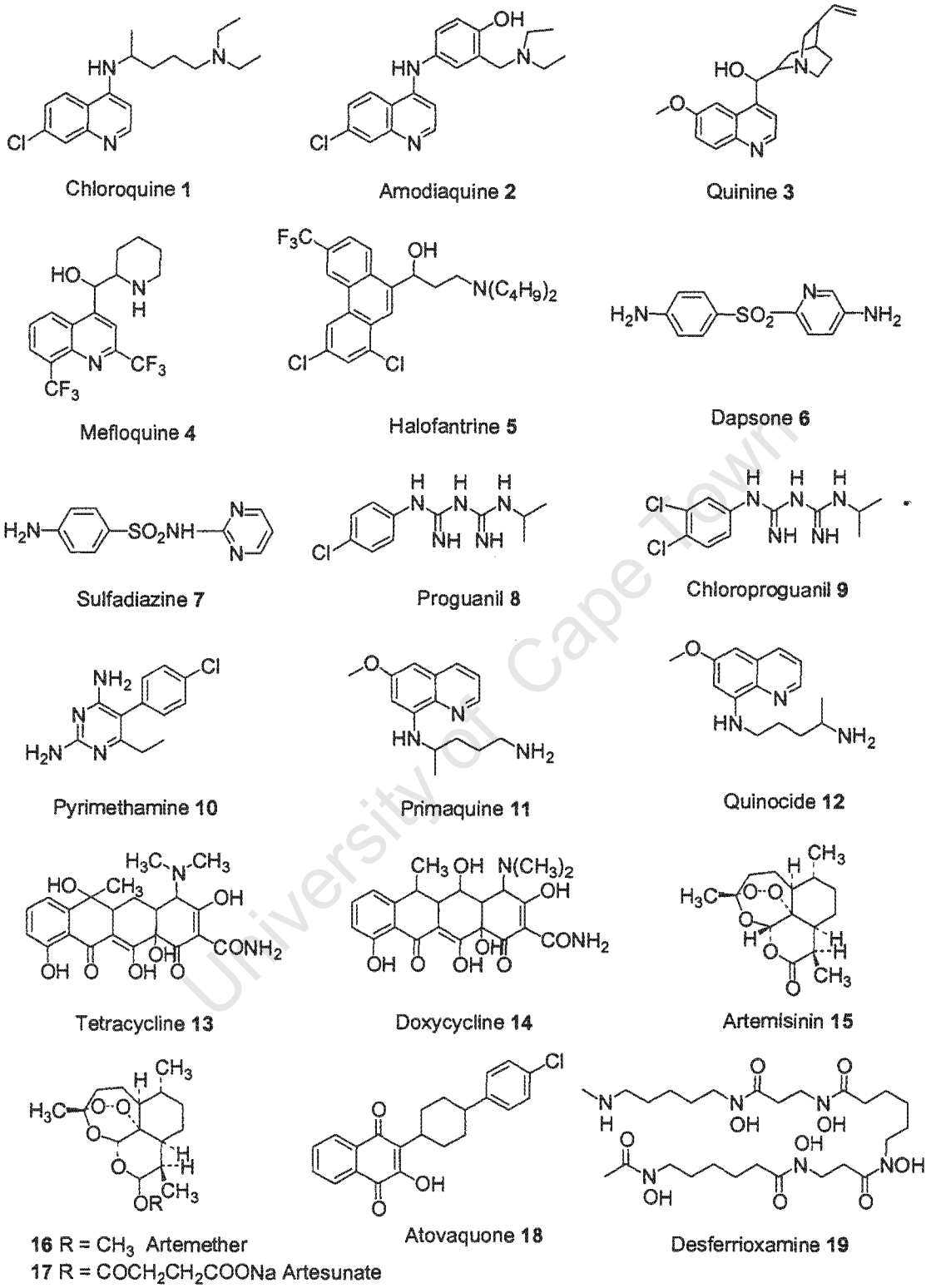


Figure 3. Chemical structures of some anti malaria drugs

formation of these adducts. The accumulation of free heme and/or of chloroquine-Fe(III)PPIX adducts is thought to generate oxidative stress, leading to peroxidation of parasite membrane lipids and parasitic death.[13] The key factors in this mechanism are the ability of chloroquine to concentrate markedly in the food vacuole and its avid binding to heme.

1.3.1.4.1.1 Resistance to Chloroquine

As with any drug, knowledge of its mode of action is an essential component of understanding how an organism becomes resistant, although its ultimate target and the molecules mediating resistance to it need not be directly connected. In resistant parasites, chloroquine is observed to accumulate to much lower levels. This could in principle arise by a lower rate of influx, a higher rate of efflux, or combination of both, and would also be influenced by any reduction in binding affinity of the drug for its ultimate target. While a higher rate of efflux was originally proposed to be the major factor, later studies indicated that changes in uptake better explained the observed kinetics.[14]

1.3.1.4.2 Aryl amino alcohols

Quinine **3** (Figure 3, p.8), an alkaloid isolated from cinchona bark in 1820, remains the best antimalarial drug for treating chloroquine-resistant *P.falciparum*. It is now taking its original place, since resistance has developed against chloroquine. Quinine is a potent blood schizonticidal drug against the four plasmodia species. It is an erythrocytic drug and has no effect on the exo-erythrocytic phase or the gametocytic phase.[15] Its mechanism of action is similar to chloroquine, it causes cytotoxicity of the parasite by inhibiting plasmodial haem polymerase with the subsequent build up of toxic haem. Side effects are numerous and could be fatal. These include: irritation of the gastric mucosa, nausea, vomiting, Cinchonism syndrome (tinnitus, headaches, blindness) and hypersensivity reactions. Sometimes hypertension occurs following really high doses, also cardiac dysrhythmias and severe CNS disturbances such as delirium and coma. Mefloquine **4** (Figure 3, p.8) is a blood schizonticidal of the erythrocytic malaria which also kills hypnozoites if given as a combination treatment with primaquine. It is used for uncomplicated chloroquine-resistant *P.falciparum* malaria and as a short-term chemoprophylaxis when entering chloroquine resistant zones. Halofantrine **5** (Figure 3,

p.8) is a blood schizonticidal against the erthrocytic *P. falciparum* resistant to chloroquine. It is used to treat the acute form of uncomplicated, multi-resistant *falciparum* malaria and as a 'stand by' drug if chemoprophylaxis fails and there is no medical aid available.

1.3.1.4.3 Folate synthesis inhibitors.

These are drugs that affect the synthesis and utilisation of folate. Examples are pyrimethamine (2,4,diaminopyrimidine) **10**, chloroproguanil **9** and proguanil **8** (Figure 3, p.8). They act by inhibiting the dihydrofolate reductase necessary for synthesis of tetrahydrofolate, a precursor in the parasite DNA synthesis.[16] Sulfonamide (sulfadiazine **7**) and sulfones (dapsone **6**) (Figure 3, p.8) act by competing for the enzyme, dihydropteroate synthetase with para-aminobenzoic acid and therefore inhibit folate synthesis.[16] They act on erythrocytic *P. falciparum*, but not on sporozoites or hypnozoites. It is a combined treatment that is given after a 7-day course of quinine in an acute attack of *P.falciparum* where there is a chloroquine resistance. It is used prophylactically against chloroquine resistant *P. falciparum* or *P. vivax*. Proguanil or pyrimethamine- dapsone (fansidar) is combined with chloroquine to prevent transmission by killing gametocytes.

1.3.1.4.4 8-Aminoquinolines

These drugs are for radical cure. Primaquine **11** and quinocide **12** (Figure 3, p.8) are potent tissue schizonticidal drugs affecting the mitochondria of the exo-erythrocytic forms and gametocytes of an avian form of *P. falciparum*. It has been suggested that 8-aminoquinolines such as primaquine acts by inhibiting the electron transport chain of the parasite, though the precise metabolic interaction is still unclear.[16] They do not have any effect on the erythrocytic forms, sporozoites or hypnozoites, therefore it is not a radical cure for *vivax* and *ovale* malaria. They are rapidly absorbed and metabolized when taken orally.

1.3.1.4.5 Antibiotics

These drugs are active against the primary exo-erythrocytic and asexual blood stages of *P. falciparum*. It has been suggested that antibiotics act on the mitochondrial ribosomes

of the parasite, inhibiting protein synthesis.[16] Tetracycline and doxycycline are often combined with pyrimethamine or quinine for 100% cure.

1.3.1.4.6 Peroxides

Artemisinin **15** (Figure 3, p.8) and related compounds are the most effective (new) drugs. Artemisinin was isolated in 1972 from *Artemisia annua*, a plant used in China for centuries to treat fever and seems to act by inhibiting protein synthesis.[16,17] Artemisinin derivatives (artesunate **17**, artemether **16**) (Figure 3, p.8) have been synthesized and have undergone extensive clinical testing. These compounds, which are already widely used in some areas, are potent, rapidly acting antimalarials that are effective against chloroquine-resistant *P. falciparum*.[18] Because recrudescences of infection after treatment are common, however, artemisinin and related compounds might best be used in combination with another drug.

1.3.1.4.7 Naphthoquinones

Atovaquone **18** (Figure 3, p.8) is a highly substituted naphthoquinone derivative and a structural analogue of coenzyme Q (ubiquinone) in the mitochondrial electron transport chain. It acts against ubiquinol-cytochrome *c* oxidoreductase (complex III), inhibits electron transport and collapses mitochondrial membrane potential, which is required for a number of parasite biochemical processes.[19] The drug has potent antimalarial activity but suffers from rapid selection of resistant parasites with mutation in the target enzyme, and so is inappropriate as a monotherapy. Atovaquone has, however, proved to be surprisingly effective in combination with the antifolate proguanil for both chemoprophylaxis and therapy of falciparum malaria.

1.3.1.4.8 Iron chelating agents

Iron chelators such as desferrioxamine (DFO) **19** (Figure 3, p.8) enhance the clearance of parasites in mild malaria and, in conjunction with quinine and Fansidar, hastens recovery from deep coma in severe falciparum malaria.[20a,b] The mechanism of action of iron chelators is still debated. Several hypotheses have been suggested: iron binding; the inhibition of iron-dependent enzymes (such as ribonucleotide reductase, phosphoenol pyruvate carboxykinase, dihydroorotate dehydrogenase, cytochrome c oxidase, or

superoxide dismutase) and the formation of free radicals.[20c-g]

1.3.2.0 African Trypanosomiasis

African trypanosomiasis is a parasitic infection transmitted to humans through the bite of a tsetse fly of the genus *Glossina*.[21] The parasite that causes African trypanosomiasis or sleeping sickness is called the trypanosome. Trypanosomes are successful parasites which manage to escape the host's immune response; this happens by a very complex mechanism of antigen switching. There are two forms of African sleeping sickness, caused by two different parasites:

Trypanosoma brucei gambiense, which causes a chronic infection lasting years and affecting countries of Western and Central Africa

Trypanosoma brucei rhodesiense, which causes acute illness lasting several weeks in countries of Eastern and Southern Africa.

African trypanosomes also infect wild and domestic animals (*T. brucei brucei*, *T. vivax* and *T. congolense*) and are responsible for "nagana" in cattle which can be transmitted to humans from these animals.

1.3.2.1 Symptoms associated with the disease

The causative parasites dwell free in the lymphatic fluid and bloodstream of their mammalian hosts and then invade the CNS and other organs. Early symptoms may include fever, weakness, headache, joint pains, rash, itching, edema, and enlargement of lymph nodes. In the late stages of the disease, when the parasite has invaded the CNS, the sufferer becomes apathetic, has difficulty concentrating, and experiences sudden mood swings. In the final stages, the victim becomes so lethargic that even simple activities such as eating and speaking require extraordinary effort. The person ultimately becomes comatose and dies.

1.3.2.2 Life cycle

The life cycles of most African trypanosomes are similar. In the vertebrate host (e.g. human) the parasite grows and reproduces as a trypomastigote form in the blood of the infected host. When a vector ingests trypomastigotes during a blood meal, the trypomastigotes transform into epimastigotes and grow and reproduce in the vector's gut

or salivary glands. The epimastigotes transform into trypomastigotes, and these infect a new host the next time a vector takes a blood meal.

1.3.2.3 Chemotherapy

The treatment of human African trypanosomiasis has always been unsatisfactory. None of the drugs discovered in the first half of the 20th century, such as Atoxyl **20**, tryparsamide **21**, suramin **22** or pentamidine **23**, are able to cross the blood-brain barrier (BBB) in sufficient quantity to prevent relapses of the late-stage cases, which are characterized by a progressive invasion of the CNS.[21,22] The introduction of melarsoprol **24** in 1949 spectacularly improved this situation, but severe adverse events frequently occur and an increasing number of infections are refractory to treatment.[23] The only drug brought to the market over the past 40 years, DL-α-difluoromethylornithine (DFMO) **25**, is not effective against *Trypanosoma brucei rhodesiense* infections and is very expensive.

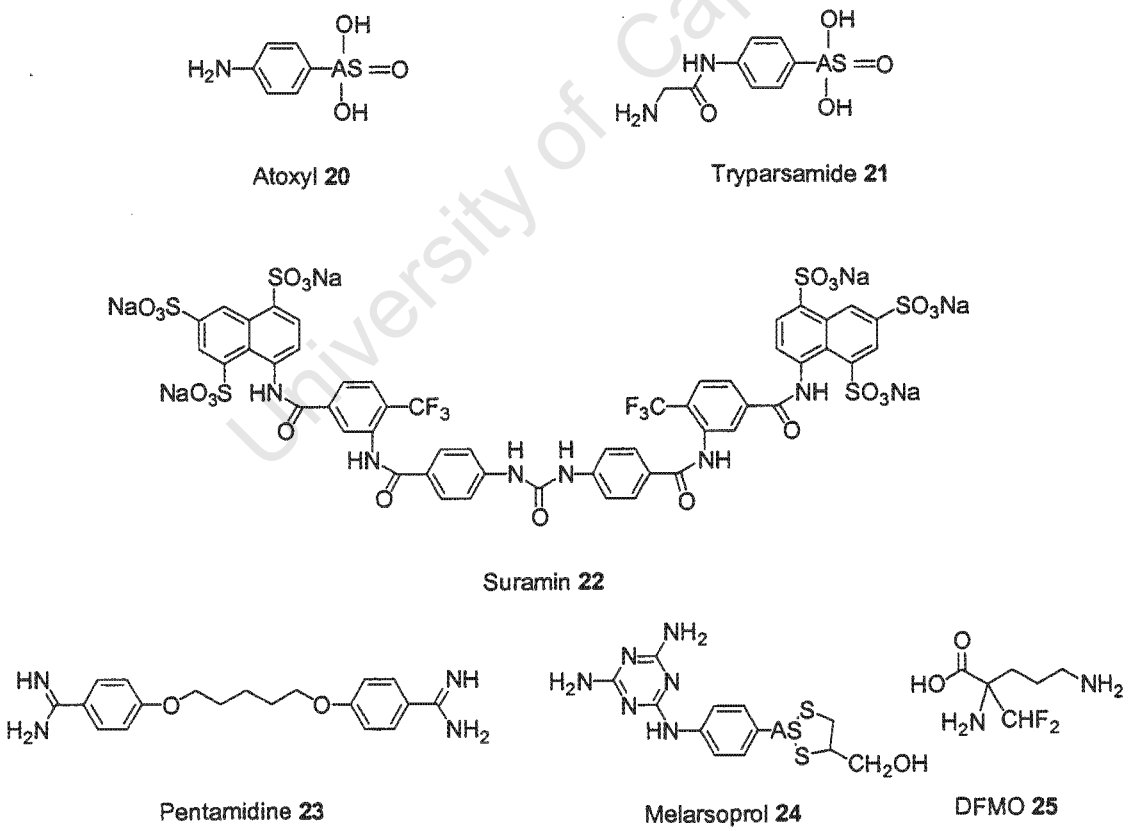


Figure 4. Chemical structures of drugs for African trypanosomiasis

Furthermore, there is a constant threat that production of the currently used drugs will be interrupted because it is not profitable. Treatment depends upon the stage of disease and many of the drugs used to treat African trypanosomiasis have significant toxicities.

Before the parasite becomes manifest in the CNS, suramin **22** and pentamidine **23** are the drugs of choice against rhodesiense and gambiense forms of the disease, respectively. Late-stage disease is treated with the melaminophenyl arsenical drug melarsoprol **24**. Eflornithine **25** is a drug of choice for gambiense. An alternative is pentamidine **23** or tryparsamide **21** with suramin **22**.

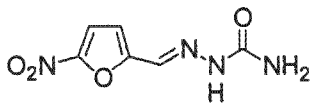
1.3.3.0 American Trypanosomiasis

The disease, also known as Chagas disease, is caused by *Trypanosoma cruzi*, a flagellated protozoan parasite which is transmitted to humans by a blood-sucking reduviid bug which deposits its infective faeces on the skin at the time of biting.[24] Chagas' disease is found throughout much of central and northern South America, Central America, and Mexico. It is characterized by an acute phase in which several symptoms occur: anemia, loss of strength, nervous disorders, chills, muscle and bone pain, and varying degrees of heart failure. Death may occur 3-4 weeks after infection. The chronic form of Chagas disease is seen usually in adults and is characterized by dysfunctions in the central and peripheral nervous systems. Many patients die of heart failure due to an enlargement of the heart and subsequent lack of cardiac muscle tone. Others suffer heart damage because the parasite damages the nervous supply to the heart.

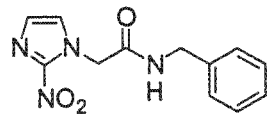
1.3.3.1 Chemotherapy

The wide tissue and cell distribution of intracellular *T. cruzi* amastigotes during both acute and chronic phases makes Chagas disease a more difficult subject for drug targeting in comparison to human African trypanosomiasis where there is a defined problem of the brain blood barrier. Perhaps because of this, little attention has been paid to drug delivery and pharmacokinetics of anti-*T.cruzi* drugs since the studies of Avila *et al.* [25]

Nifurtimox **26** and benznidazole **27** (Figure 5, p.15), which kill the parasite when it is in the blood, are the two drugs currently in use for the treatment of the disease. However, there is no known treatment for the intracellular stages of the parasite.



Nifurtimox **26**



Benznidazole **27**

Figure 5. Chemical structures of drugs for the treatment of Chagas disease.

CHAPTER 2

INTRODUCTION II

PARASITIC CYSTEINE PROTEASES

2.1.0 Proteases

Proteases or proteolytic enzymes catalyse the cleavage of peptide bonds in proteins. They are enzymes of class 3, the hydrolases, and subclass, the peptide hydrolases or peptidases. The peptidases constitute a large family divided as endopeptidases or proteinases and exopeptidases according to the point at which they break the peptide chain.[26]

2.1.1 Exopeptidases

The exopeptidases act only near the ends of polypeptide chains at the N or C terminus. Those acting at a free N terminus to liberate a single amino acid residue are called aminopeptidases whereas those acting at a free C terminus to liberate a single amino acid are known as carboxypeptidases.

2.1.2 Endopeptidases

Endopeptidases act preferentially in the inner regions of peptide chains away from the N and C termini. The presence of free α-amino or α- carboxyl groups has a negative effect on the activity of the enzyme.

2.1.3 Function of proteases

Proteases represent a class of enzymes with important roles in physiological processes. Identification and characterization of protease-mediated processes in parasitic protozoa is progressing at a rapid rate. In these organisms, proteases carry out "housekeeping" tasks common to many eukaryotes as well as functions highly specific to the parasite life cycles. Central roles have been proposed for proteases in diverse processes such as host cell invasion, catabolism of host proteins, differentiation, cell cycle progression, signaling and both stimulation and evasion of host immune responses. Although host homologues exist, parasite cysteine proteases have distinct structural and biochemical properties including, pH optima and stability, the alteration in peptide loops or domain extensions, diverse substrate specificity and cellular location. The disparate nature of parasite

cysteine proteases compared to the host orthologous proteins has opened up opportunities for chemotherapy. This makes proteases a valuable target for new pharmaceuticals.

2.1.4 Binding of a protease to an active site

Before hydrolysis of peptide bonds takes place, a protease must bind the protein or peptide substrate in its active site. The binding efficiency is a function of both the respective chemical environments that the protease sub-sites create, and the chemical nature of the peptide that interacts directly with the active site groove. Important factors affecting interactions include; size, polarity, charge, hydrophobicity and accessibility. Although a single peptide bond is cleaved during catalysis, a number of amino acids on either side of the cleavage site serves to fix the fissile bond in the correct position for cleavage to occur (Figure 6).

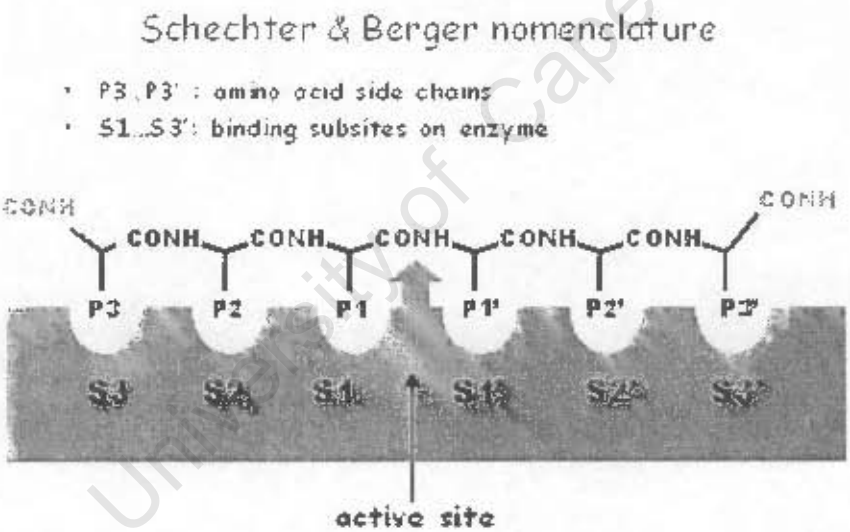


Figure 6. The Schechter and Berger nomenclature.[27]

For identifying substrate residues and sub-sites, the convention of Schechter and Berger[27] is often used. The sub-sites on the protease are called S (for sub-sites) and the substrate amino acid residues are called P (for peptide). The amino acid residues of the N-terminal side of the scissile bond are numbered P3, P2, P1 and those residues of the C-terminal side are numbered P1', P2', P3', etc. The P1 and the P1' residues are those two residues

closest to the scissile bond. The sub-sites on the protease that complement the substrate binding residues are numbered S3, S2, S1, S1', S2', S3', etc.

2.2.0 Further classification of proteases

Proteases have been further divided into groups on the basis of the catalytic mechanism used during the hydrolytic process.[26] The main catalytic types are serine, threonine, aspartate, metallo and cysteine proteases. Other 'undefined' or cryptic proteases may also exist. *This dissertation is focused on the cysteine protease class referred to as thiol or sulfhydryl proteinases.* Cysteine proteases are proteins containing two principal catalytic amino acid residues, Cys and His, which are involved in the cleavage of peptide bonds connecting the amino acid residues of a protein or peptide substrate. These proteases utilize the cysteine thiol for catalytic activity.

2.2.1 Classification of cysteine proteases

Cysteine proteases of parasitic organisms are classified into two main categories known as clans, CA and CD.[28,29] Clan CA and CD are further divided into families according to the nature and linear organization of the catalytic residues along the primary sequence. Important parasitic proteases belong to the papain superfamily or clan CA. This superfamily corresponds to the best-known cysteine peptidases and has the catalytic residues Cys-25 and His-159 conserved in all of its members. Sequence analysis has revealed that other higher plant cysteine proteinases and cathepsins B, H, L and S from mammalian lysosomes are members of the papain C1A family. In addition, bleomycin (family C1B), calpain (family C2), streptopain (family C10) and viral proteases also belong to this superfamily. Papain from from *Carica papaya* is the most studied cysteine proteinase that represents the typical member of this superfamily.

2.2.2 Active site of Papain

Two residues are involved in the catalytic process (Cys 25 and His 159). Papain is an endopeptidase and the preferred P1 residue is Arg or Lys. The thiol-group is enhanced as a nucleophile owing to the close proximity of an active site histidine which acts as a proton donor/general base. The imidazole group of the histidine polarizes the sulfhydryl group of the cysteine side chain and enables deprotonation even at neutral to weakly

acidic pH, [30, 31] producing a thiolate-imidazolium ion pair which is highly nucleophilic (Figure 7).

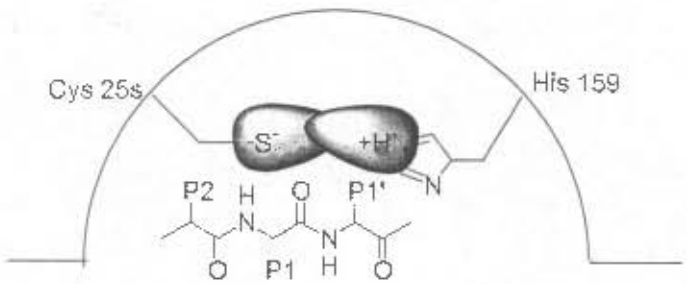


Figure 7. Schematic representation of the close spatial proximity of Cys-25 and His-159 in the active site residue of papain

2.2.3 Mechanism of hydrolysis

Cysteine proteases have mechanistic similarities with serine proteases but the thiolate ions generated during catalysis are better nucleophiles than corresponding species in serine proteases. The steps involved are as follows: (Figure 8);

Step **A**. Prior to hydrolysis the enzyme forms hydrogen bonds to the carbonyl oxygen of a substrate. The hydrogen bond donors are NH groups of the backbone or side chain (Cys, Gln) amides. This hydrogen bonding makes the carbonyl carbon more electropositive and thus more susceptible to nucleophilic attack. Then, the thiolate anion attacks the carbonyl carbon of the peptide bond to be cleaved and produces a tetrahedral intermediate. The oxyanion of the tetrahedral intermediate is stabilized by interaction with several hydrogen bond donors, in what is commonly referred to as the oxyanion hole.[32]

Step **B** and **C**. Esterification of the thiol makes the imidazolium ion sufficiently acidic (pKa = 4, general acid catalysis) to protonate the nitrogen of the leaving group and produces an acyl enzyme.[33]

Step **D**. Deacylation may occur *via* a general base-catalyzed mechanism, where the imidazole nitrogen polarizes a water molecule which then attacks the acyl enzyme at the carbonyl carbon.

Step **E**. Cleavage of the substrate (as free acid) and regeneration of the enzyme *via* a second tetrahedral intermediate takes place.

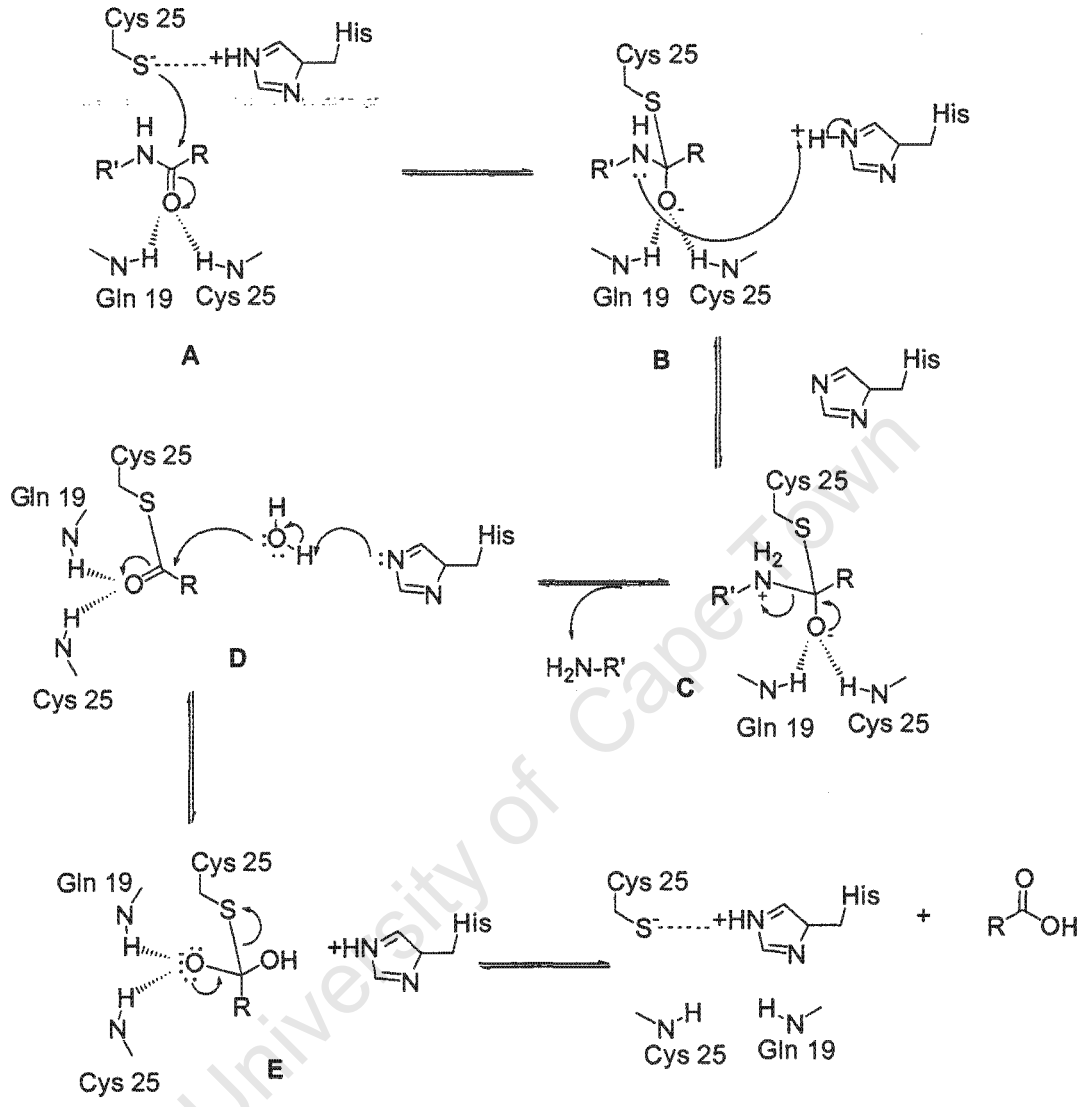


Figure 8. Proposed catalytic mechanism for cysteine proteases

2.3.0 Cysteine proteases of parasitic organisms

Parasite cysteine proteases have been shown to perform numerous indispensable roles in the biology of many species of parasites such as in replication, cell differentiation, signaling, host invasion etc.[26,34] Promising initial experiments using cysteine protease inhibitors have validated the suitability of these proteases as drug target candidates for antiparasitic chemotherapy. This section focuses on the physiological function of

papain-like cysteine proteases for three major parasitic diseases (malaria, African trypanosomiasis and American trypanosomiasis).

2.3.1 Malarial cysteine proteases

Three papain-family cysteine protease sequences named falcipain-1, falcipain-2, and falcipain-3 have been identified in *P. falciparum*.[35] In concert with two aspartic proteases named plasmepsins I and II,[36] falcipain-2 and falcipain-3 play a critical role in haemoglobin hydrolysis to provide amino acids for the development of the parasite in erythrocytes.[37-39] Falcipain-2 and falcipain-3 are principally expressed in the merozoite acidic food vacuole where haemoglobin hydrolysis occurs.[40,41]. Both proteases require a reducing environment and acidic pH for optimal activity and both prefer peptide substrates with leucine at the P2 position. The proteases differ, however, in that falcipain-3 undergoes efficient processing to an active form only at acidic pH, is more active and stable at acidic pH, and has much lower specific activity against typical papain-family peptide substrate. Thus, falcipain-3 is a second *P. falciparum* haemoglobinase that is particularly suited for the hydrolysis of native haemoglobin in the acidic food vacuole.[41]

In recent years, it has been shown that specific inhibitors of falcipain-2 block haemoglobin degradation and prevent parasite development. The degree of inhibition of falcipain-2 by fluoromethyl ketones[39] and vinyl sulfones[42] correlated with their inhibition of haemoglobin degradation and parasite development, support the hypothesis that falcipain-2 is the cysteine protease required for haemoglobin degradation and thus is a potential target for antimalarial drugs.

2.3.2 Cruzain

Efforts to characterize novel drug targets in *T. cruzi* identified a papain-like cysteine protease, named cruzain (also known as cruzipain).[43-45] Cruzain is the major proteolytic enzyme present in all stages of the life cycle of the parasite.[44,46] Biotin-labeled peptidyl diazomethane inhibitors of cruzain revealed the highest expression levels in the epimastigote.[47] At present, the structures of cruzain are the only ones solved and published among falcipain-2 and rhodesain cysteine proteases. The structure of cruzain revealed the presence of a Glu^{43} residue at the bottom of the S pocket, the major

determinant of substrate specificity.[48] As with human cathepsin B which contains Glu at the same position, the cruzain S2 specificity pocket is able to bind both P2 arginine and phenylalanine residues.[49] Inhibition of cruzain has been shown to impair *in vitro* host cell invasion and to block amastigote replication as well as trypomastigote-amastigote differentiation, thereby arresting the intracellular development.[50-53] *In vitro* studies have shown that cruzain is involved in the activation of the kinin receptors of the kinin cascade.[54,55]

2.3.3 Rhodesain

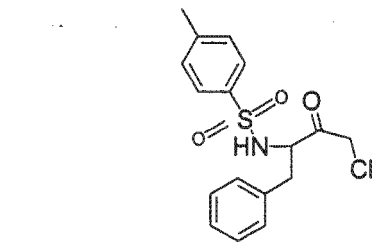
Over the past decade, studies have demonstrated the key role of trypanosomatid thiol-proteases in rational drug or vaccine design. Rhodesain is major cysteine protease of *T. b. rhodesiense* and has been identified in all life-cycle of the parasite, particularly during the infective stage of parasite development.[56-58] It has been shown that inhibition of rhodesain by small synthetic inhibitors results in the death of the parasite, demonstrating the validity of trypanosomal cysteine proteases as therapeutic drug targets. As a major proteolytic enzyme in the trypanosomatid lysosome compartment, it is likely to play a role in the degradation of phagocytozed host proteins.

2.4.0 Inhibitors of cysteine proteases

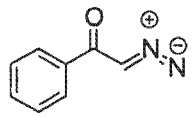
A variety of cysteine protease inhibitor templates have been discovered which may be divided broadly into three mechanistically distinct groups.[59]

2.4.1 Irreversible inhibitors

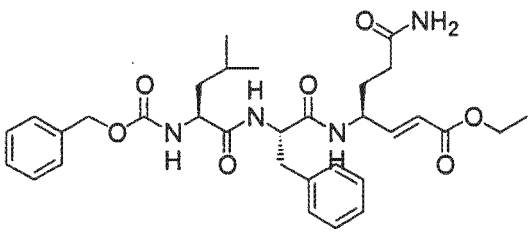
This is a series of active site titrants whose mechanism of inhibition is based upon the irreversible alkylation of the thiol moiety of the active site cysteine residue to form a thioether adduct. This inhibitor class includes peptide derived α-haloketones **28**, α-diazoketones **29**, and α, β-unsaturated ester **30** and vinyl sulfone **31** Michael acceptors (Figure 9, p.23).



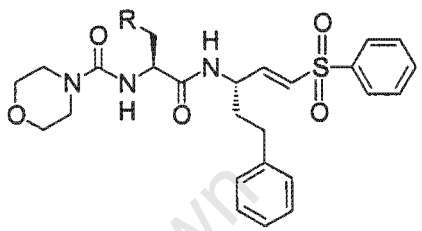
Chloromethyl ketone **28**



Diazomethyl ketone **29**



α, β–unsaturated ester **30**

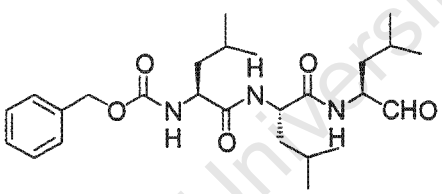


Vinyl sulfone **31**

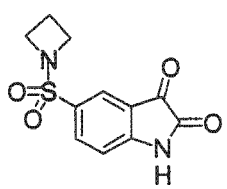
Figure 9. Chemical structures of some irreversible cysteine protease inhibitors

2.4.2 Reversible inhibitors

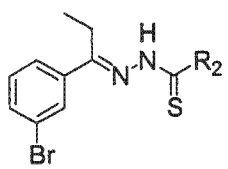
This inhibitor class forms a covalent, yet reversible, transition state-like intermediate with the active-site cysteine residues. Examples of this series include carbonyl based peptidyl aldehyde **32**, isatin sulfonamide **33**, thiosemicarbazone **34**, and urea **35** (Figure 10).



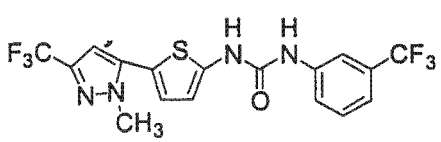
Peptidyl aldehyde **32**



Isatin sufonamide **33**



Thiosemicarbazone **34**

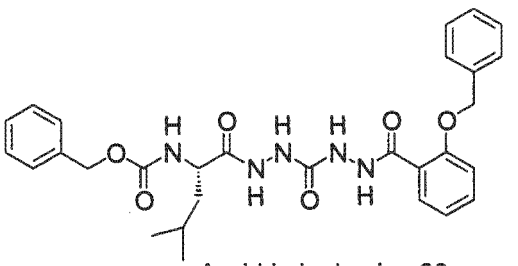


Aryl urea **35**

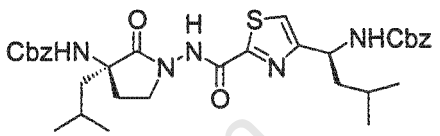
Figure 10. Chemical structures of some reversible cysteine protease inhibitors

2.4.3 Slow turnover inhibitors

This is a series of inhibitors that form a stable thioacyl-enzyme complex with the active site cysteine which, by nature of this intermediate, is slow to hydrolyze. The principle examples of these inhibitors are aza-substituted peptides acyl-bis-hydrazine **36** and diacyl hydrazine **37** (Figure 11).



Acyl-bis-hydrazine **36**



Diacyl hydrazine **37**

Figure 11. Chemical structures of some slow turnover cysteine protease inhibitors

CHAPTER 3

DESIGN AND SYNTHESIS OF MANNICH BASES

3.1.0 Introduction

This chapter is focused on the design and synthesis of four different classes of Mannich bases as potential new inhibitors of falcipain-2, cruzain and rhodesain. For each class of Mannich bases a small exploratory library of compounds was synthesized in order to primarily evaluate their inhibitory activities against the *Plasmodium falciparum* cysteine protease falcipain-2 and against chloroquine-resistant strains (W2 and K1) and a chloroquine sensitive strain (D10) of *Plasmodium falciparum*. The screens would also be extended to two other important homologous parasitic trypanosomal cysteine proteases cruzain and rhodesain. Furthermore, representative compounds would be examined in order to discern structure –activity relationships. From the information generated, one or more potential lead compounds may be identified for subsequent development.

3.1.1 The Mannich reaction

The Mannich reaction is a classic multi-component condensation reaction.[60] Basically, it requires three components: (i) ammonia, a primary or secondary amine, (ii) a nonenolizable aldehyde, usually formaldehyde and (iii) a compound with an active hydrogen atom or a reactive nucleophilic site. These components condense with concomitant release of water to produce a new base, known as a 'Mannich base', in which the active hydrogen is replaced by an aminomethyl group (equation 1). It is, therefore, also known as an aminomethylation reaction.

$$R\text{-}H + CH_2O + HNR_1R_2 \xrightarrow{(-H_2O)} R\text{-}CH_2\text{-}NR_1R_2 \qquad \text{(equation 1)}$$

The aminomethylation reaction was first described by van Marle and Tollens in 1906.[61] However, it was Carl Mannich who was the first to recognize the enormous significance of this reaction type and it was he who extended the chemistry into a broad based synthetic methodology through systematic research. Since then this reaction that now carries his name has developed into one of the most important carbon-carbon and carbon-nitrogen bond-forming reactions in organic chemistry. [62,63]

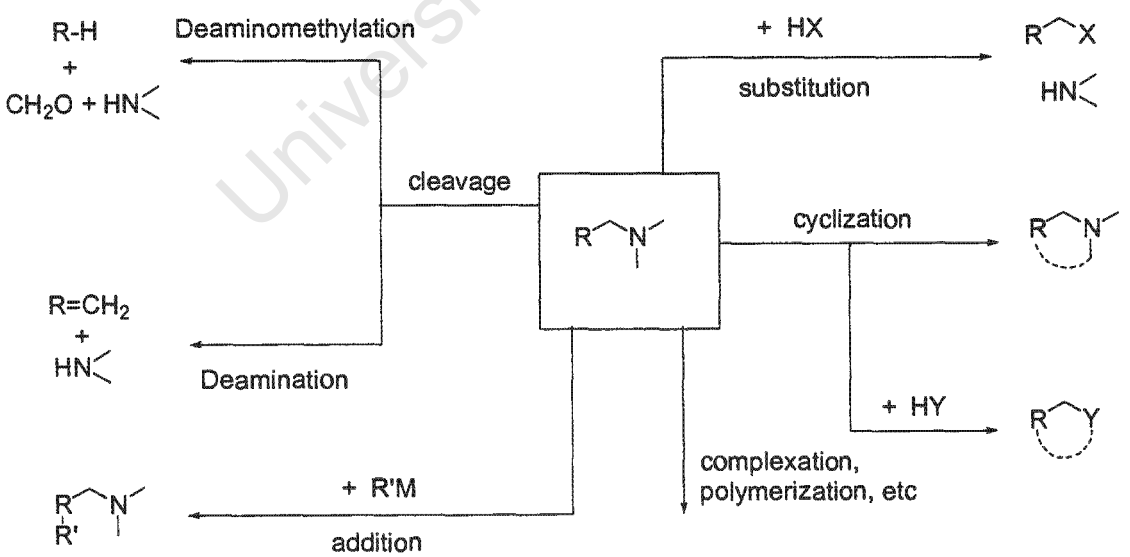
3.1.2 Classification of Mannich bases

Mannich bases are classified according to the nature (functional group) of the substrate as the amine components employed in the Mannich reactions are either commercially available or readily accessible.[62] The substrate or the active hydrogen component, in which the active hydrogen atom is replaced, may be a ketone, ester, nitrile, nitro compound, an α-alkylpyridine, hydrogen cyanide, a thiol, a phenol, tertiary aromatic amine, an indole, alkyne, enamines, heteroaromatic and heterocyclic rings. The general classification is presented in Table 1.

3.1.2 Reactivity of Mannich Bases

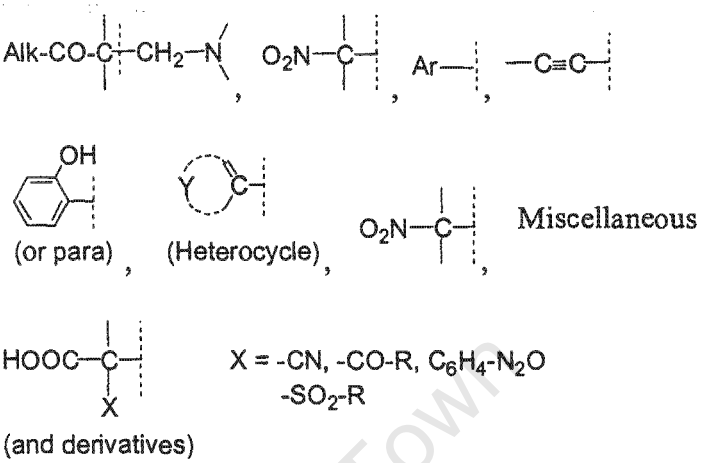
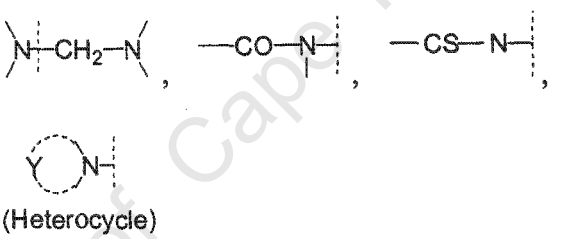
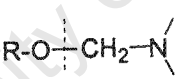
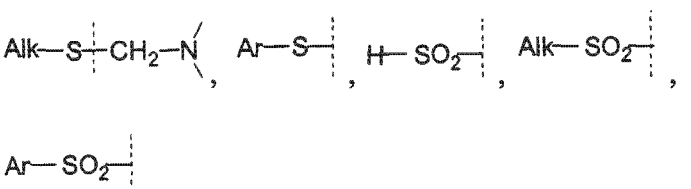
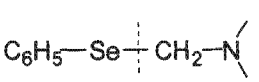
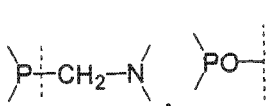
The remarkable number of reactions to which the Mannich bases can be subjected demonstrates that these compounds are particularly versatile synthetic building blocks, which can be converted into a range of useful and valuable derivatives (Scheme1).

Such derivatives include Michael acceptors (formed by deamination), 1,3-amino alcohols (produced by reduction or addition of organometallic compounds) and functionalized carbonyl compounds (formed by substitution of $-NR_2$ by nucleophiles). Mannich bases and their derivatives have many applications, however, the most important application by far is in the area of pharmaceutical products.[64,65]



Scheme1. Reactivity of Mannich bases

Table 1. General classification of Mannich bases

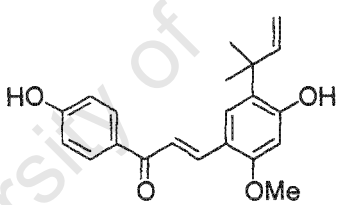
Type of Mannich Bases	Structure of Mannich Bases
C-Mannich bases	
N-Mannich bases	
O-Mannich bases	
S-Mannich bases	
Se-Mannich bases	
P-Mannich bases	

The design and synthesis of Mannich bases presented in this chapter are limited to the subclasses of C-Mannich and N-Mannich bases.

3.2.0 Mannich bases of Acetylenic Chalcones

3.2.1 Background

Chalcones constitute an important group of natural products characterized by the presence of a 1,3-diphenylprop-2-en-1-one skeleton.[66] For a structurally simple group of compounds, chalcones have displayed an impressive array of pharmacological activities. Depending on the substitution pattern on the two aromatic rings, a wide range of biological activities have been identified for various chalcones among which antileishmanial,[67] antifungal,[68] antiinflammatory,[69] antimitotic,[70] antiulcer,[71] antibacterial,[72,73] nitric oxide inhibition,[74] and modulation of P-glycoprotein-mediated multidrug resistance[75] have been cited in literature. The first antimalarial activity of chalcones was observed when licochalcone A **38** (figure 12), a natural product isolated from Chinese liquorice roots, was reported to exhibit potent *in vivo* and *in vitro* antimalarial activity.[76]



38

Figure 12. Licochalcone A

A year later, a study based upon the hypothesis that chalcones are plasmodium cysteine protease inhibitors showed that appropriate substitution of the chalcone skeleton results in derivatives with low micromolar IC_{50} values.[77] The studies also revealed that the α,β-unsaturated ketone bridge is essential for the antimalarial activity. Reduction of the carbon-carbon double bond of α,β-unsaturated ketone bridge of some selected chalcones resulted in a 10-fold decrease in antimalarial activity. In addition, the DOCK program and modeling studies showed that chalcones are more rigid than their saturated counterparts and adopt a more extended conformation because of the conjugated linker. This generates a linear, almost planar structure, which was shown to fit well into the long

cleft of the active site of the malarial cysteine protease and produces favorable interaction with the protease.[77]

Even though a variety of substitution patterns can be attempted on the two aromatic rings to give a large number of potential analogues, a sound knowledge of the structural requirements for antimalarial activity in chalcones is important in guiding and optimizing the drug design efforts. However, no comprehensive stydy of structure-activity relationships of antimalarial chalcones has been reported in literature. At this juncture, it is noteworthy that a recent article describing structure-activity relationships of antileishmanial and antimalarial chalcones has appeared.[78] A comparison of structure-activity relationships revealed that different physicochemical and structural requirements exist for these two activities. Antileishmanial activity is associated with less lipophilic chalcones, in particular those with 4'-hydroxy-substituents on the B ring and hetero/polyaromatic A rings (Figure13). In constract, chalcones with good anti-malarial activity have alkoxylated B rings and electron-deficient A rings. Visualization of the steric and electrostatic fields generated from comparative molecular field analysis indicated that ring A of chalcones makes a more significant contribution to antileishmanial activity while both rings A and B are important for antimalarial activity. These studies have created a major interest in chalcones as potential drugs for the treatment of malaria.

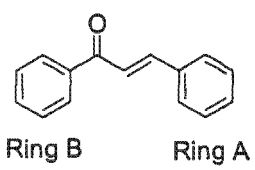


Figure 13. Rings A and B of chalcone

3.2.2 Rational Design of Target Compounds

In spite of the increased interest in chalcones as potential chemotherapeutic agents, acetylenic Mannich bases of chalcones have not been reported as potential antimalarial inhibitors of falcipain-2. This led us to design and synthesize exploratory Mannich bases of acetylenic chalcones of the type **39**, **40** and **41** in which the acetylenic moiety is incorporated in both rings A and B (Figure 14, p.30).

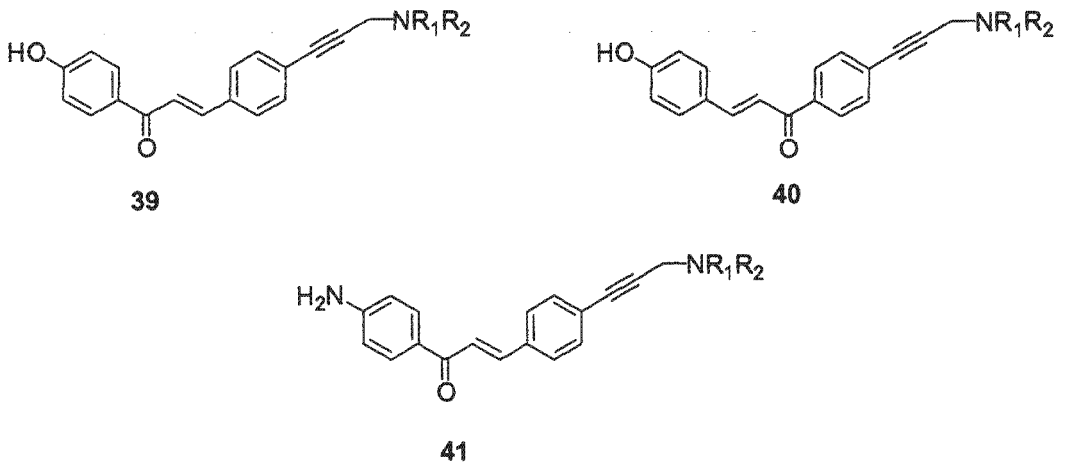


Figure 14. Exploratory Mannich bases of acetylenic chalcones

Figure 15 depicts the chemical features from which the rational behind the design are derived.

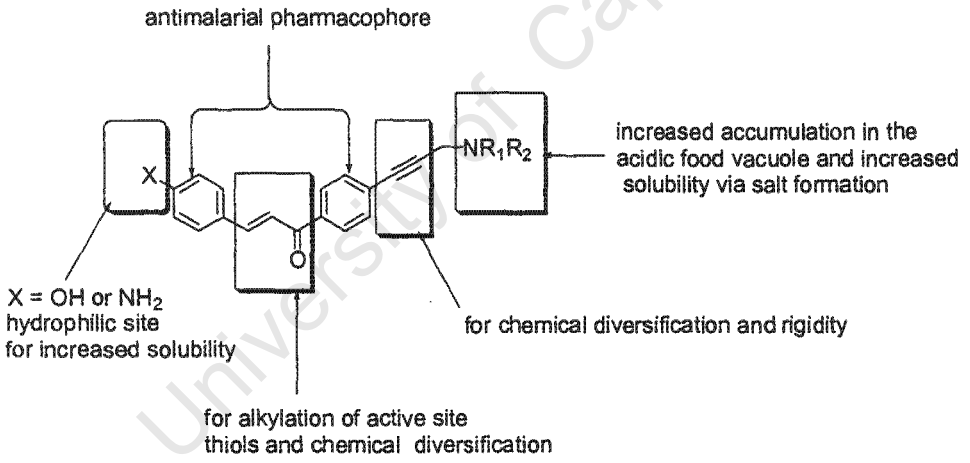


Figure 15. Chemical features depicting hypotheses for Mannich bases of acetylenic chalcones for *P. falciparum.*

Essential key features are noted below:

1. The presence of a protonatable nitrogen is beneficial to accumulation, by pH trapping, of these compounds in the acidic food vacuole of the malaria parasite where falcipain-2 is involved in the hemoglobin degradation process. Further, a protonatable nitrogen provides a site for salt formation in order to improve aqueous solubility.

2. The rigid acetylene moiety created in this reaction is frequently encountered in natural products possessing interesting biological properties such as antibacterial,[79,80] antifungal,[79,81] pesticidal,[82,83] and antitumor[83,84] activity. Molecular rigidity has been proposed to play a role in locking out access to clearance enzymes while inhibitory potency against the target is retained.[85,86] This has implications (good) for bioavailability.[86]

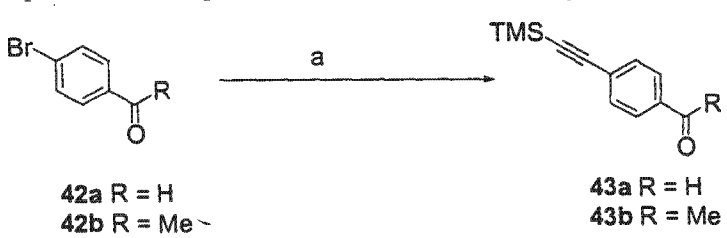
3. The β-aminoalkyne provides two extra sites (amine and alkyne) for introducing chemical diversity which is important for structure activity studies within this class of Mannich bases.[87]

4. The α,β-unsaturated and aromatic moieties of chalcones provides flexibility towards further elaboration. Coupled with the already known antimalarial activity, these two elements of diversity present further opportunities for improving upon antimalarial potencies.

5. The Mannich bases of chalcones are readily accessible in a few synthetic steps and the chemistry is very versatile in that many functional groups are compatible with the reaction conditions.

3.2.3 Synthesis of Mannich bases of acetylenic chalcones

The strategy employed in the synthesis of target Mannich bases of acetylenic chalcones involved a combination of Sonogashira,[88] Claisen-Schmidt condensation,[89] and Mannich reactions.[62] The starting point in the synthesis was the incorporation of the acetylenic moiety into derivatives **42a** and **42b**. Thus **42a** and **42b** were subjected to Pd-catalyzed Sonogashira coupling with ethynyltrimethylsilane in the presence of triethylamine and a catalytic amount of cuprous iodide to give **43a** and **43b** in 63% and 79% yields respectively after aqueous work-up and column chromatography (Scheme 2).



42a R = H
42b R = Me

43a R = H
43b R = Me

Scheme 2. *Reagents and conditions*: (a) 1.7 equiv of trimethylsilylacetylene, 0.02 equiv of $Pd(OAc)_2$, 0.02 equiv of PPh_3, 0.04 equiv of CuI, Et_3N, reflux, 4h

3.2.4.0 Proposed mechanism for the Sonogashira coupling reaction

The mechanism for this transformation is not clear. However, a tentative mechanism has been proposed. The reaction is said to proceed by the catalytic cycle similar to the Stille coupling[90] depicted in Figure 16. It involves three steps; (a) oxidative addition of an organic aryl halide to give a palladium (II) intermediate, (b) the intermediate undergoes transmetallation with copper acetylide and (c) reductive elimination with coupling of the two organic ligands gives the product and regenerates the palladium (0) catalyst.

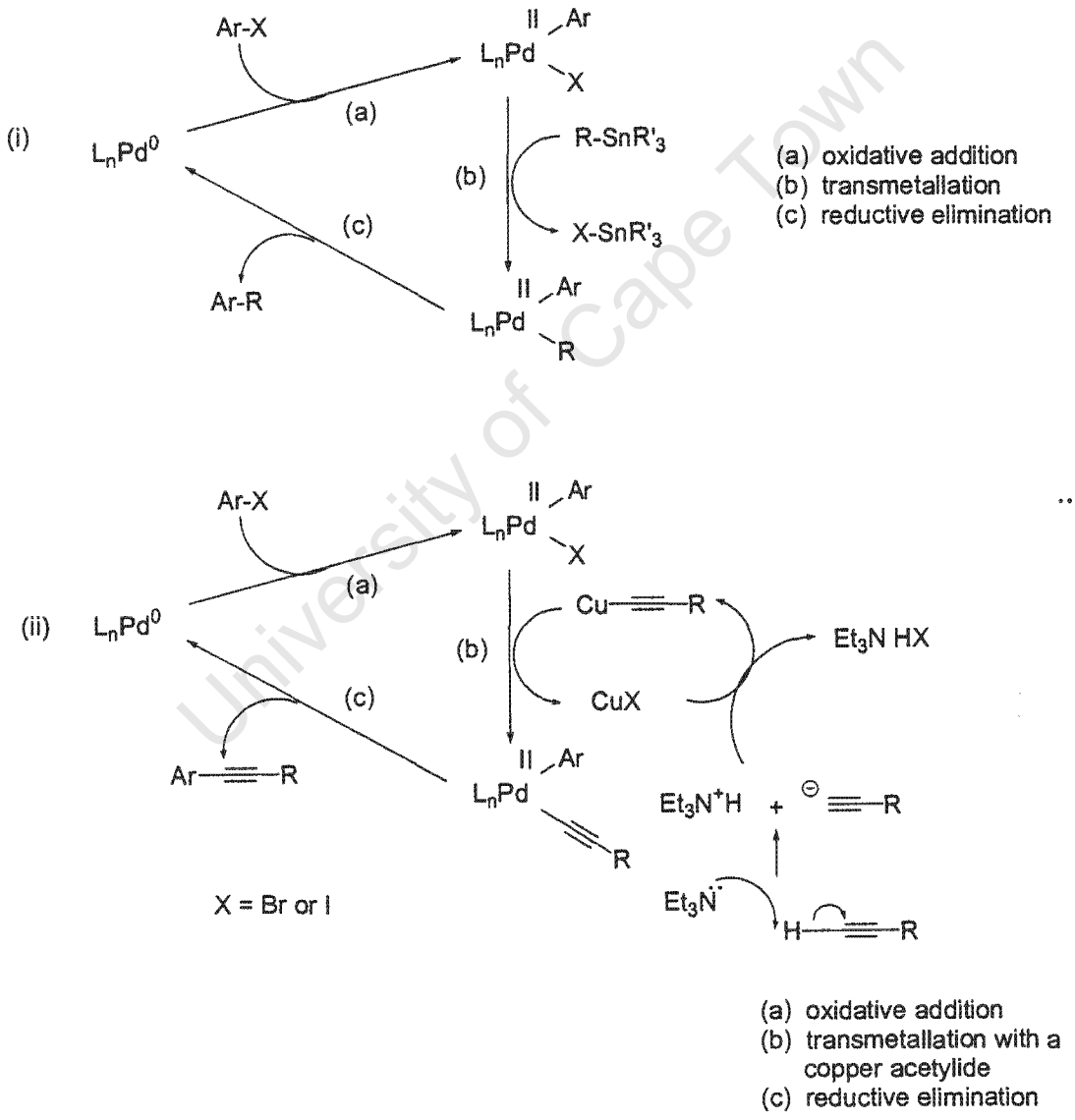


Figure 16. Proposed mechanism for (i) Stille coupling reaction (ii) Sonogashira coupling reaction

3.2.4.1 The role of copper(I)

Although alkynyl protons are more acidic than most hydrocarbon protons (pKa~ 25), they are not sufficiently acidic to undergo deprotonation in the presence of amines (pKa~10). The solution to this problem is found in the Cu(I) that cocatalyzes the reaction. Copper may form a π complex with the alkyne (Figure 17) which may be deprotonated by an amine to give a copper (I) acetylide, a species that can undergo transmetallation with Pd(II). In other words, copper activates the alkyne toward deprotonation.

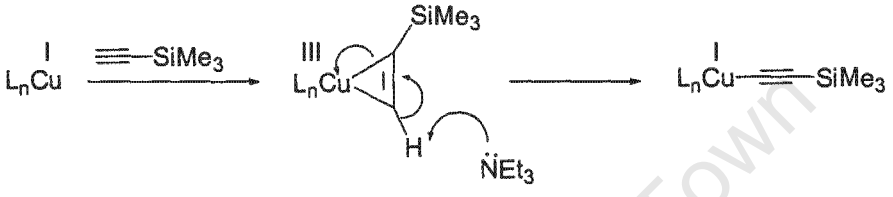
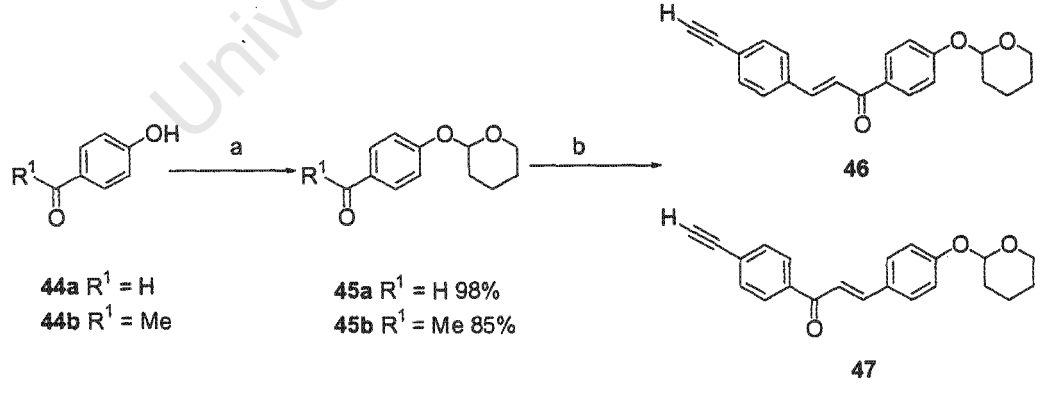


Figure 17. Deprotonation of an alkyne by triethylamine in the presence of Cu(I)

The next step was to prepare chalcone scaffolds **46** and **47** as outlined in Scheme 3 . The hydroxy groups in **44a** and **44b** were protected as tetrahydropyranyl ethers to give **45a** and **45b** which were condensed with **43b** and **43a** in the presence of 2M potassium hydroxide in methanol at 70 °C to furnish chalcone scaffolds **46** and **47** in 81% and 84% yields respectively. These reaction conditions were found to be optimal and were subsequently often used in our routine chalcone synthesis.



Scheme 3. *Reagents and conditions*: (a) 1.6 equiv of 3,4-dihydro-2H-pyran, 0.04 equiv of pyridinium *p*-toluenesulfonate, CH_2Cl_2, rt, 4h (b) 1.0 equiv of **43a** or **43b**, 2.0 equiv of KOH, EtOH, 70 °C, 3h

Protection of the phenolic groups in the starting materials is necessary for improved product yields. Under the reaction conditions above, the hydroxy group of **44a** would be

deprotonated to give species in which the electrons are fed into the carbonyl group making it less electrophilic and hence less susceptible to nucleophilic attack. This would lead to low product yields (Figure 18).

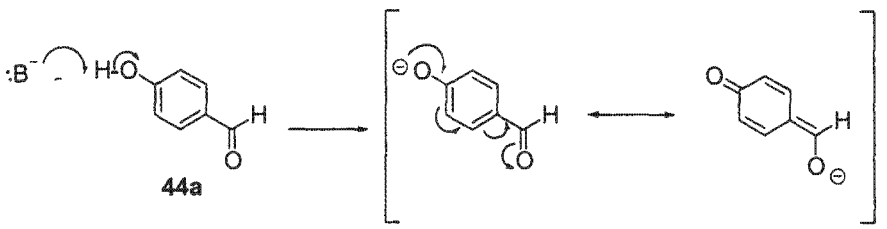
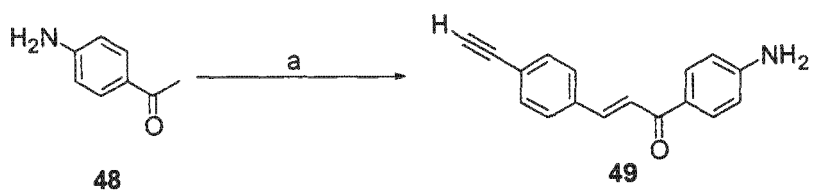


Figure 18. Resonance stabilization of phenoxide anion

Phenolic protons are quite acidic (pKa ~ 10) compared to methyl ketone protons (pKa > 20). The phenolic protons are readily abstracted to give resonance stabilized phenoxide anions (Figure 18). In the case of methyl ketone **44b**, deprotonation of the phenolic proton takes place faster (forms more stabilized intermediate) than deprotonation of the α-methyl ketone proton. This implies that only a small fraction of **44b** would be converted into enolate ions, which would react to produce chalcone products in low yields. It is also noteworthy that under these condensation conditions the TMS group that functions as a protecting group for the acetylene is removed to furnish new terminal acetylenes.

3.2.5 Synthesis of acetylenic chalcone 49

In a similar fashion **49** was prepared from trimethysilylethynyl benzaldehyde and 4-amino acetophenone in 81% yield applying the conditions described above (Scheme 3).

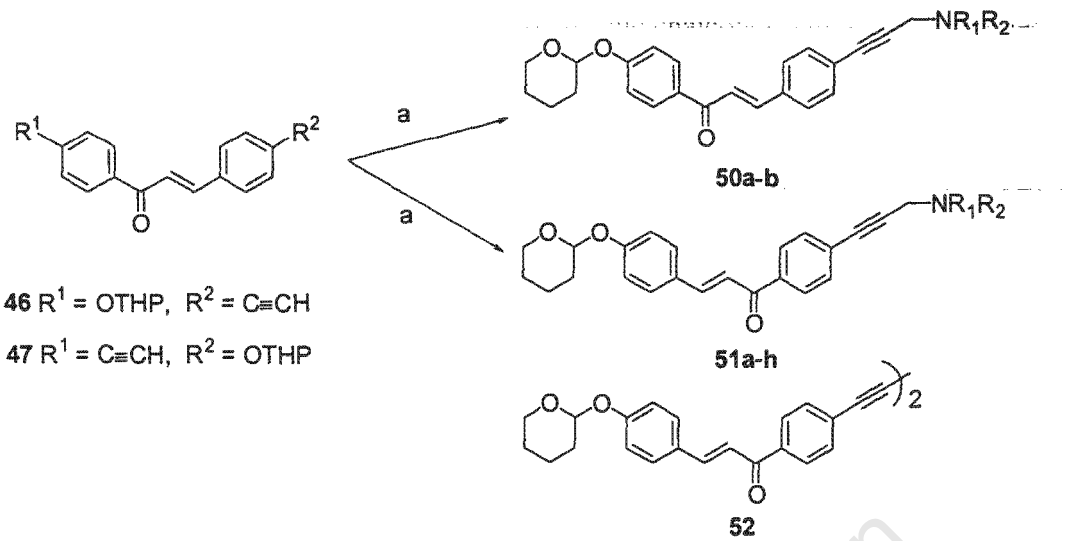


Scheme 4. *Reagents and conditions*: (a) 1.0 equiv of **48**, 2.0 equiv of KOH, EtOH, 70 °C, 3h

In the ^{1}H NMR spectra of these compounds, the protons of the α, β-unsaturated system resonated as two doublets around δ 7.50 and δ 7.76 with a coupling constant of 15.6 Hz expected for these predominantly *trans* isomers. In the ^{13}C NMR spectra the three carbon atoms associated with α, β-unsaturated system resonated at δ 122.0, 144.0 and 189.0. The alkyne proton resonances were evident at around δ 3.2 in the ^{1}H NMR while the corresponding carbon resonances were found at about δ 80.0 and δ 83.0. No *cis* isomers were observed. The rest of the protons appeared in the expected region and the molecular ion observed in the mass spectra for all compounds confirmed their molecular weights.

3.2.6.0 Mannich reactions of acetylenic chalcones with selected amines

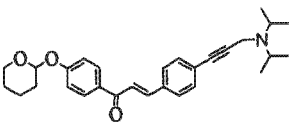
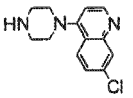
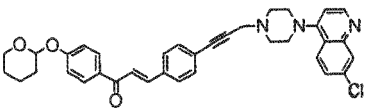
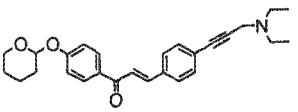
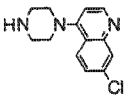
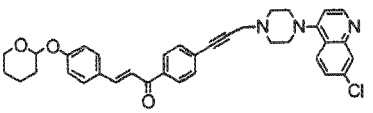
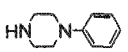
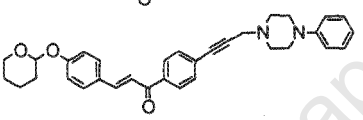
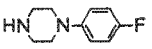
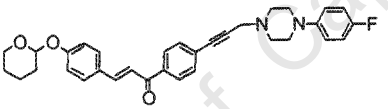
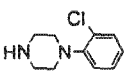
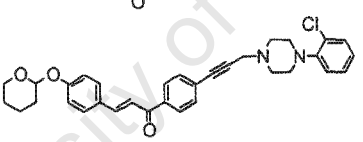
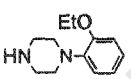
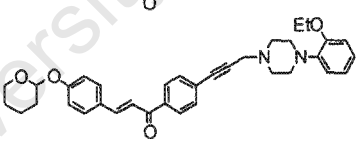
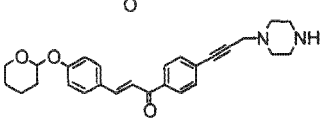
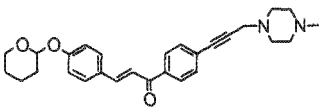
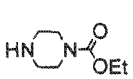
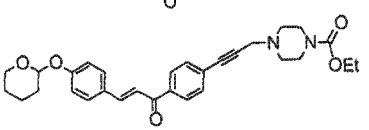
Having prepared the acetylenic chalcones the next task was to carry out the Mannich reaction. The Mannich reaction of acetylenes, aldehydes and secondary amines represents a powerful reaction for the construction of propargylamines and large numbers of these starting materials (acetylenes, amines and aldehydes) are commercially available or readily accessible. There are several methods for preparing propargylamines including the reaction of 1-(α-amino alkyl)-benzotriazoles with lithium alkenydes[91] or with sodium dialkynyldiethyl aluminates,[92] reaction of propargyl bromide with amines,[93] or reaction of geminal amino ethers with terminal acetylenes.[94] However, the most convenient method for the preparation of acetylenic Mannich bases is refluxing a solution of a terminal acetylene, a suitable amine and formaldehyde in a polar solvent, usually dioxane, in the presence of a catalytic amount of a copper salt [usually CuCl or $Cu(OAc)_2$].[95] Copper salts are frequently used in the reaction, since they have been found to increase the nucleophilicity of the acetylenic substrates towards the Mannich reactants.[62,96] The conversion of acetylenic chalcones into Mannich bases was effected by employing the latter method but using the optimized reaction conditions (Scheme 5). Thus, 1.0 equivalent of acetylenic chalcone was treated with 1.3 equivalents of formaldehyde, 1.0 equivalent of selected secondary amine and 0.5 equivalent of copper(II)acetate at reflux in dioxane to furnish Mannich bases of acetylenic chalcones **50a-c** and **51a-h** in good to excellent yields. The results are summarized in Table 2.



Scheme 5. *Reagents and conditions*: (a) 1.0 equiv of R_1R_2NH, 1.3 equiv of CH_2O, 0.5 equiv of $Cu(OAc)_2$, dioxane, 100 °C, 2.5h

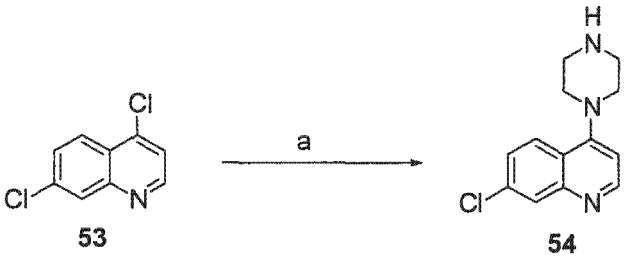
From Table 2, it is clear that the conversions of acetylenic chalcones with the acetylenic component attached to C-1 of the prop-2-en-1-one unit were free of the by-product (diyne) expected from Glaser oxidative coupling[97] whereas acetylenic component attached to C-3 of the prop-2-en-1-one component gave, in addition to the expected Mannich bases, the homocoupling product as the minor component. On the basis of these results, it is reasonable to suppose that the pKa of the acetylenic proton is the main determinant of the product distribution. The electron-withdrawing effect of the ketone carbonyl group in the acetylenic chalcones **51a-h** increases the acidity of the acetylenic proton. This results in the competing base (amine)-mediated dimerization side reaction. The proton NMR spectra of all the compounds showed a characteristic signal for the methylene group derived from formaldehyde in the vicinity of δ 3.6 and its corresponding carbon resonated at about δ 47.8. The IR spectrum showed bands at 1600, 1660 and 2250 due to the presence of the C=C, C=O and C≡C functional groups respectively. Electron impact mass spectroscopy failed to give the molecular ion for most of the compounds since the THP group was removed under these conditions.

Table 2. Products of the Mannich reaction of acetylenic chalcones with selected amines and their respective % yields

Chalcone	R_1R_2NH	Product structure	Product MB[a] (%Yield)	Dimer **52** % yield
46			**50b** (93)	-
46			**50a** (95)	-
46			**50c** (98)	-
47			**51a** (57)	14
47			**51b** (64)	14
47			**51c** (80)	16
47			**51d** (87)	12
47			**51e** (84)	13
47			**51f** (52)	13
47			**51g** (51)	21
47			**51h** (49)	18

MB[a] = Mannich base

The amine, 7-chloro-4-piperazin-1-yl-quinoline **54** employed in the synthesis of Mannich bases **50a** and **51a**, was prepared by treating 4,7-dichloroquinoline with piperazine in NMP at 140 °C (Scheme 6).



Scheme 6. *Reagents and conditions*: (a) 5.0 equiv of piperazine, 0.26 equiv of K_2CO_3, 0.25 equiv of Et_3N, NMP, reflux, 6h, 91%

3.2.6.1 Mechanism for the formation of 4,7-dichloroquinoline

The quinoline moiety requires no more than its own in-built capacity for electron withdrawal and is therefore attacked by nucleophiles at the 4-chloro position, which is closer to the nitrogen atom, which acts as an electron 'sink', than the 7-chloro position (Figure 19). Since the reaction follows a second order rate law, there is some formal resemblance to S_N2 reactions. However, it is follows a typical S_nAr mechanism. The carbon atom undergoing attack by the imino group is sp2 hybridised. The interaction of the nitrogen lonr pair is with an antibonding p-aromatic orbital orthogonal to the plane of the C-Cl bond. This part of the mechanism is similar to that of a Michael reaction.

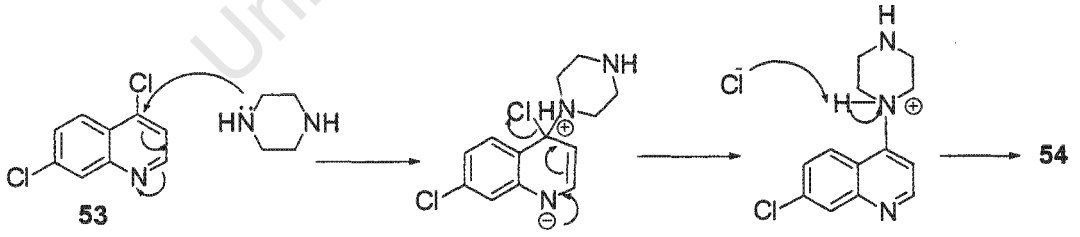


Figure 19. Mechanism for attack of 4,7-dichloroquinoline by piperazine

Attempts to prepare the amino chalcones of Mannich bases **41a** and **41b** (Figure 20) using the experimental procedure described above resulted in mixtures of products which proved difficult to separate.

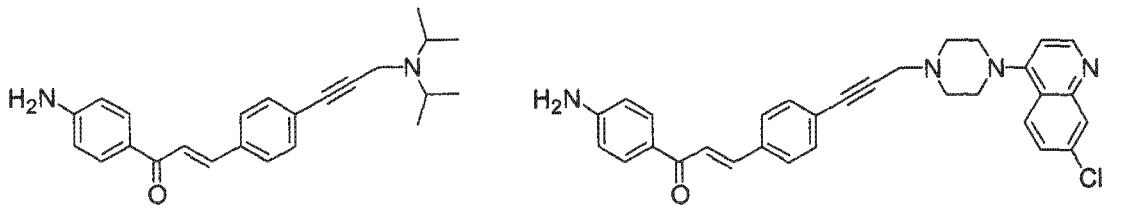
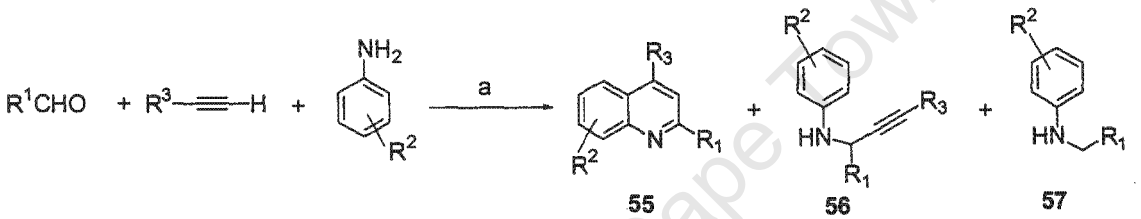


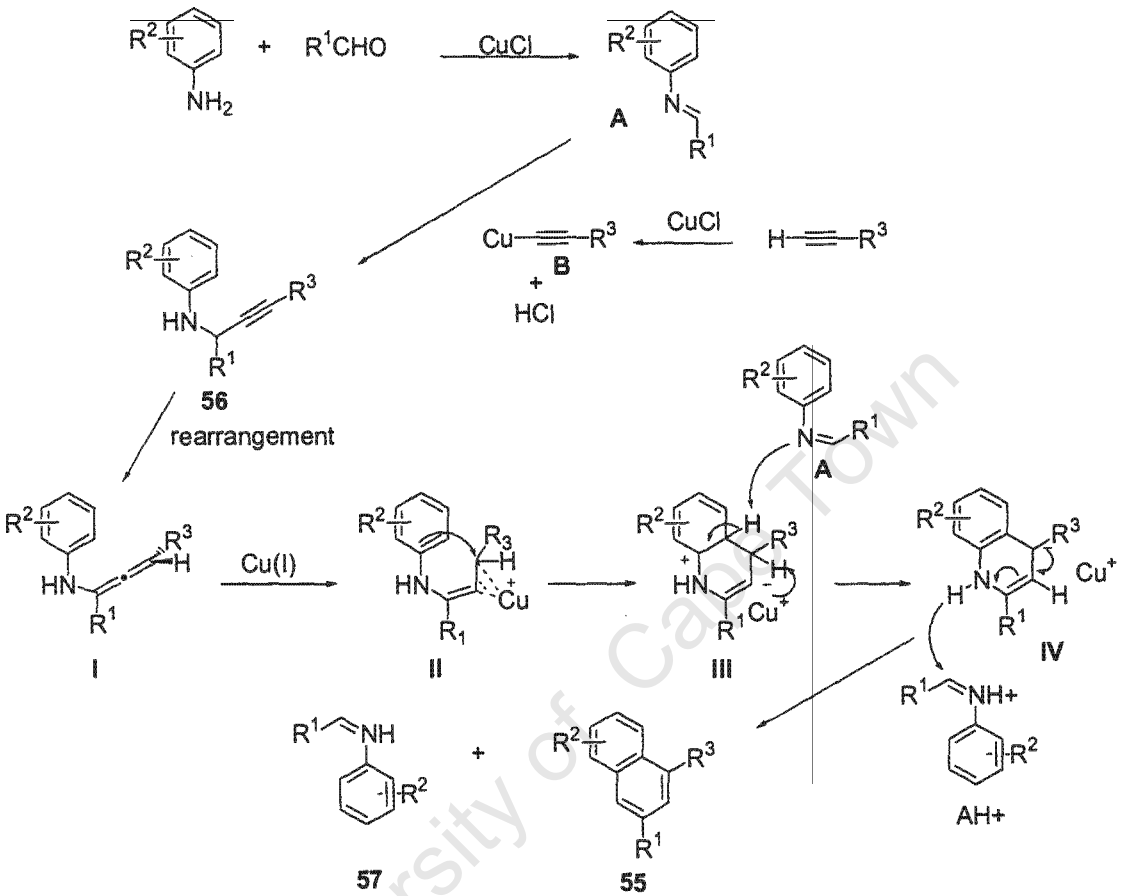
Figure 20. Amino chalcones of Mannich bases

Iqbal et al[98] have shown that amino groups on the aryl ring, under similar reaction conditions, also undergo transformation to give a mixture of three products **55**, **56** and **57** (Scheme 7). The separation problems encountered in the attempted preparation of **41a** and **41b** may have something to do with this product distribution reported by Iqbal et al.[98]



Scheme 7. *Reagents and conditions*: (a) CuCl (30 mol%), THF, reflux

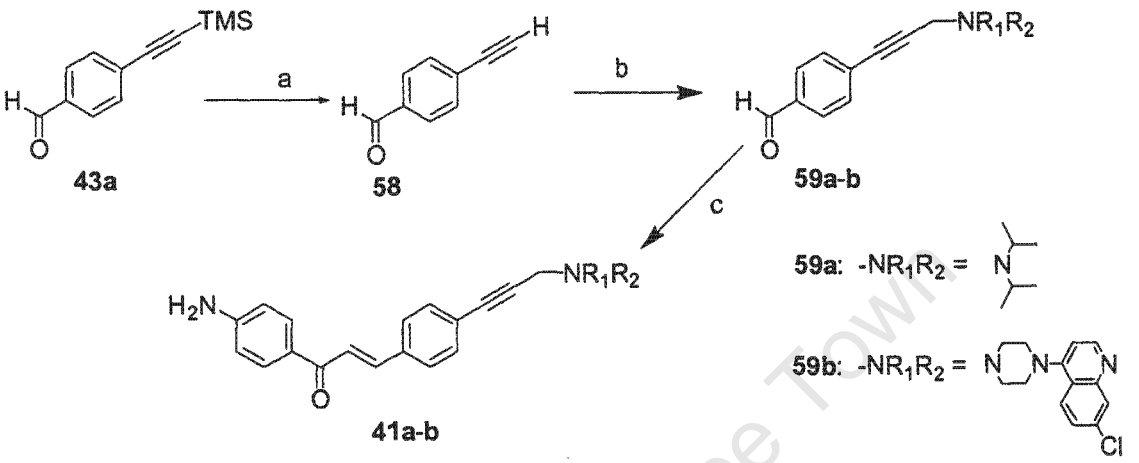
Scheme 8 gives the proposed mechanism for the conversion of the three components to **55**, **56** and **57**.



Scheme 8. Proposed mechanism for the conversion of the components to **55**, **56** and **57**

At first, a Schiff's base **A** is formed from the aldehyde and the amine under the reaction conditions. Cu-acetylide **B**, generated in situ then adds to imine **A** to give intermediate **56** which in turn undergoes propargyl-allenyl isomerization to form **I**. Next, coordination of copper(I) to the terminal bond of allene **I** would give intermediate **II** which will trigger antramolecular nucleophilic attack to produce the zwittrion **III**. The later would then isomerize via a 1,2-shift and proton transfer to **A** into the more stable zwitterionic intermediate **IV**, and protonated **A** (i.e. **AH+**). The intermediate **IV** would transform into quinoline **55** and benzylamine **57** by an oxidative process (hydride tranfer to **B**).

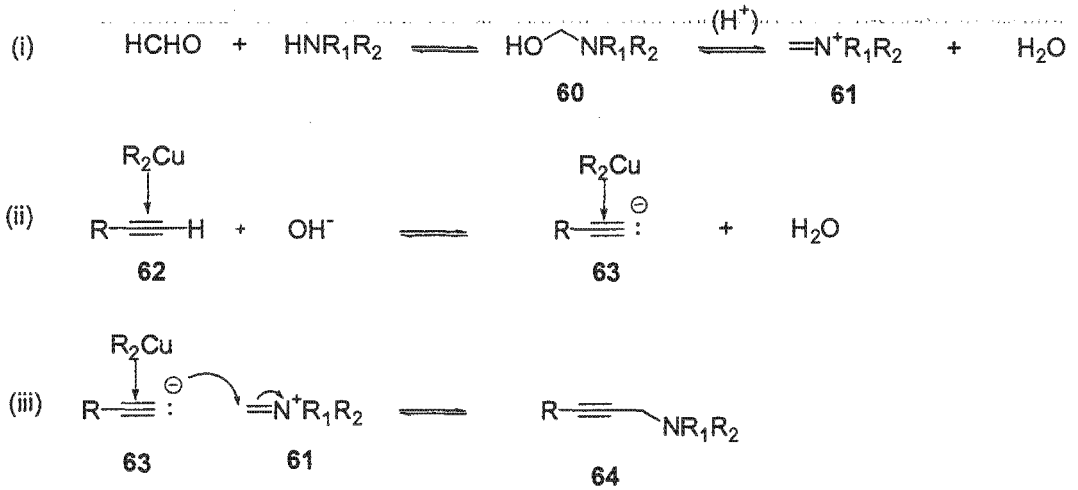
After unsuccessful attempts to isolate compounds **41a** and **41b**, an alternative method was sought. This involved first preparing the Mannich bases from ethynylbenzaldehyde **58** and appropriate amines and then condensing them with 4-amino acetophenone (Scheme 9). The reactions proceeded smoothly giving Mannich bases **41a** and **41b**.



Scheme 9. *Reagents and conditions*: (a) 2M KOH, EtOH, rt, 1h (b) 1.0 equiv of R_1R_2NH, 1.3 equiv CH_2O, 0.5 equiv of $Cu(OAc)_2$, dioxane,100 °C, 3h (c) 1.0 equiv of **48**, 2.0 equiv of KOH, EtOH, 70 °C, 3h in 95% and 90% yields respectively.

3.2.6.2 Mechanism of the Mannich reaction

The mechanism for the Mannich reaction has been well investigated[62]. The reaction consists of the condensation of a substrate (R-C≡CH) possessing at least one active hydrogen with formaldehyde (or other aldehydes) and a primary or secondary amine (or amide). The condensation occurs in two steps: first, the amine reacts with formaldehyde to give aminol **60** (Scheme 10, p.42). This undergoes dehydration to **61** which then undergoes attack by the Cu(II)-complexed acetylenic anion **63** to give the acetylenic Mannich base **64**.



Scheme 10. Mechanism for the Mannich reaction.

3.2.6.3 Deprotection of Mannich bases of acetylenic chalcones

Finally, removal of the THP protecting group (Scheme 11) under mild acidic conditions afforded our target Mannich bases of acetylenic chalcones in moderate to good yields (Table 3).

50a-b: R^1 = OTHP, R^2 = C≡CCH$_2$NR'R"

51a-e: R^1 = C≡CCH$_2$NR'R", R^2 = OTHP

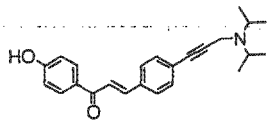
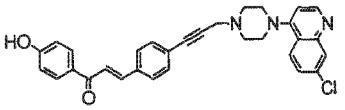
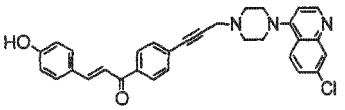
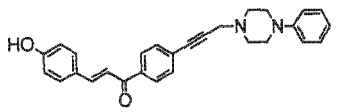
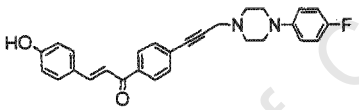
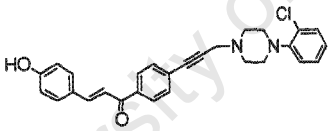
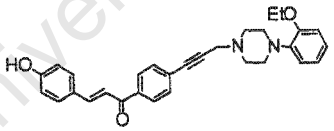
39a-b: R^3 = OH, R^4 = C≡CCH$_2$NR'R"

40a-e: R^3 = C≡CCH$_2$NR'R", R^4 = OH

Scheme 11. *Reagent and conditions*: (a) 4M HCl, MeOH, rt, 1h

^{1}H NMR, ^{13}C NMR and IR spectra were essentially those of **39a-b** and **40a-e** but without the THP ether signals in the aliphatic region. The $[M]^+$ signal in the HRMS for each compound confirmed their molecular weights.

Table 3. Deprotected Mannich bases of acetylenic chalcones and their respective % yield

Mannich base No.	Deprotected Mannich base	% Yield
39a		78
39b		68
40a		79
40b		87
40c		87
40d		68
40e		97

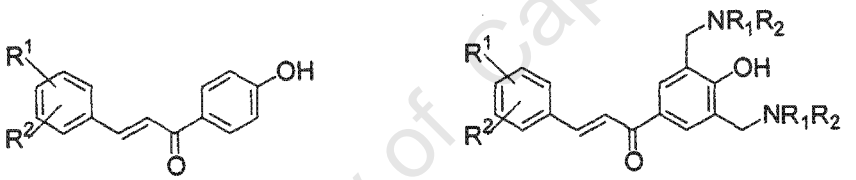
3.3.0 Phenolic Mannich bases of Thiosemicarbazones and Semicarbazones

3.3.1.0 Background

This new class of compounds has two components, the phenolic Mannich base and the thiosemicarbazone, condensed together. Therefore, in the discussion of the background, each component is treated separately.

3.3.1.1 Phenolic Mannich Base Component

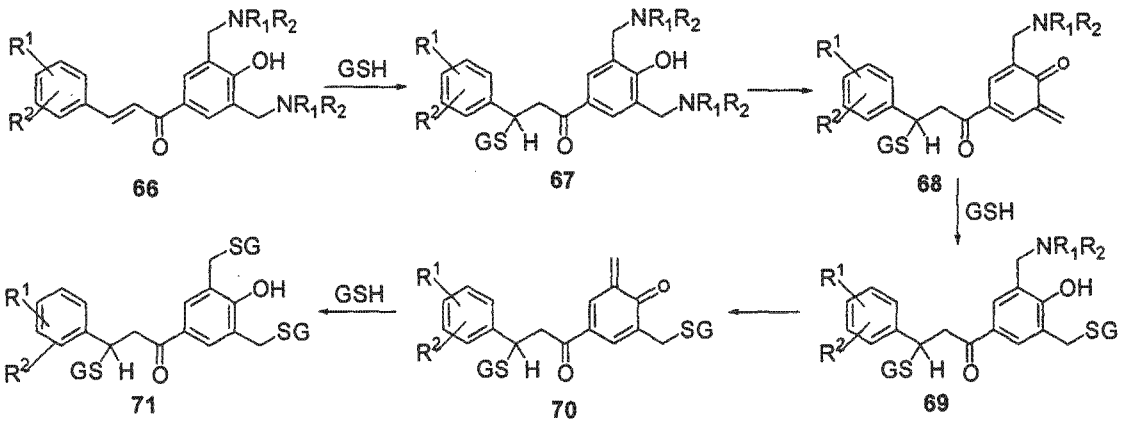
A number of compounds containing the phenolic Mannich base component have been synthesized and shown to possess significant cytotoxicity and anticancer properties.[99,100] The bioactivities have been attributed partially to the α, β-unsaturated ketones liberated from the Mannich bases by deamination. These α, β-unsaturated ketones have markedly greater affinity for thiols over amino and hydroxy nucleophiles.[101,102] Hence interactions with nucleic acids may be avoided and the problems of mutagenicity and carcinogenicity which are associated with certain alkylating agents may be absent. A few years ago, Dimmock et al[103] designed and synthesized phenolic chalcones **65** and their Mannich base derivatives exemplified by **66** (Figure 21) for cytotoxic and anticancer evaluation. Biological results obtained showed that the Mannich base derivatives were more potent against L1210 leukemia cells and human tumors than the corresponding parent phenolic chalcones when the compounds were tested with glutathione in the presence of the π-isozyme of glutathione-S-transferase.



65 **66**

Figure 21. Phenolic chalcone **65** and its Mannich base derivative **66**

The markedly improved activity of the Mannich base derivatives was attributed to the concept of sequential cytotoxicity[104] (Scheme 12) which states that chemical attack by two or more cytotoxic agents may cause greater toxicity to malignant tissues than normal cells. It was, therefore, suggested that the potency of Mannich base derivatives was due to the greater number of sites in the compounds for electrophilic attack by glutathione (GSH). The sequence of events that takes place is as follows: (i) alkylation of a cellular thiol (such as GSH) with the chalcone **66** produces the adduct **67**, (ii) deamination of **67** gives the reactive intermediate **68** which undergoes nucleophilic attack to produce the bisadduct **69**, (iii) extrusion of dialkylamine from **69** gives the reactive enone **70** which is further alkylated by a thiol to produce **71**.



Scheme 12. Illustration of the concept of sequential cytotoxicity

Amodiaquine **2** (Figure 3, p.8), chemically related and more effective than chloroquine, is a phenolic Mannich base that is now being considered as an alternative option for the management of uncomplicated *Plasmodium falciparum* malaria in Africa.[104,105] Recently, a new class of mechanism-based Mannich base inhibitors of thioredoxin reductase (TrxR) have been identified.[107] TrxR is the homodimeric flavoenzyme that catalyzes the reduction of oxidized thioredoxin (Trx) to the dithiol in the presence NADPH (equation 2) in *Plasmodium falciparum*. TrxR is an enzyme essential for survival of the parasite and has been validated as suitable target for drug development.

$$NADPH + Trx(S)_2 \xrightarrow{TrxR} NADP^+ + Trx(SH)_2 \qquad \text{(equation 2)}$$

The Mannich bases that were designed, synthesized and used in the inactivation experiment of TrxR are shown Figure 22.

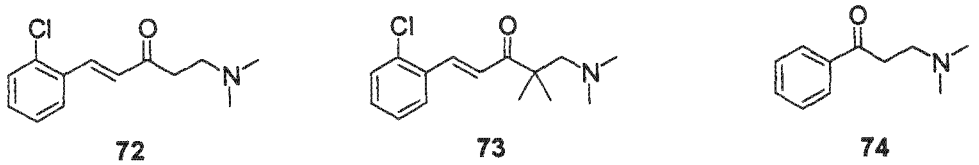


Figure 22. Mannich bases used for inactivation studies of TrxR

It was found that treatment of TrxR with **72**, compound with more than one site of alkylation, produced the most promising result compared to **73** and **74**. This was in agreement with the theory of sequential cytotoxicity.

3.3.1.2 Thiosemicarbazone Component

Compounds containing a thiosemicarbazone component have shown a broad spectrum of chemotherapeutic properties, including antimalarial,[108,109] and antitumor[110] activity *in vitro* and *in vivo*, as well as antibacterial[111], antitrypanosomal[112] and antiviral[113] activity *in vitro*. The compounds are also members of a class of iron-chelators that are Schiff bases. Since iron (Fe) is essential for the biological activity of a number of plasmodial proteins, including the rate-limiting enzyme of DNA synthesis, ribonucleotide reductase, withholding it inhibits the growth of the malaria parasite.[114,115] *In vitro* studies have shown that iron-chelating agents inhibit parasite growth and proliferation by deprivation of essential nutrients.[116]

3.3.2 Rationale

Figure 23 shows the chemical features of phenolic Mannich bases of thiosemicarbazones from which the rationale for the design are derived.

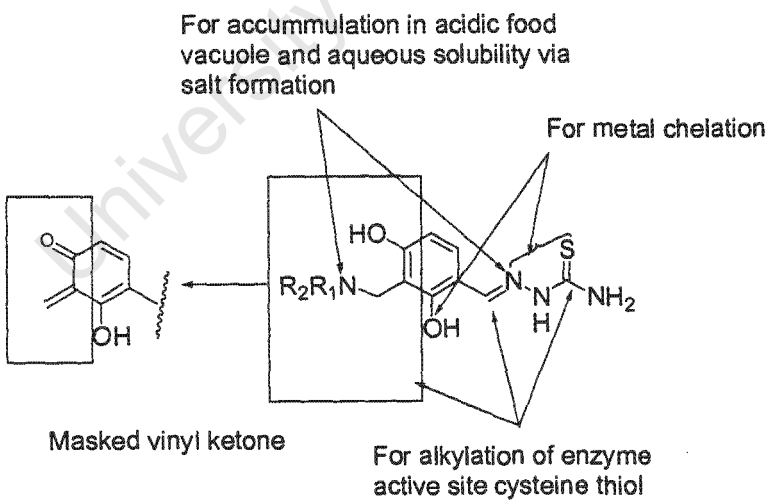


Figure 23. Chemical features of Phenolic Mannich bases of thiosemicarbazones as Antimalarial Agents

The following is a discussion of the chemical features and implications thereof:

1. The presence of an additional protonatable nitrogen atom of the phenolic Mannich base would enhance the compound's accumulation in the parasites acidic food vacuole *via* pH trapping.

2. The compounds would have three proximal ligands for metal (Fe) chelation when the conformation shown in Figure 24 is attained. This chelation would have an impact on parasite growth and proliferation. More specifically, this metal binding property could turn these thiosemicarbazones into metal-interactive cysteine protease inhibitors.[116]

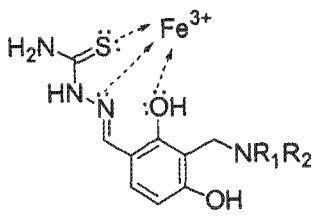


Figure 24. Appropriate orientation of ligands for iron chelation

3. The non-peptidic nature of aromatic thiosemicarbazones would make the compounds more resistant to hydrolysis than peptidic compounds. This has (good) implications for bioavailability.

4. Under the acidic conditions of the food vacuole, phenolic Mannich bases could conceivably undergo deamination to provide very reactive intermediates (enones) which would be prone to nucleophilic attack by cellular thiols (Figure 25).

Figure 25. Mechanism for the deamination of phenolic Mannich bases under acidic conditions

5. The interaction of thiosemicarbazones with thiols of cysteine proteases of the active site are reversible irrespective of the site of nucleophilic attack (i.e. imine C=N or thiocarbonyl C=S). This has implications for (low) host toxicity (high toxicity would arise if an inhibitor that is not molecularly recognized binds irreversibly to an enzyme)

and could be used as ligands for purification of the target cysteine protease.[117]

6. The thiosemicarbazone scaffold has advantageous properties: low molecular weight, reasonable *C*logP (*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), good hydrogen bond donating and accepting capabilities as well as easy and economical synthetic routes.

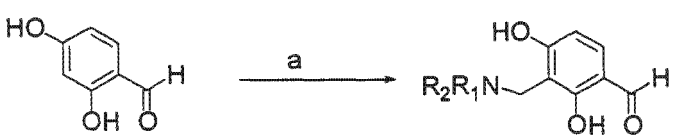
7. The imine bonds of thiosemicarbazones are more stable and less prone to hydrolysis compared to the corresponding imines with the result that thiosemicarbazones would be more bioavailable. The greater stability of thiosemicarbazones arises from delocalization, and hence, stabilization of the imine (C=N) double bond by the electronegative nitrogen substituent. This makes the imine (C=N) bond of thiosemicarbazones less susceptible to nucleophilic attack and/ or hydrolysis, Figure 26.

Figure 26. Stabilization of the imine double bond of the thiosemicarbazones by the electronegative nitrogen substituent.

Thus, it was envisaged that the combined effect of all the chemical entities would result in increased antiprotozoal activity. Issues of host toxicity are complex but can be dealt with at an appropriate stage.

3.3.3 Synthesis of phenolic Mannich bases

The synthesis of phenolic Mannich bases of thiosemicarbazones and semicarbazones is operationally simple and straight forward. The starting point in the synthesis involved preparation of phenolic Mannich bases from 2,4-dihydroxybenzaldehyde (Scheme 13).

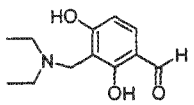
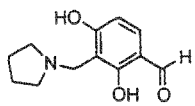
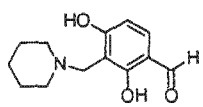
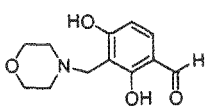
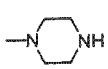
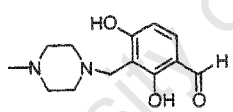
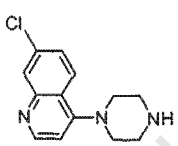
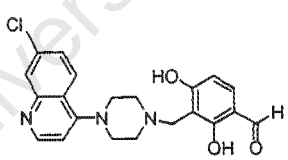


75 **76a-f**

Scheme 13. *Reagents and conditions*: 1.0 equiv of R_1R_2NH, 1.0 equiv of CH_2O, EtOH, 65 °C, 1h

Treatment of equimolar amounts of 2,4-dihydroxybenzaldehyde **75** with formaldehyde and selected secondary amines in methanol at reflux afforded phenolic Mannich bases **76a-f** in high yields. The results are presented in Table 4.

Table 4. Products of the Mannich reaction of 2,4-dihydroxybenzaldehyde with selected amines and their respective % yields

R_1R_2NH	Mannich base		% Yield
		**76a**	72
		**76b**	77
		**76c**	88
		**76d**	90
		**76e**	86
		**76f**	96

It should be noted that the aminomethyl group is regioselectively introduced at position 3 in 2,4-dihydroxybenzaldehyde. This was established from the ^{1}H NMR data of all individual compounds which showed two doublet signals corresponding to the aromatic protons having an *ortho*-coupling constant of about 8.4 Hz. The preferential condensation at one of the available *ortho* positions is dependent upon both steric and electronic factors.[118] Experimentally the preferred site for substitution is the more hindered C-3 of the two *ortho* positions. In contrast the Mannich reaction of phenols generally yield the less hindered product. Consequently the products observed in our work are not those

anticipated from steric considerations. The regioselectivity can be accounted for, however, by comparing the cationic intermediates (or transition states) for the substitution at both positions. Substitution at C-3 (Figure27a) yields more stabilized cation than substitution at C-5 (Figure 27b). Since the reaction is thermodynamically controlled, the reaction path leading to substitution at C-5 would be energetically less favorable.

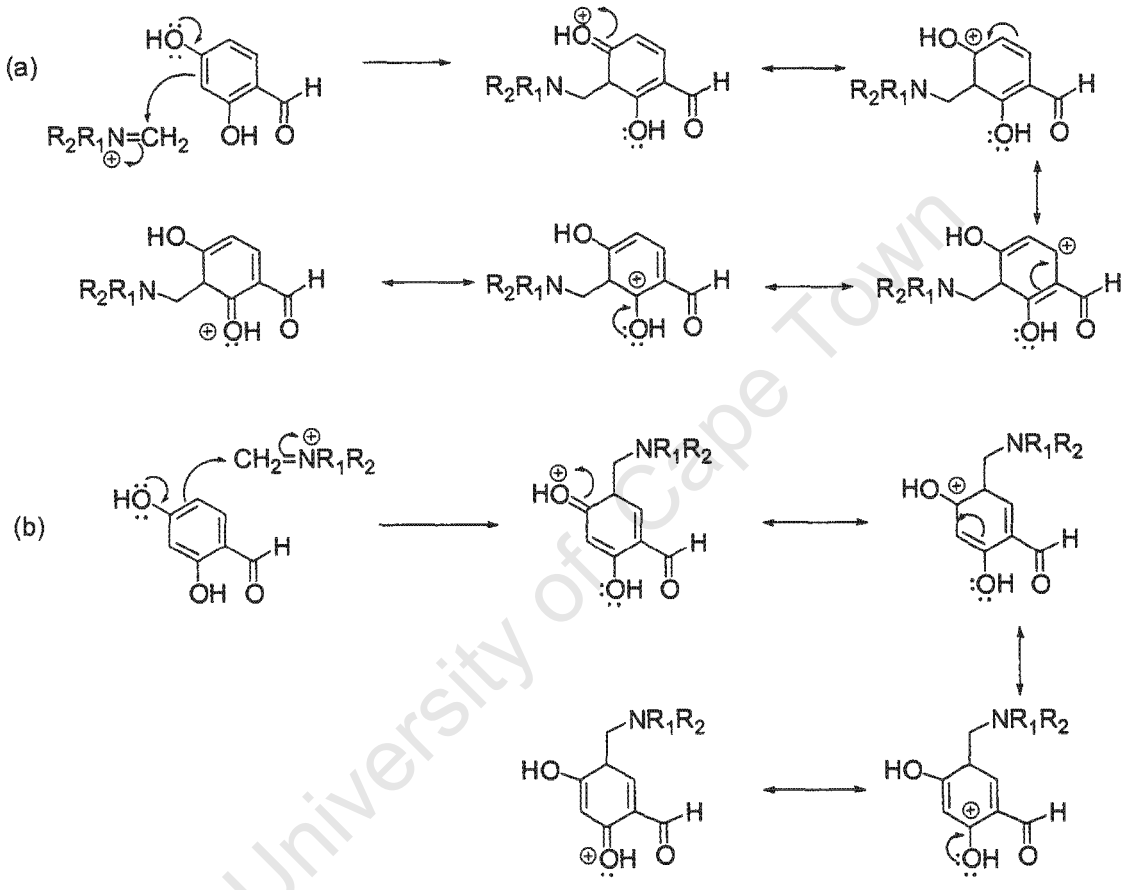


Figure 27. (a) Aminomethylation at C-3 (b) Aminomethylation at C-5

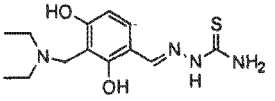
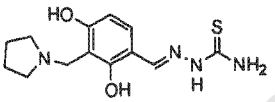
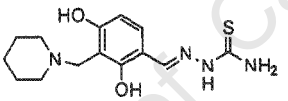
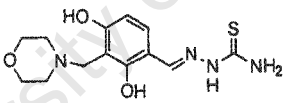
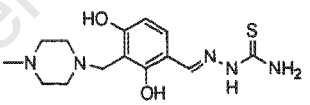
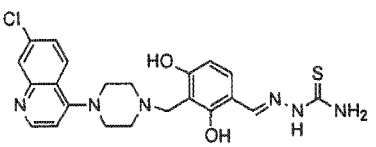
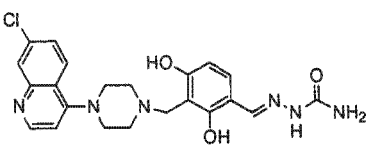
3.3.4 Synthesis of Phenolic Mannich bases of (thio)semicarbazones

Having successfully prepared the phenolic Mannich bases, the next and final task was to prepare phenolic Mannich bases of thiosemicarbazones and semicarbazones. The latter were prepared for comparative biological studies. This was achieved by treating the phenolic Mannich bases with thiosemicarbazide/semicarbazide in ethanol at reflux to provide the target thiosemicarbazones/semicarbazone (Scheme 14) in very good yields (Table 5).

76a-f **77a-g** X = O or S

Scheme 14. *Reagents and conditions*: (a) 1.0 equiv of thio/semicarbazide, MeOH, reflux, 1h

Table 5. Products of condensation phenolic Mannich bases with thiosemicarbazones/semicarbazones and their respective % yields

Mannich base	(Thio) Semicarbazone	% Yield
77a		87
77b		76
77c		78
77d		84
77e		95
77f		96
77g		92

The proton NMR data were found to be consistent with the proposed structures. Figures 28 and 29 show ^{1}H NMR spectra of representative compounds **77f** and **77g** respectively.

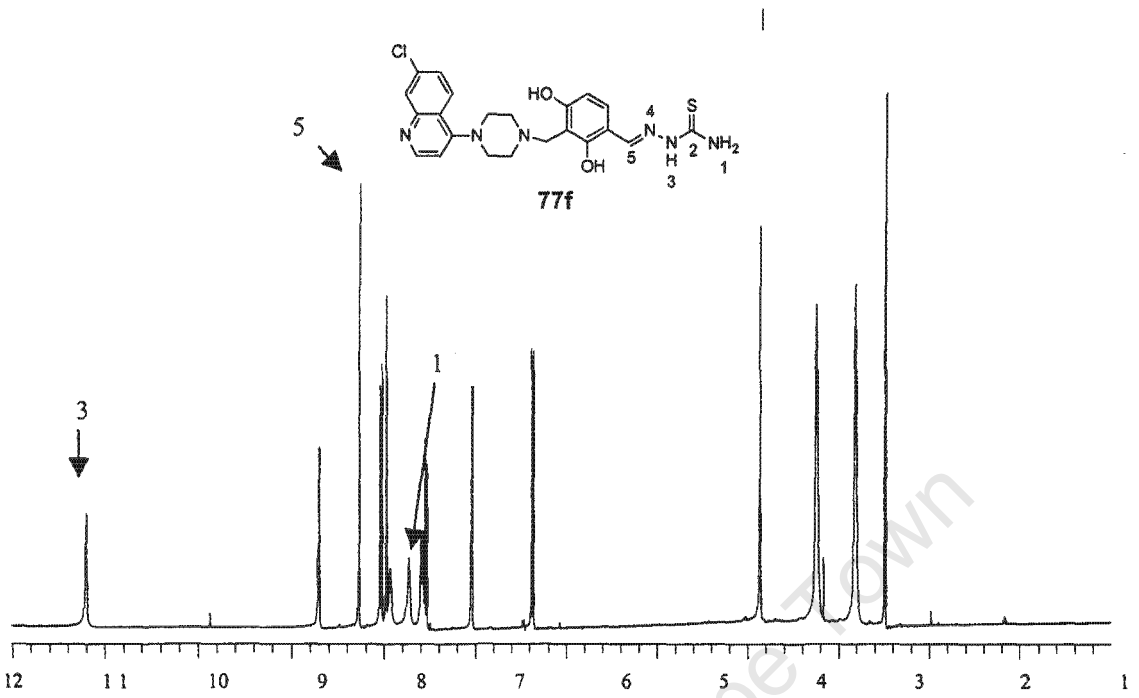


Figure 28. Spectrum of Thiosemicarbazone in DMSO-$_{d6}$

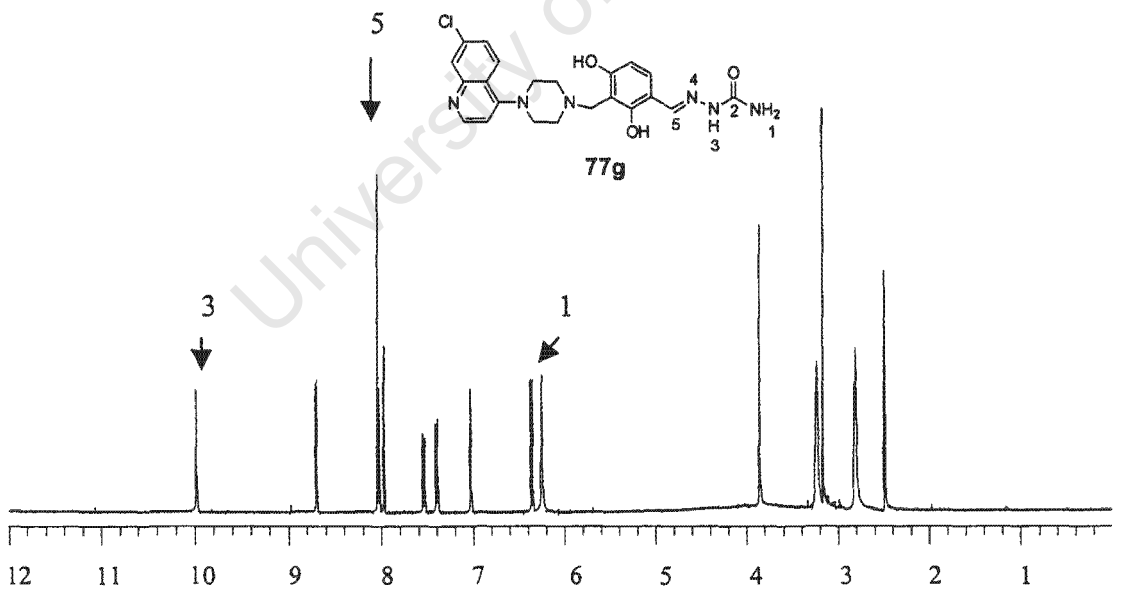


Figure 29. Spectrum of Semicarbazone in DMSO-$_{d6}$

It is noteworthy that:

1. The protons of the thiosemicarbazone side chain appear more downfield than would be expected compared to those of the corresponding semicarbazone side chain. This is due to increased electron delocalization and increased size of the sulfur atom (or competitive conjugation between C=S and C=O) leading to more double-bond character for the C=S bond.[119]

2. The protons at position 1 in the semicarbazone are already coalescing at room temperature, whereas those in the thiosemicarbazone are still sharply resolved. This is attributed to dissimilarities in the magnetic environments which in turn is due to the increased rotational barriers of the thiosemicarbazone.[119]

3. The imine proton at position 5 and aldehyde proton at position 3 were evident at about δ 8.25 and δ 11.20 in thiosemicarbazones respectively, whereas those in the semicarbazone appeared at about δ 8.03 and δ 9.97 respectively. These provided proof that the desired compounds had indeed been successfully synthesized. This was further supported HRMS data.

A phenolic thiosemicarbazone derivative was also prepared in order to investigate whether the potency of phenolic Mannich bases of thiosemicarbazones is due to the phenolic Mannich base or phenolic thiosemicarbazone component or the combined effect of the two components. Accordingly, phenolic thiosemicarbazone **78** was prepared by condensing 2,4-dihydroxyaldehyde with thiosemicarbazone as outlined in Scheme 15.

75 **78**

Scheme 15. *Reagents and conditions*: 1.0 equiv of thiosemicarbazide, MeOH, reflux, 2h, 89%

It is important to investigate the relationship between structure and antiparasitic activity within the series of compounds since the information gained may make it possible to establish the principles governing activity which can be applied to other classes of antiparasitic agents yet to be discovered. In the work reported by Du et al,[117] the

structure-activity relationships were explored in much greater detail on the thiosemicarbazone side-chain for inhibitors of cruzain. In particular, it was observed that exchange of the sulfur atom of the thiocarbonyl group for oxygen and reduction of the imine double bond resulted in significantly diminished activity. In view of these findings they concluded that the probable site of attack by the active site cysteine thiolate (S-Cys25) on the thiosemicarbazone was the thiocarbonyl group (Figure 30), and accordingly suggested binding modes using DOCK-based algorithms.

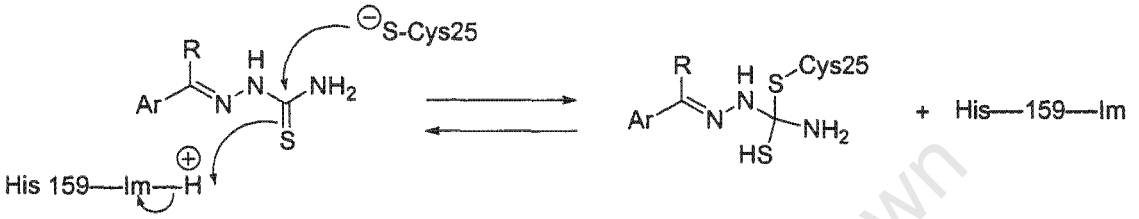


Figure 30. Alkylation of cruzain active site thiol by thiosemicarbazones

3.4.0 Phenolic Mannich base of Aminoquinoline Semicarbazones

This is a new class of compounds in which the side chain of chloroquinoline is modified by linking it to a phenolic Mannich base *via* a semicarbazone. The chemistry and pharmacological activity of the phenolic Mannich base derivative have been discussed in the previous section and need not to be overemphasized here. Therefore, the discussion in this section is limited to the semicarbazone component.

3.4.1 Urea derivatives

3.4.2 Background

Semicarbazones can also be regarded as urea derivatives. In recent years the ureido unit (-NH-CO-NH-) has gained considerable importance[120] in the design of enzyme inhibitors,[121] as a replacement for the amide (-CO-NH-) bond in peptidomimetics[122] and as a self-complementary bi-directional hydrogen bonding motif in supramolecular chemistry.[122a,b] Since peptides have poor metabolic stability and limited oral absorption, they are rarely useful drug candidates. Unlike the peptide bond, the urea linkage has remarkable resistance to proteolytic degradation by enzymes in the gastro intestinal tract, which opens perspectives for the oral delivery of these compounds.[123] In addition, the hydrogen bond forming capacity of the urea unit may assist in rendering the urea compounds more water soluble. Furthermore, an appropriately placed hydrogen-bonding

unit may cause additional affinity in the interaction. In view of the above mentioned factors, compounds which contain a urea moiety are more desirable as potential drug candidates than those that contain the peptide bond.

3.4.3 Rationale

Figure 31 illustrates the chemical features of phenolic Mannich bases of semicarbazone derivatives from which rationale for the design are derived:

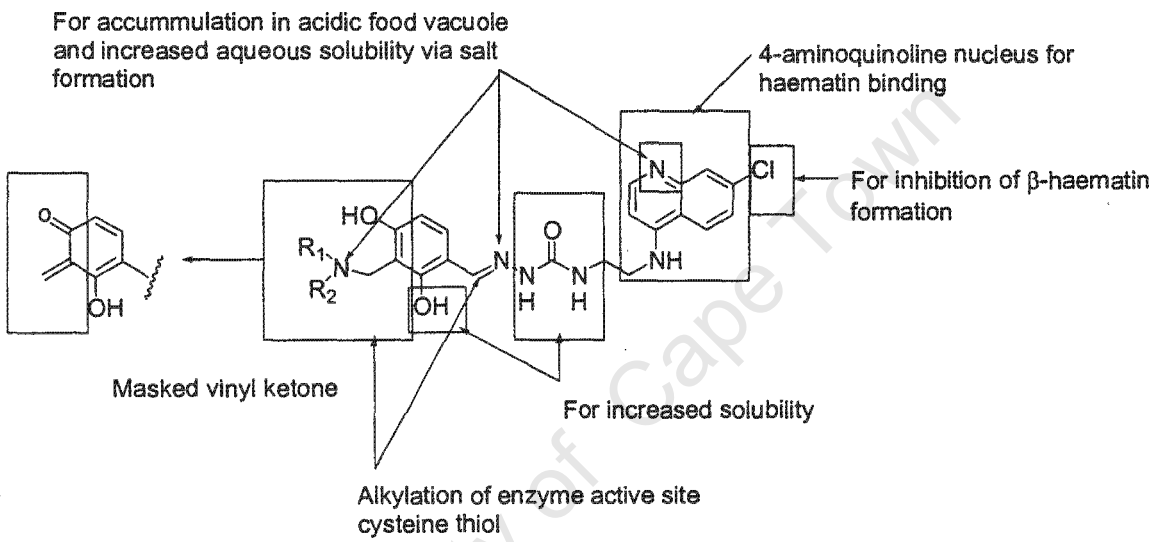


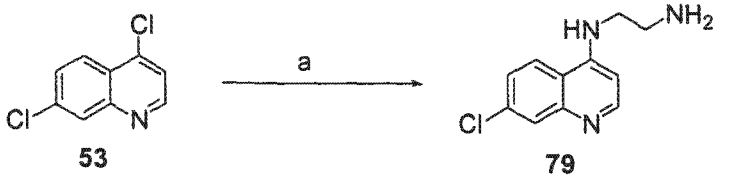
Figure 31. Chemical features for phenolic Mannich bases of aminoquinoline semicarbazones derivatives as antimalarial agents

The following is the summary for the rationale:

1. The presence of more than one protonable nitrogen atom would increase the molecule's accumulation in the acidic food vacuole by pH trapping.
2. Unlike a peptide bond, a urea linkage potentially offers more resistance towards enzymatic hydrolysis and enhanced bioavailability. In addition, the urea moiety also offers increased water solubility through hydrogen bonding.
3. The 7-chloro substituent in the 4-aminoquinoline moiety has been shown to be important for the inhibition of haemozoin formation.[13]

3.4.4 Synthesis of Phenolic Mannich Bases of an Aminoquinoline Semicarbazone

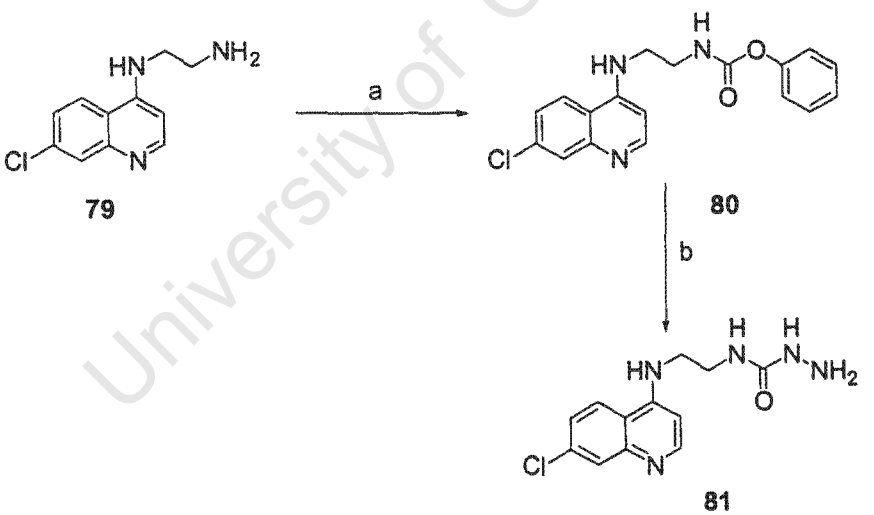
The synthesis begins with the preparation of the known precursor **79**[124] from 4,7-dichloroquinoline (Scheme 16). Thus, 4,7-dichloroquinoline was reacted with ethylenediamine at 135 °C to give **79** in 82 % after purification on silica gel.



53 **79**

Scheme 16. *Reagents and conditions*: (a) 5.0 equiv of $NH_2(CH_2)_2NH_2$, reflux, 6h, 82%

Next, the semicarbazide reactant **81** was prepared according to a known procedure[125] with slight modification. Compound **79** was treated with phenyl chloroformate **82** in the presence of DMF to give carbamate **80** in 72% yield which was reacted with hydrazine monohydrate to afford **81** in 81 % (Scheme 17).



79 **80** **81**

Scheme 17. *Reagents and conditions*: (a) 1.0 equiv of chloroformate, 1.0 equiv of Et_3N, DMF/DCM (1:1), 0 °C, 1h (b) 10 equiv of $H_2NNH_2.H_2O$, MeOH, reflux, 12h

The proton NMR spectrum was consistent with the proposed structure of **81**. The HRMS spectrum had the $[M+H]^+$ signal at m/z 280, corresponding to the molecular formula $C_{12}H_{14}ClN_5O$. The C=O signal in the ^{13}C NMR spectrum appeared at δ 162.8 ppm while

the IR spectrum showed peaks at 3320, 3225, 3150 cm^{-1} due to N-H stretching vibration and 1660 cm^{-1} due to the C=O stretch respectively.

It should be noted that DMF not only acts as a co-solvent in the reaction but also as a catalyst.[126] DMF reacts with phenylchloroformate **82** to produce an intermediate adduct which in turn is attacked by **79** to give the carbamate **80**. The reaction mechanism is illustrated in Figure 32.

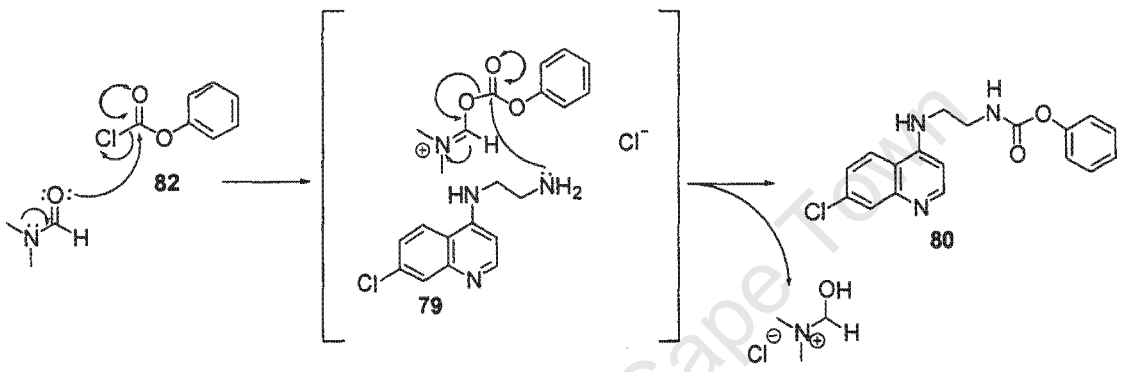
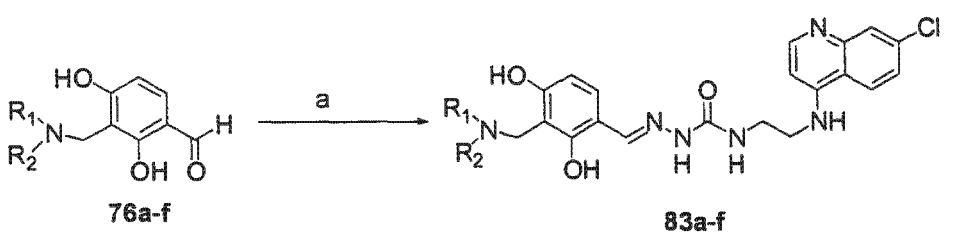


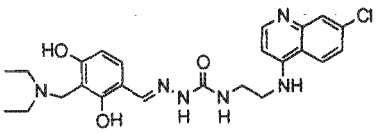
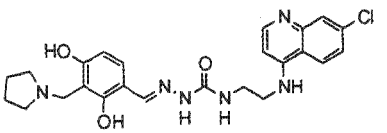
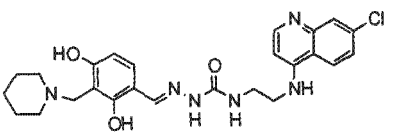
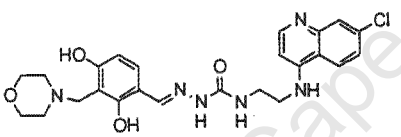
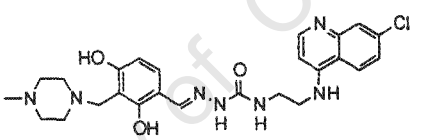
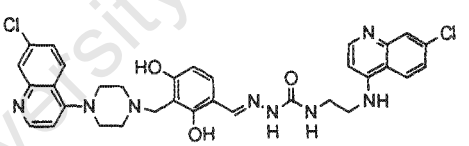
Figure 32. Reaction mechanism for the formation the carbamate catalyzed by DMF.

With the semicarbazide in hand, efforts were directed towards the preparation of target compounds (Scheme 18). The phenolic Mannich bases synthesized in section 3.3.3 were reacted with **81** in the presence of *p*-toluenesulfonic acid in methanol at room temperature to give the semicarbazone derivatives **83a-f** in moderate to good yields (Table 6).



Scheme 18. *Reagents and conditions*: (a) 1.0 equiv of **81**, 0.5 equiv of p-TsOH, MeOH, rt, 12h

Table 6. Products of the condensation of phenolic Mannich bases with an aminoquinoline semicarbazide and their respective % yields.

Mannich base	Urea derivatives	% Yield
83a		61
83b		52
83c		54
83d		69
83e		80
83f		74

3.5.0 Isatins

3.5.1 Background

The chemistry of isatin (1*H*-Indole-2,3-dione) dates as far back as 1841 when it was first obtained as a product from the oxidation of indigo by nitric and chromic acids. In nature, isatin is found in the number of plants including those of the genus *Isatis*.[127] It has also been found as a metabolic derivative in humans. Isatins are synthetically versatile substrates that open the way to the synthesis of a large variety of heterocyclic compounds such as quinolines,[128] spiropolyheterocycles,[129] indoles[130] and as novel scaffolds for drug synthesis.[131] Isatin derivatives are reported to show a variety of pharmacological

properties including antibacterial,[132] antimicrobial,[133] anti-HIV[134] and antiprotozoal[135] activities. Within the context of enzyme inhibitors, isatins have recently found application in the inhibition of cysteine and serine proteases.[136] Recently, a series of thiosemicarbazones derivatives of isatins were synthesized in our laboratory and shown to be active (with IC_{50} values in the low micromolar range) against the parasitic cysteine proteases cruzain, falcipain-2 and rhodesain.[137] Encouraged by these results, a new class of Mannich base thiosemicarbazone derivatives of 5-sulfamoyl isatins was designed and synthesized.

3.5.2 Rationale

The chemical features from which the rationale for the design of isatin derivatives are derived are shown in Figure 33.

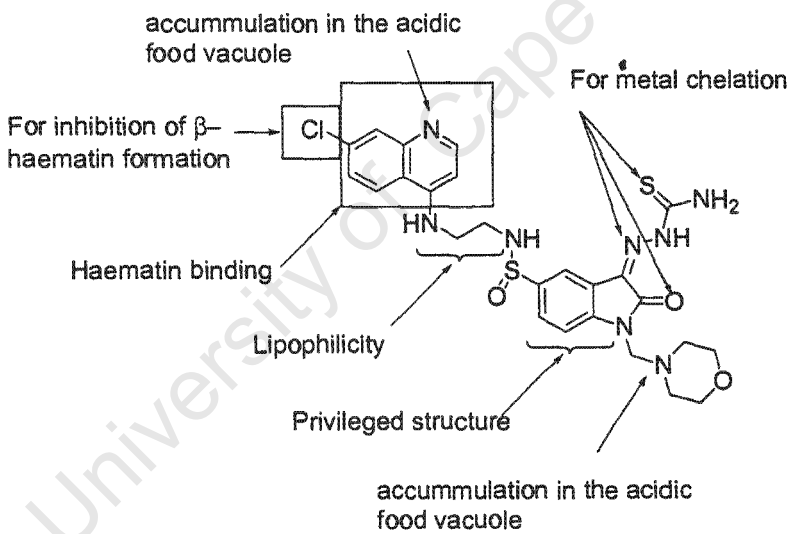


Figure 33. Chemical features for Mannich base thiosemicarbazone derivatives of isatin against *Plasmodium falciparum*

The rationale is summarized as follows:

1. A variety of drugs are available that are sulfonamide combinations, for example Sulfadoxine and Pyrimethane (Fansidar) an antimalarial agent used for the treatment and prevention of *P. falciparum* malaria

2. The biological activity and thus the effectiveness of sulfonamides and/or iron chelators is dependent on the lipophilicity. Lipophilicity is essential for the activity

because it determines the permeability of the drug into the cell and ionization at physiological pH. A recent study has highlighted the importance of lipophilicity in the antimalarial iron chelators.[138]

3. The 7-chloro-4-aminoquinoline moiety is a feature of chloroquine derivatives with antiplasmodial activity.

4. Variation of the alkyl (ethylene) spacer between the aminoquinoline and isatin moieties could lead to more active compounds that are able to circumvent aminoquinoline resistance. This has been ably demonstrated recently with aminoquinoline derivatives with shortened and lengthened alkyl spacers in the lateral side chain.[139]

5. Inhibition of parasite cysteine protease by alkylation of the active site cysteine thiol *via* nucleophilic addition to either the imine or thiocarbonyl group was envisaged.[136]

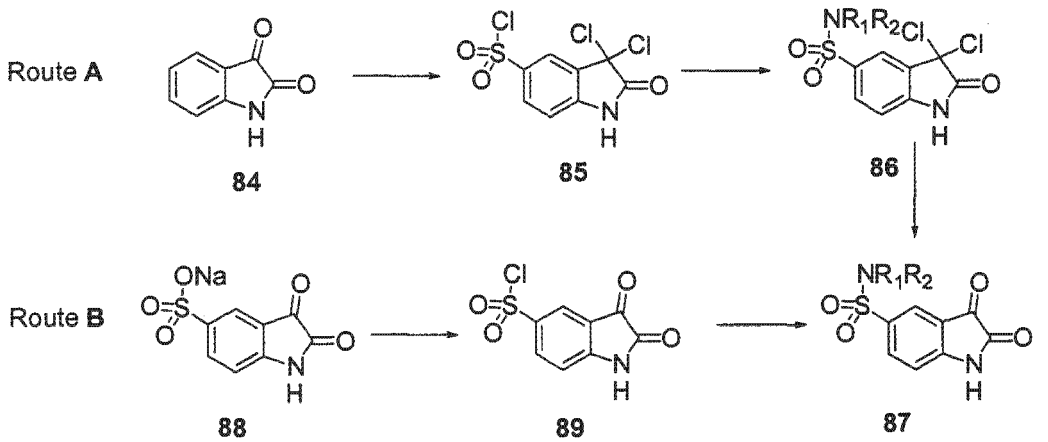
6. Isatin-3-thiosemicarbazones have been reported to bind ferric ions.[140] This was envisaged to have implication on the inhibition of the growth of *P. falciparum* as previously discussed.

7. Due to the non-peptidic nature of isatin sulfonamides, improved metabolic stability could be anticipated.

8. Privileged (isatin) structure: being a natural product, libraries generated around this basic structure should yield hits at significantly reduced library size compared to classical combinatorial chemistry libraries based on non-natural product templates.

3.5.3 Synthesis of 5-Isatin Sulfonamide Derivatives

The key step in the synthesis of target molecules involved preparation of the 5-isatin sulfonamides **87** (Scheme 19, p.61). There are two synthetic pathways to 5-isatin sulfonamide. Route **A** would involve the preparation of gemi-dichloro-5-sulfamoylindolinone **85** using chlorosulfonic acid and then reacting it with a range of primary and secondary amines to provide the intermediate **86**, which upon acid hydrolysis would afford 5-isatin sulfonamide **87**. Route **B**, which is a more straightforward pathway, involves treating commercially available sodium 5-isatin sulfonate **88** with phosphorus oxychloride to give sulfonyl chloride **89**, which is then reacted with a variety of primary and secondary amines to afford 5-isatin sulfonamide **87**.



Scheme 19. Possible routes to sulfonamide **87**

In this work, method **B** was employed, making only slight modifications to literaure protocols.[141] Accordingly, 5-chlorosulfonylisatin **89** was prepared in 56% yield by reacting sodium 5-isatin sulfonate with an excess of $POCl_3$ in sulfolane at 80 °C. It is worth mentioning that in order to obtain the product free of sulfolane, several washings with water are required during the extraction process since sulfolane (bp 284 °C) is difficult to remove under reduced pressure. The next step was to synthesize sulfonamides **87a** and **87b** depicted in figure 34. This was achieved by treating 1.0 equiv of sulfonyl chloride **89** with 1.0 equiv of selected amines in the presence of 2.0 equiv of diisopropylethylamine in THF or a mixture of THF/MeOH at ambient temperature to give **87a** and **87b** in 71% and 62% yields respectively.

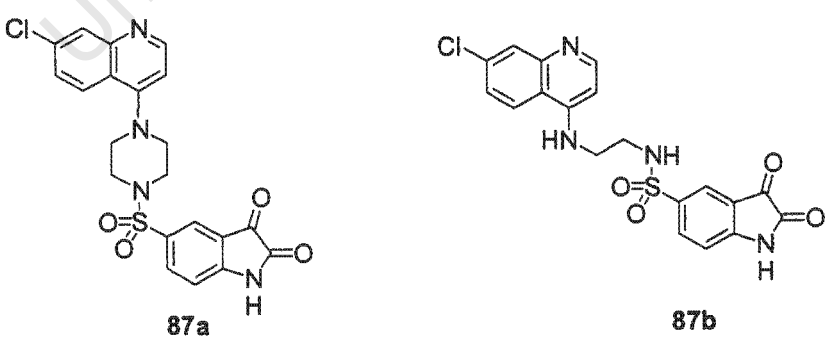
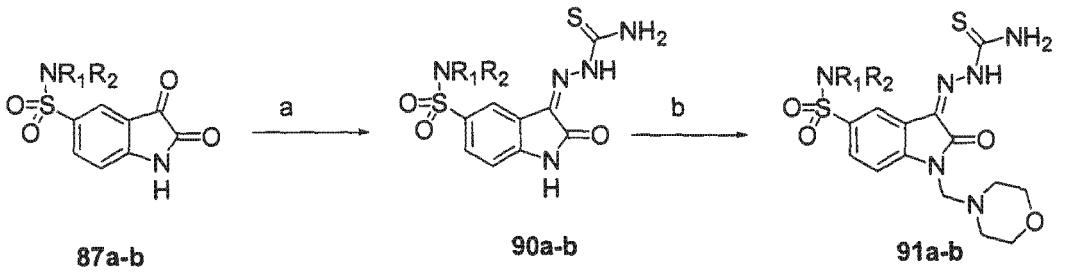


Figure 34. 5-isatin sulfonamides

Having successfully accomplished the synthesis of the 5-isatin sulfonamides **87a** and **87b** in good yields, attention was now turned to the preparation of the target molecules. This was executed as outlined in Scheme 20.



87a-b **90a-b** **91a-b**

Scheme 20. *Reagents and conditions*: (a) 1.0 equiv of $H_2NNHCSNH_2$, DMF, 70 °C, 12h (b) 1.0 equiv of morpholine, 1.3 equiv of CH_2O, DMF, 70 °C, 4h

5-Isatin sulfonamides **87a** and **87b** were reacted with thiosemicarbazide in DMF at 70 °C to afford **90a** and **90b** in 80 % and 75 % yields respectively. The nucleophilic addition predictably occurred at C-3, the more electrophilic of the two carbonyl groups available. Finally, subjecting **90a** and **90b** to the Mannich reaction furnished **91a** and **91b** in 76% and 74% yields respectively. All the compounds were characterized by ^1H and ^{13}C NMR, IR and HRMS spectra.

In summary, a limited series of Mannich bases of acetylenic chalcones, phenolic thiosemicarbazones/semicarbazone, aminoquinoline semicarbazone and isatins have been synthesized in moderate to excellent yields. The synthetic routes employed in the preparation of all the compounds are simple and straightforward. This is very important if these compounds are ever to be developed into therapeutic agents for the world's developing countries.

CHAPTER 4

BIOLOGICAL RESULTS AND DISCUSSION

4.1.0 C-Mannich Bases

4.1.1 *In vitro* activity of C-Mannich bases against falcipain-2 and a chloroquine-resistant strain (W2) of *P. falciparum*

The antimalarial and cysteine protease inhibitory effects of Mannich bases of acetylenic chalcones, thiosemicarbazones and semicarbazones on cysteine protease were assessed using two *in vitro* assays. These assays measured effects of the compounds on enzyme (falcipain-2) inhibition, and parasite development. The tests were conducted at the Department of Medicine, San Francisco General Hospital, University of California, San Francisco (USA). The description of the assays is given in the experimental section. The results are presented in Tables 7-9.

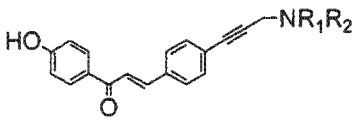
4.1.2 Results and Discussion

It is clear from Table 7 that all compounds with the exception of **40e** generally showed weak to moderate activity against both falcipain-2 and *P. falciparum* W2 strain. One of the reasons for the potencies of these compounds may generally be ascribed to the protonation of nitrogen atoms under the weakly acidic conditions within the parasite food vacuole. This may increase the interaction of these compounds with the protease at His 67 in the active sites or else protonated His 67 (free histidine has pKa of ~6[26]) may form a hydrogen bond with the inhibitors.[77] There is fairly good correlation between inhibition of falcipain-2 and antimalarial activity. This correlation is quite strong when the two compounds **40d** and **40e** (with IC_{50} values > 10 μM) are excluded. In this case inhibition of falcipain-2 alone may not be the sole determinant of *in vitro* antimalarial activity.

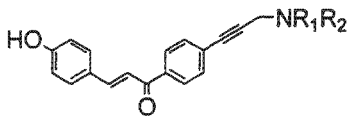
It is also clear that compounds **39b**, **41b** and **40a** containing the 4-amino-7-chloroquinolinyl moiety are more efficacious than the others regardless of the chalcone aromatic ring to which they are attached. The enhanced antimalarial activity of these 4-aminoquinoline compounds may be attributed to the presence of an additional protonatable quinoline nitrogen atom which is predicted to increase accumulation in the parasite acidic food vacuole via pH trapping.[77] In addition to increased accumulation, the

increased antimalarial activity may be may due to binding of the 4-amino-7-chloroquinolinyl moiety to hematin which then prevents dimerization of hematin to the inert β-hematin or hemozoin. The net result is a build up of the toxic hematin which kills the parasite.[142]

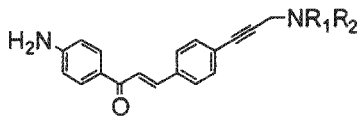
Table 7. Effects of Mannich bases of acetylenic chalcones on the activity of recombinant falcipain-2 and development of a cultured W2 strain of *P. falciparum*



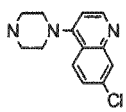
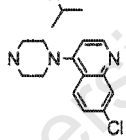
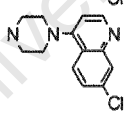
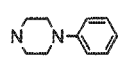
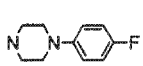
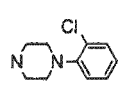
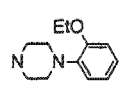
39a-b



40a-e



41a-b

Compound no.	R_1R_2N	IC_{50} (μM) Falcipain-2	IC_{50} (μM) W2[a]
39a		6.07	5.24
39b		2.01	1.70
41a		11.41	4.58
41b		2.13	1.13
40a		1.54	1.22
40b		6.96	2.95
40c		5.51	3.22
40d		3.75	10.60
40e		2.91	>10
Chloroquine			0.24

[a]No food vacuole abnormality was observed

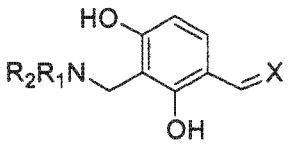
The 4-aminoquinoline compounds **39b**, **40a** and **41b** were potent against both falcipain-2 and W2. Compound **40a** with the Mannich base moiety on the ketone component showed comparable inhibitory effect both against falcipain-2 and cultured parasites to its counterpart **39b** ("reversed chalcone") with the Mannich base on the aldehyde component. Although compounds **39b**, **40a** and **41b** are less active compared to chloroquine in the W2 strain, reliable comparative studies can only be made upon screening against a wide range of parasite strains of varying degrees of resistance and/or sensitivity. This is because it is now well accepted that drug resistance in malaria is often compound specific and strain-dependent. Overall, Mannich bases of acetylenic chalcones containing the 4-amino-7-chloroquinolinyl moiety are the most promising scaffolds in this limited series of compounds for both falcipain-2 and cultured parasites.

As can be seen from the footnote (Table 7), no food vacuole abnormality that should accompany toxicity due to inhibition of falcipain-2 was observed. This abnormality in the food vacuole is indicative of a block in hemoglobin degradation. That it was not observed suggests that falcipain-2 is not the target of these compounds, which may be acting by different mechanisms, including inhibition of other cysteine proteases not contained in the screen. There are many cysteine proteases in *P. falciparum* which have not been fully characterized in terms of their role. One such cysteine protease is falcipain-1 which has recently been implicated in invasion of the host cells by the malaria parasite.[143]

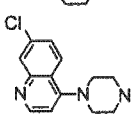
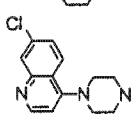
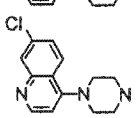
It is apparent from the results in Table 8 that all the aldehyde precursors except **76f** were ineffective against falcipain-2 and cultured parasites at the maximum concentration used. As expected for an aminoquinoline, compound **76f** showed good activity (low-micromolar IC_{50} value) against *P. falciparum* W2 parasites but exhibited poor inhibitory activity against falcipain-2. Thiosemicarbazones **77b**, **77c** and **77f** were active against falcipain-2 with IC_{50} values less than 10 μM. Since the corresponding aldehyde precursors were ineffective against falcipain-2, it is reasonable to suppose that the thiosemicarbazone moiety plays a role in the inhibition of falcipain-2. This statement is further supported by the lack of activity displayed by semicarbazone **77g** which surprisingly showed superior antimalarial activity against W2 compared to **77f**, a

thiosemicarbazone. However, of these thiosemicarbazones, only aminoquinoline **77f** showed antimalarial activity with IC_{50} less than 10 μM. On the basis of previous data, this may be due to the aminoquinoline moiety. This is further supported by **76f** (aldehyde precursor) and **77g** (semicarbazone), both aminoquinolines, which also showed antimalarial activity despite exhibiting poor inhibitory activity against falcipain-2.

Table 8. Effects of phenolic Mannich bases of aldehydes, thiosemicarbazones and semicarbazone on the activity of falcipain-2 and development of *P. falciparum*.



X = O
X = NNHC(S)NH$_2$
X = NNHC(O)NH$_2$

Compound No	R_1R_2N	X	IC_{50} (μM) Falcipain-2	W2[a]
76a		O	>20	>10
76b		O	>20	>10
76c		O	>20	>10
76d		O	>20	>10
76e		O	>20	>10
76f		O	>20	1.07
77a		NNHC(S)NH$_2$	11.07	>10
77b		NNHC(S)NH$_2$	5.85	>10
77c		NNHC(S)NH$_2$	3.80	>10
77d		NNHC(S)NH$_2$	>20	>10
77e		NNHC(S)NH$_2$	18.22	>10
77f		NNHC(S)NH$_2$	2.25	3.75
77g		NNHC(O)NH$_2$	>20	0.25
78	NMB[b]	NNHC(S)NH$_2$	>20	>10
Chloroquine				0.24

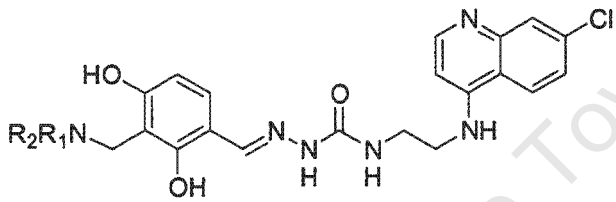
[a]No food vacuole abnormality; [b]NMB = no Mannich base

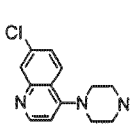
The only thiosemicarbazone from this limited series which showed activity against both falcipain-2 and cultured parasites with IC_{50} values of 2.25 μM and 3.78 μM respectively is compound **77f**. Once again due to the absence of food vacuole abnormality, these compounds do not act by inhibiting falcipain-2. Compared with chloroquine, compound **77g** was almost equipotent against W2 strain whereas **76f** and **77f** were 4 and 15 times less active than chloroquine respectively.

Thiosemicarbazones **77b**, **77c** and **77f** and to lesser extent semicarbazone **77g**, are potential metal chelators and may be acting as iron chelators in *P. falciparum*. The antimalarial action of chelators is dictated by three factors:[144] iron(III)-binding capacity, chelator ingress into parasitized erythrocytes and chelator egress from parasites after treatment. Various iron chelators have been shown to improve drug lipophilicity leading to increased access of the drug to intracellular parasites and to faster speed of action.[145,146] For example, desferrioxamine **19** does penetrate the infected red blood cell, and its antimalarial activity is dependent on this[147]. It would be predicted that an effective antimalarial iron chelator would have the ability to cross lipid membranes well, have a high affinity for iron, selectively bind iron as compared with other trace metals and selectively bind iron(III) rather than iron(II).[148] Thus, the hydrophilic/hydrophobic balance or relative lipophilicity of a compound is an important factor in movement of an agent across a lipid containing membrane to enter a cell, and in determining its usefulness.[149] There are two known ways for metal chelators to inhibit development of *P. falciparum*: (i) withholding iron from plasmodial metabolic pathways or (ii) forming complexes with iron that are toxic to the parasite. It has been shown that withholding iron can lead to malfunctioning of iron dependent enzymes such as δ-aminolevulinate synthase, an enzyme in the heme biosynthetic pathway[150] and ribonucleotide reductase, RR, an iron-dependent enzyme essential for DNA synthesis.[151,152] The main mechanism of action is thought to be a metal-mediated reaction with the tyrosyl radical in the M2 subunit of RR. Therefore binding to endogenous Fe is essential to this mode of action.[153] Tridentate chelating thiosemicarbazones have also been proposed to act by inhibiting dihydrofolate reductase.[154,155] Lastly, one study gives evidence that copper complexes of thiosemicarbazones produce significant oxidative stress by binding endogenous reducing agents such as glutathione.[156]

Since compound **78** was ineffective against both falcipain-2 and cultured parasites, this suggests that the activity of compounds **77a**, **77b**, **77c** and **77f** is not due to the thiosemicarbazone side chain alone. Coupled with data presented in Table 8, with an exception of **76f**, it can be concluded that the inhibitory activity of phenolic Mannich bases of thiosemicabazones is due to the combined effects of both Mannich base and thiosemicarbazone components.

Table 9. Effects of Mannich bases of 4-aminoquinoline semicarbazone derivatives on the activity of falcipain-2 and development of *P. falciparum* W2 strain.



Compound No	R_1R_2N	IC_{50} (μM) Falcipain-2	IC_{50} (μM) W2[a]
83a		2.60	0.44
83b		0.72	1.07
83c		0.63	0.27
83d		>20	0.11
83e		>20	0.38
83f		3.16	0.077
Chloroquine			0.24

[a]No food vacuole abnormality was observed

The results from Table 9 indicate that all compounds exhibited significant antiplasmodial activity. The most notable compound is bisquinoline **83f** with an IC_{50} value of 0.077 μM. Compounds **83a**, **83b**, **83c** and **83f** were active against both falcipain-2 and cultured parasites. There was generally no correlation between the ability of these compounds to

inhibit falcipain-2 and their antiplasmodial activity against W2 strain in *vitro*. As such, the mechanism of action of these aminoquinoline semicarbazones is unclear. Most likely, as was the case with a series of chalcones, although some of the aminoquinoline semicarbazones inhibited falcipain-2, their antimalarial effects were independent of the inhibition of this enzyme. This was supported by the absence of food vacuole abnormality. In any case, it is evident from the results that the quinolinyl moiety plays a significant role in determining the antimalarial activity of the tested quinoline semicarbazones, and this is in agreement with results obtained in Tables 8 and 9. The most potent compound against W2 strain is **83f**, a bisquinoline, with an IC_{50} value of 0.077 μM. Like chloroquine and other antimalarial bisquinolines, bisquinoline **83f** may exert its antimalarial properties by inhibiting hematin dimerization. It may bind to hematin μ-oxo dimer, shifting the equilibrium between hematin monomer and hematin μ-oxo dimer, thereby decreasing hematin monomer incorporation into hemozoin.[157] It should be noted, however, that bisquinoline-hematin μ-oxo binding may have other consequences which may or may not contribute to their antimalarial properties. One example is inhibition of both glutathione- and glutathione/hydrogen peroxide-mediated iron release from hematin and inhibition of hematin-dependent lipid peroxidation.[158] Another is inhibition of polyamine transport.[159] Compared to chloroquine, **83a**, **83b**, **83e** were slightly less active against W2 strain. Once again a wider variety of parasite strains need to be used on these compounds for meaningful comparisons to be made against chloroquine. Compound **83c** was almost equipotent with chloroquine whereas **83d** and **83f** were more active, with **83f** being 3 times more active than chloroquine. In light of the results obtained, this series of compounds particularly bisquinoline **83f**, which is a novel compound based on the semicarbazone scaffold, warrants further investigation.

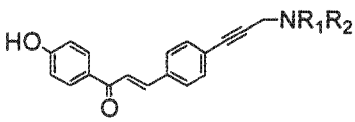
4.2.0 *In vitro* activity of C-Mannich bases against cruzain and rhodesain

The subclasses of Mannich bases presented in Tables 4, 5 and 6 were also screened against trypanosomal cysteine proteases rhodesain and cruzain. The tests were conducted at the Department of Tropical Diseases Research Unit, University of California, San Francisco (USA) by Prof. James H. McKerow and Elizabeth Hansell. The description of the assays is given in the experimental section. Results of the screens are presented in Tables 10-12.

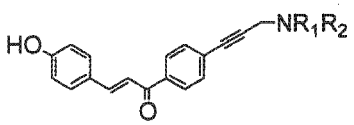
4.2.1 Results and Discussion

Percentage residual enzyme activity was determined in the presence of 10 μM inhibitor. Compounds that reduced enzyme activity to 50% or less were screened at concentrations ranging from 10 μM to 0.1μM and IC_{50} values accordingly determined.

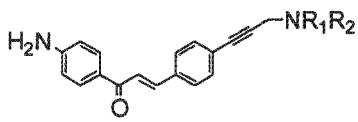
Table 10. Effects of Mannich bases of acetylenic chalcones on the activity of rhodesain and cruzain



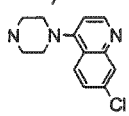
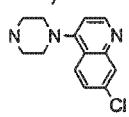
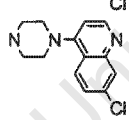
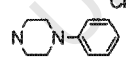
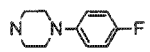
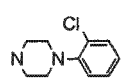
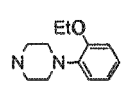
39a-b



40a-e



41a-b

		Rhodesain		cruzain	
Compound No	R_1R_2N	% residual activity at 10 μM inhibitor	IC_{50} (μM)	% residual activity at 10 μM inhibitor	IC_{50} (μM)
39a		~100	ND	83	ND
39b		89	>10	83	ND
41a		88	ND	99	ND
41b		86	>10	87	ND
40a		~100	ND	92	ND
40b		~100	ND	90	ND
40c		~100	ND	89	ND
40d		~100	ND	94	ND
40e		~100	ND	97	ND

ND = not determined

From Table 10, it is clear that all compounds generally showed poor inhibitory activity against both cruzain and rhodesain. Compared to falcipain-2, the SAR data against

cruzain and rhodesain provides some insight into the differences in the active site topology between malarial cysteine proteases and trypanosomal cysteine proteases. The data also suggests that the chalcones may not bind or bind weakly to the active sites of trypanosomal cysteine proteases because their dimensions of the pharmacophore are not complementary to the active sites.

Studies carried out by Du et al[117] showed that the most potent thiosemicarbazone inhibitor against cruzain contained a bromo or trifluoromethyl substituent at the 3′ position on the aryl ring and an ethyl group at position 5 of the thiosemicarbazone (Figure 35). They proposed a reversible covalent interaction between thiosemicarbazones and cruzain in which the aryl group and the ethyl group of the thiosemicarbazone lie in the S2 and S1 pockets of the enzyme respectively.

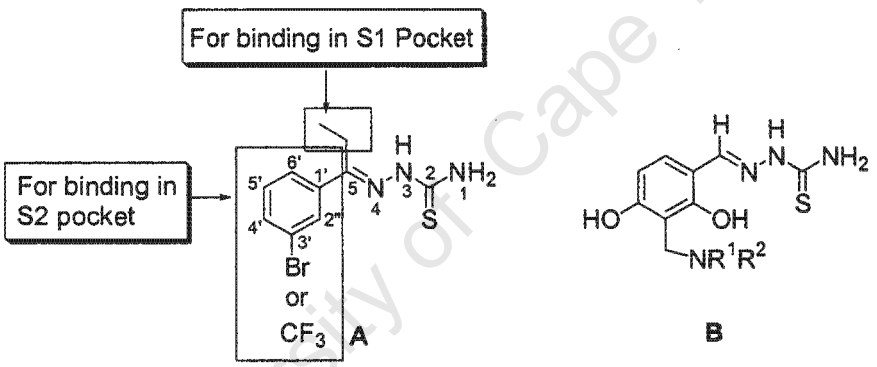
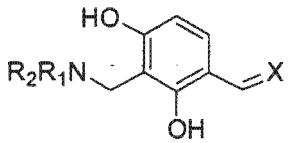


Figure 35. Comparison of known thiosemicarbazone inhibitor **A** against cruzain and potential thiosemicarbazone of Mannich base **B**

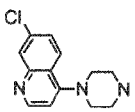
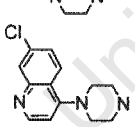
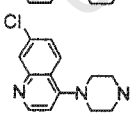
4.2.2 SAR Studies

A series of thiosemicarbazones with various Mannich base substituents at position 3 on the aryl moiety was elaborated. Compound **78**, which does not have a Mannich base substituent at position 3, was used for comparison purposes. The results from Table 11 show that all Mannich bases of 2,4-dihydroxybenzaldehyde were practically ineffective against both rhodesain and cruzain whereas Mannich bases of thiosemicarbazone showed some inhibitory activity. This clearly indicates that the semicarbazone moiety plays a significant role in the inhibition of rhodesain and cruzain. Compounds **77a**, **77b**, **77c**, **77e** and **77f** were potent against rhodesain in low to mid micromolar range. The most

Table 11. Effects of phenolic Mannich bases of aldehydes, thiosemicarbazone and semicarbazone on the activity of rhodesain and cruzain



X = O
X = NNHC(S)NH$_2$
X = NNHC(O)NH$_2$

Compound No	R_1R_2N	X	Rhodesain % residual activity at 10 μM inhibitor	Rhodesain IC_{50} (μM)	Cruzain % residual activity at 10 μM inhibitor	Cruzain IC_{50} (μM)
76a		O	~100	ND	~100	ND
76b		O	~100	ND	~100	ND
76c		O	~100	ND	~100	ND
76d		O	~100	ND	~100	ND
76e		O	~100	ND	~100	ND
76f		O	~100	ND	~100	ND
77a		NNHC(S)NH$_2$	77	4.0	64	9.0
77b		NNHC(S)NH$_2$	12	2.0	19	2.1
77c		NNHC(S)NH$_2$	26	3.0	26	5.0
77d		NNHC(S)NH$_2$	73	10	71	20
77e		NNHC(S)NH$_2$	33	7.0	88	ND
77f		NNHC(S)NH$_2$	9	1.0	87	ND
77g		NNHC(O)NH$_2$	73	>10	83	ND
78	NMB	NNHC(S)NH$_2$	~100	ND	82	ND

ND = not determined, NMB = no Mannich base

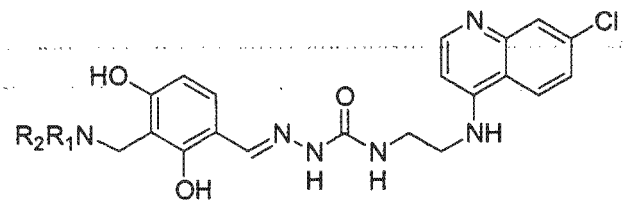
The biological results for compound **77a** are omitted in the discussion since they are misleading and need to be reconfirmed. Under normal circumstances, it is not possible for a compound with >50% residual activity at 10 μM inhibitor to have an IC_{50} above 10

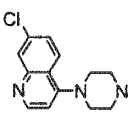
or a compound with <50% residual activity at the same concentration to have IC_{50} below 10.

Compounds **77b**, **77c**, **77e** and **77f** were potent against rhodesain in low to mid micromolar range. The most effective compound was **77f** with IC_{50} of 1.0 μM. A few compounds were active against cruzain (**77b** and **77c**) also in the low to mid micromolar range although the basic SAR was the same.

Compound **77b** was the most effective against cruzain. This indicates that the Mannich base substituent at this position may be important for binding in the S2 pocket of these cysteine proteases. Although other substituted thiosemicarbazones are missing from these preliminary SAR studies against rhodesain and cruzain, a comparison between compound **78** (unsubstituted aryl thiosemicarbazone) and compound **77b** (3-substituted aryl thiosemicarbazone) suggests that the 3-position in the benzene nucleus is a favourable position for binding against rhodesain and cruzain. From the studies conducted by Du and coworkers,[117] it was shown that the C=S of the thiosemicarbazones was important for the inhibition of cruzain. In our study, this was also confirmed against rhodesain (an homologue of cruzain) when comparing thiosemicarbazone **77f** and corresponding semicarbazone **77g**. Compared to falcipain-2, the SAR data against rhodesain and cruzain is striking in that a number of compounds were effective in the low micromolar range. In this regard, compounds **77b**, **77c**, **77e** and **77f** are noteworthy. Against cruzain, only compound **77b** was effective. Within this limited series of compounds, preliminary SAR data against the proteases gives some insight into the significant difference in the active site topology between all the three (falcipain-2, cruzain and rhodesain) closely related cysteine proteases. On one hand few inhibitors of cruzain were identified while on the other hand there were many more inhibitors of rhodesain. Based on the data for all three enzymes, rhodesain is by far the most promiscuous enzyme which can accommodate a broad range of inhibitors. Once again, these inhibitors have the ability to chelate a transition metal and it is likely that this mechanism of action is responsible for the activity of these compounds.

Table 12. Effects of Mannich bases of 4-aminoquinoline semicarbazone derivatives on rhodesain and cruzain



		Rhodesain		Cruzain	
Compound No	R_1R_2N	% residual activity at 10 μM inhibitor	IC_{50} (μM)	% residual activity at 10 μM inhibitor	Cruzain IC_{50} (μM)
83a		21.0	5.0	86	ND
83b		1.0	2.5	91	2.8
83c		1.0	1.8	95	1.8
83d		~100	ND	37	>10
83e		~100	ND	94	ND
83f		~100	ND	32	3.5

ND not determined

Compounds **83b-d** are omitted in the discussion of activity against cruzain (see comment on page 72).

From Table 12, it is clear that compounds **83a**, **83b** and **83c** were effective against rhodesain with IC_{50} values in the low-micromolar range. On the other hand, only compounds **83f** exhibited inhibitory activity against cruzain. Once again, this indicates that an aminoquinoline semicarbazone can bind to a cysteine protease target. None of Compounds was effective against all the three (falcipain-2, cruzain and rhodesain) closely related cysteine proteases.

4.3.0 N-Mannich bases

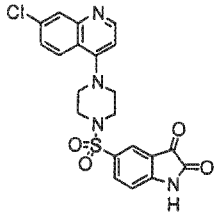
4.3.1 Antiplasmodial activity of isatin derivatives

Isatin scaffolds were screened for *in vitro* antiplasmodial activity against a chloroquine sensitive strain (D10) and chloroquine resistant (K1) strain of the parasite *P. falciparum*. It should be noted, however, that this class of compounds was not screened against the proteases as they were primarily just intended as exploratory compounds against *P. falciparum* in *vitro*. As with sulfonamides and thiosemicarbazones, targets for these compounds could be multiple. It was reasoned that an unbiased screening against *P. falciparum* might reveal promising compounds whose targets could be identified in future.

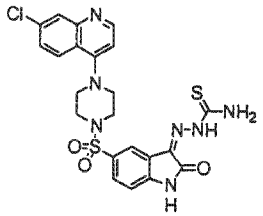
4.3.2 Results and Discussion

The compounds in the series **87a-b**, **90a-b** and **91a-b** were screened against K1 and D10 strains of *P. falciparum*. Percentage parasite viability, which gives a quick but reliable measure of inhibitory activity, was determined instead of IC_{50}. The lower the % parasite viability, the more potent a compound is as an antiplasmodial agent at a given concentration. The results are presented in Table 13.

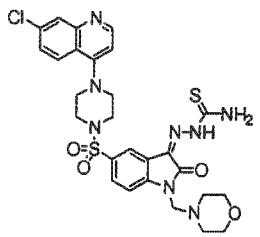
Table 13. Percentage parasite viability against *P. falciparum* D10



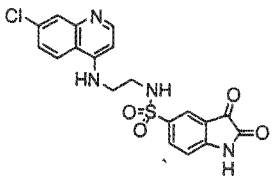
87a



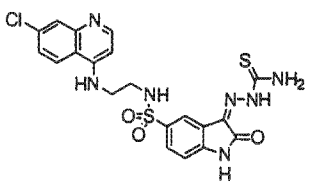
90a



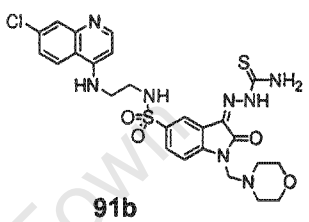
91a



87b



90b



91b

	Parasite Viability %			
Sample/Concentration	1000 ng/ml	500 ng/ml	250 ng/ml	ClogP
87b	23.67	55.17	57.95	2.95
90b	0.00	45.82	51.93	3.37
91b	0.00	51.25	52.12	4.04
87a	53.48	47.83	51.02	2.57
90a	0.00	54.22	60.11	3.00
91a	11.25	62.63	56.47	3.66
CQ Concentration	100 ng/ml	50 ng/ml	25 ng/ml	
CQ1	0.00	0.00	0.00	
CQ2	0.00	0.31	0.00	

According to the results obtained, all the compounds showed significant activity against the *P. falciparum* D10 strain. It is interesting to note that: (i) thiosemicarbazones of isatin sulfonamides **90a,b**; **91a,b**, were generally more effective against the *P. falciparum* D10 than corresponding isatin sulfonamides **87a,b**. As mention earlier, thiosemicarbazones are potential iron-chelators and may exert their biological activity either by withholding iron from plasmodia. They also possess a protonatable hydrazinic nitrogen atom which possibly increases accumulation in the acidic food vacuole relative to the isatin precursors. In addition they have an imine (C=N) and thiol carbonyl (C=S)

groups which are potential sites for interaction with a cysteine thiolate in the active site of the enzymes (cysteine proteases), (ii) compounds with the ethylene spacer (**87b** and **91b**) were more active than those with a piperazinyl spacer (**87a** and **91a**) at 1000ng/ml whereas **90b** was more active than **90a** at 500ng/ml. This is probably due to the fact that the ethylene spacer generally increases the lipophilicity of the compound. This fact is highlighted when the *C*logP values of compounds **91a** and **91b** are compared with those of compounds **90a** and **90b**. As a general rule, within a congeneric series, drug absorption usually increases rapidly as lipophilicity rises, and is maintained at a plateau for a few units of *C*logP, after which there may be steady decrease.[160] (iii) appending a Mannich base unit to the thiosemicarbazone of isatin sulfonamide was not beneficial to the antimalarial activity. This is evident when comparing results obtained from compounds **90a**, **90b** and **91a**, **91b** respectively.

Some compounds showed different parasite viability at 250 ng/ml and were retested against *P. falciparum* D10 strain (Table 14). The activity of compound **90b** could not be replicated with retesting. Compound **91a** showed variability again at 250 ng/ml. Therefore compounds **90b** and **91a** were tested for a third time against the sensitive strain of the parasite (D10).

Table 14. Compounds retested against *P. falciparum* D10

	D10-2			D10-3		
Samples Concentration	1000 ng/ml	500 ng/ml	250 ng/ml	1000 ng/ml	500 ng/ml	250 ng/ml
87b	85.42	94.88	92.48			
90b	74.54	94.87	93.21	93.84	90.86	70.80
91b	5.96	85.55	84.07			
87a	112.43	87.87	33.66			
90a	5.52	92.36	66.44			
91a	16.62	56.52	55.78	101.23	103.64	77.25
CQ Concentration	30 ng/ml	15 ng/ml	7.5 ng/ml	30 ng/ml	15 ng/ml	7.5 ng/ml
CQ1	0.00	11.81	48.83	38.07	57.48	100.56
CQ2						

D10-2 refers to the second repeat; D10-3 refers to the third repeat

Table 15. Percentage parasite viability against *P. falciparum* D10 and K1 strains

	D10-1			K1		
Samples Concentration	1000 ng/ml	500 ng/ml	250 ng/ml	1000 ng/ml	500 ng/ml	250 ng/ml
87b	23.67	55.17	57.95	80.60	87.39	90.96
90b	0.00	45.82	51.93	55.75	76.71	92.78
91b	0.00	51.25	52.12	14.87	75.78	87.19
87a	53.48	47.83	51.02	89.78	100.24	95.83
90a	0.00	54.22	60.11	47.75	89.61	88.33
91a	11.25	62.63	56.47	54.44	71.13	90.95
CQ Concentration	100 ng/ml	50 ng/ml	25 ng/ml	200 ng/ml	100 ng/ml	50 ng/ml
CQ1	0	0	0	16.36	32.49	73.39
CQ2	0	0.31	0	22.19	34.22	76.62

D10-1 refers to the first experiment

From the results, the initial activity could not be replicated for these two compounds (**90b**, **91a**) for reasons which are unclear. The interpretation of the data was made more difficult by the inconsistent data obtained from the biological testing. The expected trend was to be such that the % parasite viability should increase with decrease in the concentration of each compound. The inconsistent biological data casts doubt on the validity of such. These experiments need to be repeated and more consistent results obtained. The most active compounds against the sensitive strain were also screened against *P. falciparum* K1 strain (Table 15). The initial *P. falciparum* D10 results were used to compare with the *P. falciparum* K1 results as these experiments were performed with fresh stock solutions. Here, it should be note that the experiments D10-2 and D10-3 were not performed with solutions taken from the fresh stock solutions used in experiment D10-1. However, the same trend was observed with *P. falciparum* K1. From the data obtained compounds **90b**, **91b**, **90a** and **91a** showed some activity against both strains of *P. falciparum* at the concentrations tested. Compound **91b** was the most promising against both strains of the parasite.

4.4.0 Conclusion

From the screens, none of the Mannich bases of acetylenic chalcones were potent against either cruzain or rhodesain. What is significant, however, is that all of them were

effective against the W2 strain of *P. falciparum* and most of them were active against falcipain-2 (i.e compounds with IC_{50}<10). The most effective compound against both falcipain-2 and W2 strain is **40a**. For thiosemicarbazones, **77f** and **77g** were active against W2 strain with **77g** being almost as potent as chloroquine. Most of the thiosemicarbazones were effective against rhodesain whereas a few were active against cruzain. All 4-aminoquinoline semicarbazones were effective against W2 strain with **83f** being 3 times more active than CQ. Most of the compounds were potent against falcipain-2. Comparison within each series of compounds has shown that 4-aminoquinoline semicarbazones are the most potent compounds against falcipain-2 and rhodesain. From a therapeutical point of view, compounds that combine pharmacological activities may be beneficial or not beneficial. In this case of 4-aminoquinoline semicarbazones the pharmacological properties against homologous cysteine proteases from multiple protozoan parasites is advantageous.

We also identified the Mannich base of isatin derivative **91b** as the most promising hit against the chloroquine sensitive (D10) and chloroquine resistant (K1) strains of *P. falciparum* parasites. However, screens against *P. falciparum* should include a wide range of strains with varying degrees of resistance before any compound is dismissed. This is in view of the fact that antimalarial drug resistance is often strain specific and a compound or compounds that perform badly against one strain could perform better in other strains.

Finally, the synthetic routes employed in the preparation of the 4-aminoquinoline are simple and straightforward. This is very important if these compounds are ever to be developed into therapeutic agents for the world's developing countries. Future research efforts should be directed towards 4-aminoquinoline semicarbazones since a vast improvement in the activity of these potential cysteine protease inhibitors is possible by a rational modification of the basic structure.

CHAPTER 5

TRYPANOSOME ALTERNATIVE OXIDASE AS A TARGET FOR THE DEVELOPMENT OF ANTI-TRYPANOSOME CHEMOTHERAPEUTIC AGENTS

5.1.0 Introduction

Biological systems for energy production are essential for survival, continued growth and reproduction of all living organisms, including parasites. Parasites have developed numerous physiological functions for their survival within the specialized environment of the host. Since parasites are totally dependent on glycolysis for energy metabolism, they adapt to the low oxygen tension environment in the host using metabolic pathways that are unique to those of the host animals. Moreover, most parasites do not utilize free oxygen available within the host, but employ systems other than oxidative phosphorylation for ATP synthesis. In such systems, parasite mitochondria undergo significant changes in their morphology and composition during development and environmental adaptation.[161] These unique aspects show that the parasite mitochondria is a worthwhile target for the development of anti-trypanosomal chemotherapeutic agents.

5.2.0 Mitochondria of *T. b. brucei*

T. b. brucei is closely related to, and serves as a model for, *T. b. rhodesiense* and *T. b. gambiense*. Flagellates generally have one mitochondrion per cell, which contains RNA-editing kinetoplast DNA. Both the structure and function of *T. brucei* mitochondria change markedly during their life cycle.[161] In the insect stage, the mitochondria is fully developed and respiration phosphorylation provides the main source of energy. However, in the bloodstream form of this parasite, the stage infective for mammals, the mitochondria contain no cytochrome-mediated electron system and no tricarboxylic acid cycle and do not synthesize ATP by oxidative phosphorylation.[162] The parasite depends entirely on glycolysis for energy production. Bowman *et al* have shown that the high rate of oxygen uptake by these protozoa is not inhibited by cyanide, antimycin, rotenone, or azide.[163] It was further shown that the respiratory system is composed of glycerol-3-phosphate dehydrogenase, ubiquinone and cyanide-insensitive glycerol-3-phosphate

oxidase (Figure 36) and that respiration is a mitochondrial function in these parasites.[164,165]

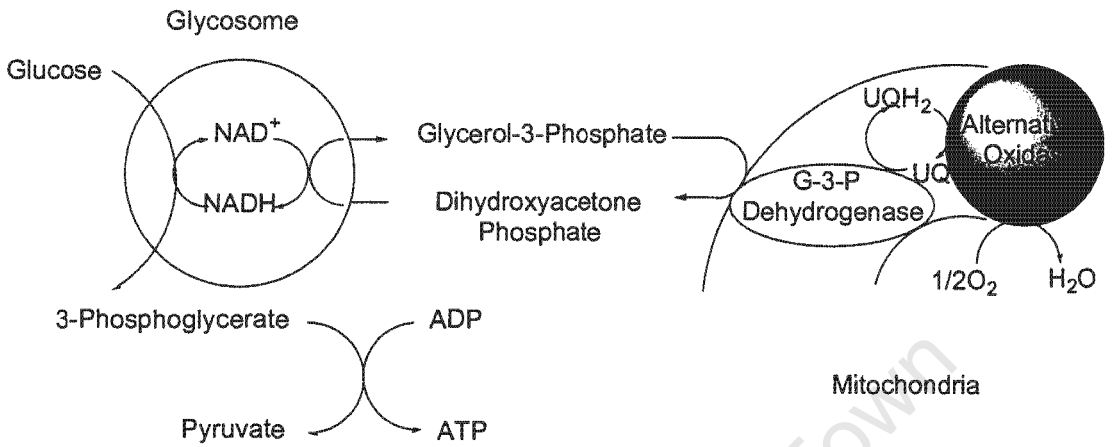


Figure 36. Glycerol-3-phosphate oxidase system in *T. brucei* mitochondria. This oxidizes glycerol-3-phosphate produced in the glycosomes using the electron transport system consisting of glycerol-3-phosphate dehydrogenase, ubiquinone, and cyanide-insensitive alternative oxidase (TAO) localized in the mitochondria inner membrane

5.3.0 Glycolytic enzymes

Bloodstream forms of *T. brucei* exclusively produce energy through glycolysis, metabolizing glucose only as far as pyruvate, making the inhibition of any of the glycolytic enzymes a potential therapeutic target.[166] A great effort has been made to exploit these enzymes; however, none of the inhibitors tested has led to promising results *in vivo*. Recently, research has focused on the trypanosomal glyceraldehyde-3-phosphate dehydrogenase, with the design of selective adenosine analogues. The promising *in vitro* activity remains to be confirmed *in vivo*.[167] The discovery that the phosphoglycerate mutase in the parasite and the host are not homologous, the presence of an ATP-dependent phosphofructokinase with a typical pyrophosphate-dependent signature and the presence of a pyruvate kinase that is uniquely regulated by fructose-2,6-bisphosphate offer additional potential for the design of selective inhibitors.[168] A different approach that has proved effective *in vivo* is the inhibition of trypanosome alternative oxidase (TAO) with benzamides or ascofuranone in combination with glycerol only.[169,170]

5.4.0 Glucose Metabolism

Glucose metabolism in the bloodstream-form trypanosomes differs from glycolysis in eukaryotes in a number of respects: (a) trypanosomes lack a lactate dehydrogenase and therefore the NADH generated in the glycolysis is reoxidised by molecular oxygen *via* a dihydroxyacetone phosphate (DHAP): glycerol-3-phosphate (G3P) shuttle combination with G3P oxidase (TAO) in the mitochondria. This oxidase reacts with oxygen without the intervention of pyridine nucleotide coenzymes or cytochromes. Its high activity for G3P is sufficient to account for the high rate of respiration of the bloodstream trypanosomes, (b) pyruvate rather than lactate is the end-product of glycolysis. It is excreted into the the bloodstream and, (c) under anaerobic conditions, glucose is converted quantitatively into equimolar amounts of pyruvate and glycerol (Figure 37).[171,172]

During aerobic respiration, the production and consumption of ATP and NAD are balanced in the glycosome. The conversion glyceraldehydes-3-phosphate to pyruvate yields 2 mol of ATP. Since the production of fructose-1,6-diphosphate consumes 2 mol of ATP and because only glyceraldehydes-3-phosphate is utilized in the glycolytic pathway there would no net ATP synthesis. Similarly, no net change in the NADH takes place in the glycosome during aerobic glycolysis because the NADH produced in the glycosome is reoxidised by glycerol-3-phosphate dehydrogenase which reduces DHAP to G3P which, in turn, is reoxidised to DHAP in the mitochondria by the terminal G3P oxidase.[172]

Under anaerobic conditions, or in the presence of a respiratory inhibitor, glycerol-3-phosphate accumulates inside the glycosome is converted to glycerol by the reverse action of glycerol kinase. In general, this enzyme functions almost exclusively in the forward direction, producing glycerol-3-phosphate from ATP and glycerol. The novel anaerobic glycolytic pathway in trypanosome leads to the synthesis of 1 mol of ATP and 1 mol of glycerol. In this system, 1 mol of glucose is dismutated into equimolar amounts of pyruvate and glycerol, with a net synthesis of 1 mol of ATP. A G3P:ADP transphosphorylase activity of glycerol kinase has indeed been found in *T. brucei* and the

relatively high specific activity of glycerol kinase, provides for the synthesis of glycerol and ATP from G3P and ADP. Moreover, glycerol kinase functions under true equilibrium conditions in the absence of oxygen, rather than catalyzing the phosphorylation of glycerol to G3P.[173] This is facilitated by the ability of the ATP/ADP ratio inside the glycosome to change independently from that in the cytosol. Therefore, under anaerobic conditions as well, no net change in ATP and ADP occurs in the glycosome during metabolism (Figure 37).

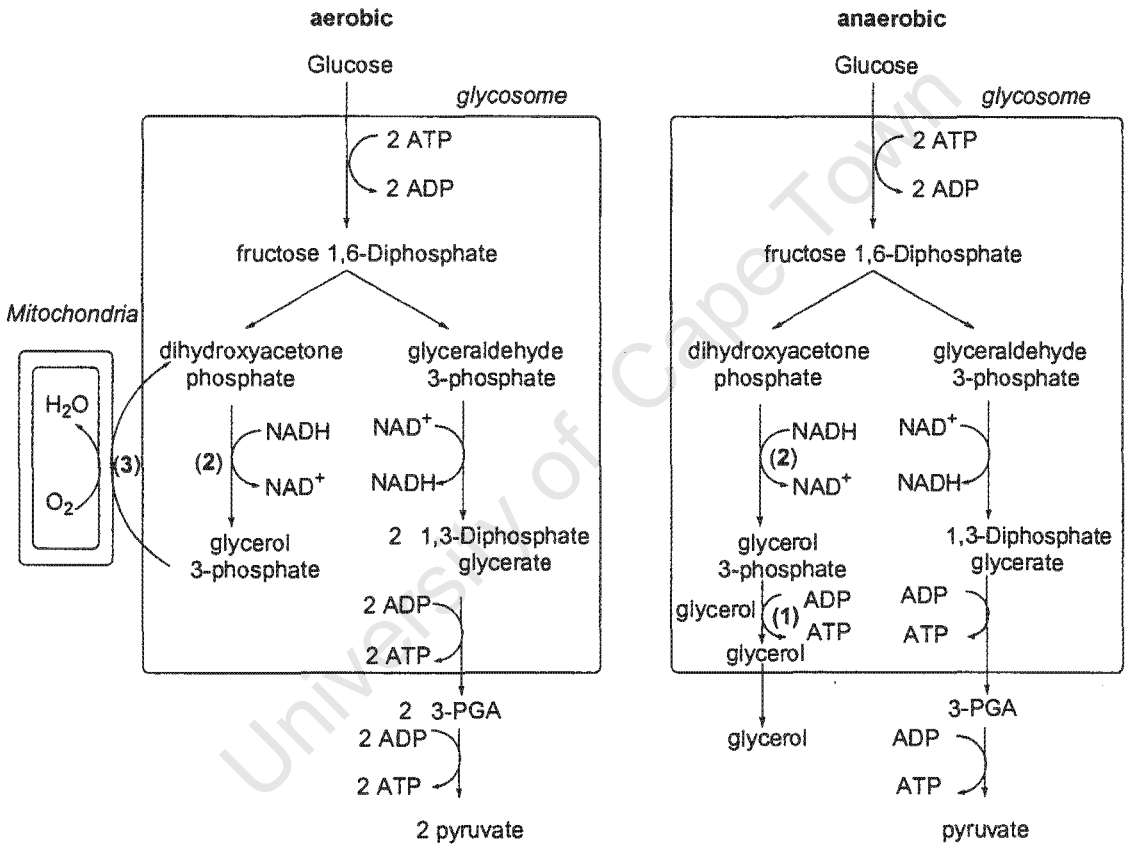


Figure 37. Glucose metabolism in the bloodstream form of *T. b. brucei* under aerobic and anaerobic conditions. (1) Glycerol kinase, (2) glycerol-3-phosphate dehydrogenase, (3) cyanide-insensitive quinol oxidase (TAO).

5.5.0 Inhibition of TAO by SHAM

Salicylhydroxamic acid (SHAM) is a potent inhibitor of the trypanosomal G3P oxidase or TAO. This was first observed by Clarkson Jr. and Brohn[174] when they treated rats that were infected with typanosomes. Inhibition, however, does not harm the bloodstream form of *T. brucei*, since the organism has the capability of switching from an aerobic to

anaerobic glycolysis. During aerobic glycolysis every glyceraldehyde-3-phosphate molecule is converted to pyruvate and one molecule of NAD$^+$ is reduced to NADH. NADH is reoxidised to NAD$^+$ by glycerol-3-phosphate dehydrogenase through the conversion of DHAP to G3P. Due to the inhibition of G3P oxidase (Figure 38), the G3P concentration rises in the glycosome because the glycosomal membrane constitutes a true barrier for the phosphorylated intermediates of the glycolytic pathway. The reversed reaction of glycerol kinase becomes important now. This causes the formation of a molecule of ATP from ADP. If excess of glycerol is present, glycerol kinase is inhibited by its own substrate and the formation of ATP ceases. Hence anaerobic-like glycolysis is no longer possible and the parasite dies because its energy supply is blocked.

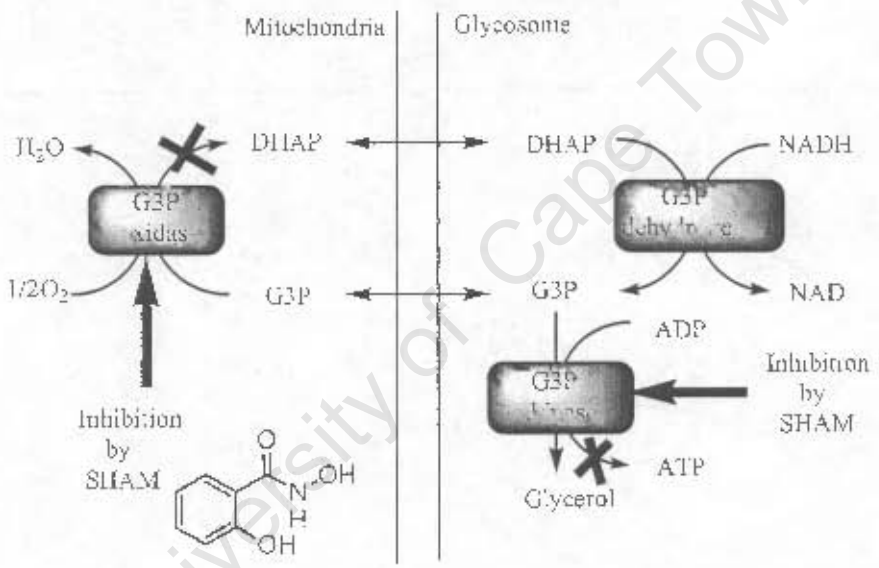


Figure 38. Inhibition of TAO by SHAM

5.5.1 Proposed model for the active site of TAO

Due to its strong association with the mitochondrial inner membrane, purification of the alternative oxidase has been difficult and pure preparations of this protein have not yet been produced. Instead the biochemical work has been performed on partially purified enzyme or mitochondrial membrane fractions. The active site of TAO is believed to contain a binuclear iron center coordinated by helices that include a Glu-X-X-His motif as well as two helices with conserved Glu or Asp residues (Figure 39).[175] SHAM could be assumed to act as a metalloenzyme (TAO) inhibitor by chelating to the proposed

active site iron atoms.[175] However, this would not account for the apparent selectivity of SHAM for TAO over other metalloenzymes.

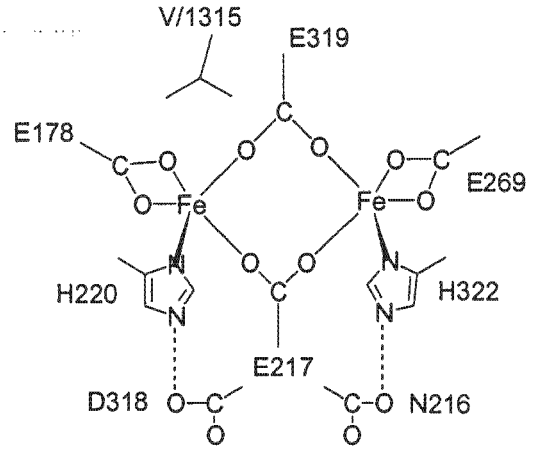
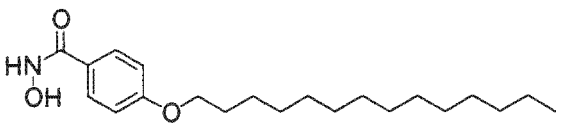


Figure 39. Proposed active site of TAO. E178, E217, E269, E319 and H322 are the ligands to the Fe center. E217 and 319 are bridging carboxylates, while E178 and E269 are terminal ligands

5.5.2 Probable mode of action of SHAM and related inhibitors of TAO.

The mode of action is best explained by the hypothesis that they interfere with ubiquinone/ubiquinol- mediated electron transport by binding to the ubiquinone receptor on the oxidase component of TAO. This hypothesis is consistent with the reversibility and the uncompetitive nature of the inhibition by SHAM which was observed by Clarkson Jr. et al[176] when glycerol-3-phosphate was the independently controlled substrate and the velocity of the glycerol-3-phosphate oxidase was measured by the rate of oxygen consumption.

This was further supported by structure-activity relationship studies. A series of *p-n*-alkoxybenzhydroxamic acids was synthesized and the ability of these compounds to kill the parasite in *vitro* was evaluated.[177] From the series *p-n*-tetradecyloxybenzhydroxamic acid **92** (Figure 40), which was 70 times more active than SHAM, was the most effective compound.



p-n-tetradecyloxybenzhydroxamic acid **92**

Figure 40. Structure of *p-n*-tetradecyloxybenzhydroxamic acid

Figure 41 shows presumed conformation of the substrates on the terminal oxidase which could account for the observed relative activities of the various *p*-n-alkyloxybenzhydroxamic acids.

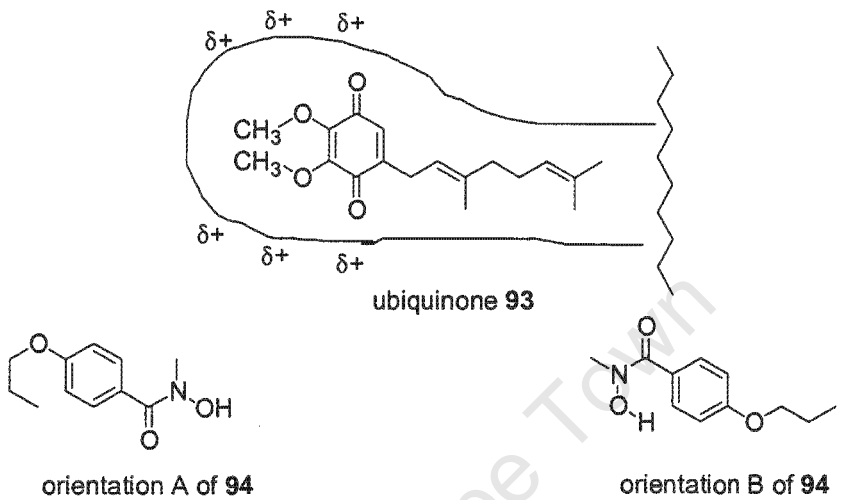


Figure 41. Possible orientation of *p*-*n*-propylbenzhydroxamic acid **94** in the receptor site for ubiquinol. Ubiquinol is shown in the upper drawing, with *p*-n-propylbenzhydroxamic acid **94** in the lower two drawings.

The short chain analogues could assume either orientation 'A' or 'B'. Orientation 'A' would be as a result of the short alkyloxy chain being attracted to the site where the methoxy groups of ubiquinol bind. When the length of the alkyloxy group is increased, orientation 'B' would be favored due to steric hindrance. The alkyloxy groups would then bind to the site usually occupied by the long lipophilic isoprenoid chain of ubiquinol. Flexibility within the active site would permit a lateral shift of the aromatic nucleus so as to assume a minimum energy conformation. For compounds in orientation 'B' it was observed that increasing the alkyl chain length increased activity of the inhibitor as the alkyl chain would better mimic the isoprenoid side chain.[177] However, this increase would be limited by the decreasing aqueous solubility associated with long chains.

The work presented in this section is part of a collaborative project with the common objective of rational development of new drugs against African trypanosomiasis in which TAO was chosen as the target. Our roles in this project were to design and synthesize

some new simple potential inhibitors of TAO. The tests for inhibitory activity *in vitro* against TAO, *T. brucei brucei* intact cells and mitochondria were conducted at the Department of Biochemistry, Vanderbilt, University, Nashiville, USA by Professor George Hill and Dr. Robert Ott.

5.6.0 Rationale for the design of prenylated amides and carbohydrate conjugates

Figure 42 illustrates the chemical feactures from which the rationale for the design are derived

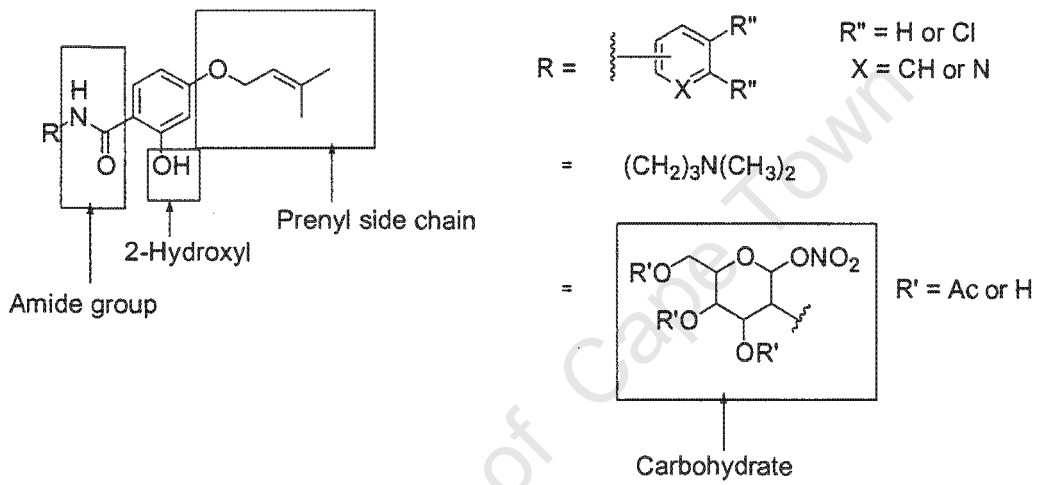
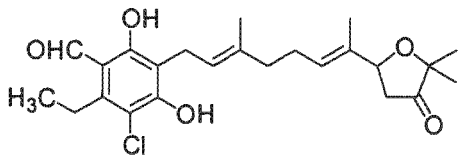


Figure 42. Chemical features of prenylated amides from which the rationale for design are derived as TAO inhibitors

The following is the summary for the rationale:

1. It was envisaged that the prenyl group would mimic the isoprenoid chain of ubiquinone.
2. The 2-hydroxy group would mimic that found in SHAM.
3. Amide analogs were chosen because of their greater solubility and resistance to serum hydrolyases *in vivo* than hydroxamates and also because the amide linkage offers more possibilities than the ether bond for further derivatization of the side chain.
4. It has recently been shown that ascofuranone **95** (Figure 43, p.88), a prenylphenol antibiotic isolated from a phytopathogenic fungus, *Ascochyta visiae*, strongly inhibited both glucose-dependent cellular respiration and glycerol-3-phosphate-dependent mitochondrial O_2 consumption of long slender bloodstream forms of *T. b. brucei*.[178]



95

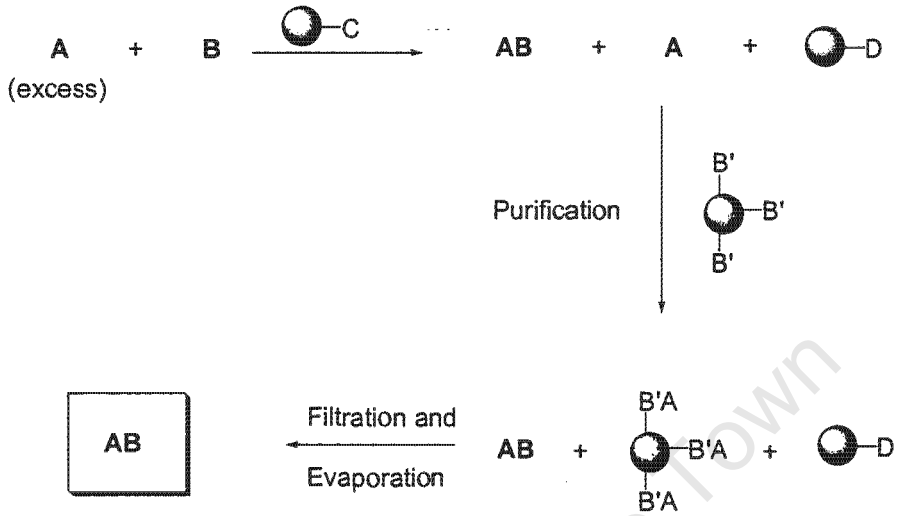
Figure 43. Structure of ascofuranone

5. As domains of natural products, carbohydrates play important roles in conferring certain physical, chemical and biological properties to their carrier molecules. Furthermore, they are implicated in many cellular processes, including cell-cell recognition, cellular transport, and adhesion: they appear in all cells in some form or another, for example, as peptido- and proteoglycans, glycoproteins, nucleic acids, lipopolysaccharides or glycolipids.[179] Some recent investigations have revealed that attachment of carbohydrate residues to peptides that are not glycosylated in nature can influence their biological functions by increasing their passage through the blood-brain barrier (central nervous system, CNS).[180-183] It has also been reported that drugs used to treat brain tumors and other conditions enter the brain better when a carbohydrate such as a glucose molecule is attached to their molecular structure. This is because glucose is actively transported into the brain by the passive glucose transporter, GLUT-1.[184] This protein transporter is located in the membrane of brain capillary endothelial cells composing the blood-brain barrier, which excludes most hydrophilic molecules. Of course this is relevant to African sleeping sickness which is a CNS infection. In addition, improved solubility was anticipated from attachment of a carbohydrate motif.

5.6.1 Synthesis of amides using polymer-supported reagents and scavengers

Since the pioneering work by Merrifield[185] polymeric supports have been identified to play a key role in organic synthesis. In classical solution phase synthesis, the final aspect of work-up and product purification can sometimes be the most problematic. In order to address some of the problems associated with classical solution phase synthesis and generate libraries of compounds rapidly, improved methods of work-up and purification must be developed as a substitute for aqueous extractions, crystallization and flash chromatography. The use of polymer-bound reagents and scavengers provides a simple and very effective means of purifying multiple solution phase reactions in combinatorial

or parallel synthesis and has recently been the subject of intense investigation.[186-189] The details of a prototypical polymer-assisted purification process are depicted in Scheme 21.



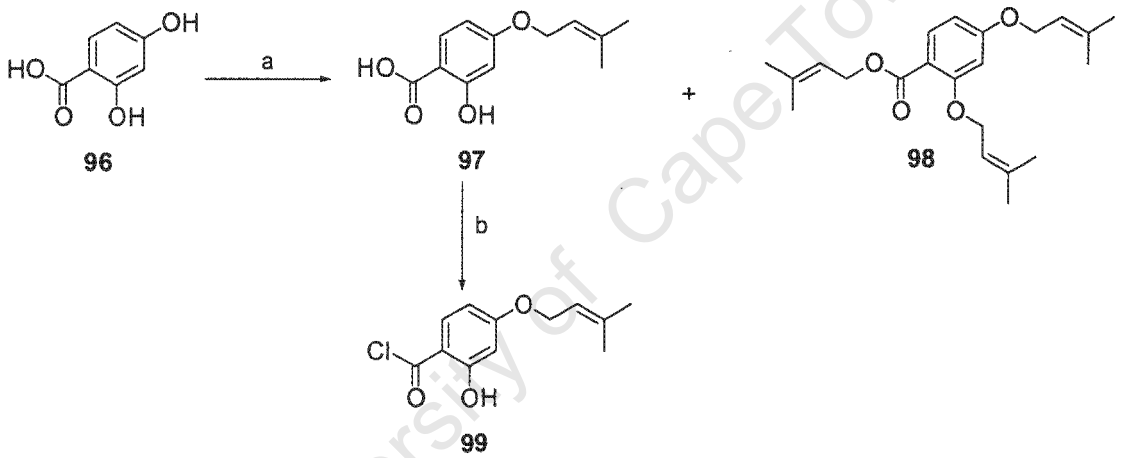
Scheme 21. Reaction of **A** and **B** employing polymer-supported reagent **C** and scavenger **B'**

In a reaction between **A** and **B** using a polymer-bound reagent **C**. a slight excess of reagent of **A** is used to drive the reaction to completion. At the end of this hypothetical reaction, a mixture of the product **AB**, excess reagent **A** and transformed polymer-bound reagent **D** exists. An excess of an insoluble polymer which bears reactive functionality that is similar to the limiting reagent **B** is added to consume the remainder of starting reagent **A**. Product isolation is achieved by filtration and solvent evaporation.

The advantages of polymer-assisted solution-phase over conventional solution-phase chemistry include (1) the ease of separation of the supported species from a reaction mixture by filtration and washing, (2) excess reagents may be used to drive the reaction to completion without workup problems, (3) the supported reagent or catalyst maybe reused, (4) the ease of adaptation to continous-flow processes and hence use in automated synthesis, (5) the reduced toxicity and odor of supported species compared with low molecular weight unsupported analogues and (6) chemical differences, such as prolonged activity or altered selectivity of a catalyst in supported form compared to its soluble analogue.[190]

5.6.2.0 Synthesis of prenylated amides

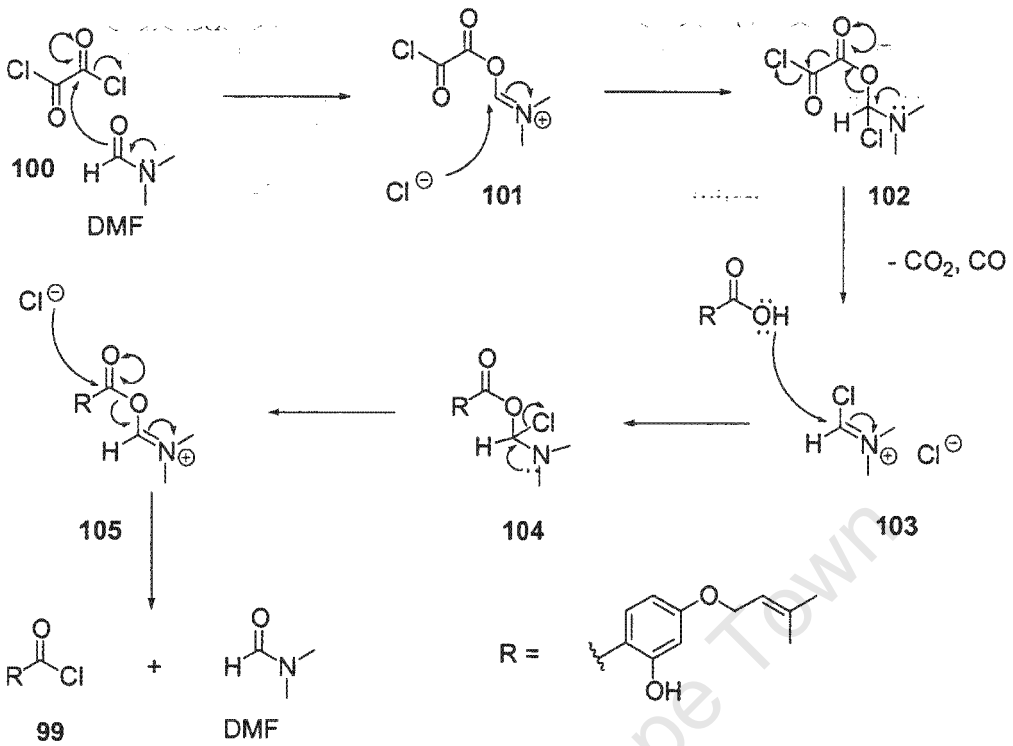
The first step in the synthesis was the preparation of the prenyl acid **97** (Scheme 22). Accordingly, 2,4-dihydroxybenzoic acid **96** was alkylated with 1-bromo-3-methyl-2-butene in the presence of anhydrous potassium carbonate as a base to afford a mixture two products that was subjected to column chromatography to give prenyl acid **97** and a triprenylated compound **98** in 67% and 28% yields respectively after hydrolysis. The ^1H NMR spectrum of compound **98** was identical to that of the prenyl acid **97**. Its molecular ion peak at 358 in the mass spectrum confirm the identity of **98**. Treatment of **97** with oxalyl chloride in the presence of a catalytic (2-3 drops) amount of DMF gave the acid chloride **99** which was treated with appropriate amines without further purification.



Scheme 22. *Reagents and conditions*: (a) 1.0 equiv of Prenyl bromide, 1.1 equiv of K_2CO_3, DMF, rt, 1.5 h (b) 3.0 equiv of Oxalyl chloride, 2 drops of DMF, CH_2Cl_2, reflux, 3 h

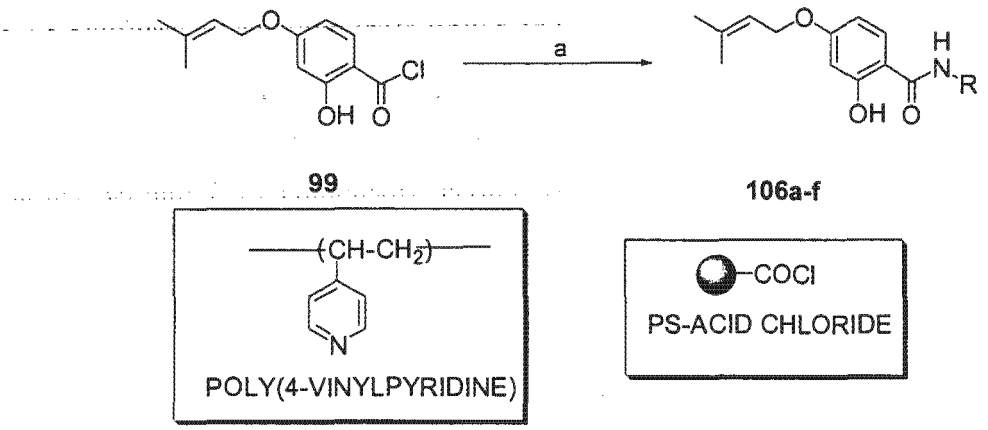
5.6.2.1 The mechanism for the formation of the acyl chloride 99

The mechanism for the formation of the acid chloride **99** is outlined in Scheme 23 on page 91. Oxalyl chloride **100** reacts with DMF to give a highly electrophilic cationic intermediate **103**, eliminating CO and CO_2 *via* the tetrahedral intermediate **102**. Nucleophiles can attack the C=N much as they might attack the C=O, an electrophile powerful enough to react even with the weak nucleophile Cl^-. Then, the carboxylic acid reacts with the reactive intermediate **103** producing another intermediate (**105**), which intercepts chloride ion to give the acyl chloride **99** and regenerates DMF.



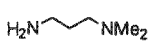
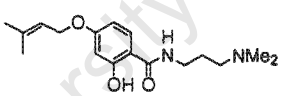
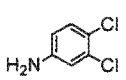
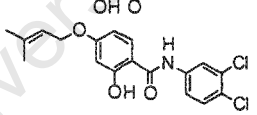
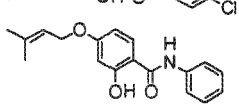
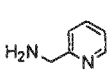
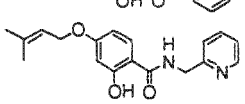
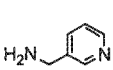
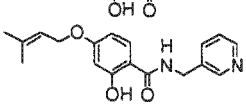
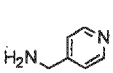
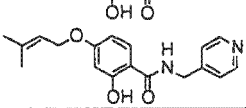
Scheme 23. Mechanism for the formation of the acyl chloride **99**

The next experimental consideration required the determination of a reliable method to form amides with various amines in a parallel or array format. Two requirements of this reaction were (i) high conversion in a reasonable timeframe and, (ii) the ability to remove HCl formed during the reaction and any unreacted reagent through sequestering with solid-phase resins. This was readily accomplished, as demonstrated in Scheme 24, through the use of polyvinylpyridine as base and a polymer-bound acid chloride as a scavenging agent. In a typical experiment compound **99** was treated with slight excess of various amines RNH_2 in the presence of polyvinylpyridine in CH_2Cl_2 for 3 h, after which 3 equivalents of polymer-bound acid chloride (relative to excess amine) was added and the reaction vessel agitated for 3 h. Removal of the resins and concentration afforded the amides in moderate to excellent yields (Table 16). The amides were judged to be of sufficient purity by ^{1}H NMR.



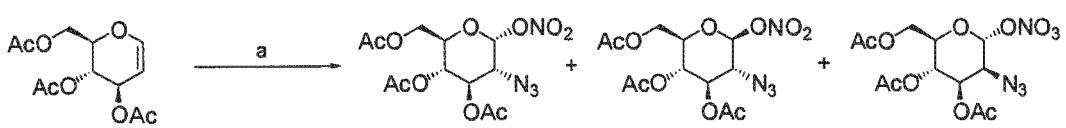
Scheme 24. *Reagents and conditions*: (a) 1.2 equiv of RNH_2, 3.0 equiv of poly(4-vinylpyridine), CH_2Cl_2, rt, 3h, then 0.6 equiv of polymer-supported acid chloride, 3h

Table 16. Products of condensation of prenylated acid chloride with amines and their respective % yields.

RNH_2	Product		% Yield
		**106a**	86
		**106b**	41
		**106c**	89
		**106d**	99
		**106e**	98
		**106f**	62

5.6.3 Synthesis of carbohydrate-linked analogues of prenylated amides

The chosen starting point in the synthesis of the carbohydrate-linked analogues was the azidonitration of 3,4,6-tri-*O*-acetyl-*D*-glucal using cerium (IV) complexes.[191] Among the various cerium (IV) complexes, cerium (IV) ammonium nitrate (CAN) is the most important oxidant in organic synthesis, since it is sufficiently stable in different solvents and is commercially available. Besides its propensity for introducing and removing protecting groups *via* single-electron transfer or Lewis acid catalysis, CAN serves as a convenient reagent for generation of radicals from CH-acidic substrates. Due to the comparable oxidation potential of CAN (+1.61 V) and manganese (III) acetate (+1.54 V), both one-electron oxidants exhibit a similar reactivity pattern.[192a,b] In general azidonitration of tri-*O*-acteyl-*D*-glucal **107** gives **108a**, **108b**, and **108c** with very poor stereoselectivity, due to lack of substrate direction at C-4 (Scheme 25) 2-azido-2-deoxy-*D*-glucose derivative **108a**.[193] However, the advantage of CAN consists in the milder reaction conditions, which allow the generation of radicals in methanol or acetonitrile at lower temperature. Due to the mild reaction conditions, CAN is superior to $Mn(OAc)_3$ for applications in carbohydrate chemistry. For the aforementioned reasons we chose to employ CAN in the azidonitration reaction. Thus, 3,4,6-tri-*O*-acetyl-*D*-glucal was treated with 1.5 equivalents of sodium azide and 3.6 equivalents of ceric ammonium nitrate in dry acetonitrile to afford a mixture of three diastereoisomers **108a**, **108b**, **108c** in 94% yield after aqueous work-up (Scheme 25). Selectivity of the reaction has been found to depend upon the reaction temperature, ratio of the reactants and the protecting groups employed.[194]



107 **108a** **108b** **108c**

Scheme 25. *Reagents and conditions*: (a) 1.5 equiv of NaN_3, 3.7 equiv of CAN, CH_3CN, -15 °C, 10 h , 94%

The diastereoisomeric products showed doublet signals at δ 6.30, 6.20 and 5.60 ppm in the 300 Hz ^{1}H NMR spectrum, which were assigned to the anomeric protons of **108a**,

108b and **108c** on the basis of coupling constants. Based on literature data,[194] diastereoisomer **108a** which had a coupling constant of 4.2 Hz was assigned as α-*D* glucopyranosyl nitrate. Diastereoisomer **108b** had a coupling constant of 1.8 Hz was and assigned as β-*D*-glucopyranosyl nitrate while **108c** which had a coupling constant of 8.7 Hz was assigned as α-*D*-mannopyranosyl nitrate. The proportion of diastereomers was found to be 1: 2: 1 (Figure 44). The azidonitration reaction is presumably initiated by addition of an azide radical and proceeds regioselectively to yield products. The stereoselectivity of this preparatively very useful transformation was found to be 3:1 in favor of the 2-azido-2-deoxy-*D*-glucose derivatives **108a** and **108b** to 2-azido-2-deoxy-*D*-mannose derivative **108c**. Previous studies have suggested that steric effects influence the selectivity of reaction involving glucals. [195] However, modeling of tri-*O*-acetyl protected *D*-glucals showed that neither face of the molecule appeared particularly hindered, thus supporting the lack of selectivity observed experimentally.

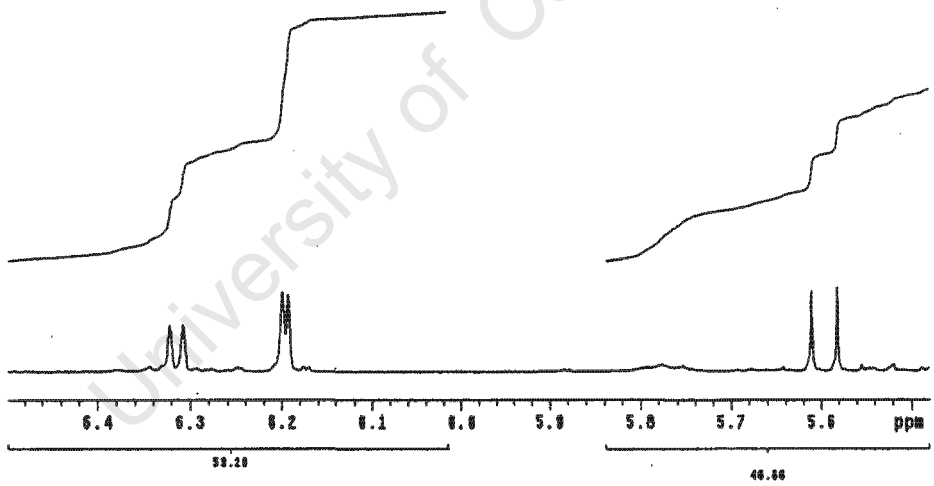
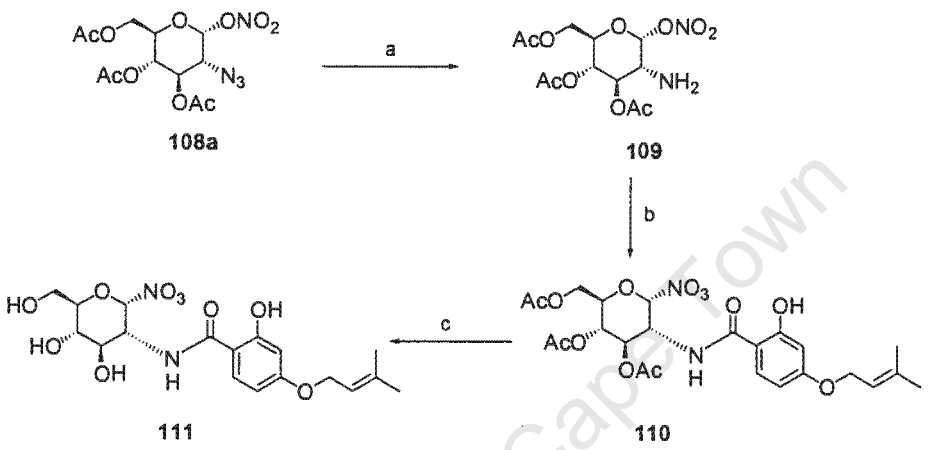


Figure 44. Partial ^{1}H NMR spectrum of diastereomeric products showing the ratio of anomeric protons

After trituration of the mixture with diethyl ether 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-*D*-glucopyranosyl nitrate **108a** was obtained as a white crystalline powder in 20% yield based on the starting material. Since we were only interested in **108a**, the mixture of **108a** and **108b** was not separated.

The synthesis of target carbohydrate-linked prenylated amides **110** and **111** is summarized in Scheme 26. Reduction of the azido group in **108a** *via* catalytic hydrogenation afforded **109** in 98% yield. This was subsequently coupled with prenylated acid chloride **99** to furnish **110** in 81% yield after removal of excess glucosamine **109** with polymer supported isocyanate. Deacetylation of **110** with 10% NaOMe in MeOH gave **111** in 84% yield.



Scheme 26. *Reagents and conditions*: (a) H_2, 10% (w/v) Pd/C, absolute EtOH, 1h (b) 0.8 equiv of prenylated acid chloride 7, 3.0 equiv of poly(4-vinylpyridine), CH_2Cl_2, rt, 3h and then 3.0 equiv of polymer-supported isocyanate (equivalent to excess amine), 3h (c) 10% NaOMe, MeOH, 1h

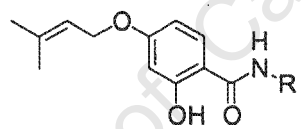
5.7.0 Biological Results and Discussion

5.7.1 Effects of Prenylated amides on TAO.

As can be seen from Table 17, all compounds with the exception of **106c** showed very weak to moderate inhibitory activity against TAO at 20-40 μg/ml. The most promising compounds from this limited series are **106e** and**106f**. When the percentage inhibition of **106b** is compared with that of **106c**, a compound without chlorine substituents on the benzene ring, it is clear that compound **106c** displayed a significantly weakened inhibitory effect on TAO, suggesting a possibility that the chloride groups might be playing some role in the specific binding or in the inhibition mechanism. Although the results suggest a role of the chloride groups in the inhibition, further information is unavailable for lack of additional derivatives. Extended studies on structure-activity relationship studies of compound **106b** might help shed light on the role of the chloride groups in the interaction with the ubiquinone redox site.

Comparison between **106d**, **106e** and **106f**, compounds with a heteroaromatic (pyridine) ring showed that the effect of changing the position of the substituent on the pyridine ring was not linear. A peak of activity was reached when the substituent was at position 4, **106d** and **106e** being less active at 20 μM compared to **106f**. These observations may suggest that **106f** binds much strongly to the active site of the enzyme compared to **106d** and**106e**. In other words the planar orientation of the aromatic ring (pyridinyl) might place the nitrogen atom of **106f** in a position within the active site of the enzyme suitable for formation of a charge-transfer complex while **106d** and **106e** could cause some instances of binding to be ineffective with respect to formation of such a complex. While further studies must be conducted before any definite conclusions can be drawn regarding the interactions, apparent differences between them appear to be significant. Compound **106a** produced a moderate inhibitory effect on TAO.

Table 17. Effects of prenylated amides on the activity of TAO



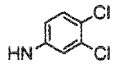
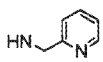
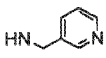
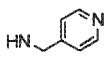
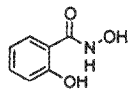
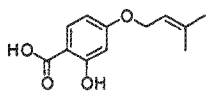
Compound	R	% inhibition at			
		10 μg/ml	20 μg/ml	40 μg/ml	80 μg/ml
106a		36.8	38.4	45.0	39.5
106b		30.0	30.0	40.0	52.0
106c		-	8.3	8.3	8.3
106d		-	26.0	33.0	66.0
106e		-	37.5	61.0	-
106f		-	61.0	80.0	-

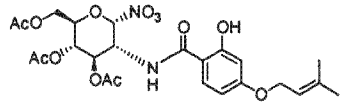
Table 18. Comparative effect of SHAM, prenylated acid **97** and carbohydrate-linked conjugates of prenylated amides (**110** and **111**) on the activity of TAO in the presence of two substrates.



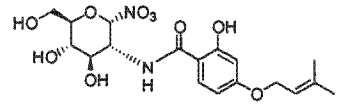
SHAM



97



110



111

	% Inhibition at					
	240 μM		484 μM		762 μM	
	αGP	Q1	αGP	Q1	αGP	Q1
SHAM	91.0	100	95.0	100	100	-
97	82.3	100	100	100	100	-
110	69.0	93.0	94.3	100	100	-
111	94.0	94.0	100	100	100	-

Table 18 presents the results of the screens of potential parasite respiratory inhibitors against the TAO in the presence of two substrates (alpha glycerophosphate αGP or Q1 donor). αGP is a substrate for the cytochrome pathway. If it is used as the substrate, TAO utilizes its own endogenous Q1 that has been activated by the flow of electrons through the cytochrome pathway whereas TAO can utilize Q1 directly bypassing the cytochrome pathway. One of the objectives of this experiment was to determine if these potential respiratory inhibitors interfere with the ubiquinone/ubiquinol-mediated electron transport or with the flow of electrons through the cytochrome pathway. The data were also expected to be informative regarding the degree of inhibitory activity of these compounds in comparison with SHAM. If a compound does not inhibit Q1-supported respiration but does inhibit αGP-supported respiration, this would suggest that it operates on the cytochrome oxidase since it does not interfere with the reduction of ubiquinone. However, all four compounds studied did inhibit Q1-mediated respiration as well as αGP-mediated respiration. This suggests that the compounds interfere with ubiquinone/ubiquinol-mediated electron transport, more specifically that they bind to the

ubiquinol receptor on the oxidase component of the TAO. At 240 μM concentration, the degree of inhibition of αGP-mediated respiration was less than that of Q1-mediated respiration, markedly so for compounds **97** and **110**. This suggests that there are multiple sites of inhibition but these data do not allow one to determine the relative contribution of each. They do suggest that the effect is not a specific one.

Table 19. Effect of **111** on *T. b. brucei* Intact Cells and Mitochondria

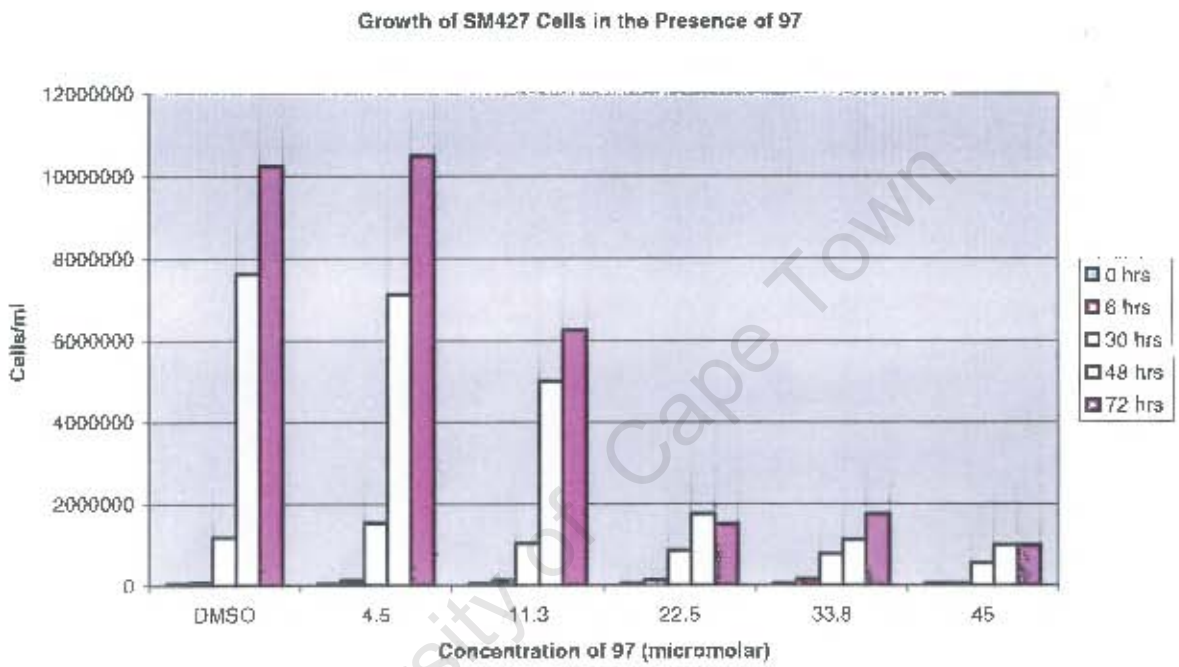
	% Inhibition			
	111		SHAM	
Concentration (nM)	Intact cells	Mitochondria	Intact cells	Mitochondria
25	0	92	99	100
121	28	94	-	-
290	96	92	-	-

Table 19 shows the inhibitory effect of **111** on the respiration of *T .b. brucei* intact cells and mitochondria. On the basis of the results from Table 3, **111** was chosen because it was more active than **110** and somewhat more soluble. The objective of the experiment was to evaluate its potential as a trypanocidal drug. The assay with intact cells was carried out to determine if the cell membrane would act as a barrier to permeability whereas the assay with mitochondria was chosen as a primary screen in order to focus on the TAO itself, thereby obviating effects due to membrane transport and tissue distribution within the host. It is clear from Table 4 that **111** was less active than SHAM. On the other hand, besides being potent against TAO in mitochondria preparation, **111** was ineffective against intact cells at low concentration (25 nM). However, as the concentration was increased the activity of **111** was enhanced. Two possible explanations for these observations are: (1) the cell membrane was acting a barrier to permeability and, (2) the effective trypanocidal level of **111** does not reach TAO at low concentrations.
The decrease in the inhibitory activity of **111** at 290 nM in mitochondria might be due to a decrease in the rate of oxygen consumption caused by both loss of enzyme activity due to degradation and by the approach to equilibrium of the enzyme-inhibitor complex.

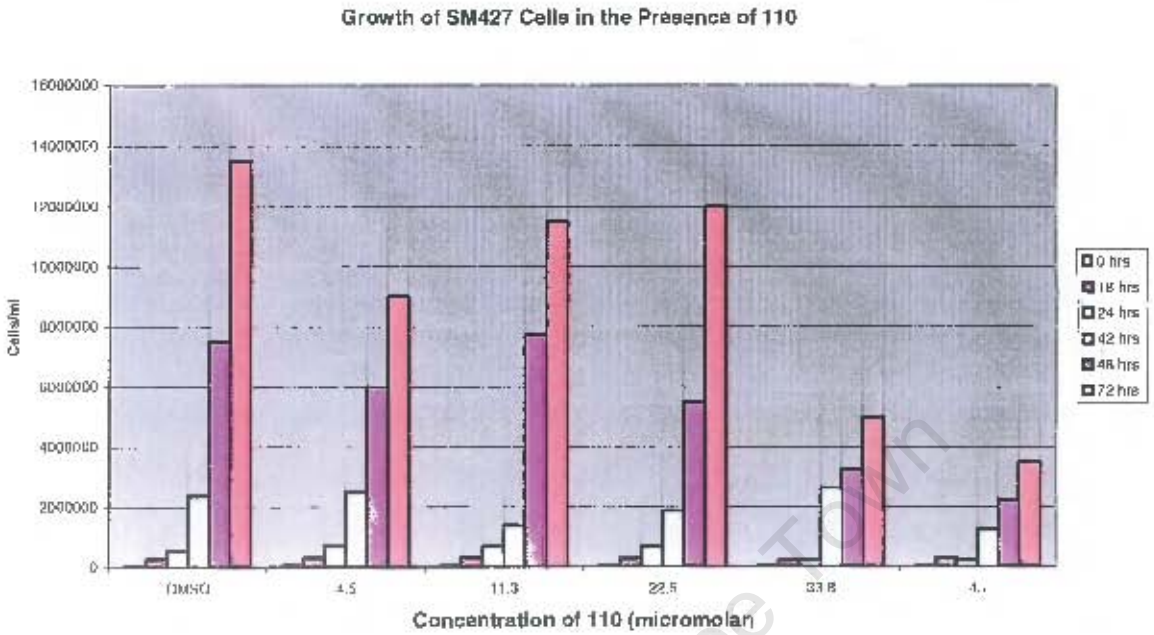
5.7.2 Cell growth of *T. b. brucei* in the presence of increasing amounts of 97, 110 and 111

The growth of bloodstream cells of *T. b. brucei* in the presence of increasing amounts of **97**, **110** and **111** was investigated and data are presented as bar graphs A, B and C.

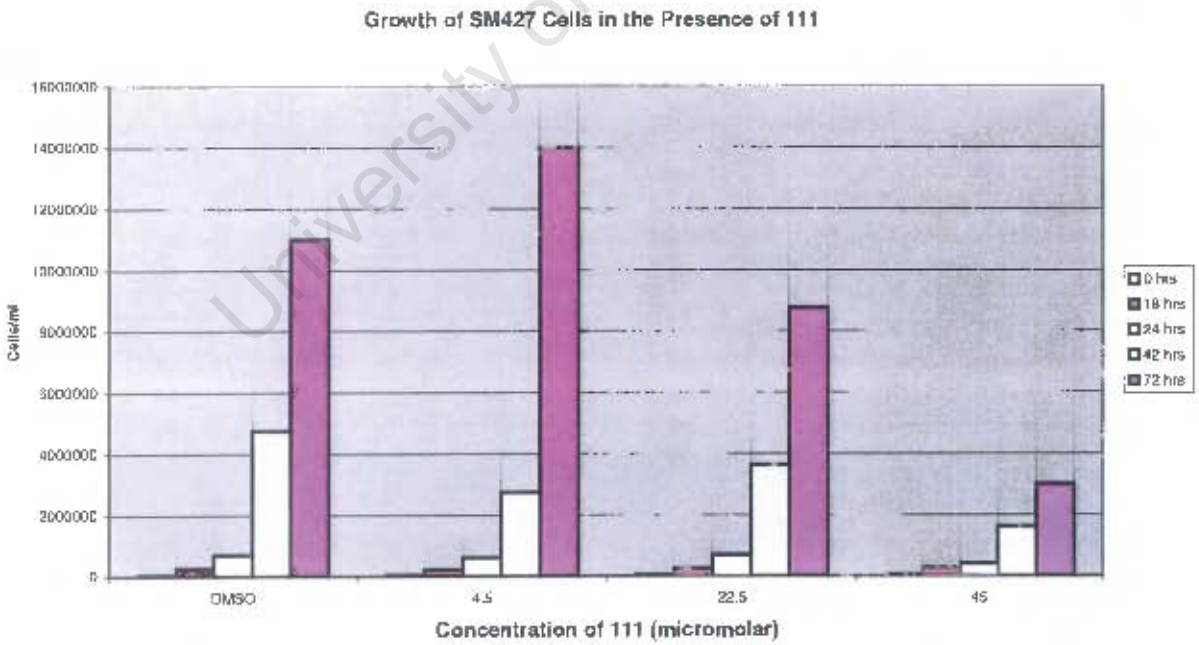
Graph A.



Graph B.



Graph C.



In each experiment, a control flask was incubated in the presence of DMSO. Growth was monitored in each at regular intervals by directly counting the number of cells per ml.

It is clear from the graphs that **97** was the most active compound while **110** was the least active. The fluctuation in the number of cells per ml as the concentration was increased may suggest that the parasite has a way of interacting with these compounds more effectively than others or it can metabolize these compounds to a less potent form.

In summary, none of the compounds studied was more effective than SHAM. Studies conducted on the carbohydrate-linked prenylated amides showed that these compounds act by interfering with ubiquinone/ubiquinol electron transport, more specifically by binding to the ubiquinol receptor on the oxidase component of the TAO. This was supported by the fact that the carbohydrate-linked prenylated amides inhibited both Q1-mediated respiration as well as αGP-supported respiration. For these compounds, the parasite cell membrane is not a barrier, however, high concentrations are required for an effective trypanocidal level to reach the TAO. The degree of inhibition was comparable to SHAM at high concentration. These compounds are not likely clinical candidates but the results presented could be used to guide future efforts to identify compounds more likely to be of clinical utility.

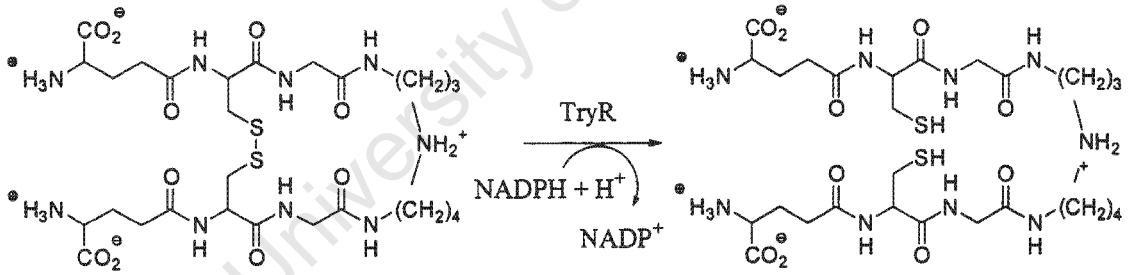
CHAPTER 6

SYNTHETIC AND METHODOLOGICAL STUDIES

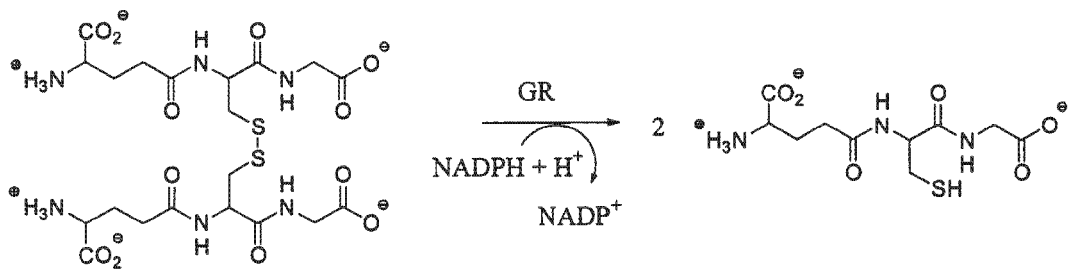
6.1.0 Convenient Synthesis Of Disulfide Substrate For Trypanothione Reductase Using Polymer-Supported Reagents

6.1.1 Introduction

The enzymes of thiol metabolism, and in some cases thiols themselves, of parasitic protozoa differ in many interesting ways from those of mammals. Trypanosomes and *Leishmania* are most remarkable in that they employ trypanothione [N^1,N^8-*bis*(glutathionyl)spermidine] $T[SH]_2$ to maintain an intracellular reducing environment, which is important for the reduction of disulfides and detoxification of peroxides.[196] $T[SH]_2$ is a molecular variant of glutathione GSH in which the glycyl residues are cross-linked by spermidine (Figure 1). The redox interconversion of the oxidized and reduced forms of trypanothione is catalyzed by a flavoprotein trypanothione reductase (TryR) which is an equivalent of the NADPH-dependent glutathione reductase (GR) that maintains the GSSG-GSH equilibrium in the mammaliam host cells (Figure 46).



Trypanothione disulfide ($T[S]_2$) **112** Trypanothione ($T[SH]_2$) **113**



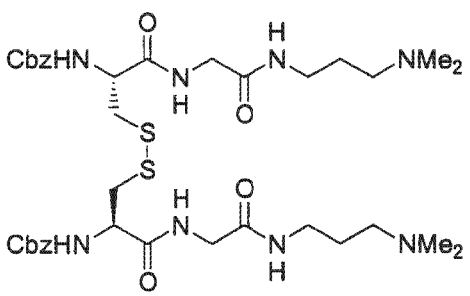
Glutathione disulfide (GSSG) **114** Glutathione (GSH) **115**

Figure 46. Reactions catalyzed by human glutathione reductase and parasite trypanothione reductase.

TryR has a vital role in protecting these parasites against oxidative damage that occurs both internally as a result of aerobic metabolism and externally because of immune response of the mammalian host and displays a substrate specificity distinct from that of glutathione reductase. A comparison of structures of $T[S]_2$ and GSSG suggests the basis for this selectivity. Binding of GSSG by GR requires accommodation of two negatively charged glutamate carboxylate residues present in this molecule, whereas TryR must bind a substrate with a positively charged amino chain. A crystal structure for TryR has been reported.[197b] It revealed five nonconservative changes at the active site between the two enzymes that convey this difference in substrate specificity. TryR possesses an enlarged active site with an overall negative charge to accommodate the larger, positively charged T[S]2, while GR's smaller active site has an overall positive charge required for GSSG.[197a,b] Knowledge of these differences coupled with the crucial role of TryR for thiol homeostatasis and its absence from mammalian host cells have led to the development of selective TryR inhibitors.

6.1.2 Solution phase synthesis of *N,N'*-bis(benzyloxycarbonyl)-L-cysteinylglycyl-3-dimethylaminopropylamide disulfide 116

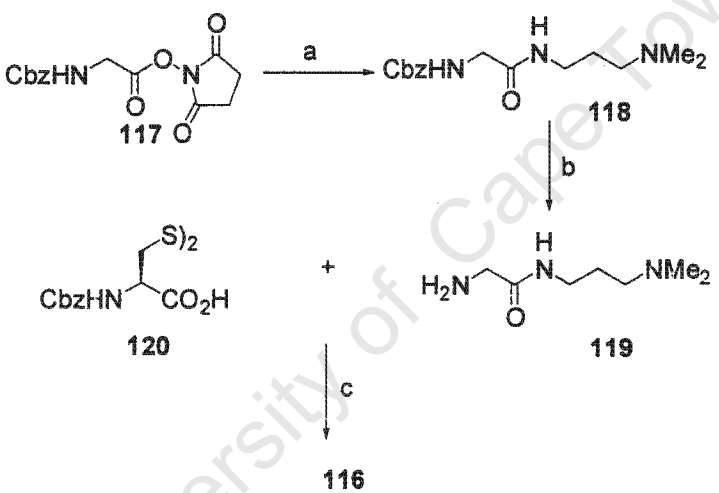
The isolation, crystallization and study of recognition determinants of TryR require a specific assay and to date this has required trypanothione. However, the limiting factors are that $T[SH]_2$ is expensive to purchase and difficult to synthesize.[198] A few years ago, an alternative substrate *N,N'*-bis(benzyloxycarbonyl)-L-cysteinylgylcyl-3-dimethylamino propylamide disulfide **116** (Figure 47) that is easy to use and is specific to TryR was synthesized using simple and straight forward solution phase chemistry.[198]



116

Figure 47. *N,N'*-bis(benzyloxycarbonyl)-L-cysteinylglycyl-3-dimethylaminopropylamide disulfide

The synthesis of compound **116** using solution phase chemistry is outlined in Scheme 27. *N*-Benzyloxycarbonylglycyl-*N*-oxosuccinimide ester **117** was condensed with 3-dimethylaminopropylamine in THF to afford *N*-benzyloxycarbonylglycyl-3-dimethylaminopropylamide **118** in 74% yield after chromatography over neutral alumina. Compound **118** was subjected to Pd catalyzed hydrogenation to give **119** in 92% yield. The synthesis of the symmetrical disulfide **116** was achieved in 46% yield by coupling commercial *N,N*-bis(benzyloxycarbonyl)-L-cysteine disulfide **120** with two molar equivalents of the free amino moiety, Gly-DMAPA **119**, following the procedure of Konig and Geiger.[199]

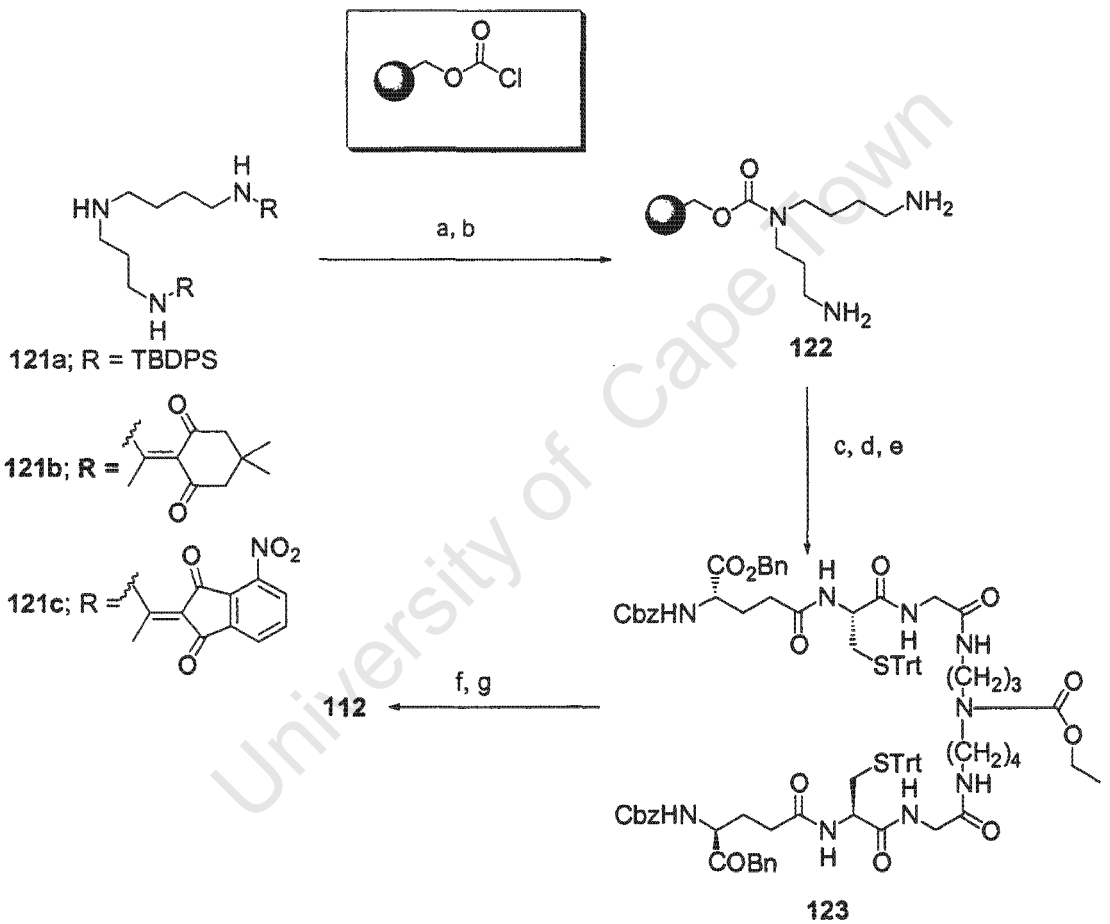


Scheme 27. ***Reagents and conditions*****: (a)** 1.0 equiv of $NH_2(CH_2)_3N(CH_3)_2$, THF, overnight, then alumina-flash chromatography (EtOAc/MeOH, 3/1, v/v), 74% (b) H_2, 10% (w/v) Pd/charcoal, absolute EtOH, 92% (c) 1.0 equiv of 120, 2 equiv of 119, 2.0 equiv of DCC, 2.0 equiv of HOBt, DMF, alumina-flash chromatography (dichloromethane/MeOH, 6/1, v/v), 45%

6.1.3 Solid phase synthesis of trypanothione disulfide 112

In order to overcome problems associated with work-up and product purification in solution phase synthesis of trypanthione disulfide **112**, solid phase organic synthesis methodologies have been developed.[200] The first such method involved attaching tert-butyldiphenylsilyl (TBDPS)-protected spermidine **121a** to a chloroformate resin followed deprotection under acidic conditions to give resin bound spermidine **122** (Scheme 28, p105). Elongation of the peptide chain by successive coupling of suitably protected

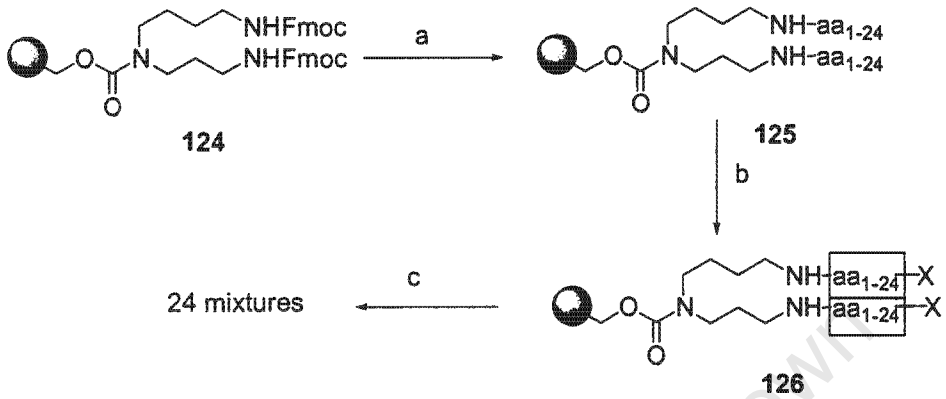
amino acids resulted in resin-bound protected trypanothione **123**, which after removal of the protecting groups afforded **112**.[201] Similarly, another strategy was employed starting from spermidine protected as N-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) **121b** and N-1-(4-nitro-1,3-dioxan-2-ylidene)ethyl (Nde) **121c** groups respectively,[202] (Scheme 27). However, the low reproducibility of these methodologies-probably due to the high lability of the employed protected groups[203] prompted other groups to explore more reliable methods of attaching various amino acids onto polyamine based resins.



Scheme 28. *Reagents and conditions*: (a) PS-OCOCl, *i*-Pr_2NEt, CH_2Cl_2, rt; (b) 0.5N HCl/THF (2/1 v/v); (c) (i) Fmoc-Gly-OH, HOBt, BOP, *i*-Pr_2NEt, DMF (ii) Piperidine/DMF (1/4 v/v); (d) (i) Fmoc-Cys(Trt)-OH, HOBt, BOP, *i*-Pr_2NEt, DMF (ii) Piperidine/DMF (1/4 v/v); (e) (i) Cbz-Glu-Obn, HOBt, BOP, *i*-Pr_2NEt, DMF (ii) Piperidine/DMF (1/4 v/v); (g) HF, *p*-MePhOH, *p*-MePhSH, 4°C; (f) air oxidation, NH_4OAc.

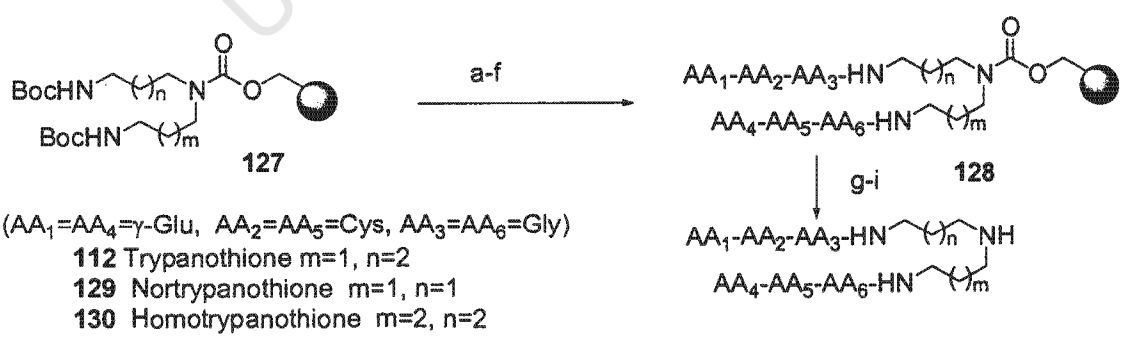
In the first synthesis of polyamine-based libraries directed against TryR, a variety of suitably diprotected polyamines were anchored onto a polystyrene amino methyl resin to give resin bound diprotected polyamines **124** in a short and efficient protocol. The library

of 24 mixtures was synthesized from a number of aminoacids (aa) via a split/mix approach, (Scheme 29).[204] A library of 10,000 analogues of trypanothione synthesized based on this methodology has been reported.[205,206]



Scheme 29. ***Reagents and conditions***: (a) Split, 20% piperidine, 24 amino acids, DIC, HOBt; (b) Mix, split, 20% piperidine, 24 carboxylic acids and sulfonyl chlorides (A-X), DIC, HOBt; (c) TFA-H_2O-$HS(CH_2)_2SH$-$SiH(I\text{-}Pr)_3$, 37:1:1:1

Results obtained from the screening of these mixtures led to some false positive hits being identified after deconvolution of an apparently active mixture. Nevertheless, the methodology developed, (Scheme 30), was useful in the preparing a number of polyamine conjugates including trypanothione disulfide **112** and related substrates, nortrypanothione and homotrypanothione disulfides **129** and **130** respectively (figure 48, p.107).[207]



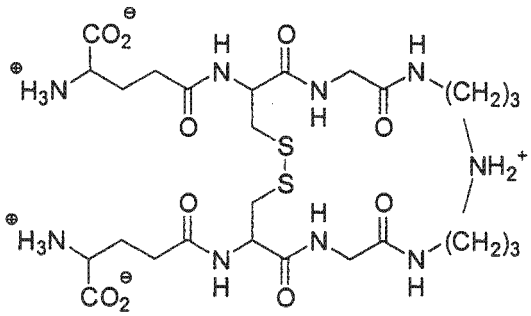
(AA_1=AA_4=γ-Glu, AA_2=AA_5=Cys, AA_3=AA_6=Gly)

112 Trypanothione m=1, n=2

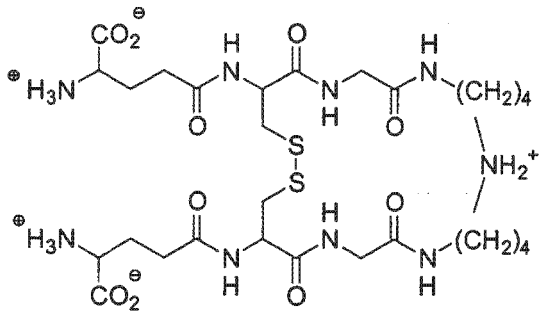
129 Nortrypanothione m=1, n=1

130 Homotrypanothione m=2, n=2

Scheme 30. ***Reagents and conditions***: (a) 50% TFA; (b) Fmoc-Gly, DIC, HOBt; (c) 20% piperidine; (d) Fmoc-Cys(Trt), DIC, HOBt; (e) 20% piperidine; (f) Bob-Glu(OH)-OtBu, DIC, HOBt; (g) 10 equiv of TFMSA, TFA, Edt, PhSMe; (h) MeOH, I_2; (i) HPLC



129



130

Figure 48.

6.1.4 Synthesis of compound 116 using polymer-supported reagents and scavengers

While the synthetic route employed in the solution phase synthesis only required a few steps, isolation and purification of products required laborious and time-consuming chromatography. Accordingly, we became interested in the synthesis of this substrate using polymer-supported reagents and scavengers for convenience and ease of purification.

Also, we were interested in preparing structural analogues of this new substrate in which the glycyl moiety of the molecule is omitted or replaced by an *O*-protected seryl moiety. In addition to these, we were also interested in replacing the disulfide bridge with the sulfide bridge to prepare a lanthionine derivative.

A lanthionine bridge represents an unusual conformational constraint. It has a stronger stabilizing effect on conformations of short peptides than analogous disulfide bridge. As a result higher bioactivities are frequently observed.[208] In addition, lanthionine peptides generally exhibit increased stability towards enzymatic degradation.[209] Two methods were considered for the preparation of these analogues. The first one involved lengthening the *N*-Z-cystine (Figure 49a, p.108) while the second strategy involved the oxidation of the two cysteine residues, already incorporated in the appropriate peptide chain, thus affording the corresponding cystine peptide (Figure 49b, p.108).

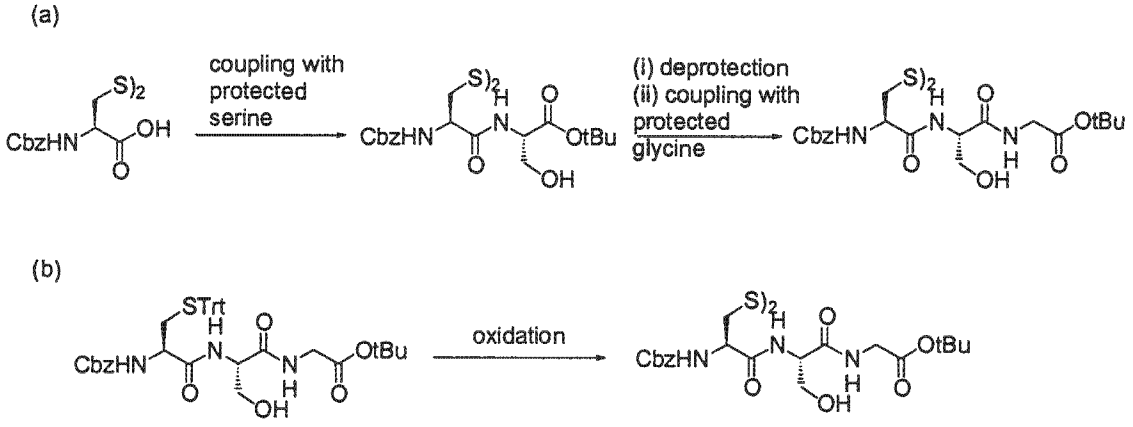
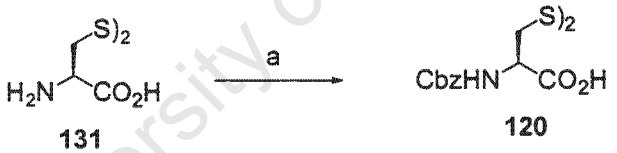


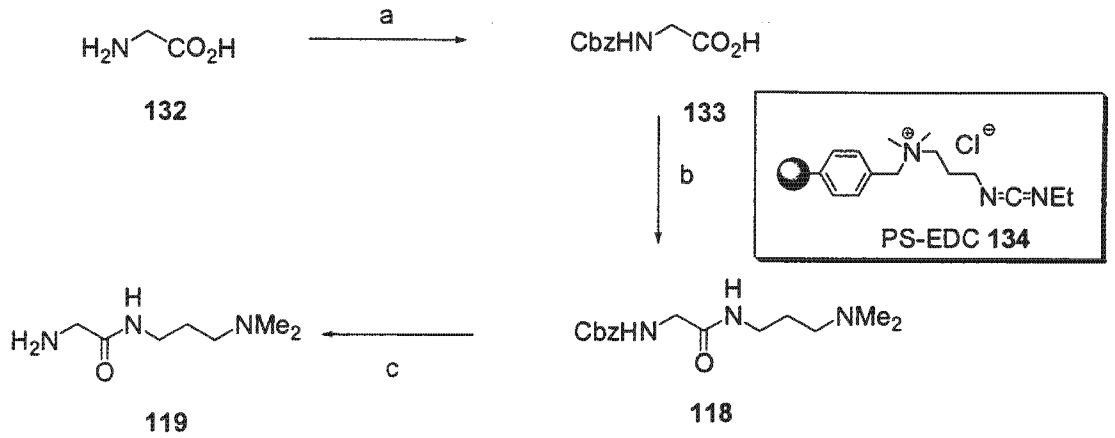
Figure 49.

Our syntheses started with the preparation of *N,N'*-dibenzyloxycarbonyl-*L*-cystine **120** by employing the method described by Wolfe et al.[210] Thus, commercially available L-cystine **131** was treated with benzyl chloformate and 1M sodium hydroxide in dioxane at 5°C for 2 h to afford **120** in 65% yield after acidification with dilute hydrochloric acid (Scheme 31).



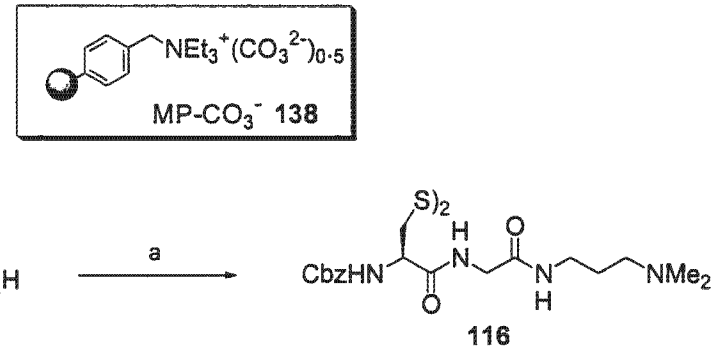
Scheme 31. *Reagents and conditions*: (a) 2.2 equiv of CbzCl, 1M NaOH, Toluene, 5 °C, 2h, 65%

Having prepared **120** our next task was to synthesize **119**, another precursor required for the formation of **116**. This was achieved by coupling **133** (prepared from glycine **132** and benzyl chloroformate) with *N,N*-dimethylaminopropylamine in the presence of an activating polymer-supported 1-ethyl-3-(3-dimethyaminopropyl)carbodiimide (PS-EDC),[211] followed by removal of Cbz protecting group from **118** under catalytic hydrogenation as shown in Scheme 32 below.



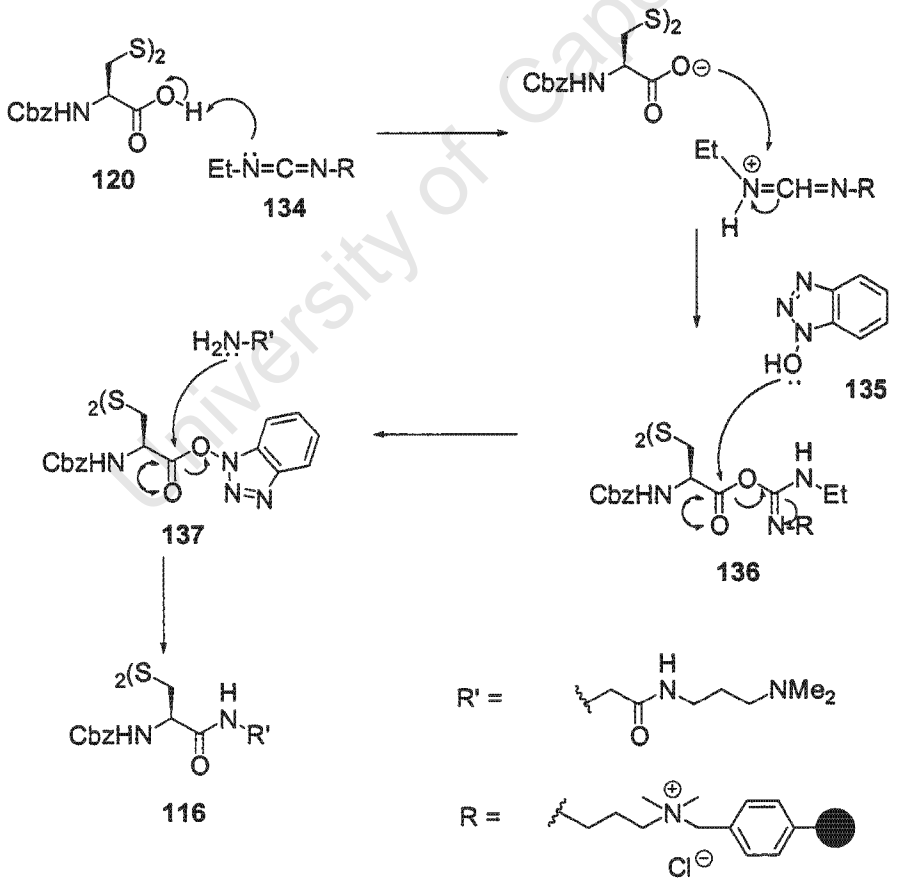
Scheme 32. *Reagents and conditions*: 2.2 equiv of CbzCl, 1M NaOH, Toluene, 0 °C, 1h, 81% (b) 1.0 equiv of $H_2N(CH_2)_3N(CH_3)_2$, 2.5 equiv of PS-EDC, CH_2Cl_2, rt, 16h, 91% (c) 10% (w/v) Pd/charcoal, MeOH, r.t, 2h, 100%

With **119** and **120** in place, the final coupling reaction was executed by using 1-hydroxybenzotriazole (HOBt) as a peptide additive in combination with PS-EDC. Thus, a mixture of 2.5 equivalents of PS-EDC, 1.0 equivalent of **119**, **120** and HOBt (**135**) in dry chloroform were shaken at room temperature for 16 h. Then, 3.0 equivalents of macroporous triethylammonium methylpolystyrene carbonate (MP-carbonate **138**)[212] were added and the resultant mixture shaken for a further 3 h by which time the reaction mixture showed no HOBt. Filtration and concentration afforded **116** in 79% yield (Scheme 33). The ^{1}H NMR and MS data of compound **116** were consistent with those reported in literature.[198]



Scheme 33. *Reagents and conditions*: (a) 2.0 equiv of **119**, 1.2 equiv of **120**, 2.5 equiv of PS-EDC, 1.0 equiv of HOBt, $CHCl_3$, rt, 16h, then 3.0 equiv of MP-CO_3^-, 3h , filtration, 79%

Several points are worth mentioning about this reaction; (1) HOBt was added to preserve the chiral integrity of the cystyl moiety of the molecule and at the same time to enhance the rate of reaction. The mechanism for this reaction is depicted in Scheme 34. PS-EDC reacts with cysteine to form an activated ester which can be attacked by any nucleophile to produce a very stable urea as a leaving group. The problem with attacking this ester directly with an amino group of an amine or a second amino acid is that some racemization of the active ester is often observed. The answer to this problem is found in HOBt, which intercepts the activated ester **136** first and forms a new intermediate that does not racemize mainly because the reaction is highly accelerated by additon of HOBt. The amine or second amino acid then attacks the HOBt ester **137** to afford the product in a very fast reaction without racemization. (2) MP-carbonate was added to purify the product by mopping up the HOBt and (3) the excess carboxylic acid **120**, used to drive the reaction to completion remained on the polymer support.



Scheme 34. Mechanism EDC and HOBt-mediated coupling reaction

Having successfully prepared **116**, several attempts were made to extrude sulfur to give the thioether **139** by treating **116** with 25.0 equivalent of tris(diethylamino)phosphine (Figure 50). Unfortunately, this exercise proved futile and was therefore abandoned.

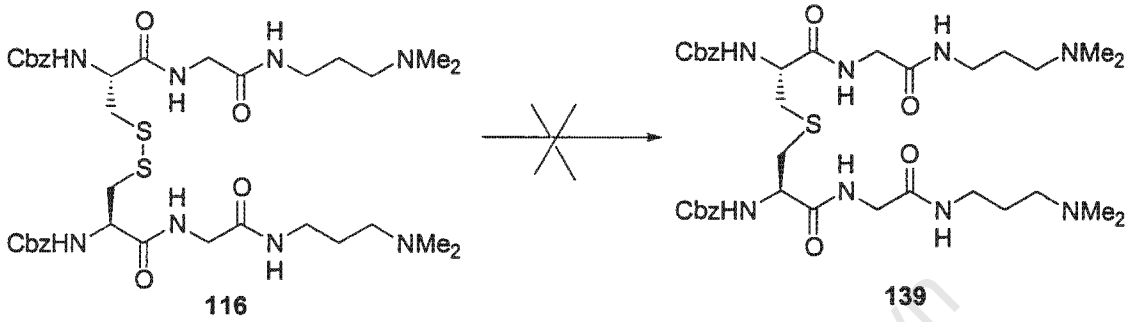
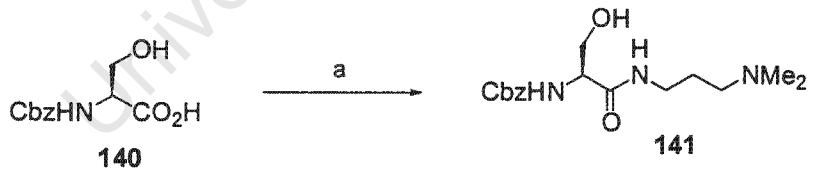


Figure 50. Attempted conversion of **116** to thioether **139**

6.1.5 Synthesis of compound 147

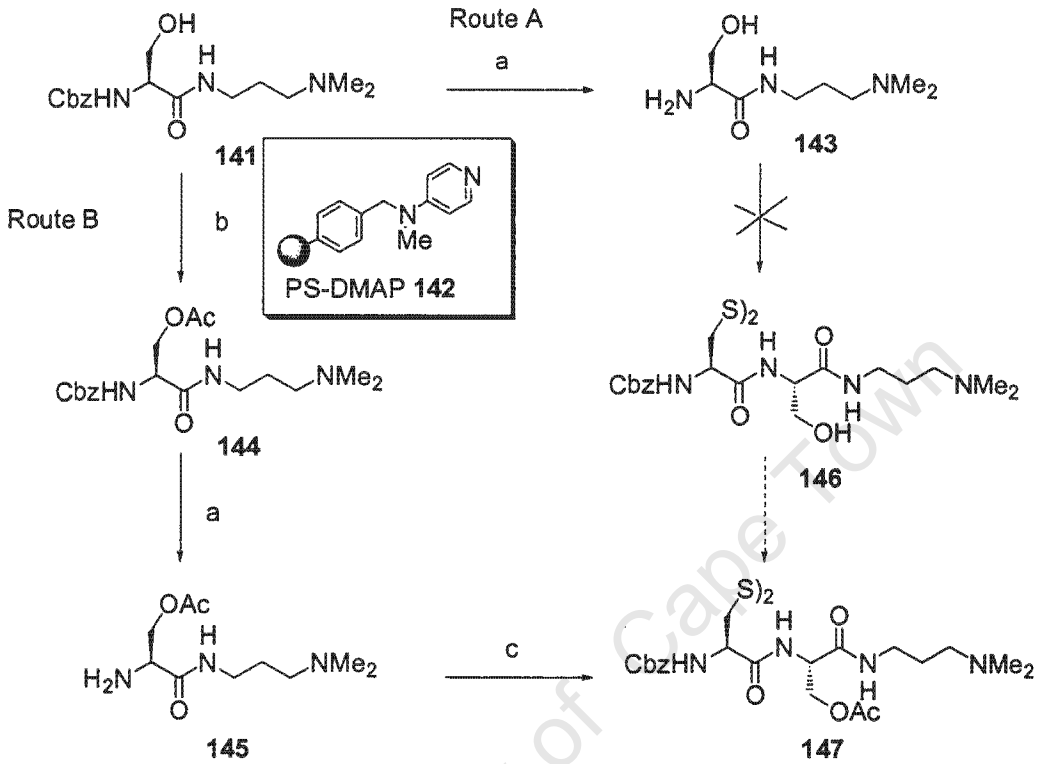
Two routes to prepare this compound were examined. The starting point in both cases was the preparation of **141** (Scheme 35) using the coupling conditions described for the synthesis compound **116**. Thus, Cbz-serine **140** was condensed with *N,N*-dimethylaminopropylamine in the presence of PS-EDC and HOBt to afford **141** in 96% yield.



Scheme 35. *Reagents and conditions*: (a) 1.0 equiv of $H_2N(CH_2)_3N(CH_3)_2$, 2.5 equiv of PS-EDC, 1.0 equiv of HOBt, $CHCl_3$, rt, 16h, then 3.0 equiv of MP-CO_3^-, 3h , filtration, 96%

As summarized in Scheme 36 below, attempts to prepare **147** via route **A** did not give meaningful results after the coupling reaction between **120** and **143**. Therefore, we decided to employ route **B** which later proved to be more efficient. Compound **141** was treated with acetyl chloride in the presence of *N*-(methylpolystyrene)-4-(methylamino)pyridine (PS-DMAP)[213] **142** to give **144** in 97% yield. Subsequent

deprotection of **144** and condensation of **145** with **120** afforded the desired product **147** in 74% yield.

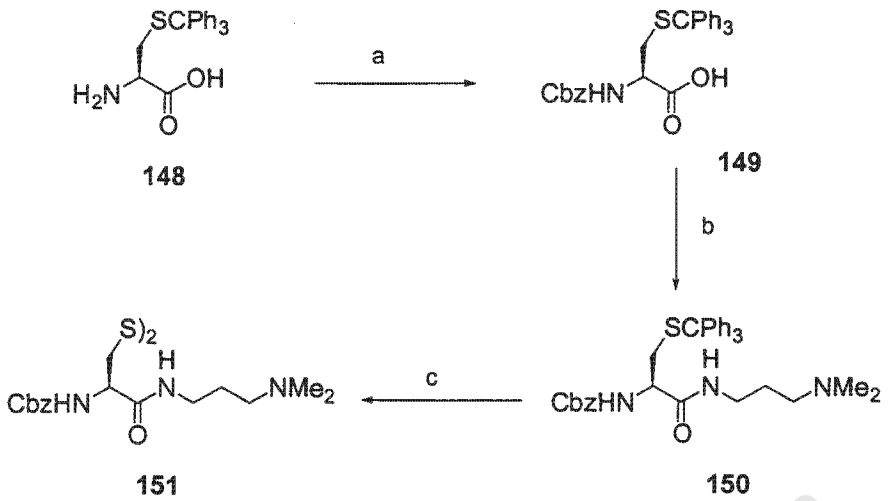


Scheme 36. *Reagents and conditions*: (a) 10% (w/v) Pd/charcoal, MeOH, r.t, 2h, 100% (b) 2.0 equiv of AcCl, 4.0 equiv of PS-DMAP, CH_2Cl_2, rt, 12h, 97% (c) 1.0 equiv of **120**, 2.0 equiv of **145**, 2.5 equiv of PS-EDC, 1.0 equiv of HOBt, $CHCl_3$, rt, 16h, then 3.0 equiv of MP-CO_3^-, 3h , filtration, 74%

6.1.6 Synthesis of disulfide 151

Disulfide bridge formation can be carried out in either one or two steps.[214] In the two-step route, the S-protecting groups are first smoothly removed. A further oxidation gives the disulfide bridge. In the one-step route, a well-known method in peptide synthesis is to directly oxidize, using iodine, S-protected cysteine residues to the corresponding cystines. Although the two-step route gives high yields, starting materials are often recovered. Therefore, the one-step route is preferred.

The synthesis of disulfide **151** was achieved as outlined in Scheme 37 below.



Scheme 37. *Reagents and conditions*: 2.2 equiv of CbzCl, 1M NaOH, toluene, 0 °C, 1h, 72% (b) 1.0 equiv of $H_2N(CH_2)_3N(CH_3)_2$, 2.5 equiv of PS-EDC, 1.0 equiv of HOBt, $CHCl_3$, rt, 16h, then 3.0 equiv of MP-CO_3^-, 3h , filtration, 86% (c) I_2, MeOH, rt, 1h, 57%

Protection of the *S*-trityl-L-cysteine**148** with a benzyloxycarbonylgroup gave **149** in 72% yield. This compound (**149**) was then condensed with the diamine, using the coupling conditions described for preparation of **135**, to furnish **150** in 86% yield, which was used without further purification. Finally, exposure of **150** to a solution of iodine in methanol at room temperature afforded **151** in 57 % yield. The ^{1}H NMR and Mass spectra were consist with the structure of **151**.[215]

In summary the methodologies for the synthesis of the alternative substrate **116** and its analogues **147** and **151** using polymer-supported reagents and scavengers proved to be superior in terms simplicity, efficiency and yields compared to previously reported solution phase syntheses. Coupled with the reported solid phase synthesis of the natural substrate,[203,204,209] our approach to the synthesis of compound **116** should provide options for synthesizing these disulfides and related compounds.

6.2.0 Solid Phase Synthesis of a Biaryl Chalcone

6.2.1 Introduction

Solid phase chemistry has been used for the synthesis of molecules for over 40 years, first for peptide,[185,216] and then nucleotide[217] synthesis. Three decades ago, the synthesis of

small organic molecules on the solid phase was investigated by a number of groups.[218,219] However, the wide range of chemical reactions that needed to be optimized on the solid phase greatly reduced any advantage gained using this approach for the synthesis of the small number of molecules required. The advent of combinatorial synthesis of pharmaceutically interesting compounds has sparked a major revival in the study of solid phase organic synthesis. Large numbers of reactions have been translated from solution phase to solid phase[220-222] and more continue to be developed. Central to the success of any solid phase synthesis strategy is a straightforward and general method for coupling the initial starting material onto the solid support. This is achieved through a cleavable linker.[223,224] A linker has been described as a bifunctional protecting group (Figure 51)- it is attached to the molecule being synthesized through a bond labile to the cleavage conditions (e.g silyl ethers, esters, carbamates, etc) and is attached to the solid phase polymer through a more stable bond (alkyl ethers, amides or alkanes).

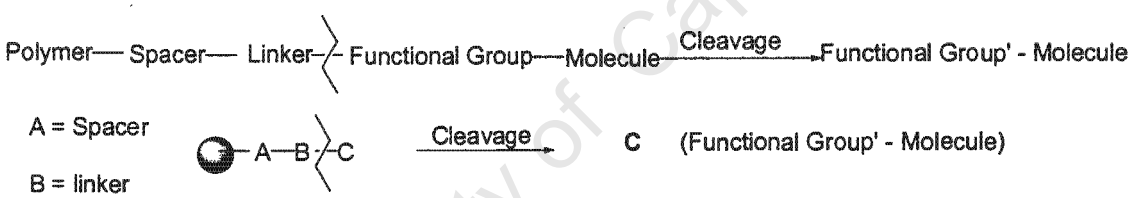


Figure 51. Linker can perform a similar function to traditional protecting groups

The ideal linker would fulfill the following important criteria:-

1. It should be cheap and readily available.
2. The attachment of a starting material should be readily achieved in high yield.
3. The linker should be stable to the chemistry used in the synthesis.
4. Cleavage should be efficient under conditions that do not damage the final product(s).
5. The cleavage method should be easily worked up on large numbers (for libraries of compounds) and should not introduce impurities that are difficult to remove.

6.2.2 Principle of solid phase organic synthesis

In this process, the substrates remain attached to the solid support during a chemical transformation until, in the final step, it is cleaved from the support (Figure 52). The starting materials (A) are immobilized onto a polymer and the reactions are performed with other reactants (B). Then, the resulting polymer bound-products (A-B) are detached from the polymer.

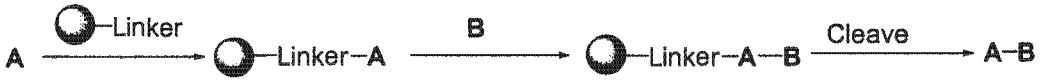


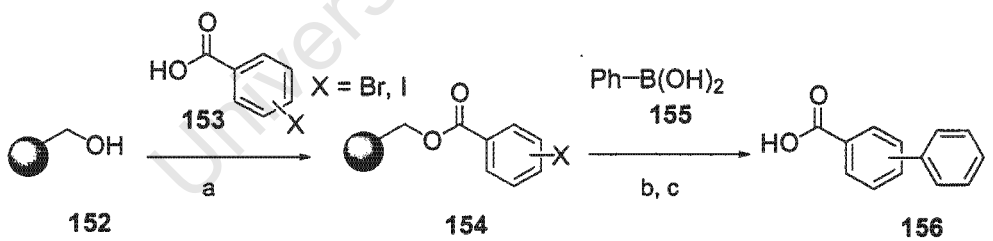
Figure 52. Synthesis on the polymer surface

The advantages of this methodology have been well described in literature.[225] Excess reagents can be used to drive reactions to completion, impurities and excess reagents can be removed by simple washing of the solid phase, and enormous numbers of compounds can be created using the mix and split technique. Limitation to SPOS may include (a) the presence of a resin vestige in the final molecules (the point of attachment of the molecule to the solid support, (b) the need for two extra steps (attaching the starting material to the solid support, and removing the material from the support, (c) a potential scale limitation imposed by the loading level of the solid support, and (d) the need to re-optimize solution phase chemistry on the desired solid support.

Newly emerging solid phase synthesis techniques for the formation of non-peptidic C-C bonds include 1,3-cycloaddition,[226] palladium-catalyzed coupling,[227] Mitsunobu coupling,[228] enolate alkylation,[229] and reductive amination.[230] The biaryl subunit is an important pharmacophore that is present in a variety of biologically active compounds.[231] The Suzuki coupling reactions of aryl halides with arylboronic acids or esters provides ready access to biaryl compounds and has become a valuable tool for the organic chemists.[232-235] The low toxicity, stability and ease of handling of boronic acids have made them the most popular intermediates for cross-coupling reactions with a variety of substrates. A number of reactions of boronic acids catalyzed by palladium have been reported.[236] Numerous catalysts have been investigated to effect the coupling of aryl

halides and boronic acids. Commercial Pd(0) sources, tris-(dibenzylideneacetone)dipalladium $\{Pd_2(dba)_3\}$ or tetrakis (triphenylphosphine)palladium $\{Pd(PPh_3)_4\}$ have been found to be very effective catalysts whereas Pd(II) or Ni(II) catalysts such as [1,1'-bis(diphenylphosphino)ferrocene]-palladium chloride, {PdCl2(dppf)}and [1,1'-bis(diphenylphosphino)propane]nickel chloride $\{NiCl_2(dppp)\}$ were ineffective.

A palladium-mediated three-component coupling reaction involving both the Suzuki and Heck reactions has been developed for the solid phase synthesis employing a variant of Kenner's safety-catch linker.[237] Kenner's safety-catch linker is an acylsufonamide linker developed by Kenner. Kenner's safety-catch linker is well suited for carbon-carbon bond formation on solid support due to its stability under a range of reaction conditions, yet can be cleaved with nucleophiles upon activation. A representative example of solid phase Suzuki cross coupling reaction is shown in Scheme 38. Commercially available resin (SASRIN[238] or Wang[239]) **152** was coupled with an iodo- or a bromo-benzoic acid such as **153** via standard protocol with EDCI and HOBt to provide resin-bound product **154**. Phenylboronic acid **155** was then coupled with **154** to afford **156** after cleavage of the polymer-bound product.



Scheme 38. *Reaction conditions and Reagents*: (a) EDCI, Et_3N, HOBt, CH_2Cl_2, rt, 20 h (b) Pd(0) cat., DMF, rt (c) TFA/ RT, 30 min

6.2.3 Mechanism of Suzuki reaction

The mechanism of the Suzuki reaction is closely related to that of the Stille coupling reaction, and is also best described by a catalytic cycle (Figure 53, p.117).[240] The mechanism involves three steps:

(a) Oxidative addition-reaction of the halide component **157** with a palladium-(0) complex **161** to give apalladium(II) species **162**.

(b) Transmetallation-transfer of substituent R′ from boron to the palladium center, thus generating a palladium(II) species that contains both the substituent R and R′; that are to be coupled.

(c) Reductive elimination- to yield the coupling product **159** and the regenerated catalytically active palladium(0) complex **161**.

The boronic acid **158** is first converted to an active species **164** containing a tetravalent boron center by reaction with a nucleophile. Halides or trifluoromethanesulfonate are used as coupling partners R-X for boronic acids.

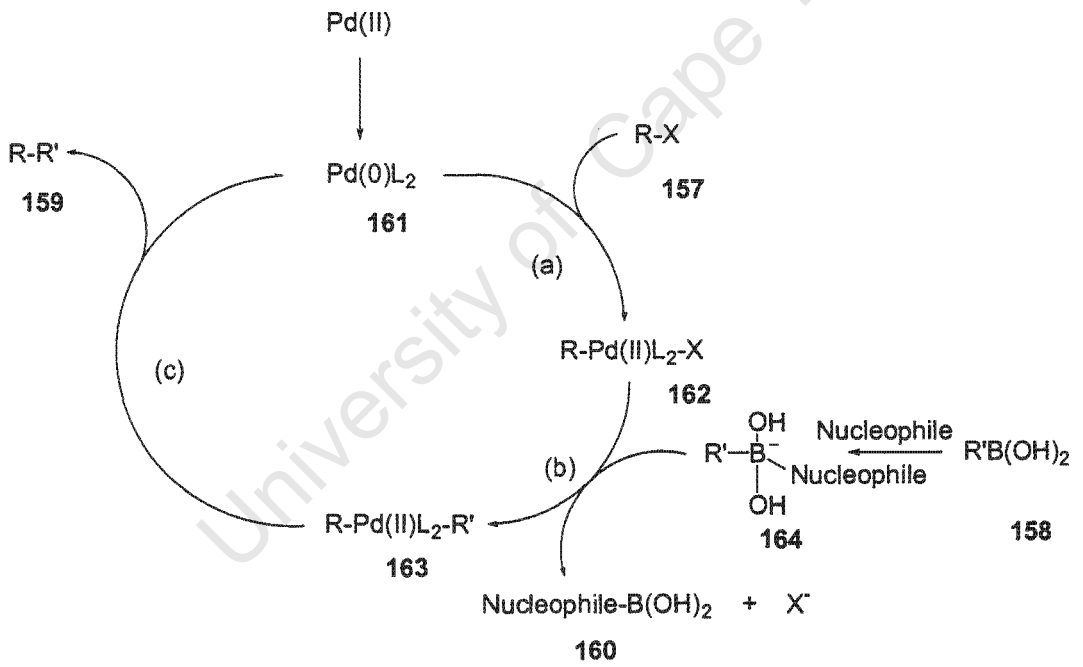
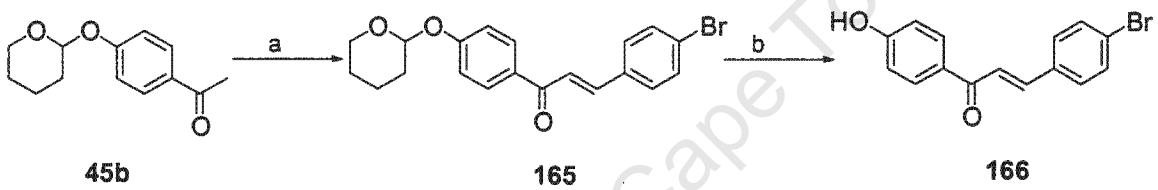


Figure 53. Mechanism of the Suzuki cross coupling reaction

6.2.4 Solution phase synthesis of biaryl chalcone 168

In order to optimize the conditions for adapting to the envisaged solid phase synthesis, biaryl chalcone **168** was synthesized in solution *via* Suzuki cross coupling using tetrahydropyranyl (THP) as a protecting group (Scheme 39, p.118). The THP protecting

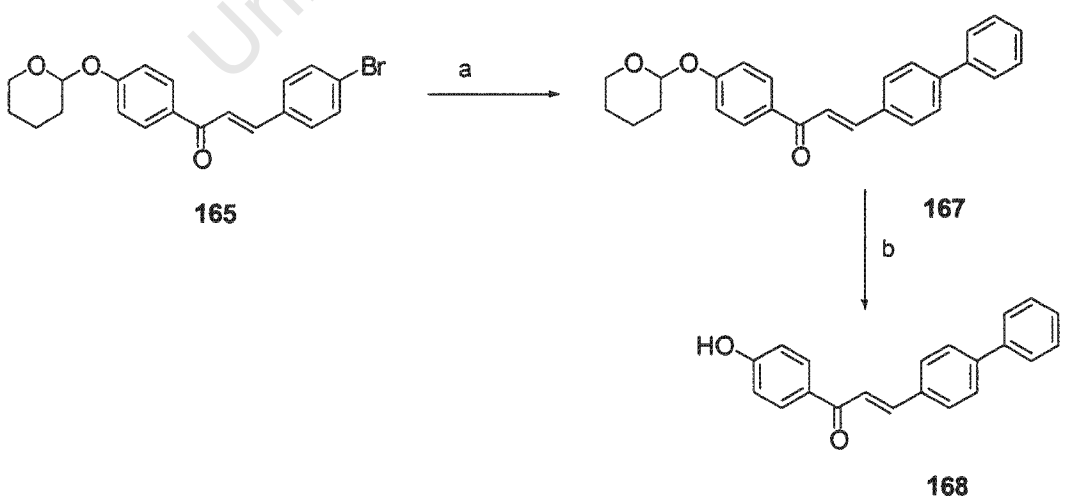
group has been employed extensively in organic synthesis. Dihydropyran provides a general and straightforward method for alcohols and/or phenols protection. Even hindered alcohols can be coupled to dihydropyran in high yield under mild conditions and the THP ether product is stable to most basic reagents. The starting point for the synthesis of **168** involved the preparation of chalcone **165** by the Claisen-Schmidt condensation reaction. Accordingly, THP-protected 4-hydroxyacetophenone **45b** (prepared by employing the reaction conditions described in Chapter 3) was treated with 1.1 equivalents of 4-bromobenzaldehyde in the presence of 2.0 equivalents of KOH in MeOH at 70 °C to give the chalcone **165** in 99% yield. Deprotection of compound **165** using 4M HCl afforded **166** in 97% yield.



45b **165** **166**

Scheme 39. *Reagents and conditions*: (a) 2.0 equiv of NaOMe, THF:MeOH 1:1, 70 °C, 3h (b) 4M HCl, MeOH, rt, 30 min

The synthesis of the biaryl chalcone **167** was accomplished in 38% yield by subjecting 1.0 equivalent of compound **165** and 1.5 equivalents of phenylboronic acid to Pd-catalyzed Sukuzi cross coupling conditions (Scheme 40).



165 **167** **168**

Scheme 40. *Reagents and conditions*: (a) 1.5 equiv of phenylboronic acid, 0.05 equiv of $Pd_2(dba)_3$, DMF, 65 °C, 24 h (b) 4M HCl, EtOH, rt, 30 min

Removal of the THP protecting group gave **168** in 93% yield. The proton NMR and MS data of all the compounds were consistent with the proposed structures.

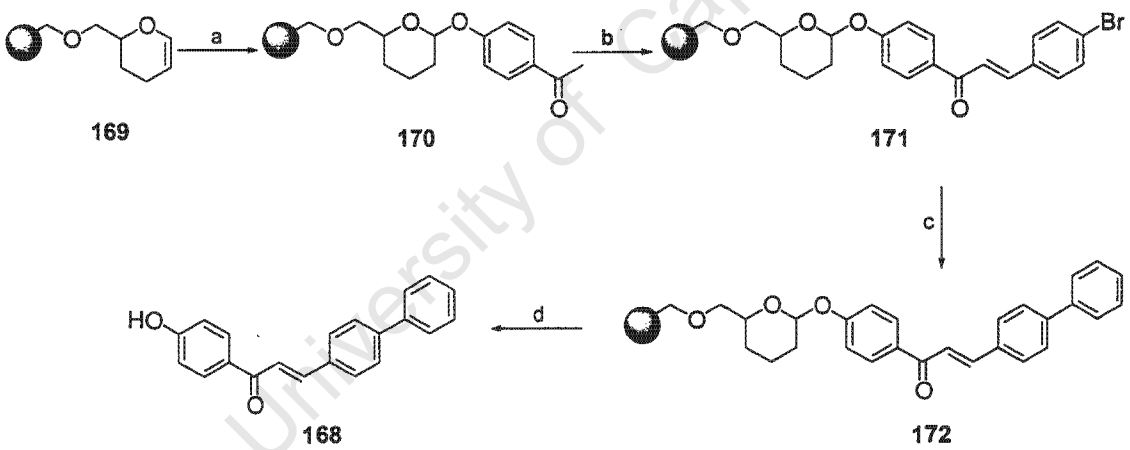
6.2.5 Solid phase synthesis of biaryl chalcone 168

Attachment to the solid support through the alcohol functionality provides one of the most versatile strategies of protection. Accordingly, a number of methods have been developed to couple alcohols to the solid phase through ester,[241] silyl ether[242] and trityl ether[243] functionalities. While these methods have been very useful for the synthesis of biopolymers, their utility in the solid phase synthesis of many organic compound libraries is limited by the stability of the attachment functionality to nucleophilic or basic reagents, and/or difficulties in obtaining useful loading levels particularly for secondary alcohols. The dihydropyran-functionalized support, which provides a general and straightforward method for alcohol and/or phenol attachment through the base-stable tetrahydropyranyl ether linkage was employed in the synthesis of biaryl chalcone **168**. 4-Hydroxyacetophenone was attached to the resin (tetrahydropyranyl [THP] linker) employing PPTS in dichloroethane at 80 °C for 16 h to afford polymer-supported **170** in 68% yield based on the loading level (Scheme 41, p.120). The loading level was determined by measuring the increase in mass of the resin after washing and drying to a constant weight. To ensure a high loading level, 5.0 equivalents of 4-hydroxyacetophenone and 5.0 equivalents of PPTS were used. It is noteworthy that in solution-based synthesis, the addition of the THP group to a molecule introduces a stereocenter, which for chiral molecules, results in diastereomers that often complicate chromatographic purification and NMR evaluation. However, on solid support these complications do not occur with a tetrahydropyranyl-based linkage agent since product isolation is accomplished simply by washing away excess reagent from the support-bound material, and analytical evaluation predominantly is performed on compounds after cleavage of the compounds from the solid support.

Next, the Claisen-Schmidt condensation reaction was carried out by adding 5.0 equivalents of 4-bromobenzaldehyde to a mixture of polymer-supported **170** (PS-**170**) and 2.0 equivalents of sodium methoxide solvated in THF:MeOH (1:1) at 70 °C and then shaken for 24 h. After filtering, washing and drying to a constant weight, **PS-171** was

obtained in 90 % yield. To ascertain whether the condensation reaction had taken place or not, a few milligrams of **PS-171** was treated with TFA:H_2O (95:5) for 30 minutes. After filtration and evaporation of the acid, the residue was re-dissolved in MeOH and then subjected to thin layer chromatography against compound **166** (obtained from the solution phase synthesis). The R_f value of the cleaved product was the same as that of compound **166** which confirmed that the condensation reaction had indeed taken place. Further proof was to follow after detachment of the final product from the resin.

Finally, the Suzuki cross coupling reaction was conducted. Accordingly, 2.0 equivalents of phenylboronic acid, 0.05 equivalent of $Pd_2(dba)_3$ and 2.0 equivalent of Na_2CO_3 were added to the **PS-171** solvated in dichloroethane. The resultant was shaken at reflux for 24 h and then cooled, filtered, washed and dried to give **PS-172.** Treatment of **PS-172** with TFA (95:5) afforded **168** in 9% yield (2 steps) after filtration and evaporation of the acid.



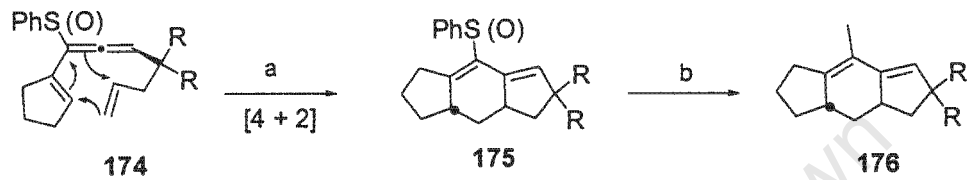
Scheme 41. *Reagents and conditions*: (a) 5.0 equiv of 4-hydroxyacetophenone, 5.0 equiv of PPTS, $ClCH_2CH_2Cl$, rt, 16 h (b) 5.0 equiv of 4-bromobenzaldehyde, 2.0 equiv of NaOMe, THF:MeOH 1:1, 70 °C, 24 h (c) 2.0 equiv of phenylboronic acid, 0.05 equiv of $Pd_2(dba)_3$, 2.0 equiv of Na_2CO_3, $ClCH_2CH_2Cl$, reflux, 24 h (d) TFA:H_2O 95:5, 30 min

6.3.0. Homologation of Terminal Aryl Acetylenes to Allenes *via* Mannich Bases

6.3.1. Introduction

Allenes are an important class of unsaturated hydrocarbons which contain two cumulated double bonds in an orthogonal geometry. They represent a versatile functional group that can be utilized as a useful building block in a variety of synthetic transformations,

leading to complex structures that are useful for constructing natural and un-natural products.[244] The versatility and synthetic utility of the allene moiety in organic chemistry has been extensively documented in the recent literature. For instance, allenes participate in a variety of cycloaddition and electrocyclic reactions affording products such as sterpurenes **176** that are not easily accessible by other synthetic methods (Scheme 42).[245,246]



174 **175** **176**

Scheme 42. *Reagents and Conditions*: (a) CH_2Cl_2, rt, 5 h, 85% (b) MeMgBr, Ni(dppp)Cl_2, THF, reflux, 19 h (57%)

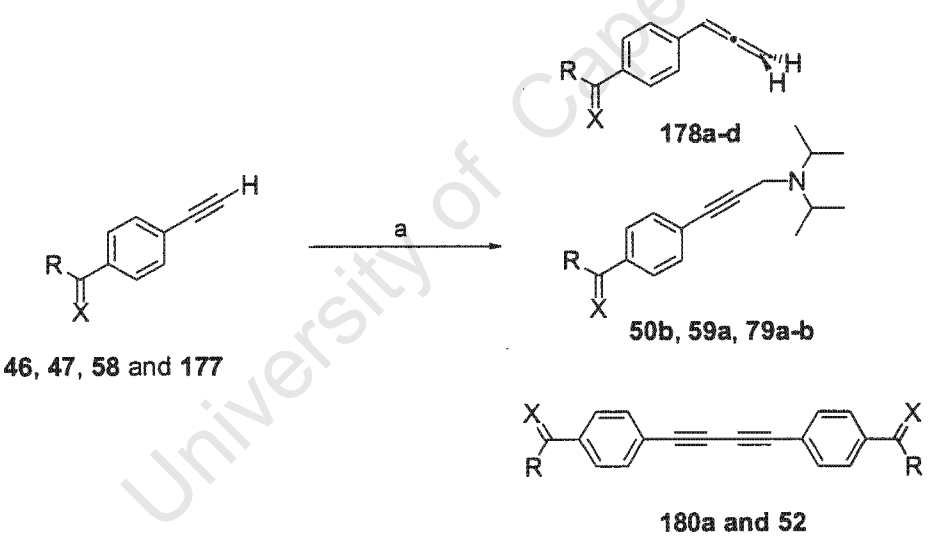
In addition, the allene moiety can be transformed into a variety of other function groups such as olefins, α,β-unsaturated carbonyl compounds and alkynes.[247] Moreover, allene axial chirality has been used to transfer asymmetry in cycloaddition reactions (Scheme 42).[248] Allenes have also received considerable attention due to the discovery of interesting biological properties[249,250] as well as to the development of improved synthetic methods.

Our interest in the synthesis of biologically active aryl acetylenic Mannich bases led us to explore the homologation of aryl acetylenes to allenes. In particular we sought to elucidate the effect that the nature of aryl substituents and variation of the catalyst component would have on product composition and yield distribution. Homologation of acetylenes to allenes *via* Mannich bases has been previously reported.[251-256] However, these reports have only addressed a limited range of acetylene systems. In addition, the effects of the nature of aryl substituents as well as variation of the catalyst component on the pattern of product composition and yield distribution have to date not been investigated. Thus we decided to undertake such an investigation. Aryl acetylenes prepared in Chapter 3 were employed as substrates for the homologation while Cu(I)Br, Cu(II)Cl and Cu(II)acetate were employed as catalysts.

6.3.2 Variation of the catalyst component

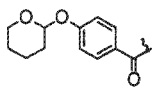
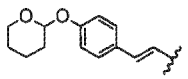
Homologation of alkynes to allenes were carried out by systematically replacing the anionic part of the copper-catalyst since it had been shown that the real catalyst is the Cu(I) species.[257] In case of Cu(II)-catalyst, Cu(I) is formed by the reduction of the initial Cu(II) complex.

First, the reaction of aryl acetylenes with diisopropylamine and paraformaldehyde in the presence of Cu(I)Br under the reaction conditions described by Crabbe and co-workers were investigated (Scheme 43).[258] Allenes **178a-d**, Mannich bases **50b**, **59a**, **179a-b** and homocoupling products **52** and **180a** were obtained and the results are summarized in Table 20. These product compositions are similar to those reported by Crabbe etal for phenyl and non-aromatic acetylenic substrates. [258]



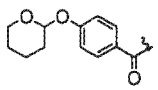
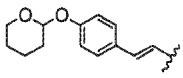
Scheme 43. *Reagents and conditions*: (a) 2.5 equiv of CH_2O, 2.0 equiv of $[(CH_3)_2CH]_2NH$, 0.5 equiv of CuBr, dioxane, reflux, 3h

Table 20. Cu(I)Br catalyzed reaction of **46**, **47**, **58** and **177** with diisopropylamine and paraformaldehyde

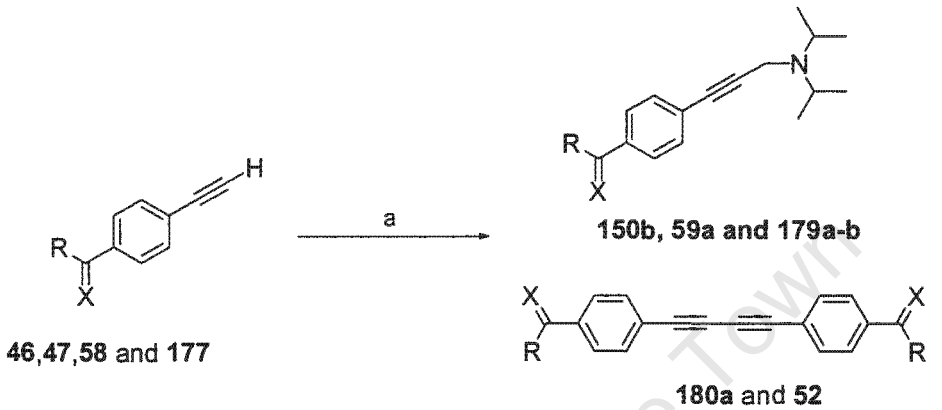
Compound	R	X	Products (%Yield)
58	H	O	**178a**(14) **59a**(58) -
177	Me	O	**178b**(35) **179a**(24) **180a**(11)
46	H		**178c**(30) **50b**(20) -
47		O	**178d**(12) **179b**(36) **52**(13)

As shown in Table 20, the product composition varied depending upon the nature of the substituent on the aryl acetylenic component. Notably, no homocoupling product was obtained when the acetylenic moiety was on the benzaldehyde component **58** or attached to the C-1 of the pro-2-en-1-one unit **46** (Table 20). The reactions of aryl acetylenes with diisopropylamine and paraformaldehyde in the presence of Cu(II)Cl gave the same product composition and similar yield distribution as those obtained with Cu(I)Br (Table 21).

Table 21. Cu(II)Cl catalyzed reaction of **46**, **47**, **58** and **177** with diisopropylamine and paraformaldehyde

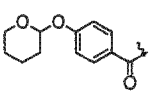
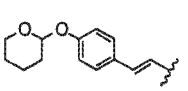
Compound	R	X	Products (%Yield)
58	H	O	**178a**(16) **59a**(51) -
177	Me	O	**178b**(29) **179a**(27) **180a**(7)
46	H		**178c**(34) **50b**(18) -
47		O	**178d**(20) **179b**(47) **52**(8)

Next, the reactions of **46**, **47**, **58** and **177** with diisopropylamine and paraformaldehyde in the presence of $Cu(OAc)_2$ as the catalyst were performed. In this case only **50b**, **52**, **59a**, and **180a** were obtained exclusively (Scheme 44). Interestingly, no allenes were obtained. The results are presented in Table 22.



Scheme 44. *Reagents and conditions*: (a) 2.5 equiv of CH_2O, 2.0 equiv of $[(CH_3)_2CH]_2NH$, 0.5 equiv of $Cu(OAc)_2$, dioxane, reflux, 3h

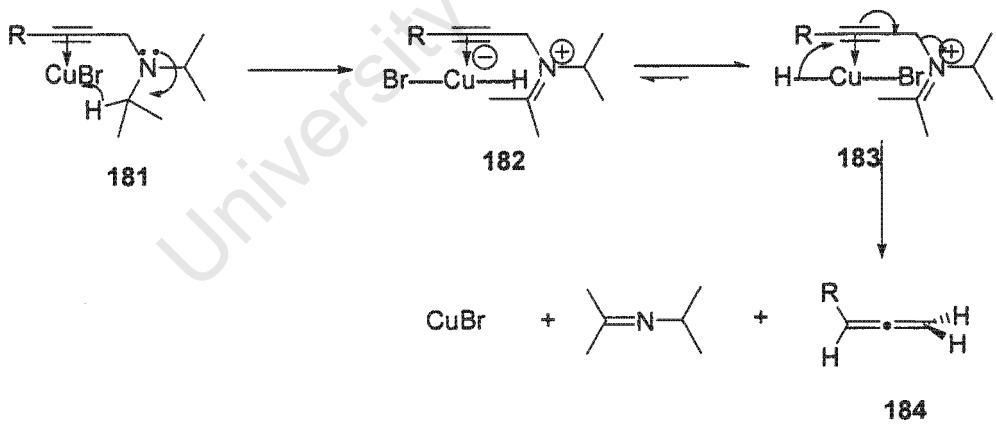
Table 22. $Cu(OAc)_2$ catalyzed reaction of **46**, **47**, **58** and **177** with diisopropylamine and paraformaldehyde

Compound	R	X	Products (%Yield)
58	H	O	**59a**(63) -
177	Me	O	**179a**(36) **180a**(21)
46	H		**50b** (97) -
47		O	**179b**(42) **52**(30)

From the results in Tables 20-22, it is clear that the product composition and yield distribution are influenced by both the anionic component of the catalyst and the nature of the aryl substituent. Comparable yields are obtained for catalysts [Cu(I)Br and Cu(II)Cl] which gave same product composition (Tables 20 and 21).

6.3.3 Mechanism for the formation of allenes from Mannich bases

The formation of allenes from Mannich bases and mechanistic studies have been reported.[258] The mechanism involves an intramolecular transfer of an α-hydrogen atom in an *N*-alkyl group of the intermediate Mannich base, to the carbon terminus of the allene. First, the copper(I) forms a 1:1 π complex **181** with the Mannich base in which the hydrogen is well positioned to be lost from the α-position of an isopropyl group (Scheme 45). Hydride ion shift occurs to give a hydridocopper(I) complex **182** which is similar to the cuprate complex as postulated by Pasto and co-workers for the reaction of a lithium cuprate with prop-2-ynylic halides.[259] Then, rotation of the copper atom takes place making the hydrogen available for transfer to the R-substituted terminus of the alkyne and electronic shifts to the allene **184**, thus releasing the diisopropylimine and the catalyst. It is important to note that the ability of copper to serve as both a supplier and sink for electrons, is the key to this process, as with most other cuprous-catalyzed reactions. Mannich base formation is kinetically controlled since this first occurs, even at 50 °C, whereas formation of allene is thermodynamically controlled requiring the reaction to be driven to 100 °C (refluxing dioxane).



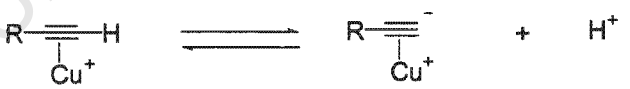
Scheme 45. Mechanism for the formation of an allene from a Mannich base

6.3.4 Trend in the product composition

The non-formation of allenes from copper(II) acetate catalyzed reactions may be explained by considering the mechanism for the formation of allenes from the Mannich bases. Formation of **183** (Scheme 45) may not have occurred due to structural reasons

(steric effects) when the acetate ion is the anionic component of the copper(I) catalyst. Unlike CuBr and CuCl-acetylene complexes, the CuOAc-acetylene complex does not have a linear chain structure. The bulky carboxylate ion may have prevented rotation of the copper atom from delivering the hydrogen in a favorable position for transfer to the R-substituted terminus of the alkyne.

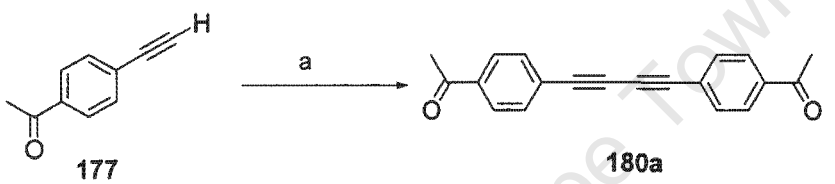
On the other hand, non-formation of the homocoupling products when the acetylenic component is attached to the benzaldehyde component or to the C-1 of prop-2-en-1-one unit may be explained on electronic grounds. In studies carried out by Bohlmann *et al*[260] on the rate of dimerization as influenced by the electronic nature of conjugated acetylenes, they found that more acidic acetylenes underwent more rapid dimerization under alkaline conditions, which was consistent with results obtained by Klebansky *et al*.[261] However, under acidic conditions, an inverse relationship was observed, and addition of a copper(I) salt became necessary. This also agrees well with the recent results obtained by Balcioglu *et al*.[257] These observations were explained on the basis of the formation of the π complexes between Cu^{I} ion and the triple bond, which would activate the alkyne toward deprotonation (Scheme 46). This complexation would be expected to be weaker for the diffuse π system of polyconjugated substrates, which indeed react more slowly. The reason for low yields in the experiments involving electron-withdrawing groups may be due to the low nucleophilicity of the generated copper acetylides.



Scheme 46. Acetylene activation by π-complex formation postulated by Bohlmann *et al*[260]

The rate of reaction of substrates **58** and **46** with the acetylenic moiety attached to the benzaldehyde component or to the C-1 of prop-2-en-1-one was apparently slower than those with the acetylenic moiety attached to the acetophenone component or to the C-3 of the prop-2-en-1-one component (**177** and **47**). Since dimerization reactions were competing with the rapid Mannich base formation no homocoupling products were

obtained from reactions involving **58** and **46**. In order to verify these observations, the issue of trend in yield distribution was addressed by embarking on a study of the dimerization reactions. First, the reaction conditions were optimized using **177** as a model substrate in the presence of $Cu(OAc)_2$ as the catalyst and triethylamine as a base (Scheme 47). It was found that the use of 0.5 equiv of $Cu(OAc)_2$ and 1.0 equiv of triethylamine in dioxane effectively catalyzed formation of **180a** even though prolonged reaction times were necessary. This result is worth noting since typical Eglinton-Glaser Cu-mediated reactions require an excess amount (10 equiv) of $Cu(OAc)_2$ in the absence of dioxygen. Attempts to reduce the amount of $Cu(OAc)_2$ resulted in incomplete reactions.



Scheme 47. *Reagents and conditions*: (a) 0.5equiv of $Cu(OAc)_2$, 1.0 equiv of Et_3N, dioxane, reflux, 16h, 72%

Using the optimized conditions, **46**, **47** and **56** were successfully dimerized. The results are summarized in Table 23.

Table 23. $Cu(OAc)_2$ catalyzed dimerization of **46**, **47**, **58** and **177**

Compound	Product (% Yield)
58	**180b** (32)
177	**180a** (72)
46	**180c** (62)
47	**52** (99)

As can be seen from Table 23, a similar trend in the yield distribution of homocoupling products was observed as those in Tables 20, 21 and 22. Compounds with the acetylenic moiety to the benzaldehyde component or attached to C-1 of the prop-2-en-1-one unit (**58** and **46**) gave low yields compared to their counterparts **47** and **177**, compounds with the acetylenic moiety attached to the acetophenone component or to the C-3 of the prop-2-

en-1-one component. The dimerization conditions were extended to a few selected arylacetylenic compounds with varying electronic properties of ring substituents (Scheme 48). The results are presented in Table 24.

185a-d **186a-d**

Scheme 48. *Reagents and conditions*: (a) 0.5equiv of $Cu(OAc)_2$, 1.0 equiv of Et_3N, dioxane, reflux, 16h

Table 24. $Cu(OAc)_2$ catalyzed dimerization of selected aryl acetylenic compounds

Compound	R	Product (% Yield)
185a	H	**186a** (100)
185b	CH_3	**186b** (97)
185c	NO_2	**186c** (47)
185d	Br	**186d** (29)

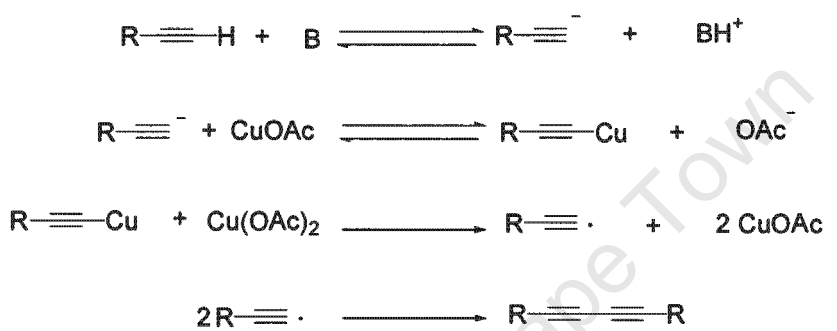
A similar trend in yield distribution (Table 24) was observed as that in Table 23. It was evident from Table 24 that under the reaction conditions employed, compounds with electron-withdrawing substituents gave low yields compared to compounds with electron-donating groups. This may be due to the low nucleophilicity of the generated acetylides. The very low yield in case the **186d** compound may be attributed to low solubility of the cuprous derivatives.

6.3.5.0 Mechanistic investigations.

There have been surprisingly few mechanistic investigations of alkyne couplings, given their wide applicability in the synthesis, and the potential for improved conditions that a more complete mechanistic understanding could provide. The two most common mechanisms that have been proposed for the copper-catalyzed couplings are: (i) radical mechanism and (ii) dimeric copper acetylide mechanism.

6.3.5.1 Radical mechanism

This was proposed by Clifford and Waters who showed that copper(I) ions were required for the coupling using $Cu(OAc)_2$ in pyridine in the absence of dioxygen.[262] The mechanism involves formation of copper(I) acetylides which are rapidly oxidized by the transfer of a single electron to copper(II) through and an acetate ligand bridge. Then, decomposition of the resultant copper(II) acetylide and recombination of the free radicals would give the coupled products (Scheme 49).

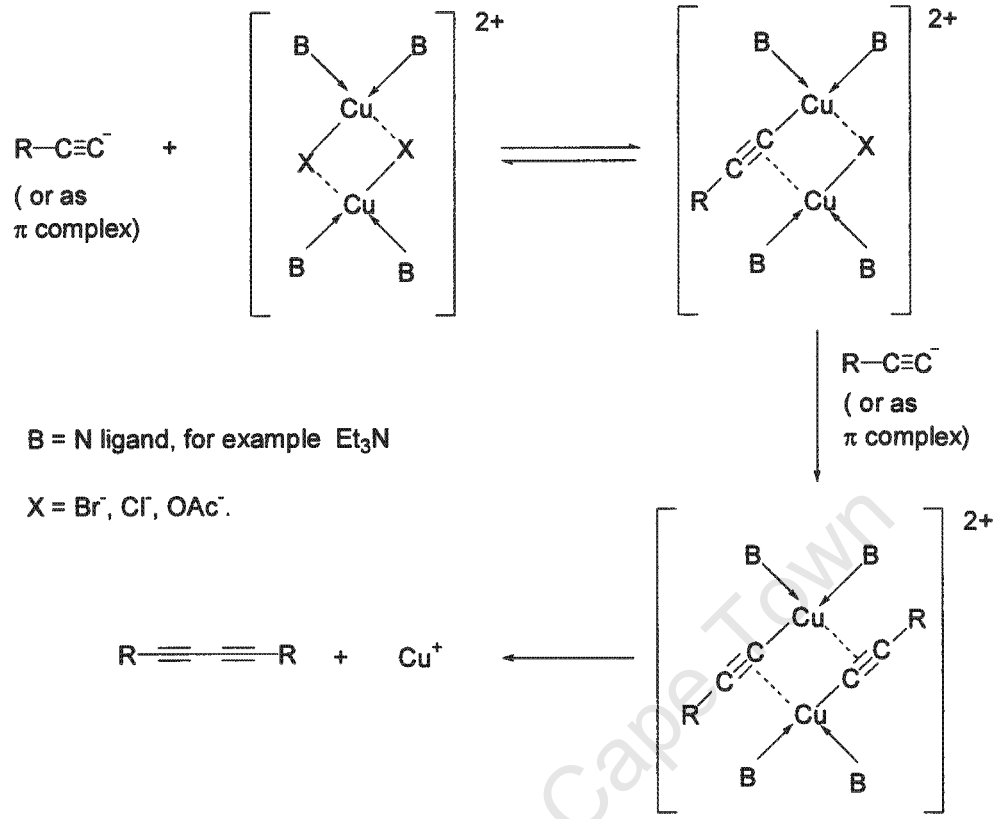


Scheme 49. Mechanism proposed for the oxidative coupling using $Cu(OAc)_2$ in pyridine in the absence of O_2. The coordinating pyridine molecules are omitted.

6.3.5.2 Dimeric copper acetylide mechanism

This mechanism was postulated by Bohlmann and co-workers.[260] They suggested that the mechanism involved the formation of dinuclear copper(II) acetylide that collapses directly to the homocoupling product (Scheme 50).

In an attempt to rationalize the formation of the homocoupling products, the dimerization reaction of **177** was repeated, but this time in the presence of 2,2,6,6-tetramethylpiperidine-N-oxyl, (TEMPO$^{\bullet}$), a radical scavenger.[263] There was no inhibition of dimerization. This suggested that no free radicals were present as intermediates in the formation of the homocoupling products. It was, however, suggested that dimerization reaction proceeds by the mechanism proposed by Bohlmann and co-workers.



Scheme 50. Dimeric copper acetylides were first proposed by Bohlmann *et al* as intermediates in the oxidative acetylenic coupling.

In summary, the product composition depends on both the nature of the aryl substituent and the anionic component of the catalyst. No homocoupling products are produced from substrates with strong electron withdrawing groups due to weaker complexation with the Cu(I) ion which leads to slow formation of the homodimers compared to the Mannich bases. On the other hand, the catalyst, $Cu(OAc)_2$, with a bulky non-linear anionic component does not give allene products because the carboxylate ion prevents rotation of copper atom from delivering the hydrogen in a favorable position for transfer to the R-substituted terminus of the alkyne. Finally, the yield distribution depends only on the nature of the aryl substituent. The yields of homocoupling products decrease as the strength of the electron withdrawing group increases presumably due to the decrease in the nucleophilicity of the intermediate acetylide ion.

EXPERIMENTAL

^{1}H NMR spectra were determined in $CDCl_3$, CD_3OD or $(CD_3)_2SO$ solution on a Varian Gemini (300 MHz) or a Varian Unity Spectrometer (400 MHz), using TMS as an internal standard. The chemical shift (δ) are given in ppm relative to TMS (δ = 0.00). The coupling constant *J* is given in Hz. ^{13}C NMR spectra were recorded on the same instruments at 75 MHz or 100 MHz in the same deuterated solvents using TMS as an internal inference.

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 shifts values are simply listed without specific assignment to carbon atoms.

Mass spectra (EI) were recorded on a VG Micromass 16F spectrometer at 70eV with accelerating voltage of 4 Kv and accurate mass determinations were measured on a Kratos Limited MS9/50 spectrometer (at Cape Technikon, SA) and VG (Micromass) 70-SE magnetic sector mass spectrometer (at Kent, UK).

Melting points were determined using a Reichert-Jung hot stage apparatus and are uncorrected

Infrared (IR) spectra were recorded in Nujol on a satellite FTIR spectrometer.

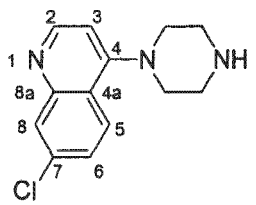
Microanalyses were performed on a Fisons EA 1108 CHNS-O instrument.

Reactions were monitored by thin layer chromatography (tlc) using Merck F254 silica gel plates and visualized with uv light and either by staining with iodine vapors or spraying with 1% aqueous potassium permanganate solution containing 5% sodium hydroxide or 7% anisaldehyde in ethanol containing 1% sulfuric acid.

Column chromatography and preparative layer chromatography (plc) were performed using Merck Kieselgel 60.

All materials and dry solvents used in reactions were purchased from commercial suppliers and used without further purification unless otherwise stated.

7-Chloro-4-piperazin-1-yl-quinoline 54



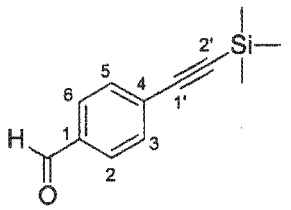
A mixture of piperazine (10.8g, 126.4 mmol), 4,7-dichloroquinoline (5.00g, 25.2 mmol), K_2CO_3 (1.04g, 7.57 mmol), triethylamine (1.0 ml, 7.57 mmol) and 1-methyl pyrrolidinone (18.0 ml) under nitrogen was heated at 135 0° C for 3 hours. The reaction mixture was cooled to room temperature, diluted with dichloromethane (200 ml), washed with brine, dried ($MgSO_4$) and concentrated to give a yellow crude oil which was columned on silica gel to afford **54** as a pale yellow solid. Recrystallisation from chloroform-hexane gave white crystals (5.61g, 91%).

mp 113-115 °C (from chloroform-hexane) (Lit. 113-115 °C);[264] R_f = 0.26 (20% MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 3420 , 1612, 1580; δ_H(300 MHz; $CDCl_3$) 3.16 (8H, m, [CH_2CH_2]$_2$NH), 6.85 (1H, d, *J* 5.1, H-3), 7.43 (1H, dd, *J* 2.1, 9.0, H-6), 7.93 (1H, d, *J* 9.0, H-5), 8.02 (1H, d, *J* 2.1, H-8), 8.70 (1H, d, *J* 5.1, H-2).

General Method A for the preparation of compounds 43a-b

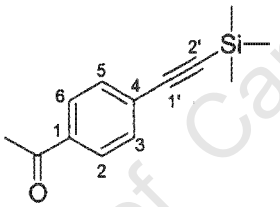
A solution of aldehyde or ketone (27.0 mmol), ethynyltrimethylsilane (47.0 mmol), palladium (II) acetate (0.50 mmol), triphenylphosphine (1.0 mmol) and copper (I) iodine (1.0 mmol) in triethylamine (54.0 ml) was heated to gentle reflux under nitrogen at 100°C for 4 hours. The resulting slag was cooled to room temperature and filtered. The filtrate was concentrated and then mixed with 50ml of aqueous sodium hydrogen carbonate and extracted with dichloromethane (3× 50ml). The combined organic fractions were dried (magnesium sulphate), concentrated and then purified by column chromatography on silica gel (10% ethylacetate: hexane) to afford the title compounds **43a-b**.

4-(Trimethylsilanylethyny)l-benzaldehyde 43a



The conditions employed for the preparation of this compound were those described in General Method A, which afforded **43a** as a yellow solid (4.15g, 76%); mp 67-69 °C (from ethyl acetate-hexane) (Lit. mp 66-67 °C);[265] R_f = 0.45 (10% EtOAc/hexane); δ_H(300 MHz; $CDCl_3$) 0.26 (9H, s, Si[CH_3]$_3$), 7.58 (2H, d, *J* 8.4, H-2/6), 7.80 (2H, d, *J* 8.4, H-3/5) 9.99 (1H, s, C*H*O).

1-(4-Trimethylsilanylethynyl-phenyl)-ethanone 43b

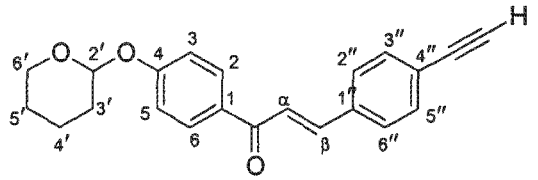


The conditions employed for the preparation of this compound were those described in General Method A, which afforded **43b** as a light brown oil (5.01g, 86%); R_f = 0.44 (10% EtOAc/hexane); δ_H(300 MHz; $CDCl_3$) 0.27 (9H, s, Si[CH_3]$_3$), 2.59 (3H, s, COCH_3), 7.55 (2H, d, *J* 8.7, H-3/5), 7.89 (1H, d, *J* 8.7, H-2/6).

General Method B for the preparation of compounds 41a-b, 46, 47, 49 and 165

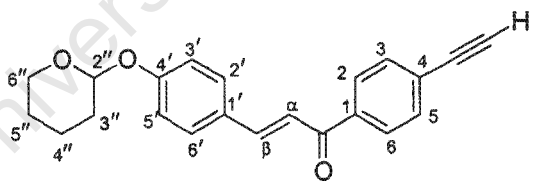
To a solution of methyl ketone (0.37 mmol) and 2.0 equivalents KOH in dry methanol (5.0 ml) under nitrogen at 70 °C was added aldehyde (0.37 mmol) in methanol (5.0 ml). The resultant mixture was stirred for 3h, diluted with water (10 ml) and extracted with ethyl acetate (3 x 20 ml). The extracts were combined, washed with brine (20 ml) and concentrated to give the title compounds **41a-b**, **46**, **47**, **49** and **165**.

3E-(4-Ethynyl-phenyl)-1-(4-(tetrahydro-pyran-2-yloxy-phenyl]-propenone **46**



The conditions employed for the preparation of this compound were those described in General Method B, which gave **46** as a pale yellow solid (0.11g, 90%); mp 138-140 °C (from ethylacetate-hexane); R_f = 0.43 (30% EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1584 (C=C), 1680 (C=O), 2255 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.68 (3H, m, H-5a′/4′), 1.90 (2H, m, H-3a′/5b′), 2.00 (1H, m, H-3b′), 3.20 (1H, s, C≡C*H*), 3.63 (1H, m, H-6a′), 3.87 (1H, m, H-6b′), 5.54 (1H, t, *J* 3.0, H-2′), 7.14 (2H, d, *J* 9.0, H-3/5), 7.52 (2H, d, *J* 9.0, H-2″/6″), 7.23 (1H, d, *J* 15.6, H-α), 7.59 (2H, d, *J* 9.0, H-3″/5″), 7.76 (1H, d, *J* 15.6, H-β), 8.01 (2H, d, *J* 9.0, H-2/6); δ_c(75 MHz; $CDCl_3$) 18.5, 25.0, 30.1, 62.0, 79.2, 83.2, 96.1, 116.4 (2C), 122.9 , 123.9, 128.2 (2C), 130.7, 131.6 (2C), 132.6 (2C), 135.5, 142.7, 161.0, 188.6; Anal. Found C79.28, H 6.03% Calc. For $C_{22}H_{20}O_3$: C 79.50, H 6.06%; LRMS (EI) m/z (%), 332 (M^+, 0.26), 248 (M^+-THP-H, 10), 85 ($[THP+H]^+$, 100).

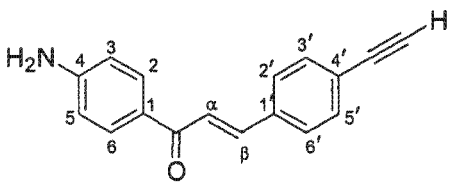
1-(4-Ethynyl-phenyl)-3E-(4-(tetrahydro-pyran-2-yloxy-phenyl]-propenone **47**



The conditions employed for the preparation of this compound were those described in General Method B, which gave **47** as a yellow solid (0.098g, 84%); mp 125-128 °C (from ethylacetate-hexane); R_f = 0.47 (30% EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1593 (C=C), 1680 (C=O), 2245 (C≡C); δ_H(400 MHz; $CDCl_3$) 1.66 (3H, m, H-5a″/4″), 1.88 (2H, m, H-3a″/5b″), 2.01 (1H, m, H-3b″), 3.23 (1H, s, C≡C*H*), 3.64 (1H, m, H-6a″), 3.88 (1H, m, H-6b″), 5.49 (1H, t, *J* 3.2, H-2″), 7.09 (2H, d, *J* 8.8, H-3′/5′) 7.38 (1H, d, *J* 15.6, H-α), 7.59 (2H, d, *J* 8.8, H-2′/6′), 7.60 (2H, d, *J* 8.8, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.96 (2H, d, *J* 8.8, H-2/6); δ_c(100 MHz; $CDCl_3$) 18.8, 25.3, 30.4, 62.3, 80.3, 83.1, 96.4,

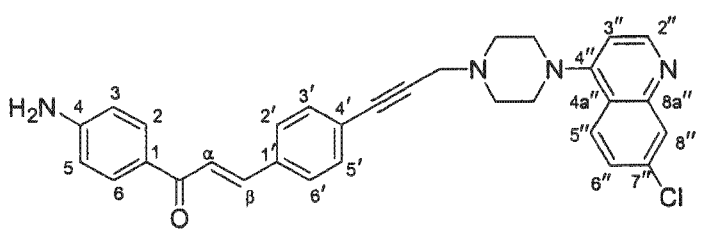
117.0 (2C), 119.9, 126.6, 128.2 (2C), 128.5, 130.4 (2C), 132.5 (2C), 138.6, 145.3, 159.6, 189.7; Anal. Found C79.18, H 5.92% Calc. For $C_{22}H_{20}O_3$: C 79.50, H 6.06%. LRMS (EI) m/z (%), 333 ($[M+H]^+$, 2), (M^+-THP-H, 10), 85 ($[THP+H]^+$, 100).

***1-(4-Amino-phenyl)-3E-(4-ethynyl-phenyl)-propenone* 49**



The conditions employed for the preparation of this compound were those described in General Method B, which gave **49** as a yellow solid (0.070g, 81%); mp 165-168 °C (from ethylacetate-hexane); R_f = 0.14 (30% EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1596 (C=C), 1680 (C=O), 2250 (C≡C); 3278 (N-H), 3319 (N-H); δ_H(300 MHz; $CDCl_3$) 3.19 (1H, s, C≡C*H*), 4.18 (2H, br s, NH_2) 6.69 (2H, d, *J* 8.4, H-3/5), 7.51 (2H, d, *J* 8.1, H-2′/6′), 7.53 (1H, d, *J* 15.6, H-α), 7.58 (2H, d, *J* 8.1, H-3′/5′), 7.74 (1H, d, *J* 15.6, H-β), 7.92 (2H, d, *J* 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 79.0, 83.3, 113.9 (2C), 120.8, 123.0, 128.0 (2C), 128.4, 131.1 (2C), 132.6 (2C), 135.7, 141.9, 151.2, 187.7; Anal. Found C81.64, H 5.18, N 6.09% Calc. For $C_{17}H_{13}NO$: C 82.51, H 5.30, N 5.66%; LRMS (EI) m/z (%), 247 (M^+, 100), 120 ($C_7H_6NO^+$, 85).

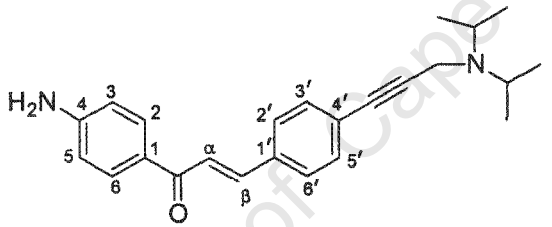
***1-(4-Amino-phenyl)-3E-(4-{3-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)- propenone* 41b**



The conditions employed for the preparation of this compound were those described in General Method B, which gave **41b** as orange crystals (0.080g, 90%); mp 110-112 °C (from chloroform-hexane); R_f = 0.56 (MeOH/$CHCl_3$/17%NH_4OH 2:2:1);

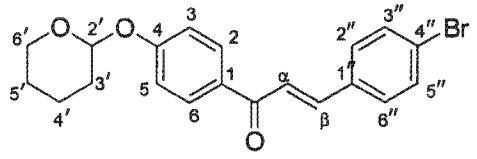
IRν_{max}(Nujol)/cm^{-1} 1596 (C=C), 1660 (C=O), 2250 (C≡C), 3375 (N-H), 3402 (N-H); δ_H(300 MHz; $CDCl_3$) 2.95 (4H, t, *J* 4.8 Hz, $CH_2N[CH_2CH_2]_2N$), 3.34 (4H, t, *J* 4.8, $CH_2N[CH_2CH_2]_2N$), 3.70 (2H, s, $C{\equiv}CCH_2$), 4.4 (2H, br s, NH_2), 6.68 (2H, d, *J* 9.0, H-3/5), 6.85 (1H, d, *J* 5.1, H-3″), 7.42 (1H, d, *J* 2.1, 9.0, H-6″), 7.49 (2H, d, *J* 8.4, H-2/6′), 7.52 (1H, d, *J* 15.6, H-α), 7.57 (2H, d, *J* 8.4, H-3′/5′), 7.74 (1H, d, *J* 15.6, H-β), 7.96 (2H, d, *J* 9.0, H-2/6), 7.97 (1H, d, *J* 9.0, H-5″), 8.04 (1H, d, *J* 2.1, H-8″), 8.72 (1H, d, *J* 5.1, H-2″); δ_c(75 MHz; $CDCl_3$) 47.8, 52.0 (2C), 52.1 (2C), 85.3, 87.2, 109, 116.4 (2C), 118.9, 121.8, 125.2, 125.3, 126.4, 126.9 (2C), 127.2, 128.4, 130.6 (2C), 131.9 (2C), 135.3, 138.0, 145.5, 149.9, 151.5, 157.2, 159.5, 189.8; HRMS (EI) *m/z* Found: M^+, 506.18752 Calc. For $C_{31}H_{27}ClN_4O$: M, 506.18734.

1-(4-Amino-phenyl)-3E-[4-(3-Diisopropylamino-prop-1-ynyl)-phenyl]-propenone **41a**



The conditions employed for the preparation of this compound were those described in General Method B, which gave **41a** as yellow crystals (0.13g, 95%); Decomposed above 180 °C (from chloroform-hexane); R_f = 0.79 ($MeOH/CH_2Cl_2$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1592 (C=C), 1649 (C=O), 2249 (C≡C), 3250 (N-H), 3429 (N-H); δ_H(300 MHz; $CDCl_3$) 1.14 (6H, d, *J* 6.6, $N[CH(CH_3)_2]_2$), 1.15 (6H, d, *J* 6.6 Hz, $N[CH(CH_3)_2]_2$), 3.26 (2H, m, $N[CH(CH_3)_2]_2$), 3.66 (2H, s, $C{\equiv}CCH_2$), 4.16 (2H, br s, NH_2), 6.70 (2H, d, *J* 8.7, H-3/5), 7.40 (2H, d, *J* 8.4, H-2′/6′), 7.50 (1H, d, *J* 15.6, H-α), 7.54 (2H, d, *J* 8.4, H-3′/5′), 7.73 (1H, d, *J* 15.6, H-β), 7.91 (2H, d, *J* 8.7, H-2/6); δ_c(75 MHz; $CDCl_3$) 20.8 (4C), 35.0 (2C), 49.0, 83.7, 90.8, 115.7 (2C), 122.3, 123.2, 128.1 (2C), 128.3 , 130.7 (2C), 131.9 (2C), 134.6, 143.0, 161.2, 188.6; Anal. Found C79.71, H 8.11, N 7.25% Calc. For $C_{24}H_{28}N_2O$: C 79.96, H 7.83, N 7.77%; LRMS (EI) m/z (%), 361 ($[M+H]^+$, 100), 345 (M^+- CH_3, 20),

3-E(4-Bromo-phenyl)-1-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **165**

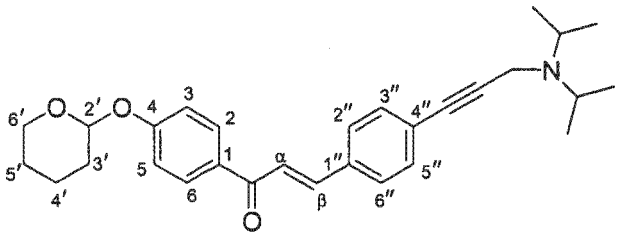


The conditions employed for the preparation of this compound were those described in General Method B, however, 4.50 mol were used, which gave **165** as a pale yellow solid (1.74g, 99%); mp 120-122 °C (from ethylacetate-hexane); R_f = 0.6 (30% EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1580 (C=C), 1650 (C=O), ; δ_H(300 MHz; $CDCl_3$) 1.66 (3H, m, H-5a′/4′), 1.92 (2H, m, H-3a′/5b′), 2.00 (1H, m, H-3b′), 3.20 (1H, s, C≡C*H*), 3.63 (1H, m, H-6a′), 3.87 (1H, m, H-6b′), 5.54 (1H, t, *J* 3.0, H-2′), 7.14 (2H, d, *J* 9.0, H-3/5), 7.52 (2H, d, *J* 9.0, H-2″/6″), 7.23 (1H, d, *J* 15.6, H-α), 7.59 (2H, d, *J* 9.0, H-3″/5″), 7.76 (1H, d, *J* 15.6, H-β), 8.01 (2H, d, *J* 9.0, H-2/6); δ_c(75 MHz; $CDCl_3$) 18.5, 25.1, 30.1, 62.1, 96.1, 116.4, 122.9, 123.9, 128.2 (2C), 130.7 (2C), 131.6, 132.6 (2C), 135.5, 142.7, 161.0, 188.2; HRMS (EI) *m/z* Found: M^+, 386,05169 Calc. For $C_{20}H_{19}BrO_3$: M, 386.05176.

General Method C for the preparation of compounds 50a-c, 51a-h, 52, and **59a-b**

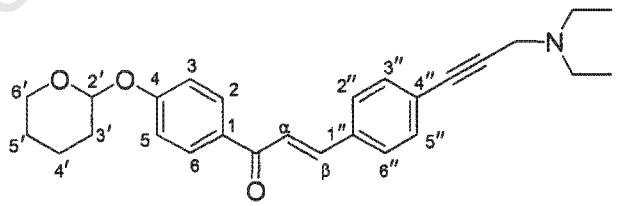
A mixture of acetylenic chalcone (0.35 mmol), paraformaldehyde (0.46 mmol), amine (0.35 mmol) and copper (II) acetate (0.35 mmol) in dry 1,4-dioxane (5.0 ml) was heated under reflux at 100°C for 3 h. The reaction mixture was cooled to room temperature and then filtered. The filtrate was poured into water and extracted with ether (3×10 ml). The combined ether extracts were concentrated to give a light brown solid residue, which was subjected to column chromatography on silica gel (10% EtOAc: hexane and MeOH:$CHCl_3$: 17% NH_4OH 2: 2: 1) to afford chalcone Mannich bases **50a-c, 51a-h, 52,** and **59a-b**.

3E-[4-(3-Diisopropylamino-prop-1-ynyl)-phenyl)-1-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **50b**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **50b** as a yellow solid (0.065g, 93%); mp 66-68 °C (from ethyl acetate-hexane); R_f = 0.14 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1030 (C-O), 1600 (C=C), 1680 (C=O), 2251 (C≡C); δ_H(400 MHz; $CDCl_3$) 1.17 (12H, d, *J* 6.4 Hz, NCH[C*H*$_3$]$_2$), 1.69 (3H, m, H-5a′/4′), 1.90 (2H, m, H-3a′/5b′), 2.01 (1H, m, H-3b′), 3.29 (2H, sep, *J* 6.4 Hz, N[C*H*(CH$_3$)$_2$]), 3.65 (1H, m, H-6a′), 3.68 (1H, s, C≡CC*H*$_2$), 3.88 (1H, m, H-6b′), 5.54 (1H, t, *J* 3.2, H-2′), 7.13 (2H, d, *J* 8.8, H-3/5), 7.42 (2H, d, *J* 8.0, H-2″/6″), 7.50 (1H, d, *J* 15.6, H-α), 7.55 (2H, d, *J* 8.0, H-3″/5″), 7.75 (1H, d, *J* 15.6, H-β), 8.01 (2H, d, *J* 8.8, H-2/ 6); δ_c(75 MHz; $CDCl_3$) 18.5, 20.8 (4C), 25.3, 30.3, 35.2 (2C), 49.0, 62.3, 80.2, 83.6, 96.1, 116.4 (2C), 122.5, 126.0, 128.2 (2C), 130.8, 131.9 (2C), 132.9 (2C), 136.7, 143.3, 161.2, 188.9; HRMS (EI) *m/z* Found: M$^+$, 445.26078 Calc. For $C_{29}H_{35}O_2N$: M,445.26169.

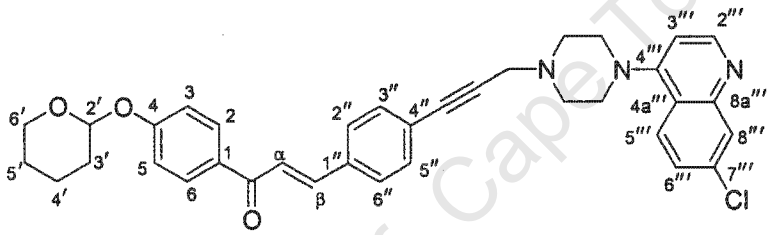
3E-[4-(3-Diethylamino-prop-1-ynyl)-pheny]-1-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **50c**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **50c** as a yellow solid (0.050g, 98%); mp 66-68 °C (from ethyl acetate-hexane); R_f = 0.14 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1025 (C-O), 1594 (C=C), 1680 (C=O), 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.13 (6H, t, *J* 7.2,

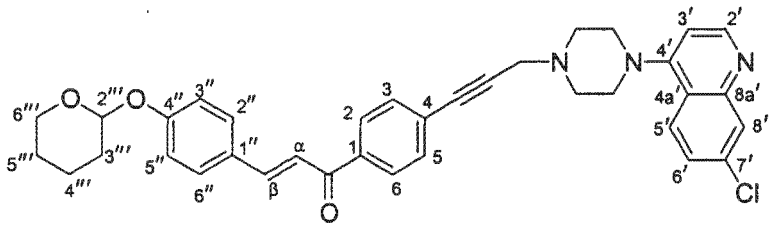
$N[CH_2CH_3]_2$), 1.66 (3H, m, H-5a′/4′), 1.88 (2H, m, H-3a′/5b′), 1.99 (1H, m, H-3b′), 2.64 (1H, q, *J* 7.2, $N[CH_2CH_3]_2$), 3.63 (1H, m, H-6a′), 3.65 (1H, s, $C{\equiv}CCH_2$), 3.88 (1H, m, H-6b′), 5.52 (1H, t, *J* 3.2, H-2′), 7.08 (2H, d, *J* 9.0, H-3/5), 7.38 (1H, d, *J* 15.6, H-α), 7.52 (2H, d, *J* 8.4, H-3″/5″), 7.58 (2H, d, *J* 9.0, H-2″/6″), 7.78 (1H, d, *J* 15.6, H-β), 7.94 (2H, d, *J* 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 12.6 (2C), 18.6, 25.1, 30.2, 41.6 (2C), 47.4, 62.0, 84.5, 88.1, 96.2, 116.8 (2C), 119.7, 127.8, 128.2 (2C), 128.3, 130.1 (2C), 131.8 (2C), 137.5, 144.9, 159.3, 189.6; HRMS (EI) *m/z* Found: $[M-H]^+$, 416.22251 Calc. For $C_{27}H_{30}NO_3$: M, 416.22257.

3E-(4-{3-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-1-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **50a**



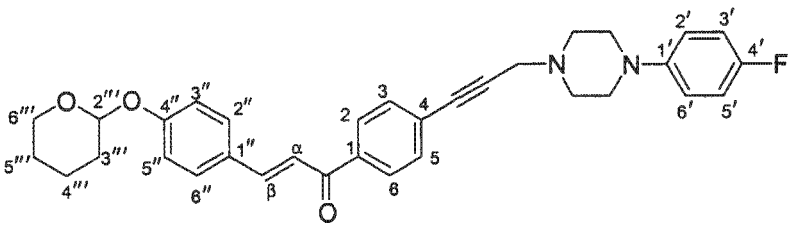
The conditions employed for the preparation of this compound were those described in General Method C, which afforded **50a** as a light brown solid (0.087g, 95%); mp 82-85 °C (from ethyl acetate-hexane); R_f = 0.89 ($MeOH/CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1023 (C-O), 1603 (C=C), 1651 (C=O), 2251 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.71 (3H, m, H-5a′/4′), 1.91 (2H, m, H-3a′/5b′), 2.01 (1H, m, H-3b′), 2.95 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.32 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.67 (3H, m, H-$C{\equiv}CCH_2$/6a′), 3.87 (1H, m, H-6b′), 5.54 (1H, t, *J* 3.0, H-2′), 6.86 (1H, d, *J* 5.1, H-3‴), 7.14 (2H, d, *J* 9.0, H-3/5), 7.43 (1H, d, *J* 9.0, H-6‴), 7.51 (2H, d, *J* 8.4, H-2″/6″), 7.53 (1H, d, *J* 15.6, H-α), 7.60 (2H, d, *J* 8.4, H-3″/5″), 7.76 (1H, d, *J* 15.6, H-β), 7.97 (1H, d, *J* 9.0, H-5‴) 8.01 (2H, d, *J* 9.0, H-2/6), 8.05 (1H, d, *J* 2.1, H-8‴), 8.72 (1H, d, *J* 5.1, H-2‴); δ_c(75 MHz; $CDCl_3$) 18.5, 25.1, 30.1, 47.8, 52.0 (2C), 52.1 (2C), 62.1, 85.5, 86.4, 96.2, 112.1, 116.1 (2C), 122.6, 123.1, 124.8, 125.2 (2C), 126.2, 128.2, 128.3, 130.6, 131.6 (2C), 132.2 (2C), 133.0, 135.0, 142.8, 150.5, 152.5, 157.0, 161.0, 188.6; LRMS (EI) m/z (%), 507 ($[M-THP]^+$ 18), 261 ($C_{18}H_{13}O_2^+$, 20), 121 ($C_7H_5O_2^+$, 87) .

1-(4-{3-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **51a**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51a** as a pale yellow solid (0.052g, 57%); mp 130-132 °C (from ethyl acetate-hexane); R_f = 0.95 (MeOH/$CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1030 (C-O), 1600 (C=C), 1651 (C=O), 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.71 (3H, m, H-5a‴/4‴), 1.91 (2H, m, H-3a‴/5b‴), 2.01 (1H, m, H-3b‴), 2.94 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.32 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.68 (3H, m, $C{\equiv}CCH_2$/6a‴), 3.87 (1H, m, H-6b‴), 5.54 (1H, t, *J* 3.0, H-2‴), 6.87 (1H, d, *J* 5.1, H-3′), 7.14 (2H, d, *J* 8.4, H-3″/5″), 7.43 (1H, dd, *J* 2.1, 9.0, H-6′), 7.50 (2H, d, *J* 8.4, H-2″/6″), 7.53 (1H, d, *J* 15.3, H-α), 7.59 (2H, d, *J* 8.4, H-3/5), 7.76 (1H, d, *J* 15.3, H-β), 7.96 (1H, d, *J* 9.0, H-5′), 8.01 (2H, d, *J* 8.4, H-2/6), 8.05 (1H, d, *J* 2.1, H-8′), 8.74 (1H, d, *J* 5.1, H-2′); δ_c(75 MHz; $CDCl_3$) 18.5, 25.1, 30.1, 47.8, 52.0 (2C), 52.1 (2C), 62.1, 85.5, 86.4, 96.2, 109.0, 116.1 (2C), 122.6, 124.8, 125.2, 126.2, 128.2, 128.3 (2C), 128.9, 130.7, 131.6 (2C), 132.3 (2C), 135.0, 136.5, 142.9, 150.2, 151.9, 157.2, 161.1, 188.6; LRMS (EI) *m/z* (%), 592 (M^++H, 0.2) 507 (M^+-THP-H, 33) 85 ($[THP+H]^+$, 100).

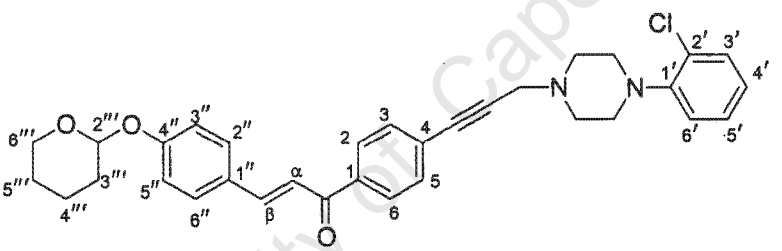
1-(4-{3-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **51c**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51c** as a brown solid (0.063g, 98%); mp 89-90 °C (from ethyl acetate-hexane); R_f = 0.26 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1040

(C-O), 1594 (C=C), 1660 (C=O), 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.71 (3H, m, H-5a‴/4‴), 1.87 (2H, m, H-3a‴/5b‴), 2.03 (1H, m, H-3b‴), 2.82 (4H, t, *J* 4.8, $CH_2N[CH_2CH_2]_2N$), 3.19 (4H, t, *J* 4.8, $CH_2N[CH_2CH_2]_2N$), 3.62 (3H, m, H-6a‴/ $C{\equiv}CCH_2$), 3.87 (1H, m, H-6b‴), 5.48 (1H, t, *J* 3.0, H-2‴), 6.90 (4H, m, H-2′/3′/5′/6′), 7.08 (2H, d, *J* 8.7, H-3″/5″), 7.38 (1H, d, *J* 15.6, H-α), 7.56 (2H, d, *J* 8.7, H-2″/6″), 7.58 (2H, d, *J* 8.7, H-3/5), 7.77 (1H, d, *J* 15.6, H-β), 7.95 (2H, d, *J* 8.7, H-2/6); δ_c(75 MHz; $CDCl_3$) 18.5, 25.1, 30.1, 47.7, 50.1 (2C), 52.1 (2C), 62.0, 85.1, 87.5, 96.2, 115.3 (2C), 116.7 (2C), 117.9 (2C), 119.6, 127.3, 128.2 (2C), 128.3, 130.1 (2C), 131.8 (2C), 137.7, 145.0, 147.8, 155.6, 159.4, 189.7; HRMS (EI) *m/z* Found: M^+, 524.24752 Calc. for $C_{33}H_{33}N_2O_3F$: M,524.24792.

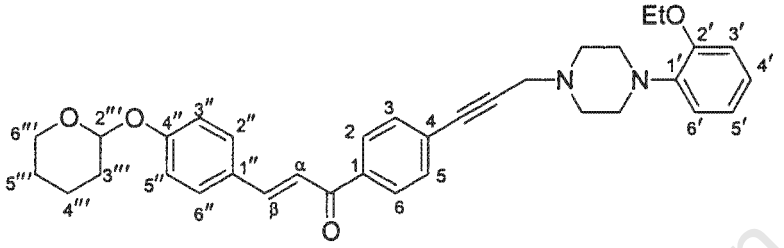
1-(*4-{3-[4-(2-Chloro-phenyl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone* 51d



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51d** as a brown solid (0.063g, 87%); mp (from ethyl acetate-hexane); R_f = 0.22 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1596 (C=C), 1662 (C=O), 2251 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.69 (3H, m, H-5a‴/4‴), 1.88 (2H, m, H-3a‴/5b‴), 2.01 (1H, m, H-3b‴), 2.86 (4H, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.19 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.60 (3H, m, H-6a‴/ $C{\equiv}CCH_2$), 3.87 (1H, m, H-6b‴), 5.48 (1H, t, *J* 3.0, H-2‴), 6.97 (1H, dd, *J* 1.8, 7.8, H-6′), 7.06 (1H, ddd, *J* 1.8, 7.8, 9.3, H-4′), 7.08 (2H, d, *J* 8.7, H-3″/5″), 7.22 (1H, ddd, *J* 1.8, 7.8, 9.3 H-5′), 7.36 (1H, dd, *J* 1.8, 7.8, H-3′), 7.39 (1H, d, *J* 15.6, H-α), 7.56 (2H, d, *J* 8.7, H-2″/6″), 7.58 (2H, d, *J* 8.7, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.95 (2H, d, *J* 8.7, H-2/6); δ_c(75 MHz; $CDCl_3$) 18.6, 25.1, 30.2, 47.8, 51.1 (2C), 52.5 (2C), 62.0, 84.9, 87.9, 96.3, 112.6, 116.8 (2C), 119.7, 120.4, 123.8, 127.4, 127.6 (2C), 128.2, 128.3, 130.1 (2C), 130.7, 131.9

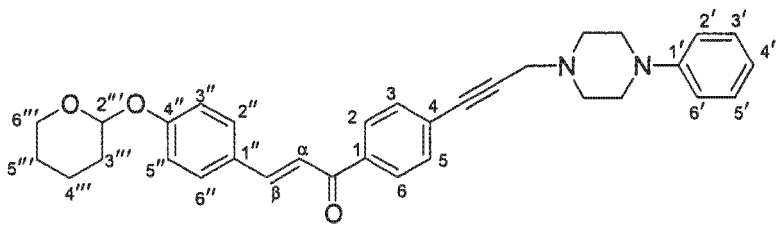
(2C), 137.7, 145.0, 149.1, 159.3, 189.7; HRMS (EI) *m/z* Found: M^+, 540.21860 Calc. For $C_{33}H_{33}ClN_2O_3$: M, 540.21799.

1-(4-{3-[4-(2-Ethoxy-phenyl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **51e**



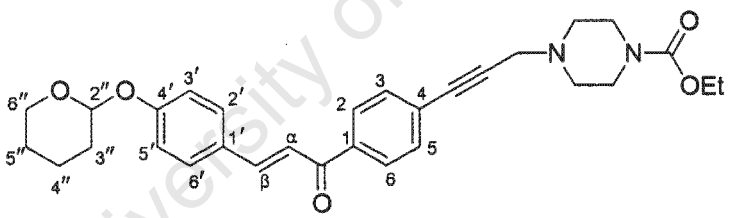
The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51e** as a brown solid (0.070g, 84%); mp 42-44 °C (from ethyl acetate-hexane); R_f = 0.26 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1018 (C-O), 1584 (C=C), 1649 (C=O) , 2249 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.46 (3H, t, *J* 6.9, OCH_2CH_3), 1.69 (3H, m, H-5a‴/4‴), 1.88 (2H, m, H-3a‴/5b‴), 2.01 (1H, m, H-3b‴), 2.89 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.20 (4H, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.62 (3H, m, H-6a‴/ $C{\equiv}CCH_2$), 3.87 (1H, m, H-6b‴), 4.07 (2H, q, *J* 6.9, OCH_2CH_3), 5.48 (1H, t, *J* 3.0, H-2‴), 6.97 (4H, m, H-3′/4′/5′/6′), 7.08 (2H, d, *J* 8.4, H-3″/5″), 7.39 (1H, d, *J* 15.6, H-α), 7.56 (2H, d, *J* 8.4, H-2″/6″), 7.58 (2H, d, *J* 8.4, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.95 (2H, d, *J* 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 14.9, 18.6, 25.1, 30.2, 47.8, 50.3, 52.4 (4C), 62.0, 63.6, 85.0, 87.7, 96.2, 112.6, 116.8 (2C), 118.2, 119.7, 121.0, 122.9, 127.4, 127.6 (2C), 128.2, 128.3, 130.1 (2C), 131.9 (2C), 137.7, 141.1, 145.0, 159.3, 189.7; LRMS (EI) *m/z* (%), 466 ([M^+-THP, 0.5), 261 ($C_{18}H_{13}O_2^+$, 11), 147 ($C_9H_7O_2^+$, 8.7) 85 ([THP+H]$^+$, 100).

1-{4-[3-(4-phenyl-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **51b**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51b** as a brown solid (0.049g, 64%); mp 88-90 °C (from ethyl acetate-hexane); R_f = 0.26 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1017 (C-O), 1588 (C=C), 1651 (C=O), 2231 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.69 (3H, m, H-5a‴/4‴), 1.88 (2H, m, H-3a‴/5b‴), 2.01 (1H, m, H-3b‴), 2.58 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 2.71 (4H, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.58 (3H, m, H-6a‴ / C≡CCH_2), 3.88 (1H, m, H-6b‴), 5.49 (1H, t, *J* 3.2, H-2‴), 7.06 (2H, d, *J* 8.8, H-3″/5″), 7.29 (5H, m, H-2′/3′/4′/5′/6′), 7.38 (1H, d, *J* 15.6, H-α), 7.52 (2H, d, *J* 8.8, H-2″/6″), 7.58 (2H, d, *J* 8.4, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.94 (2H, d, *J* 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 18.7, 25.2, 30.3, 47.8, 52.2 (2C), 52.9 (2C), 62.2, 85.0, 88.0, 96.4, 116.6 (2C), 116.8 (2C), 119.9, 127.2, 127.6, 128.3 (2C), 128.4, 129.4 (2C), 130.3 (2C′), 131.9 (2C), 137.8, 141.1, 145.0, 159.4, 189.8; HRMS (EI) *m/z* Found: M^+, 506.25673 Calc. For $C_{33}H_{33}N_2O_3$: M, 506.25694.

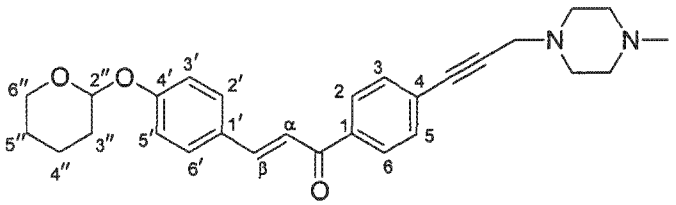
***4-[3E-(4-{3-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-acryloyl}-phenyl)-prop-2-ynyl]-piperazine-1-carboxylic acid ethyl ester* 51h**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51h** as a light brown solid (0.070g, 49%); (from ethyl acetate-hexane); Decomposed above 126 °C; R_f = 0.95 (MeOH/$CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1586 (C=C), 1651 (C=O), 2250 (C≡C); δ_H(400 MHz; $CDCl_3$) 1.26 (3H, t, *J* 7.2, OCH_2CH_3), 1.65 (3H, m, H-5a″/4″), 1.88 (2H, m, H-3a″/5b″), 2.00 (1H, m, H-3″), 2.60 (8H, br s, $CH_2N[CH_2CH_2]_2N$), 3.56 (3H, m, H-6a″/ C≡CCH_2), 3.87 (1H, m, H-6b″), 4.14 (2H, q, *J* 7.2, OCH_2CH_3), 5.48 (1H, t, *J* 2.8, H-2″), 7.01 (2H, d, *J* 8.8, H-3′/5′), 7.38 (1H, d, *J* 15.6, H-α), 7.53 (2H, d, *J* 8.4, H-3/5), 7.58 (2H, d, *J* 8.8, H-2′/6′), 7.78 (1H, d, *J* 15.6, H-β), 7.94 (2H, d, *J* 8.4, H-2/6); δ_c(100 MHz; $CDCl_3$) 14.6 (C- OCH_2CH_3), 18.5, 25.0, 30.1, 47.8, 51.7 (4C), 61.3, 62.0, 85.1, 88.0, 96.2, 116.7 (2C), 119.6, 127.2, 128.3 (2C), 128.9, 130.1 (2C), 131.8 (2C), 137.7, 145.0, 155.0,

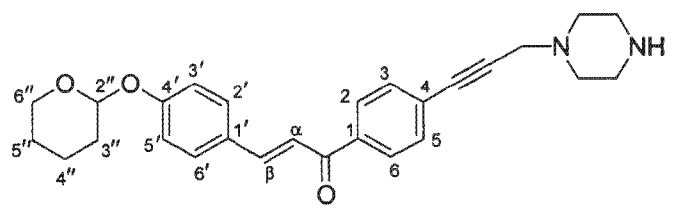
159.2, 189.6; LRMS (EI) *m/z* (%), 502 (M^+, 2.3), 418 (M^+-THP, 4.2), 85 ($[THP+H]^+$, 100).

1-{4-[3-(4-Methyl-piperazin-1-yl)-prop-1-ynyl]-phenyl}-3E-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **51g**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51g** as a light brown solid (0.034g, 51%); mp 106-108 °C (from ethyl acetate-hexane); R_f = 0.20 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1596 (C=C), 1659 (C=O), 2250 (C≡C); δ_H(400 MHz; $CDCl_3$) 1.64 (3H, m, H-5a″/4″), 1.86 (2H, m, H-3a″/5b″), 2.00 (1H, m, H-3b″), 2.64 (3H, s, NC*H*$_3$), 2.96 (8H, m, H-CH_2N[C*H*$_2$C*H*$_2$]$_2$N), 3.60 (3H, m, H-6a″/C≡CC*H*$_2$), 3.86 (1H, m, H-6b″), 5.47 (1H, t, *J* 3.2, H-2″), 7.07 (2H, d, *J* 8.8, H-3′/5′), 7.37 (1H, d, *J* 15.6, H-α), 7.55 (2H, d, *J* 8.0, H-3/5), 7.57 (2H, d, *J* 8.8, H-2′/6′), 7.76 (1H, d, *J* 15.6, H-β), 7.92 (2H, d, *J* 8.0, H-2/6); δ_c(100 MHz; $CDCl_3$) 18.7, 25.2, 30.3, 44.2, 47.4, 49.8 (2C), 54.0 (2C), 62.2, 85.7, 86.5, 96.3, 116.9 (2C′), 119.8, 127.0, 128.4 (2C), 128.5, 130.3 (2C), 132.1 (2C), 138.0, 145.2, 159.4, 189.8; HRMS (EI) *m/z* Found: M^+, 444.24175 Calc. For $C_{28}H_{32}N_2O_3$: M, 444.24129.

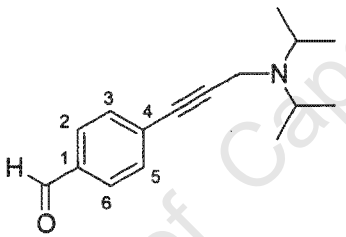
1-[4-(3-piperazin-1-yl-prop-1-ynyl)-phenyl]-3E-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **51f**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **51f** as a brown solid (0.028g, 52%); (from ethyl acetate-hexane); R_f = 0.80 ($MeOH/CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1583

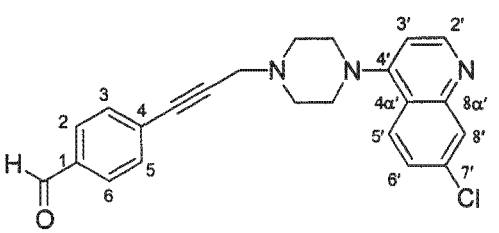
(C=C), 1650 (C=O), 2251 (C≡C), 3380 (N-H); δ_H(400 MHz; $CDCl_3$) 1.68 (3H, m, H-5a″/4″), 1.88 (2H, m, H-3a″/5b″), 2.02 (1H, m, H-3b″), 2.79 (8H, br s, $CH_2N[CH_2CH_2]_2N$), 3.60 (3H, m, H-6a″/C≡CCH_2), 3.86 (1H, m, H-6b″), 5.49 (1H, t, *J* 3.2, H-2″), 7.09 (2H, d, *J* 8.8, H-3′/5′), 7.38 (1H, d, *J* 15.6, H-α), 7.53 (2H, d, *J* 8.4, H-3/5), 7.58 (2H, d, *J* 8.8, H-2′/6′), 7.78 (1H, d, *J* 15.6, H-β), 7.94 (2H, d, *J* 8.4, H-2/6); δ_c(100 MHz; $CDCl_3$) 18.7, 25.2, 30.3, 47.7, 52.1 (2C), 53.8 (2C), 62.2, 85.2, 87.7, 96.3, 116.9 (2C), 121.5, 127.5, 127.9 (2C), 128.4, 130.2 (2C), 132.0 (2C), 137.8, 145.1, 159.4, 189.8; HRMS (EI) *m/z* Found: $[M-H]^+$, 429.21859 Calc. For $C_{27}H_{29}N_2O_3$: M, 429.21782.

4-(3-Diisopropylamino-prop -1-ynyl)-benzaldehyde **59a**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **59a** as brown crystals (0.077g, 90%); mp 142-144 °C (from chloroform-hexane); R_f = 0.34 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1053 (C-O), 1594 (C=C), 1680 (C=O), 2253 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.13 (12H, d, *J* 6.6, $N[CH(CH_3)_2]_2$), 3.23 (2H, sep, *J* 6.6, $N[CH(CH_3)_2]_2$), 3.65 (2H, s, C≡CCH_2), 7.50 (2H, d, *J* 8.1, H-3/5a, 7.77 (2H, d, *J* 8.1, H-2/6), 9.96 (1H, s, C*H*O); δ_c(75 MHz; $CDCl_3$) 20.6 (4C), 34.9, 48.6 (2C), 82.7, 93.9, 129.4, 130.1 (2C), 131.9 (2C), 135.1, 191.3; HRMS (EI) *m/z* Found: M^+, 243.16143 Calc. For $C_{16}H_{21}NO$: M, 243.16231.

4-{3-[4-(7-Chloro-quinoloin-4-yl)-piperazin-1-yl]-pro-1-ynyl}-benzaldehyde **59b**

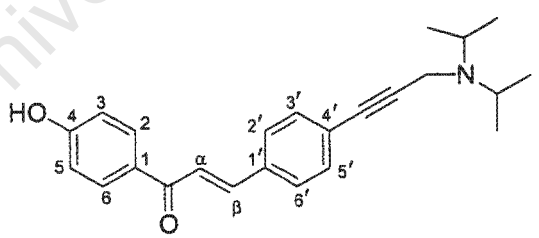


The conditions employed for the preparation of this compound were those described in General Method C, which afforded **59b** as brown crystals (0.117g, 86%); mp 156-158°C (from chloroform-hexane); Rf = 0.49 (10% MeOH/DCM); IRν_{max}(Nujol)/cm^{-1} 1045 (C-O), 1596 (C=C), 1680 (C=O), 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 2,94 (4H, br s, $CH_2N[CH_2CH_2]_2N$), 3.31 (4H, br s, $CH_2N[CH_2CH_2]_2N$), 3.67 (2H, s, $C{\equiv}CCH_2$), 6.87 (1H, d, *J* 5.2, H-3′), 7.41 (1H, d, *J* 8.8, H-5′), 7.61 (2H, d, *J* 8.0, H-3/5), 7.84 (2H, d, *J* 8.0, H-2/6), 7.96 (1H, dd, *J* 2.0, 8.8, H-6′), 8.04 (1H, d, *J* 2.0, H-8′), 8.74 (1H, d, *J* 5.2, H-2′), 10.00 (1H, s, CHO); δ_c(75 MHz; $CDCl_3$) 47.8, 52.1 (4C), 84.4, 88.5, 109.1, 122.0, 125.1, 126.0, 128.8, 129.0, 129.5 (2C), 132.3 (2C) 134.9, 135.6, 150.2, 151.9, 157.3, 191.3; HRMS (EI) *m/z* Found: M^+, 389.12943 Calc. For $C_{23}H_{20}ClN_3O$: M, 389.12949.

General Method D for the preparation of compounds 39a-b, 40a-e, 166 and 168

To a stirred solution of Mannich base (0.074 mmol) in ethanol (2.0 ml) was added 4M hydrochloric acid (1.0 ml) and the resulting mixture was stirred for 15 min and then diluted with water (10 ml). The reaction mixture was extracted with ethyl acetate (3x20 ml), washed with brine (20 ml) and concentrated to give a residue which was purified by column chromatography on silica gel ($MeOH/CHCl_3$/17% NH_4OH 2: 2: 1) to afford the title compounds **39a-b, 40a-e, 166** and **168**.

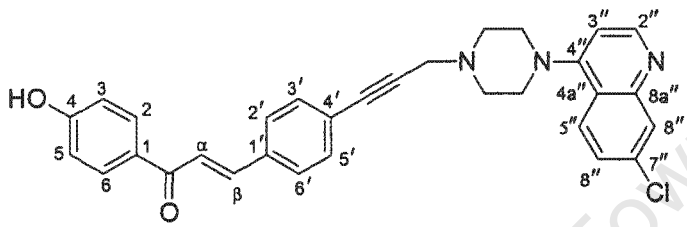
***3E-[4-(3-Diisopropylamino-prop-1-ynyl)-phenyl]-1-(4-hydroxy-phenyl)-propenone* 39a**



The conditions employed for the preparation of this compound were those described in General Method D, which gave **39a** as yellow crystals (0.030g, 78%); mp = 133-135 °C (from chloroform-hexane); R_f = 0.79 ($MeOH/CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1053 (C-O), 1594 (C=C), 1680 (C=O), 2253 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.13 (12H, d, *J* 6.6, $N[CH(CH_3)_2]_2$), 3.23 (2H, sep, *J* 6.6, $N[CH(CH_3)_2]_2$), 3.65 (2H, s, $C{\equiv}CCH_2$), 6.93 (2H, d, *J* 8.7, H-3/5), 7.40 (2H, d, *J* 8.4, H-2′/6′), 7.50 (1H, d, *J* 15.9, H-α), 7.51 (2H, d, *J* 8.4, H-3′/5′), 7.74 (1H, d, *J* 15.9, H-β), 7.97 (2H, d, *J* 8.7, H-

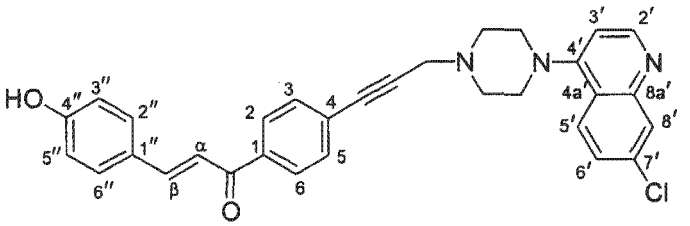
2/6), 9.96 (1H, s, C*H*O); δ_c(75 MHz; $CDCl_3$) 20.6 (4C), 34.9 (2C), 48.6, 82.7, 93.9, 115.7 (2C), 122.3, 123.2, 128.1 (2C), 129.4, 130.1 (2C), 131.9 (2C), 135.1, 143.0, 161.2, 191.3; Anal. Found C 75.00, H 7.33, N 2.37% Calc. For $C_{24}H_{27}NO_2.1H_2O$: C 75.89, H 7.64, N 3.69%; HRMS (EI) *m/z* Found: M^+, 361.20512 Calc. For $C_{24}H_{27}NO_2$: M, 361.20418.

3E-(4-{3-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-1-(4-hydroxy-phenyl)-propenone **39b**



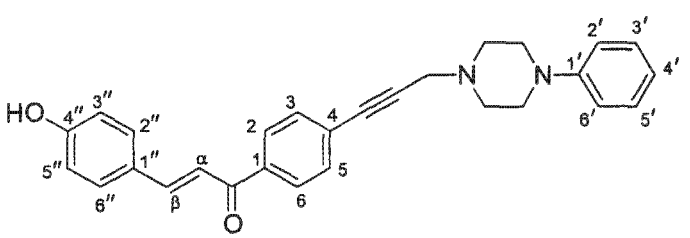
The conditions employed for the preparation of this compound were those described in General Method D, which gave **39b** as orange crystals (0.023g, 68%); mp = 130-132 °C (from chloroform-hexane); R_f = 0.62 (MeOH/$CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1600 (C=C), 1655 (C=O), 2245 (C≡C); δ_H(400 MHz; $CDCl_3$) 2.95 (4H, t, *J* 4.4, $CH_2N[CH_2CH_2]_2N$), 3.34 (4H, t, *J* 4.4, $CH_2N[CH_2CH_2]_2N$), 3.68 (2H, s, $C{\equiv}CCH_2$), 6.87 (1H, d, *J* 4.8, H-3″), 6.96 (2H, d, *J* 8.4, H-3/5), 7.43 (1H, dd, *J* 1.2, 9.0, H-6″), 7.48 (2H, d, *J* 8.4, H-3′/5′), 7.52 (1H, d, *J* 15.6, H-α), 7.57 (2H, d, *J* 8.4, H-2′/6′), 7.74 (1H, d, *J* 15.6, H-β), 7.96 (2H, d, *J* 8.4, H-2/6), 7.97 (1H, d, *J* 9.0, H-5″), 8.05 (1H, d, *J* 1.2, H-8″), 8.70 (1H, d, *J* 4.8, H-2″); δ_c(100 MHz; $CDCl_3$) 48.0, 52.2 (2C), 52.3 (2C), 80.3 (*C*, 86.5, 109.3, 116.4 (2C), 122.1, 122.9, 123.4, 124.9 (2C), 125.5, 126.6, 128.4, 130.4, 131.1 (2C), 132.4 (2C), 135.3, 135.5, 142.8, 149.9, 151.8, 157.4, 162.1, 188.7; Anal. Found C 70.82, H 4.85, N 7.41% Calc. For $C_{31}H_{26}ClN_3O_2$: C 70.72, H 5.51, N 7.90%; HRMS (EI) *m/z* Found: M^+, 507.17127 Calc. For $C_{31}H_{26}ClN_3O_2$: M, 507.17135.

1-(4-{3-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-hydroxy-phenyl)-propenone **40a**



The conditions employed for the preparation of this compound were those described in General Method D, which gave **40a** as orange crystals (0.013g, 79%); mp 207-209 °C (from chloroform-hexane); R_f = 0.56 ($MeOH/CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1600 (C=C), 1655 (C=O), 2255 (C≡C); δ_H(300 MHz; $CDCl_3$) 2.95 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.34 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.70 (2H, s, $C{\equiv}CCH_2$), 6.88 (1H, d, *J* 5.1, H-3′), 6.91 (2H, d, *J* 8.7, H-3″/5″), 7.35 (1H, d, *J* 15.6, H-α), 7.43 (1H, dd, *J* 2.0, 9.0, H-6′), 7.53 (2H, d, *J* 8.7, H-2″/6″), 7.57 (2H, d, *J* 8.7, H-3/5), 7.77 (1H, d, *J* 15.6, H-β), 7.96 (2H, d, *J* 8.7, H-2/6), 7.97 (1H, d, *J* 9.0, H-5′), 8.06 (1H, d, *J* 2.0, H-8′), 8.71 (1H, d, *J* 5.1, H-2′); δ_c(75 MHz; $CDCl_3$) 47.8, 51.9 (2C), 52.0 (2C), 85.3, 87.2, 109.0, 116.4 (2C), 118.9, 121.8, 125.3, 125.3, 126.4, 126.9 (2C), 127.2, 128.4, 130.6 (2C), 131.9 (2C), 135.3, 138.0, 145.5, 149.9, 151.5, 157.2, 159.5, 189.8; Anal. Found C 70.82, H 4.85, N 7.91% Calc. For $C_{31}H_{26}ClN_3O_2.1.5H_2O$: C 73.29, H 5.16, N 8.27%; HRMS (EI) *m/z* Found: M^+, 507.17128 Calc. For $C_{31}H_{26}ClN_3O_2$: M, 507.17135.

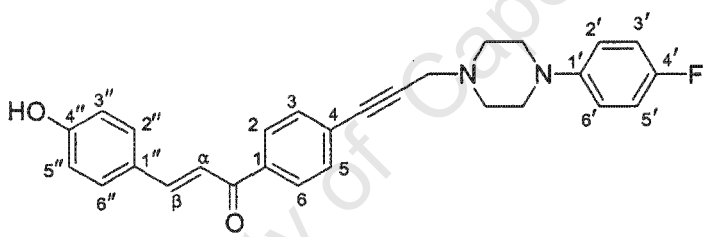
3E-(4-hydroxy-phenyl)-1-{4-[3-(4-phenyl-piperazin-1-yl)-prop-1-ynyl]-phenyl}-propenone **40b**



The conditions employed for the preparation of this compound were those described in General Method D, which gave **40b** as orange crystals (0.023g, 87%); (recrystallized from chloroform-hexane); Decomposed above 170 °C; R_f = 0.71

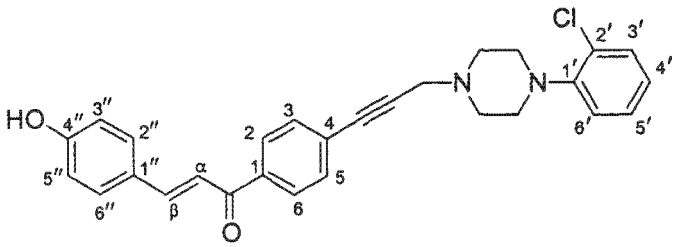
(MeOH/$CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1595 (C=C), 1660 (C=O), 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 2.86 (4H, t, *J* 5.1, $CH_2N[CH_2CH_2]_2N$), 3.30 (4H, t, *J* 5.1, $CH_2N[CH_2CH_2]_2N$), 3.63 (2H, s, $C{\equiv}CCH_2$), 6.87 (2H, d, *J* 8.4, H-3″/5″), 6.92 (3H, m, H-2′/4′/6′), 7.27 (2H, m, H-3′/5′), 7.35 (1H, d, *J* 15.6, H-α), 7.52 (2H, d, *J* 8.4, H-2″/6″), 7.53 (2H, d, *J* 8.4, H-3/5), 7.76 (1H, d, *J* 15.6, H-β), 7.93 (2H, d, *J* 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 47.9, 50.8 (2C), 52.2 (2C), 86.1, 86.6, 116.4 (2C), 116.6 (2C), 117.4 (2C), 119.2, 127.2, 128.5, 130.8 (2C), 132.1 (2C), 132.9 (2C), 136.3, 138.1, 145.8, 151.1, 159.4, 190.1; Anal. Found C 65.89, H 5.03, N 5.24% Calc. For $C_{28}H_{26}N_2O_2.5H_2O$: C 65.55, H 7.02, N 5.46%; HRMS (EI) *m/z* Found: M^+, 422.19927 Calc. For $C_{28}H_{26}N_2O_2$: M, 422.19942.

1-(4-{3-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-hydroxy-phenyl)l]-propenone **40c**



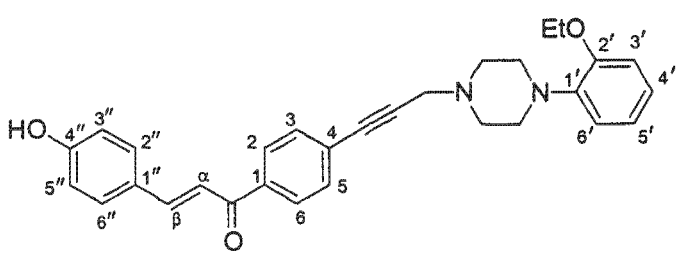
The conditions employed for the preparation of this compound were those described in General Method D, which gave **40c** as orange crystals (0.019g, 87%); mp 115-117 °C (from chloroform-hexane);R_f = 0.71 (MeOH/$CHCl_3$/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1594 (C=C) , 1660 (C=O) , 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 2.85 (4H, t, *J* 5.1, $CH_2N[CH_2CH_2]_2N$), 3.21 (4H, t, *J* 5.1, $CH_2N[CH_2CH_2]_2N$), 3.63 (2H, s, $C{\equiv}CCH_2$), 6.92 (6H, m, H-3″/5″, 2′/3′/5′/6′), 7.38 (1H, d, *J* 15.6, H-α), 7.53 (2H, d, *J* 8.7, H-2″/6″), 7.56 (2H, d, *J* 8.4, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.94 (2H, d, J 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 47.8, 50.1 (2C), 52.1 (2C), 85.1, 87.5, 115.4 (2C), 115.7 (2C), 116.0 (2C), 119.4, 127.6, 128.3, 130.5 (2C), 131.5 (2C), 131.8 (2C), 136.1, 137.8, 145.0, 149.9, 159.2, 189.7; Anal. Found C 64.74, H 4.71, N 5.19% Calc. For $C_{28}H_{25}FN_2O_2.4H_2O$: C 64.79, H 6.36, N 5..39%; HRMS (EI) *m/z* Found: M^+, 440.19127 Calc. For $C_{28}H_{25}FN_2O_2$: M, 440.19001.

1-(4-{3-[4-(2-chloro-phenyl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-hydroxy-phenyl)l]-propenone **40d**



The conditions employed for the preparation of this compound were those described in General Method D which gave **40d** as yellow crystals (0.013g, 68%); mp 81-83 °C (chloroform-hexane); R_f = 0.64 (MeOH/$CHCl_3$/17%NH_4OH 2: 2: 1); IRν_{max}(Nujol)/cm^{-1} 1600 (C=C), 1655 (C=O) , 2245 (C≡C); δ_H(300 MHz; $CDCl_3$) 2.89 (4H, t, *J* 4.2, $CH_2N[CH_2CH_2]_2N$), 3.17 (4H, t, *J* 4.2, $CH_2N[CH_2CH_2]_2N$), 3.62 (2H, s, $C{\equiv}CCH_2$), 6.88 (2H, d, *J* 8.4, H-3″/5″), 6.98 (1H, dd, *J* 1.8, 7.8, H-6′), 7.06 (1H, ddd, *J* 1.8, 7.8, 9.3, H-4′), 7.22 (1H, ddd, *J* 1.8, 7.8, 9.3, H-5′), 7.35 (1H, d, *J* 15.6, H-α), 7.36 (2H, dd, *J* 1.8 7.8, H-3′), 7.51 (2H, d, *J* 8.4, H-2″/6″), 7.53 (2H, d, *J* 8.4, H-3/5), 7.76 (1H, d, *J* 15.6, H-β), 7.93 (2H, d, *J* 8.4, H-2/6); δ_c(75 MHz; $CDCl_3$) 48.1, 51.2 (2C), 52.8 (2C), 84.9, 87.9, 116.0, 116.5 (2C), 119.2, 120.7, 123.7, 127.4, 127.6, 127.8 (2C), 128.6, 129.9 (2C), 130.9, 132.1 (2C), 138.0, 145.7, 149.2, 159.5, 190.7; Anal. Found C 69.91, H 5.32, N 5.90% Calc. For $C_{28}H_{25}ClN_2O_2$. $1.5H_2O$: C 73.59, H 5.51, N 6.13%; HRMS (EI) *m/z* Found: M^+, 456.16273 Calc. For $C_{28}H_{28}ClN_2O_2$: M, 456.16045.

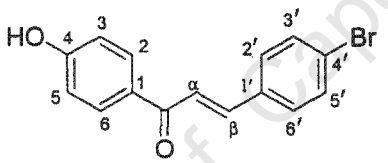
1-(4-{3-[4-(2-Ethoxy-phenyl)-piperazin-1-yl]-prop-1-ynyl}-phenyl)-3E-(4-hydroxy-phenyl)l]-propenone **40e**



The conditions employed for the preparation of this compound were those described in General Method D which gave **40e** as pale yellow crystals (0..035g, 68%); mp 159-161°C (chloroform-hexane); R_f = 0.64 (MeOH/$CHCl_3$/17%NH_4OH 2:2:1);

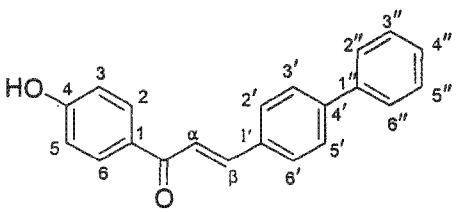
IRν_{max}(Nujol)/cm^{-1} 1603 (C=C), 1651 (C=O), 2250 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.45 (3H, t, *J* 6.9, OCH_2CH_3), 2.91 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.22 (4H, t, *J* 4.5, $CH_2N[CH_2CH_2]_2N$), 3.63 (2H, s, $C{\equiv}CCH_2$), 4.08 (2H, q, *J* 6.9, OCH_2CH_3), 6.85 (2H, d, *J* 8.4, H-3″/5″), 6.95 (4H, m, H- 3′/4′/5′/6′), 7.34 (1H, d, *J* 15.6, H-α), , 7.50 (2H, d, *J* 8.4, H-2″/6″), 7.53 (2H, d, *J* 9.0, H-3/5), 7.75 (1H, d, *J* 15.6, H-β), 7.93 (2H, d, *J* 9.0, H-2/6); δ_c(75 MHz; $CDCl_3$) 14.3, 47.6, 51.7 (2C), 56.2 (2C), 63.6, 85.8, 88.1, 114.3, 116.3 (2C), 119.0, 121.2, 123.4, 126.0, 128.1, 128.8 (2C), 129.6, 129.9 (2C), 131.2, 132.2 (2C), 138.1, 146.0, 152.2, 159.7, 189.5 (C=O); Anal. Found C 73.06, H 6.00, N 5.65% Calc. For $C_{30}H_{30}N_2O_3$. $1.5H_2O$: C 82.51, H 5.30, N 5.66%; HRMS (EI) *m/z* Found: M^+, 466.22657 Calc. For $C_{30}H_{30}N_2O_3$: M, 466.22564.

***3E-(4-Bromo-phenyl)-1-(4-hydroxy-phenyl)-propenon* 166**



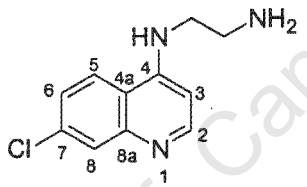
The conditions employed for the preparation of this compound were those described in General Method D, however, 2.58 mmol were used, which gave **166** as a pale yellow solid (0.76g, 97%); mp 129-131 °C (Methanol); R_f = 0.15 (30% EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1580 (C=C), 1650 (C=O), ; δ_H(300 MHz; $CDCl_3$) 6.98 (2H, d, *J* 8.7, H-3/5), 7.63 (2H, d, *J* 8.7, H-2′/6′), 7.69 (1H, d, *J* 15.6, H-α), 7.77 (2H, d, *J* 8.7, H-3′/5′), 7.89 (1H, d, *J* 15.6, H-β), 8.09 (2H, d, *J* 8.7, H-2/6); δ_c(75 MHz; $CDCl_3$) 116.3 (2C), 122.5, 123.3, 128.2 (2C), 129.4, 130.7 (2C), 131.6 (2C), 134.5, 142.3, 163.0, 188.3; HRMS (EI) *m/z* Found: M^+, 301.99419 Calc. For $C_{15}H_{11}BrO_2$: M, 301.99424.

***3E-Biphenyl-4-yl-1-(4-hydroxy-phenyl)-propenone* 168**



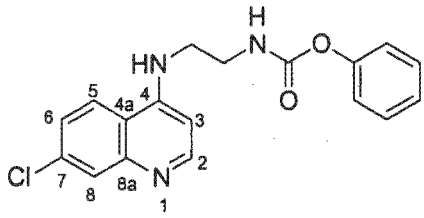
The conditions employed for the preparation of this compound were those described in General Method D, which gave **168** as a pale yellow solid (0.028g, 93%); mp 117-119 °C (Methanol); R_f= 0.6 (30% EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1585 (C=C), 1660 (C=O), ; δ_H(400 MHz; $CDCl_3$) 6.94 (2H, d, *J* 9.2, H-3/5), 7.38 (1H, t, *J* 8.0, H-4″), 7.46 (2H, t, *J* 8.0, H-3″/5″), 7.54 (1H, d, *J* 15.6, H-α), 7.62 (2H, d, *J* 8.4, H-2′/6′), 7.65 (2H, d, *J* 8.4, H-3′/5′), 7.71 (2H, d, *J* 8.0, H-2″/6″), 7.84 (1H, d, *J* 15.6, H-β), 8.01 (2H, d, *J* 9.2, H-2/6); δ_c(100 MHz; $CDCl_3$) 116.(2C), 122.9, 127.4 (2C), 127.7 (2C), 128.6, 129.7 (2C), 129.9 (2C), 130.4, 131.4 (2C), 134.8, 140.0, 142.5, 142.9, 162.9, 187.8; HRMS (EI) *m/z* Found: M^+, 300.11500 Calc. For $C_{21}H_{16}O_2$: M, 300.11503.

N'-(7-Chloro-quinolin-4-yl)-ethane-1,2-diamine 79



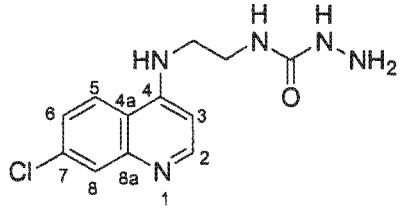
A mixture of 1,3-diaminoethane (5.00g, 25.0 mmol), 4,7-dichloroquinoline (2.25g, 5.0 mmol), K_2CO_3 (0.52g, 3.78 mmol), triethylamine (0.50 ml, 3.78 mmol) and 1-Methyl pyrrolidinone (9.0 ml) under nitrogen was heated at 135 0° C for 4 hours. The reaction mixture was cooled to room temperature and then aqueous sodium hydroxide (1M, 50 ml) was added. The resultant mixture was extracted with hot ethylacetate (3 x 100 mm). The combined extracts were washed with brine, dried ($MgSO_4$) and concentrated to give a cream white solid (2.06g, 82%) which recrystallised from methanol to give **79** as a white crystalline powder, mp 137-139 °C (from methanol), (Lit. mp 137-139 °C);[267] R_f= 0.35 (20%MeOH/DCM); δ_H(300 MHz; $CDCl_3$) 3.46 (2H, t, *J* 5.6, $NCH_2CH_2NH_2$), 3.71 (2H, t, *J* 5.6, $NCH_2CH_2NH_2$), 6.40 (1H, d, *J* 5.2, H-3), 7.36 (1H, dd, *J* 2.4, 8.8, H-6), 7.77 (1H, d, *J* 2.4, H-8), 7.97 (1H, d, *J* 8.8, H-5), 8.34 (1H, d, *J* 5.2, H-2).

1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester **80**



Phenyl chloroformate (1.41g, 9.02 mmol) was added to a stirred and cooled (0 °C), solution of *N'*-(7-chloro-quinolin-4-yl)-ethane-1,2-diamine (2.00g, 9.02 mmol) and triethylamine (1.26 ml, 9.02 mmol) in DMF (10 ml). The mixture was stirred at room temperature for 45 min, diluted with water (50 ml) and extracted with chloroform (3 x50 ml). The combined organic layers were washed with water (3 x50 ml), brine (50 ml), dried ($MgSO_4$), and concentrated to give a yellow residue. Column chromatography on silica (MeOH:DCM 1:19) afforded **80** as a white solid (2.22g, 72%) which was recrystallised from chloroform-hexane. mp 125-127 °C; R_f= 0.43 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1060 (C-O), 1580 (C=N), 1720 (C=O), 3225 (N-H); ^{1}H NMR δ_H(300 MHz; $CDCl_3$) 3.47 (2H, q, *J* 6.0, NCH_2CH_2NHCO), 3.70 (2H, q, *J* 6.0, NCH_2CH_2NHCO), 6.02 (1H, brs, $HNCH_2CH_2NHCO$), 6.21 (1H, br s, $HNCH_2CH_2NHCO$), 6.40 (1H, d, *J* 5.1, H-3), 7.22 (5H, m, Ph), 7.26 (1H, dd, *J* 2.1, 9.0, H-6), 7.66 (1H, d, *J* 9.0, H-5), 7.93 (1H, d, *J* 2.1, H-8), 8.34 (1H, d, *J* 5.1, H-2); δ_c(75 MHz; $CDCl_3$) 40.0, 45.1, 98.5, 117.2, 121.5, 121.6 (2C), 125.5, 125.7, 128.3 (2C), 126.6, 135.2, 148.8, 150.0, 150.8, 151.7, 156.9; Anal. Found C 62.64, H 4.74, N 12.15%. Calc. For $C_{18}H_{16}ClN_3O_2$: C 63.25, H 4.72, N 12.29%; HRMS (EI) *m/z* Found: $[M+H]^+$, 342.100089 Calc. For $C_{18}H_{16}ClN_3O_2$: M, 342.10093.

***Compound* 81**



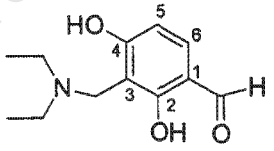
To a solution of [2-(7-chloro-quinolin-4-ylamino)-ethyl]-carbamic acid phenyl ester (1.26g, 3.70 mmol) in dry methanol (10 ml) was added hydrazine monohydrate (1.85, 37 mmol) and the resulting mixture was stirred at 90 °C for 12 h. The reaction mixture was

concentrated to give a white residue which was subjected to column chromatography on silica gel (10%MeOH/DCM followed by MeOH/DCM/17%NH_4OH 2:2:1) to give **81** as white crystals (0.87g, 84%); mp 177-179 °C (from methanol); R_f = 0.12 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1080 (C-O), 1140 (C-N), 1550 (C=N), 1600 (C=C), 1660 (C=O), 3150 (N-H), 3325 (N-H); δ_H(400 MHz; CD_3OD) 3.41 (2H, t, *J* 6.0, NCH_2CH_2NHCO), 3.52 (2H, t, *J* 6.0, NCH_2CH_2NHCO), 6.52 (1H, d, *J* 5.4, H-3), 7.35 (1H, dd, *J* 2.4, 8.8, H-6), 7.74 (1H, d, *J* 2.4, H-8), 7.99 (1H, d, *J* 8.8, H-5), 8.32 (1H, d, *J* 5.4, H-2); δ_c(100 MHz; CD_3OD) 38.1, 44.3, 98.3, 117.2, 123.0, 124.9, 126.3, 135.2, 148.3, 151.2, 151.6, 162.8; HRMS (EI) *m/z* Found: $[M+H]^+$, 280.09658 Calc. For $C_{12}H_{15}ClN_5O$: M, 280.09651.

General Method E for the preparation of compounds 76a-f

Amine (10.86 mmol) was treated with paraformaldehyde (10.86 mmol) in methanol (7.5 ml) at 65 °C for 1 h. To the reaction mixture, a solution of 2,4-dihydroxybenzaldehyde (10.86 mmol) in methanol (5.0 ml) was then added. After stirring for an additional 1 hour, the reaction mixture was cooled, concentrated and then subjected to column chromatography on silica gel.

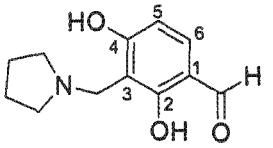
***3-Dimethylaminomethyl-2, 4-dihydroxy-benzaldehyde* 76a**



The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (ethylacetate) afforded **76a** as a dark brown solid (1.75g, 72%); mp 74-76 °C (from chloroform-hexane); R_f= 0.40 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1050 (C-O), 1150 (C-N), 1640 (C=O), 3450 (OH); δ_H(400 MHz; $CDCl_3$) 1.16 (6H, t, *J* 7.2, H- $N[CH_2CH_3]_2$), 2.71 (4H, q, *J* 7.2, $N[CH_2CH_3]_2$), 3.92 (2H, s, $PhCH_2N$-), 6.40 (1H, d, *J* 8.4, H-5), 7.30 (1H, d, *J* 8.4, H-6), 9.58 (1H, s, CHO); δ_c(100 MHz; $CDCl_3$) 10.8 (2C), 46.6, 49.3 (2C), 106.7, 110.1, 113.2, 134.5, 161.5, 169.2, 190.7; Anal. Found C 64.29, H 7.79, N 6.18% Calc. For $C_{12}H_{17}NO_3$: C 64.55, H

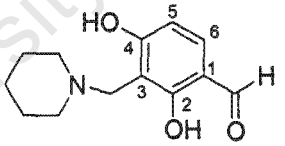
7.67, N 6.27%; HRMS (EI) *m/z* Found: $[M+H]^+$, 224.12861 Calc. For $C_{12}H_{18}NO_3$: M, 224.12866.

***2,4-dihydroxy-3-pyrrolidin-1-ylmethyl-benzaldehyde* 76b**



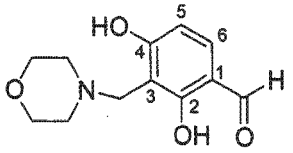
The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (ethylacetate) followed by 10%MeOH/DCM) afforded **76b** as a brown oil (1.91g, 79%); R_f = 0.28 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1060 (C-O), 1210 (C-N), 1670 (C=O) 3450 (OH); δ_H(400 MHz; $CDCl_3$) 1.91 (4H, m, N[CH_2CH_2]$_2$), 2.71 (4H, m, N[CH_2CH_2]$_2$), 3.99 (2H, s, PhCH_2N), 6.40 (2H, d, *J* 8.4, H-5), 7.31 (1H, d, *J* 8.4, H-6), 9.60 (1H, s, *C*HO); δ_c(100 MHz; $CDCl_3$) 23.6 (2C), 50.9, 53.4 (2C), 107.4, 109.9, 113.4, 134.6, 161.2, 168.6, 193.8; HRMS (EI) *m/z* Found: $[M+H]^+$, 222.11270 Calc. For $C_{12}H_{15}NO_3$: M, 222.11301.

***2,4-dihydroxy-3-piperidin-1-ylmethyl-benzaldehyde* 76c**



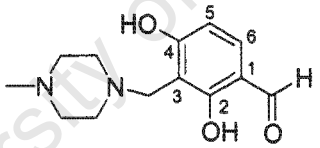
The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (ethylacetate) afforded **76c** as a light brown solid (2.26g, 88%); mp 107-109 °C (from chloroform-hexane); R_f = 0.40 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1050 (C-O), 1220 (C-N), 1660 (C=O), 3400 (OH); δ_H(400 MHz; $CDCl_3$) 1.67 (10H, m, HN[CH_2CH_2]$_2$CH_2), 3.82 (2H, s, PhCH_2N), 6.40 (2H, d, *J* 8.4, H-5), 7.29 (1H, d, *J* 8.4, H-6), 9.59 (1H, s, *C*HO); δ_c(100 MHz; $CDCl_3$) 23.6, 25.5 (2C), 53.7, 60.0 (2C), 106.5, 109.9, 113.4, 134.6, 161.6, 168.5, 193.8; Anal. Found C 66.09, H 7.36, N 5.82% Calc. For $C_{13}H_{17}NO_3$: C 66.36, H 7.28, N 5.96%; HRMS (EI) *m/z* Found: $[M+H]^+$, 236.12860 Calc. For $C_{13}H_{18}NO_3$: M, 236.12866.

2,4-dihydroxy-3-morpholin -1-ylmethyl-benzaldehyde **76d**



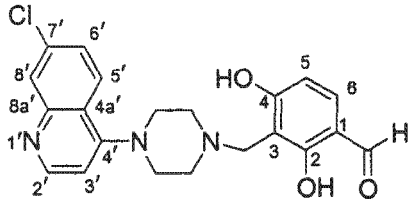
The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (10%MeOH/DCM) afforded **76d** as a cream white solid (2.48g, 96%); mp 150-152 °C (from chloroform-hexane); R_f = 0.62 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1050 (C-O), 1250 (C-N), 1660 (C=O) 3440 (OH); δ_H(300 MHz; $CDCl_3$) 2.63 (4H, m, N[CH_2CH_2]$_2$O), 3.71 (4H, m, N[CH_2CH_2]$_2$O), 3.84 (2H, s, PhCH_2N-), 6.43 (1H, d, *J* 8.4, H-5), 7.32 (1H, d, *J* 8.4, H-6), 9.62 (1H, s, *C*HO); δ_c(75 MHz; $CDCl_3$) 52.8 (2C), 53.5, 66.6 (2C), 106.5, 109.6, 114.0, 134.8, 161.8, 166.8, 194.2; Anal. Found C 64.29, H 7.79, N 6.18% Calc. For $C_{12}H_{15}NO_4$: C 64.55, H 7.67, N 6.27%; HRMS (EI) *m/z* Found: $[M+H]^+$, 238.10793 Calc. For $C_{12}H_{16}NO_4$: M, 238.10799.

2,4-dihydroxy-3-(4-methyl-piperazin -1-ylmethyl)-benzaldehyde **76e**



The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (ethylacetate) afforded **76e** as a pale brown solid (2.35g, 86%); mp 106-108 °C (from chloroform-hexane); R_f = 0.42 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1060 (C-O), 1130 (C-N), 1665 (C=O), 3400 (OH); δ_H(300 MHz; $CDCl_3$) 1.67 (3H, m, HN[CH_2CH_2]$_2$NCH_3), 2.60 (8H, m, HN[CH_2CH_2]$_2$NCH$_3$), 3.82 (2H, s, PhCH_2N-), 6.40 (2H, d, *J* 8.7, H-5), 7.29 (1H, d, *J* 8.7, H-6), 9.60 (1H, s, C*H*O); δ_c(75 MHz; $CDCl_3$) 45.8, 52.4 (2C), 53.1, 54.7 (2C), 106.7, 109.7, 113.8, 134.7, 161.7, 167.3, 194.1; Anal. Found C 61.41, H 7.45, N 10.82% Calc. For $C_{13}H_{18}N_2O_3$: C 62.38, H 7.25, N 11.19%; HRMS (EI) *m/z* Found: $[M+H]^+$, 251.13958 Calc. For $C_{13}H_{19}N_2O_3$: M, 251.13956.

3-[4-(7-Chloro-quinolin-4-yl) piperazin-1-ylmethyl]-2,4-dihydroxy benzaldehyde **76f**

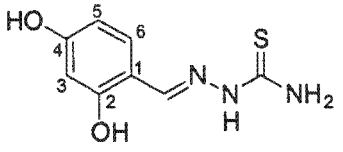


The conditions employed for the preparation of this compound were those described in General Method E. Column chromatography (ethylacetate) afforded **76f** as a cream white solid (4.06g, 94%); mp 167-169 °C (from chloroform-hexane); R_f = 0.62 (10%MeOH/DCM); IR ν_{max}(Nujol)/cm^{-1}; 1040 (C-O), 1540 (C=N), 1600 (C=C), 1660 (C=O), 3350 (OH); δ_H(400 MHz; $CDCl_3$) 2.94 (4H, br s, N[CH_2CH_2]$_2$NCH), 3.30 (4H, br s, N[CH_2CH_2]$_2$NCH), 3.99 (2H, s, PhCH_2N), 6.47 (1H, d, *J* 8.7, H-5), 6.85 (1H, d, *J* 5.1, H-3′), 7.36 (1H, d, *J* 8.7, H-6), 7.43 (1H, dd, *J* 2.1, 9.3, H-6′), 7.91 (1H, d, *J* 9.3, H-5′), 8.15 (1H, d, *J* 2.1, H-8′), 8.74 (1H, d, *J* 5.1, H-2′), 9.65 (1H, s, CHO); δ_c(100 MHz; $CDCl_3$) 51.8 (2C), 52.4 (2C), 53.0, 106.2, 109.2, 109.6, 114.0, 122.2, 124.7, 126.5 , 129.0, 134.9, 150.5, 151.9, 156.2, 160.5, 166.6, 194.2; Anal. Found C 63.30, H 5.25, N 10.31% Calc. For $C_{21}H_{20}ClN_3O_3$: C 63.40, H 5.07, N 10.56%; HRMS (EI) *m/z* Found: $[M+H]^+$, 398.12734 Calc. For $C_{21}H_{21}ClN_3O_3$: M, 398.12714.

General Method F for the preparation of compounds 77a-g and 78

A mixture of aldehyde (1.65 mmol) and thiosemicarbazide/semicarbazide (1.65 mmol) was dissolved in dry methanol (10 ml) under nitrogen. The resultant heated at reflux for 3 h until the reaction was completed (monitored by TLC). The solvent was removed under reduced pressure, and resulting solid was recrystallised from methanol to provide title compounds **77a-g** and **78**.

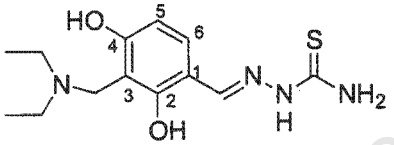
***Compound* 78**



The conditions employed for the preparation of this compound were those described in General Method F, which gave **78** as a cream white solid (2.90g, 89%); mp 237-239 °C

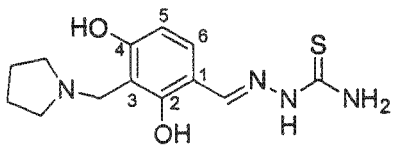
(from methanol); R_f = 0.50 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1}; 1150 (C=S), 1260 (C-N), 1540 (C=S), 1580 (C=C), 3150 (N-H), 3325 (N-H) 3450 (O-H/N-H); δ_H(300 MHz; DMSO-d_6) δ 6.26 (1H, dd, *J* 2.4, 8.4, H-5), 6.30 (1H, d, *J* 2.4, H-3), 7.65 (1H, d, *J* 8.4, H-6), 7.72 (1H, br s, NHCSNH_2), 7.92 (1H, s, NHCSNH_2), 8.24 (1H, s, PhC*H*=NNH), 9.72 (2H, s, O*H*), 11.14 (1H, s, PhCH=NN*H*); δ_c(75 MHz; DMSO-d_6) 102.3, 107.7, 111.7, 128.4, 140.0, 158.0, 160.4, 177.1; Anal. Found C 45.51, H 4.34, N 19.97, S 15.59% Calc. For $C_8H_9N_3O_2S$: C 45.49, H 4.29, N 19.89, S 15.18%; HRMS (EI) *m/z* Found: $[M+H]^+$, 212.04980 Calc. For $C_8H_{10}N_3O_2S$: M,212.14937.

***Compound* 77a**



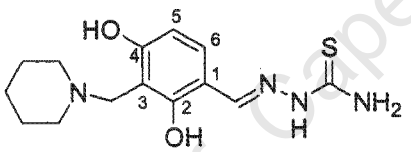
The conditions employed for the preparation of this compound were those described in General Method F, which gave **77a** as a yellow solid (0.46g, 87%) (recrystallised from methanol); decomposse above 180 °C; R_f = 0.60 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1140 (C=S), 1580 (C=C), 3130 (N-H), 3245 (N-H) 3400 (O-H/N-H); δ_H(300 MHz; $CDCl_3$) 1.05 (6H, t, *J* 7.2, N[CH_2CH_3]$_2$), 2.58 (4H, t, *J* 7.2, N[CH_2CH_3]$_2$), 3.80 (2H, s, PhCH_2N), 6.29 (1H, d, *J* 8.4, H-5), 7.55 (1H, d, *J* 8.4, H-6), 7.70 (1H, br s, NHCSNH_2), 7.91 (1H, br s, NHCSNH_2), 8.25 (1H, br s, PhC*H*=NNH), 12.34 (1H, s, PhCH=NN*H*); δ_c(75 MHz; $CDCl_3$) 10.8 (2C), 45.8 (2C), 49.2, 107.5, 111.7, 113.1, 126.1, 141.0, 158.9, 160.4, 177.0; LRMS (EI) *m/z*, M^+ 332; Anal. Found C51.45, H 6.76, N 18.88, S 11.24% Calc. For $C_{13}H_{20}N_4SO_2$: C 52.68, H 6.80, N 18.90, S 10.82%; HRMS (EI) *m/z* Found: $[M+H]^+$, 297.13858 Calc. For $C_{13}H_{21}N_4SO_2$: M, 297.13852.

***Compound* 77b**



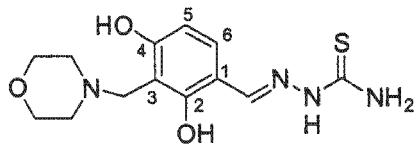
The conditions employed for the preparation of this compound were those described in General Method F, which gave **77b** as a yellow solid (0.39g, 76%) (recrystallised from methanol); decomposes above 180 °C; R_f = 0.56 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1150 (C=S), 1580 (C=C), 3400 (OH/N-H); δ_H(300 MHz; $CDCl_3$) 1.77 (4H, m, $N[CH_2CH_2]_2$), 2.58 (4H, m, $N[CH_2CH_2]_2$), 3.84 (2H, s, $PhCH_2N$), 6.29 (1H, d, *J* 8.4, H-5), 7.53 (1H, d, *J* 8.4, H-6), 7.69 (1H, br s, $NHCSNH_2$), 7.89 (1H, br s, $NHCSNH_2$), 8.24 (1H, br s, PhCHNNH), 11.54 (1H, s, PhCH=NNH); δ_c(75 MHz; $CDCl_3$) 23.9 (2C), 51.6 (2C), 53.5, 107.8, 110.7, 112.3, 127.1, 159.3, 161.3, 177.7; Anal. Found C 53.02, H 6.22, N 19.05, S 10.82% Calc. For: $C_{13}H_{18}N_4SO_2$ C 53.04, H 6.16, N 19.03, S 10.89%; HRMS (EI) *m/z* Found: $[M+H]^+$, 295.12291 Calc. For $C_{13}H_{19}N_4SO_2$: M, 295.12287.

***Compound* 77c**



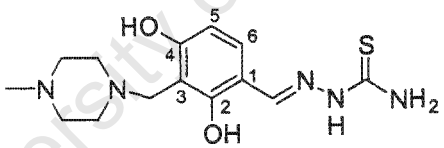
The conditions employed for the preparation of this compound were those described in General Method F, which gave **77c** as a yellow solid (0.40g, 78%) (recrystallised from methanol); decomposes above 126 °C; R_f = 0.48 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1}; 1030 (C-O), 1130 (C-N), 1590 (C=C), 3150 (N-H), 3250 (N-H), 3400 (OH/N-H); δ_H(400 MHz; DMSO-d_6) 1.43 (2H, m, $N[CH_2CH_2]_2CH_2$), 1.52 (4H, m, $N[CH_2CH_2]_2CH_2$), 2.48 (4H, m, $N[CH_2CH_2]_2CH_2$), 3.69 (2H, s, $PhCH_2N$), 6.28 (1H, d, *J* 8.8, H-5), 7.53 (1H, d, *J* 8.8, H-6), 7.70 (1H, br s, $NHCSNH_2$), 7.90 (1H, br s, $NHCSNH_2$), 8.23 (1H, br s, PhCH=NNH), 11.52 (1H, s, PhCH=NNH); δ_c(100 MHz; DMSO-d_6) 23.6 (2C), 25.5, 53.3, 54.2 (2C), 107.1, 111.1, 113.0, 125.3, 140.5, 158.7, 159.0, 177.2; HRMS (EI) *m/z* Found: $[M]^+$, 308.13071 Calc. For $C_{14}H_{20}N_4SO_2$: M, 308.13070.

***Compound* 77d**



The conditions employed for the preparation of this compound were those described in General Method F, gave **77d** as a yellow solid (0.43g, 84%) (recrystallised from methanol); decomposes above 200 °C; R_f = 0.48 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1150 (C=S), 1590 (C=O), 3150 (N-H), 3260 (N-H), 3400 (O-H/N-H); δ_H(400 MHz; DMSO-d_6) 2.48 (4H, br s, N[C*H*$_2$CH$_3$]$_2$O), 3.60 (4H, br s, N[CH$_2$C*H*$_2$]$_2$O), 3.80 (2H, s, PhC*H*$_2$N), 6.20 (1H, d, *J* 8.4, H-5), 7.55 (1H, d, *J* 8.4 , H-6), 7.69 (1H, br s, NHCSN*H*), 7.89 (1H, br s, NHCSN*H*), 8.24 (1H, s, PhC*H*=NNH), 11.16 (1H, s, PhCH=NN*H*); δ_c(100 MHz; DMSO-d_6) 53.1, 54.0 (2C), 66.7 (2C), 107.8, 108.1, 112.5, 127.6, 141.9, 158.7, 159.3, 177.; Anal. Found C 50.88, H 6.06, N 17.96, S 9.53% Calc. For $C_{13}H_{18}N_4SO_3$: C 50.31, H 5.85, N 18.05, S 10.33%; HRMS (EI) *m/z* Found: $[M+H]^+$, 311.11802 Calc. For $C_{13}H_{19}N_4SO_3$: M,311.11778.

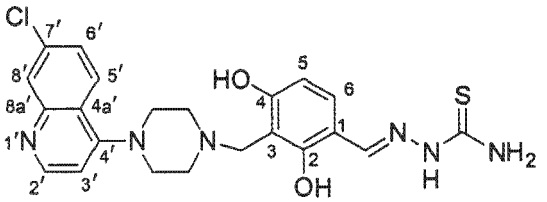
***Compound* 77e**



The conditions employed for the preparation of this compound were those described in General Method F, which gave **77e** as a yellow solid (0.52g, 95%) (recrystallised from methanol); decomposes above 200 °C; R_f = 0.52 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1}; 1060 (C-O), 1140 (C=S), 1590 (C=C), 3150 (N-H), 3300 (N-H), 3400 (O-H/N-H); δ_H(300 MHz; DMSO-d_6) 2.19 (3H, s, N(CH$_2$CH$_2$)$_2$NC*H*$_3$), 2.38 (4H, br s, N[CH$_2$C*H*$_2$]$_2$NCH$_3$), 2.49 (4H, br s, N[C*H*$_2$CH$_2$]$_2$NCH$_3$), 3.74 (2H, s, PhC*H*$_2$N), 6.32 (1H, d, *J* 8.4, H-5), 7.58 (1H, d, *J* 8.4, H-6), 7.71 (1H, br s, NHCSN*H*), 7.91 (1H, br s, NHCSN*H*), 8.26 (1H, s, PhC*H*=NNH), 11.17 (1H, s, PhCH=NN*H*); δ_c(75 MHz; DMSO-d_6) 45.4 (2C), 51.8, (2C), 53.1, 54.4, 107.1, 107.2, 111.7, 126.6, 140.9, 158.2, 158.4, 177.1; Anal. Found C 50.86, H 6.44, N 17.99, S 9.55% Calc. For $C_{14}H_{21}N_5O_2S$: C

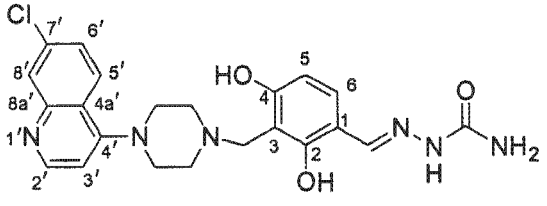
51.99, H 6.54, N 21.65, S 9.91%; HRMS (EI) *m/z* Found: $[M+H]^+$, 324.14950 Calc. For $C_{14}H_{22}N_5O_2S$: M,324.14941.

***Compound* 77f**



The conditions employed for the preparation of this compound were those described in General Method F, which gave **77f** as a yellow solid (075g, 96%) (recrystallised from methanol); decompose above 240 °C; R_f = 0.56 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1} (C-O), 1150 (C=S), 1560 (C=N), 1600 (C=C), 3140, 3275 (N-H), 3450 (O-H/N-H); δ_H(400 MHz; DMSO-d_6) 2.81 (4H, br s, N[CH_2CH_2]$_2$NCH), 3.24 (4H, br s, N[CH_2CH_2]$_2$NCH), 3.86 (2H, s, PhCH_2N), 6.36 (1H, d, *J* 8.4, H-5), 7.03 (1H, d, *J* 4.8, H-3′), 7.54 (1H, dd, *J* 2.0, 9.2, H-6′), 7.58 (1H, d, *J* 8.4, H-6), 7.73 (1H, s, NHCSNH_2), 7.93 (1H, s, NHCSNH_2), 7.97 (1H, d, *J* 2.0, H-8′), 8.02 (1H, d, *J* 9.2, H-5′), 8.27 (1H, s, PhC*H*=NNH), 8.70 (1H, d, *J* 4.8, H-2′), 11.20 (1H, s, PhCH=NN*H*); δ_c(100 MHz; DMSO-d_6) 52.3 (2C), 52.6 (2C), 53.5, 108.2, 109.0, 110.4, 112.5, 122.1, 126.6, 126.7, 127.7, 128.8, 134.3, 141.9, 150.3, 152.9, 156.7, 158.7, 159.3, 177.8; Anal. Found C 55.96, H 5.10, N 17.71, S 6.26% Calc. For $C_{22}H_{23}ClN_6O_2S$: C 56.10, H 4.92, N 17.84, S 6.81%; HRMS (EI) *m/z* Found: $[M]^+$, 470.12910 Calc. For $C_{22}H_{23}ClN_6SO_2Cl$: M, 470.129174.

***Compound* 77g**



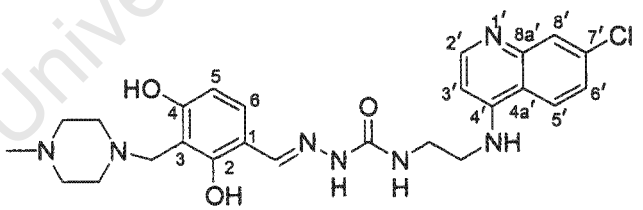
The conditions employed for the preparation of this compound were those described in General Method F, which gave **77g** as a yellow solid (0.69g, 92%) (recrystallised from methanol); decompose above 240 °C; R_f = 0.08 (MeOH/DCM/17%NH_4OH 2:2:1); IR

ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1560 (C=N), 1600 (C=C), 1650 (C=O), 3150 (N-H), 3300 (N-H), 3450 (O-H/N-H); δ_H(400 MHz; DMSO-d_6) 2.80 (4H, br s, N[CH_2CH_2]$_2$NCH), 3.22 (4H, br s, N[CH_2CH_2]$_2$NCH), 3.85 (2H, s, PhCH_2N), 6.24 (2H, s, CONH_2), 6.35 (1H, d, *J* 8.8, H-5), 7.02 (1H, d, *J* 4.8, H-3′), 7.40 (1H, d, *J* 8.8, H-6), 7.53 (1H, dd, *J* 2.0, 9.2, H-6′), 7.97 (1H, d, *J* 2.0, H-8′), 8.02 (1H, d, *J* 9.2, H-5′), 8.03 (1H, s, PhC*H*=NNH), 8.69 (1H, d, *J* 4.8, H-2′), 9.97 (1H, s, PhCH=NN*H*); δ_c(100 MHz; DMSO-d_6) 52.3 (2C), 52.7 (2C), 53.3, 108.0, 108.3, 110.3, 112.6, 122.1, 126.5, 126.7, 127.9, 128.8, 134.3, 140.0, 150.3, 152.9, 156.7, 157.1, 157.8, 158.9; Anal. Found C 58.05, H 4.68, N 18.55, % Calc. For $C_{22}H_{23}ClN_6O_3$: C 58.09, H 5.10, N 18.47%. HRMS (EI) *m/z* Found: $[M+H]^+$, 455.15970 Calc. For $C_{22}H_{24}ClN_6O_3$: M, 455.15984.

General Method G for the preparation of compounds 83a-f

To a solution of semicarbazide (0.54 mmol) in methanol (10 ml) were added a mixture of *p*-toluenesulfonic acid and aldehyde. The resultant was stirred at room temperature for 16 h. The reaction mixture was diluted with chloroform (20 ml). The organic layer was separated, washed with 5% aqueous sodium carbonate (3 x 10 ml), brine (2 x 10 ml), dried ($MgSO_4$) and concentrated under reduced pressure to afford a solid residue, which was column chromatographed on silica gel.

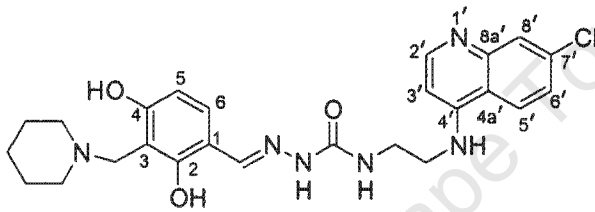
***Compound* 83e**



The conditions employed for the preparation of this compound were those described in General Method G. Column chromatography (10%MeOH/DCM followed by MeOH/DCM/17%NH_4OH 2:2:1) afforded **83e** as a cream white solid (0.22g, 80%), (recrystallised from methanol); decomposes above 140 °C; R_f = 0.33 (17% NH_4OH:MeOH:DCM 1:2:2); IR ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1130 (C-N), 1580 (C=N), 1650 (C=O) 3400 (N-H); δ_H(400 MHz; $CDCl_3$) 2.30 (3H, s, NCH_3), 2.60 (8H, br s, N[CH_2CH_2]$_2$N), 3.39 (2H, m, CONHCH$_2$CH_2N), 3.80 (2H, m, CONHCH_2CH$_2$N), 3.83

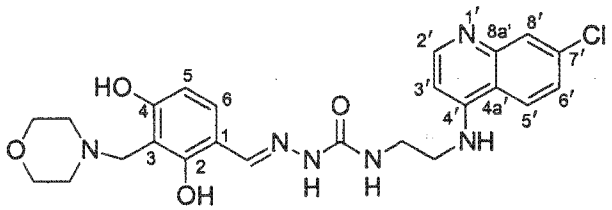
(2H, s, PhCH_2N), 6.11 (1H, br s, NHNCONH), 6.24 (1H, d, *J* 4.8, H-3′), 6.37 (1H, d, *J* 8.4, H-5), 6.84 (1H, br s, NHNCONH), 6.96 (1H, d, *J* 8.4, H-6), 7.17 (1H, d, *J* 2.0, 8.8, H-6′), 7.68 (1H, d, *J* 8.8, H-5′), 7.79 (1H, s, PhC*H*=NNH), 7.86 (1H, d, *J* 2.0, H-8′), 8.42 (1H, d, *J* 4.8, H-2′); δ_c(100 MHz; $CDCl_3$) 39.2 , 45.8, (2C), 46.0 (2C), 52.6, 53.6, 54.8, 98.2, 107.1, 109.0, 109.4 , 117.2, 121.8, 125.2, 128.0, 131.1, 134.8, 147.2, 148.6, 150.2, 151.6, 156.5, 157.5, 162.2; Anal. Found C 54.59, H 5.80, N 17.48% Calc. For $C_{25}H_{30}ClN_7O_3$: C 54.74, H 6.20, N 17.88%; HRMS (EI) *m/z* Found: $[M+2H]^+$, 513.22541 Calc. For $C_{25}H_{32}ClN_7O_3$: M, 513.22551.

***Compound* 83c**



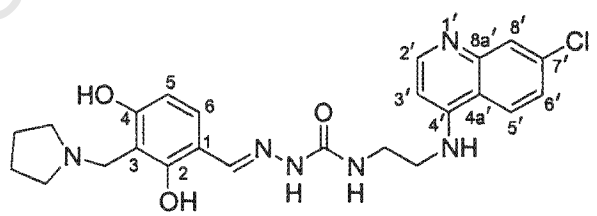
The conditions employed for the preparation of this compound were those described in General Method G. Column chromatography (10%MeOH/DCM followed by MeOH/DCM/17%NH_4OH 2:2:1) afforded **83c** as a greenish yellow solid (0.14g, 54%), (recrystallised from methanol); decomposes above 200 °C; R_f = 0.30 (17% NH_4OH:MeOH:DCM 1:2:2); IR ν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1580 (C=N), 1650 (C=O), 3370 (N-H); δ_H(400 MHz; CD_3OD) 1.42 (2H, m, N[CH_2CH_2]$_2$CH_2), 1.58 (4H, m, N[CH_2CH_2]$_2$$CH_2$), 2.42 (2H, m, N[C$H_2CH_2$]$_2$$CH_2$), 3.30 (2H, t, *J* 5.2, CONHCH_2CH_2N), 3.68 (2H, t, *J* 5.2, CONHC$H_2$$CH_2$N), 3.98 (2H, s, PhC$H_2$N), 6.13 (1H, d, *J* 5.2, H-3′), 6.16 (1H, br s, NHNCONH), 6.23 (1H, d, *J* 8.4, H-5), 6.84 (1H, d, *J* 8.4, H-6), 6.85 (1H, br s, NHNCONH), 7.05 (1H, dd, *J* 2.0, 8.8, H-6′), 7.60 (2H, d, *J* 8.8, H-5′), 7.68 (1H, d, *J* 2.0, H-8′), 7.80 (1H, s, PhC*H*=NNH), 8.25 (1H, d, *J* 5.2, H-2′); δ_c(100 MHz; $CDCl_3$) 23.8, 25.5 (2C), 26.3 (2C), 53.4, 54.3, 54.5, 99.6, 107.1, 108.8, 111.8, 116.2, 124.5, 126.8, 129.9, 132.2, 136.7, 146.5, 148.5, 151.5, 153.3, 158.7, 159.2, 162.2; HRMS (EI) *m/z* Found: $[M+H]^+$, 497.20614 Calc. For $C_{25}H_{29}ClN_6O_3$: M, 497.20679.

***Compound* 83d**



The conditions employed for the preparation of this compound were those described in General Method G. Column chromatography (MeOH:DCM 1:9 followed by 17% NH_4OH:MeOH:DCM 1:2:2) afforded **83d** a cream white solid (0.18g, 69%), (recrystallised from methanol); decomposes above 220 °C; R_f = 0.33 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1570 (C=N) 1660 (C=O), 3350 (N-H); δ_H(300 MHz; $CDCl_3$) 2.62 (4H, br s, N[CH_2CH_2]$_2$O), 3.50 (2H, t, *J* 5.1, $CONHCH_2CH_2N$), 3.58 (2H, t, *J* 5.1, $CONHCH_2CH_2N$), 3.65 (4H, br s, N[CH_2CH_2]$_2$O), 3.81 (2H, s, $PhCH_2N$), 6.11 (1H, br s, NHNCONH), 6.38 (1H, d, *J* 8.7, H-5), 6.89 (1H, d, *J* 6.9, H-3′), 6.84 (1H, br s, NHNCONH), 7.11 (1H, d, *J* 9.0, H-5′), 7.39 (1H, d, *J* 8.7, H-6), 7.68 (1H, dd, *J* 2.4, 9.0, H-6′), 7.88 (1H, d, *J* 2.4, H-8′), 8.05 (1H, s, PhCH=NNH), 8.51 (1H, d, *J* 6.9, H-2′); δ_c(75 MHz; $CDCl_3$) 38.4, 44.5, 53.1 (2C), 53.3, 66.3 (2C), 99.4, 107.4, 107.9, 112.5, 116.5, 121.8, 126.1, 128.7, 129.9, 137.7, 141.7, 145.8, 155.2, 156.8, 157.9, 159.0, 162.0; Anal. Found C 54.82, H 5.18, N 12.57% Calc. For $C_{24}H_{27}ClN_6O_4$: C 54.75, H 5.70, N 15.97%; HRMS (EI) *m/z* Found: $[M+]^+$, 498.17798 Calc. For $C_{24}H_{27}ClN_6O_4$: M, 498.17823.

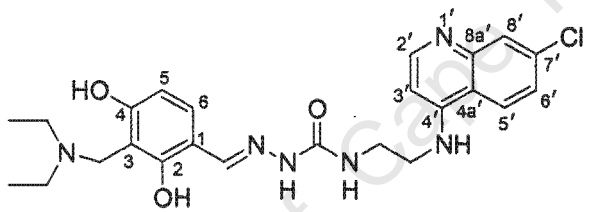
***Compound* 83b**



The conditions employed for the preparation of this compound were those described in General Method G. Column chromatography (MeOH:DCM 1:9 followed by 17% NH_4OH:MeOH:DCM 1:2:2) afforded **83b** as a brown solid (0.14g, 52%), (recrystallised from methanol); decomposes above 100 °C; R_f= 0.30 (MeOH/DCM/17%NH_4OH 2:2:1); IR ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1580 (C=N), 1650 (C=O), 3400 (N-H); δ_H(300 MHz;

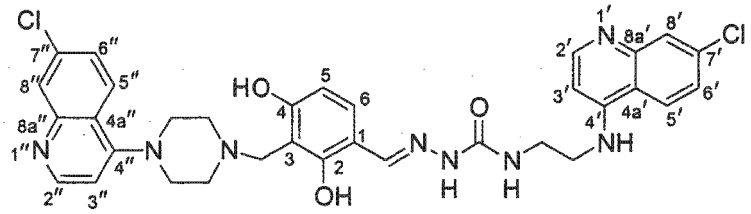
$CDCl_3$) 1.87 (4H, m, $N[CH_2CH_2]_2$), 2.69 (4H, m, $N[CH_2CH_2]_2$), 3.42 (2H, t, *J* 5.4, $CONHCH_2CH_2N$), 3.79 (2H, t, *J* 5.4, $CONHCH_2CH_2N$), 3.95 (2H, s, $PhCH_2N$), 6.01 (1H, br s, NHNCONH), 6.30 (1H, d, *J* 5.4, H-3′), 6.38 (1H, d, *J* 8.4, H-5), 6.83 (1H, br s, NHNCONH), 6.95 (1H, d, *J* 8.4, H-6), 7.28 (1H, dd, *J* 2.1, 9.0, H-6′), 7.77 (1H, d, *J* 9.0, H-5′), 7.85 (1H, s, PhCH=NNH), 7.88 (1H, d, *J* 2.1, H-8′), 8.42 (1H, d, *J* 5.4, H-2′); δ_c(75 MHz; $CDCl_3$) 23.3 (2C), 48.5, 50.0 (2C), 52.0, 54.0, 98.3, 106.9, 107.8, 108.0, 114.8, 121.8, 125.2, 128.0, 130.9, 135.8, 145.8, 148.6, 150.2), 151.8, 156.7, 157.4, 163.; Anal. Found C 54.56, H 5.85, N 14.75% Calc. For $C_{24}H_{27}ClN_6O_3$: C 54.54, H 5.87, N 15.90%; HRMS (EI) *m/z* Found: $[M+H]^+$, 483.18312 Calc. For $C_{24}H_{28}ClN_6O_3$: M, 483.18331.

***Compound* 83a**



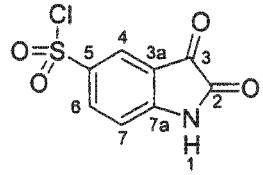
The conditions employed for the preparation of this compound were those described in General Method G. Column chromatography (10%MeOH/DCM followed by MeOH/DCM/17%NH_4OH 2:2:1) afforded **83a** pale green solid (0.16g, 61%) (recrystallised from methanol); decomposes above 150 °C; R_f = 0.30 (17% NH_4OH:MeOH:DCM 1:2:2); IR ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1570 (C=N), 1650 (C=O), 3380 (N-H); δ_H(300 MHz; DMSO-d_6) 1.06 (6H, t, *J* 7.2, $N[CH_2CH_3]_2$), 2.64 (4H, t, *J* 7.2, $N[CH_2CH_3]_2$), 3.40 (2H, t, *J* 5.4, $CONHCH_2CH_2N$), 3.45 (2H, t, *J* 5.4, $CONHCH_2CH_2N$), 3.84 (2H, s, $PhCH_2N$), 6.32 (1H, d, *J* 8.7, H-5), 6.60 (1H, d, *J* 5.4, H-3′), 7.09 (1H, br s, NHNCONH), 7.37 (1H, d, *J* 8.7, H-6), 7.43 (1H, dd, *J* 2.1, 8.7, H-6′), 7.66 (1H, br s, NHNCONH), 7.79 (1H, d, *J* 8.7, H-5′), 8.04 (1H, s, PhCH=NNH), 8.24 (1H, d, *J* 2.1, H-8′), 8.40 (1H, d, *J* 5.7, H-2′); δ_c(100 MHz; DMSO-d_6) 11.3 (2C), 46.8, 49.4 (2C), 52.4, 54.6, 98.3, 106.9, 107.7, 108.0, 116.8, 121.8, 125.0, 127.6, 130.7, 134.4, 145.6, 148.8, 151.2, 152.0, 156.9, 157.5, 162.1. Anal. Found C 57.90, H 6.14, N 15.09% Calc. For $C_{24}H_{29}ClN_6O_3$: C 57.30, H 6.17, N 16.72%; HRMS (EI) *m/z* Found: $[M]^+$, 484.19892 Calc. For $C_{24}H_{29}ClN_6O_3$: M,484.19897.

***Compound* 83f**



The conditions employed for the preparation of this compound were those described in General Method G. Column chromatography (10%MeOH/DCM followed by MeOH/DCM/17%NH_4OH 2:2:1); afforded **83f** as a cream white solid (0.26g, 74%) (recrystallised from methanol); decomposes above 140 °C; R_f = 0.13 (17% NH_4OH:MeOH:DCM 1:2:2); IR ν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1580 (C=N) 1650 (C=O), 3400 (N-H); δ_H(300 MHz; DMSO-d_6) 2.82 (4H, br s, $N[CH_2CH_2]_2NCH_2Ph$), 3.45 (4H, br s, $N[CH_2CH_2]_2NCH_2Ph$), 3.41 (2H, t, *J* 5.4, $CONHCH_2CH_2N$), 3.47 (2H, t, *J* 5.4, $CONHCH_2CH_2N$), 3.87 (2H, s, $PhCH_2N$), 6.38 (1H, d, *J* 8.4, H-5), 6.62 (1H, d, *J* 5.1, H-3′), 7.04 (1H, d, *J* 5.1, H-3″), 7.15 (1H, br s, NHNCONH), 7.43 (1H, d, *J* 8.4, H-6), 7.50 (1H, dd, *J* 2.1, 9.0, H-6′), 7.55 (1H, dd, *J* 2.1, 9.0, H-6″), 7.59 (1H, br s, NHNCONH), 7.79(1H, d, *J* 2.1, H-8′), 7.98 (1H, d, *J* 2.1, H-8″), 8.02 (1H, d, *J* 9.0, H-5′), 8.07 (1H, s, PhCH=NNH), 8.21 (1H, d, *J* 9.0, H-5″), 8.41 (1H, d, *J* 5.1, H-2′), 8.71 (1H, d, *J* 5.1, H-2″); δ_c(75 MHz; DMSO-d_6) 37.7, 40.3, 51.5 (2C), 51.9, 52.2 (2C), 98.6, 107.2, 107.5, 109.5, 111.9, 117.2, 121.3, 123.8, 124.1, 125.7, 125.9, 127.1, 128.0, 133.4, 133.5, 139.8, 148.5, 149.6, 150.3, 151.4, 152.1, 155.9, 156.1, 156.2, 157.1, 158.1; Anal. Found C 54.76, H 4.71, N 14.70% Calc. For $C_{33}H_{32}Cl_2N_8O_3$: C 54.79, H 5.39, N 15.49%; HRMS (EI) *m/z* Found: M^+, 658.19784 Calc. For $C_{33}H_{32}Cl_2N_8O_3$: M, 658.19744.

2,3-Dioxo-2,3-dihydro-1*H*-indole-5-sulfonyl chloride 89



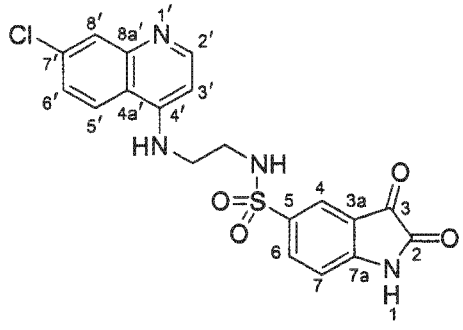
To a mixture of 5-isatin –sulfonic acid, sodium salt dihydrate (10g, 35.1 mmol), and 50 ml of tetramethylene sulfone was added phosphorus oxychloride (16.5 ml, 177 mmol).

The resulting mixture was heated at 60 °C for 3 h. The mixture was cooled to 0 °C, and 120 ml of water cautiously added. The resulting green solid was filtered and washed with water. The solid was dissolved in 100 ml of EtOAc and washed three times with 50 ml of water. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow solid. The solid was recrystallized from an EtOAc/hexane mixture to give **89** as an orange solid (4.83g, 56%); Decomposed above 215 °C; IRν_{max}(Nujol)/cm^{-1} 1150 (S=O), 1660 (C=O), 1710 (C=O); δ_H(400 MHz; $CDCl_3$) 6.87 (1H, d, *J* 8.0, H-7), 7.58 (1H, d, *J* 1.6, H-4), 7.80 (1H, dd, *J* 1.6, 8.0, H-6); δ_c(100 MHz; $CDCl_3$) 112.2, 117.8, 122.1, 136.1, 143.7, 151.4, 160.3, 184.9; HRMS (EI) *m/z* Found: M^+, 144.05753 Calc. For $C_8H_4ClNO_4S$: M, 144.05751.

General Method H for the preparation of compounds 87a-b

To a solution of 5-chlorosulfonylisatin (2.33 mmol) in 24 ml of a 1:1 THF/$CHCl_3$ mixture at 0 °C was added dropwise, via syringe pump, a solution of selected amine (3.01 mmol) and *N*, *N*-diisopropylethylamine (4.64 mmol) in 10 ml of MeOH (this was done in error to increase solubility). The reaction mixture was stirred at 0 °C for 1.0 h and allowed to stand at room temperature for 24 h. The precipitate was filtered off and washed with water (3 x 20 ml). The solid was recrystallized from methanol-water to afford the title compounds.

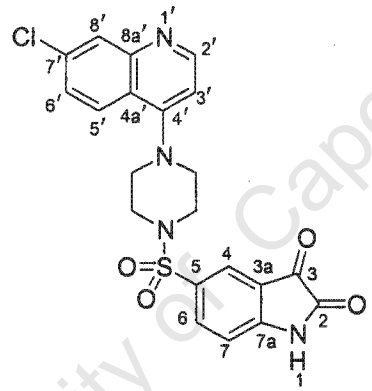
2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid [2-(7-chloro-quinolin-4-ylamino)-ethyl]-amide **87b**



The conditions employed for the preparation of this compound were those described in General Method H, which afforded **87b** as orange crystals (0.62g, 62%); decomposed

above 215 °C; IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1140 (S=O), 1300 (S=O), 1560 (C=N), 1660 (C=O), 1720 (C=O), 3250 (N-H); δ_H(300 MHz; $CDCl_3$) 3.24 (2H, q, *J* 5.1, HNC*H*$_2$CH$_2$NHSO$_2$), 3.25 (2H, q, *J* 5.1, HNCH$_2$C*H*$_2$NHSO$_2$), 7.03 (1H, d, *J* 4.8, H-3′), 7.17 (1H, d, *J* 8.4, H-7), 7.48 (1H, dd, *J* 2.1, 9.0, H-6′), 7.77 (1H, d, *J* 2.1, H-8′), 7.92 (1H, d, *J* 9.0, H-5′), 7.96 (1H, d, *J* 2.1, H-4), 8.00 (1H, dd, *J* 2.1, 8.4, H-6), 8.71 (1H, d, *J* 4.8, H-2′); δ_c(75 MHz; $CDCl_3$) 46.6, 51.7, 106.3, 113.6, 119.0, 121.9, 124.2, 126.6, 127.8, 128.7, 129.6, 134.4, 137.9, 143.0, 150.2, 152.8 154.7, 156.4, 183.6; HRMS (EI) *m/z* Found: $[M+H]^+$, 431.05815 Calc. For $C_{19}H_{16}N_4SO_4Cl$: M, 431.05808.

5-[4-(7-Chloro-quinolin-4-yl)-piperazine-1-sulfonyl]-1H-indole-2,3-dione **87a**

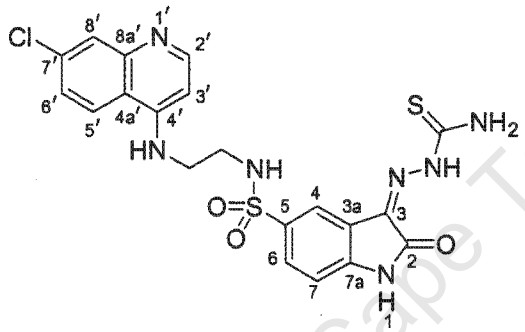


The conditions employed for the preparation of this compound were those described in General Method H, which afforded **87a** as yellow crystals (0.75g, 71%); decomposed above 215 °C; IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1160 (S=O), 1300 (S=O), 1560 (C=N), 1610 (C=O), 1730 (C=O), 3270 (N-H); δ_H(400 MHz; $CDCl_3$) 3.22 (8H, br s, N[C*H*$_2$CH$_2$]$_2$NHSO$_2$), 7.01 (1H, d, *J* 4.8, H-3′), 7.15 (1H, d, *J* 8.4, H-7), 7.46 (1H, dd, *J* 2.0, 8.8, H-6′), 7.75 (1H, d, *J* 2.0, H-8′), 7.90 (1H, d, *J* 8.8, H-5′), 7.96 (1H, d, *J* 2.0, H-4), 8.00 (1H, dd, *J* 2.0, 8.4, H-6), 8.71 (1H, d, *J* 4.8, H-2′), 11.50 (1H, br s, CON*H*); δ_c(75 MHz; $CDCl_3$) 45.6, 50.9, 109.9, 112.9, 118.3, 121.2, 123.5, 125.8, 125.9, 128.0, 128.8, 133.7, 137.2, 149.5, 152.2, 153.9, 155.6, 159.4, 182.9; HRMS (EI) *m/z* Found: $[M+H]^+$, 457.07369 Calc. For $C_{21}H_{18}N_4SO_4Cl$: M, 457.07373.

General Method I for the preparations of compounds 90a-b

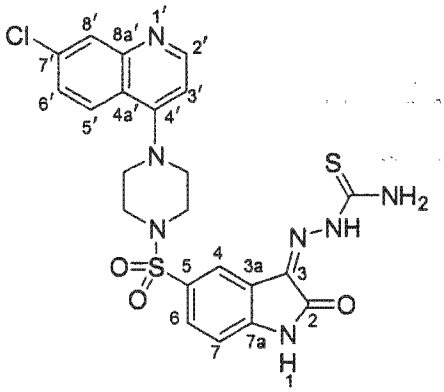
Equimolar quantities of isatin and thiosemicarbazone (2.33 mmol) in were dissolved in warm DMF (10 ml). The resulting mixture was stirred at 70 °C for 12h. The reaction mixture was cooled to room temperature and water was added to initiate product precipitation. The precipitate was filtered off, washed with water (3 x 20 ml) and dried. The solid was recrystallized from DMF-H_2O to give the title compounds **90a-b**.

***Compound* 90b**



The conditions employed for the preparation of this compound were those described in General Method I, which afforded as **90b** orange crystals (0.88g, 75%); Decomposed above 205 °C; IRν_{max}(Nujol)/cm^{-1} 1065 (C-O), 1150 (C=S), 1300 (S=O), 1560 (C=N), 1600 (C=C), 1680 (C=O), 3250 (N-H), 3375 (N-H), 3440 (N-H); δ_H(400 MHz; $CDCl_3$) 3.20 (2H, q, *J* 5.2, HNC*H*$_2$CH$_2$NHSO$_2$), 3.25 (2H, q, *J* 5.2, NCH$_2$C*H*$_2$NHSO$_2$), 7.02 (1H, d, *J* 4.8, H-3′), 7.18 (1H, d, *J* 8.4, H-7), 7.46 (1H, dd, *J* 2.0, 8.8, H-6′), 7.77 (1H, dd, *J* 2.0, 8.4, H-6), 7.92 (1H, d, *J* 8.8, H-5′), 7.94 (1H, d, *J* 2.0 , H-8′), 8.16 (1H, d, *J* 2.0, H-4), 8.70 (1H, d, *J* 4.8, H-2′) 8.98 (1H, s, NCSN*H*$_2$), 9.13 (1H, s, NCSN*H*$_2$), 11.63 (1H, s, N*H*CSNH$_2$), 12.32 (1H, br s, CON*H*); δ_c(75 MHz; $CDCl_3$) 46.6, 51.7, 106.3, 112.2, 119.2, 121.2, 121.7, 121.9, 126.7, 128.7, 129.6, 134.4, 137.4, 146.6, 150.2, 151.8, 152.9 156.4, 163.4, 190.0; HRMS (EI) *m/z* Found: $[M+H]^+$, 504.06702 Calc. For $C_{20}H_{19}N_7S_2O_3Cl$: M, 504.06793.

***Compound* 90a**

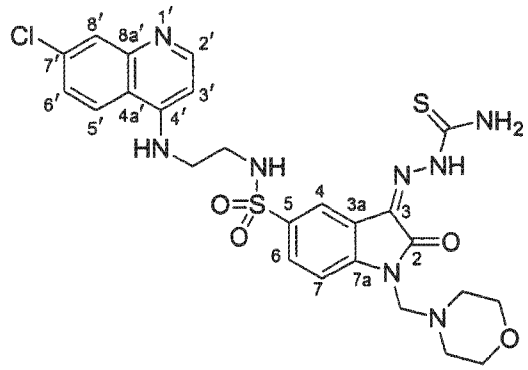


The conditions employed for the preparation of this compound were those described in General Method I, which afforded as **90a** orange crystals (0.99g, 80%); decomposed above 215 °C; IRν_{max}(Nujol)/cm^{-1} 1065 (C-O), 1150 (C=S), 1300 (S=O), 1560 (C=N), 1605 (C=C), 1680 (C=O), 3170 (N-H), 3375 (N-H), 3440 (N-H); δ_H(400 MHz; $CDCl_3$) 3.09 (4H, br s, N[C*H*$_2$CH$_2$]$_2$NHSO$_2$), 3.29 (4H, br s, N[CH$_2$C*H*$_2$]$_2$NHSO$_2$), 6.44 (1H, d, *J* 5.4, H-3′), 6.93 (1H, d, *J* 8.1, H-7), 7.42 (1H, dd, *J* 2.1, 9.3, H-6′), 7.71 (1H, dd, *J* 2.1, 8.1, H-6), 7.75 (1H, d, *J* 2.1, H-8′), 8.08 (1H, d, *J* 2.1, H-4), 8.10 (1H, d, 9.3, H-5′), 8.71 (1H, d, *J* 4.8, H-2′), 8.89 (1H, s, NCSN*H*$_2$), 9.09 (1H, s, NCSN*H*$_2$) 11.50 (1H, s, N*H*CSNH$_2$), 12.35 (1H, br s, CON*H*); δ_c(75 MHz; $CDCl_3$) 46.6, 51.7, 110.6, 112.2, 121.2, 121.7, 121.9, 126.7, 128.7, 129.3, 131.1, 131.5, 134.4, 146, 150.1, 152.8, 153.9, 156.4, 159.4, 182.9; HRMS (EI) *m/z* Found: $[M+H]^+$, 530.08325 Calc. For $C_{22}H_{2i}N_7S_2O_3Cl$: M, 530.08358.

General Method J for the preparation of compounds 91a-b

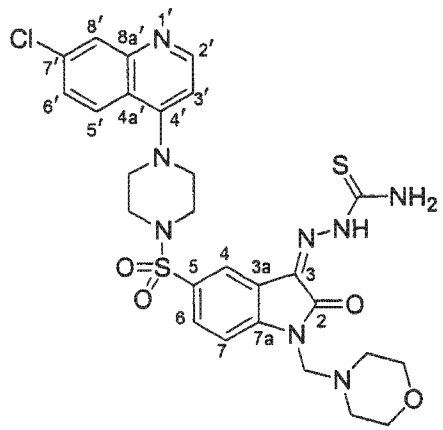
A mixture of paraformaldehyde (0.52 mmol) and morpholine (0.52 mmol) in DMF (10 ml) was stirred 70 °C for 1.0 h. To the resultant was added isatin thiosemicarbazone derivative (0.40 mmol) in DMF (5.0 ml) and the reaction mixture was stirred at 70 °C for a further 3.0 h. The reaction mixture was cooled to room temperature and water was added to initiate product precipitation. The precipitate was filtered off, washed with water (3 x 20 ml) and dried. The solid was recrystallized from DMF-H_2O to provide the title compounds **91a-b**.

***Compound* 91b**



The conditions employed for the preparation of this compound were those described in General Method J, which afforded **91b** as orange crystals (0.18g, 74%); Decomposed above 200 °C; IRν_{max}(Nujol)/cm^{-1} 1070 (C-O), 1150 (C=S), 1300 (S=O), 1570 (C=N), 1600 (C=C), 1680 (C=O), 3160 (N-H), 3375 (N-H), 3440 (N-H); δ_H(300 MHz; $CDCl_3$) 2.59 (4H, br s, N[CH_2CH_2]$_2$O), 3.21 (2H, q, *J* 5.1, HNCH_2CH_2NHSO$_2$), 3.24 (2H, q, *J* 5.1, HNCH$_2CH_2$NHSO$_2$), 3.54 (4H, br s, N[CH$_2CH_2$]$_2$O), 4.55 (2H, br s, NCH_2N), 7.01 (1H, d, *J* 5.1, H-3′), 7.18 (1H, d, *J* 8.1, H-7), 7.46 (1H, dd, *J* 2.1, 9.0, H-6′), 7.77 (1H, dd, *J* 1.8, 8.1, H-6), 7.92 (1H, d, *J* 9.0, H-5′), 7.94 (1H, d, *J* 2.1, H-8′), 8.16 (1H, d, *J* 1.8, H-4), 8.70 (1H, d, *J* 5.1, H-2′) 8.98 (1H, s, CSNH_2), 9.13 (1H, s, CSNH_2), 11.63 (1H, s, N*H*CSNH$_2$); δ_c(75 MHz; $CDCl_3$) 45.8, 50.4, 51.0 (2C), 65.9 (2C), 72.0, 109.9, 111.4, 120.4, 121.0, 121.1, 125.9, 128.0, 128.6, 129.4, 130.4, 133.6, 145.9, 146.9, 149.5, 152.1 155.6, 162.7, 178.8; HRMS (EI) *m/z* Found: [M+H]$^+$, 604.06702 Calc. For $C_{22}H_{28}N_8S_2O_4Cl$: M, 604.06793.

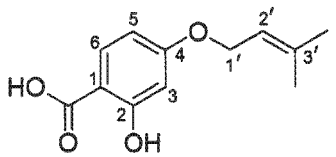
***Compound* 91a**



The conditions employed for the preparation of this compound were those described in General Method J, which afforded **91a** as orange crystals (0.19g, 76%); decomposed above 215 °C; IRν_{max}(Nujol)/cm^{-1} 1065 (C-O), 1150 (C=S), 1300 (S=O), 1560 (C=N), 1605 (C=O), 1690 (C=O), 3170 (N-H), 3370 (N-H), 3450 (N-H); δ_H(400 MHz; $CDCl_3$) 2.59 (4H, br s, N[C*H*$_2$CH$_2$]$_2$O), 3.21 (4H, br s, N[C*H*$_2$CH$_2$]$_2$NHSO$_2$), 3.54 (4H, br s, N[CH$_2$C*H*$_2$]$_2$O), 3.29 (4H, br s, N[CH$_2$C*H*$_2$]$_2$NHSO$_2$), 4.55 (2H, br s, NC*H*$_2$N), 7.01 (1H, d, *J* 4.8, H-3′), 7.17 (1H, d, *J* 8.0, H-7), 7.46 (1H, dd, *J* 2.0, 8.8, H-6′), 7.77 (1H, dd, *J* 1.6, 8.4, H-6), 7.91 (1H, d, *J* 8.8, H-5′) 7.94 (1H, d, *J* 2.0, H-8′), 8.16 (1H, d, *J* 1.6, H-4), 8.70 (1H, d, *J* 4.8, H-2′), 9.00 (1H, s, NCSN*H*$_2$), 9.12 (1H, s, NCSN*H*$_2$) 11.63 (1H, s, N*H*CSNH$_2$); δ_c(100 MHz; $CDCl_3$) 46.6 (2C), 51.2 (2C), 51.7 (2C), 63.8 (2C), 66.7, 110.6, 112.2, 121.9, 125.9, 126.7, 128.7, 129.3, 131.1, 131.5, 134.4, 135.0, 146.6, 150.2, 152.9, 156.4 162.5, 163.4, 179.2; HRMS (EI) *m/z* Found: $[M]^+$, 628.14351 Calc. For $C_{22}H_{28}N_8S_2O_4Cl$: M, 628.14417.

To a solution of 2,4-dihydroxybenzoic acid (5.00g, 32.44 mmol) in dry DMF (20 ml) were added K_2CO_3 (4.92g, 35.68 mmol) followed by prenyl bromide (5.32g, 35.68 mmol, 10 ml). The resultant was stirred at room temperature for 1.5h, filtered and diluted with water (10 ml). The diluted filtrate was extracted with ethyl acetate (3 x 20 ml). The combined extracts were washed with water (3 x 20 ml), brine (20 ml) and concentrated to give a light brown oil which was purified by column chromatography on silica gel to give **97** as a white solid (2.40g, 67%) and **98** as colourless oil (3.25, 28%).

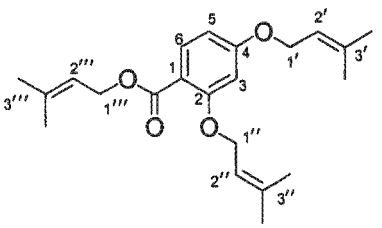
***2-Hydroxy-4-(3-methyl-but-2-enyloxy)-benzoic acid* 97**



mp = 142-143 °C (from ethyl acetate-hexane); R_f = 0.33 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1710 (C=O), 3500 (OH). δ_H(300MHz; $CDCl_3$) 1.76 (3H, s, C[C*H*$_3$]$_2$), 1.79 (3H, s, C[C*H*$_3$]$_2$), 4.81 (2H, d, *J* 7.2, H-1′), 5.44 (1H, t, *J* 7.2, H-2′), 6.36 (1H, dd, *J* 2.1, 8.7, H-5), 6.40 (1H, d, *J* 2.1, H-3), 7.74 (1H, d, *J* 8.7, H-6), 11.1 (1H, s, COO*H*); δ_c(75 MHz; $CDCl_3$) 18.1, 25.8, 62.0, 103.1, 106.2, 107.8, 118.1, 132.0, 139.9,

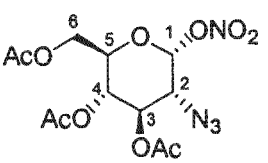
161.9, 163.5, 170.0; HRMS (EI) *m/z* Found: $[M]^+$, 222.08949 Calc. For $C_{12}H_{16}N_4O_{10}$: M, 222.08921.

***2,4-Bis(3-methyl-but-2-enyloxy)-benzoic acid –3-methyl-but-enyl ester* 98**



R_f = 0.50 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1710 (C=O), 3500 (OH). δ_H(300MHz; $CDCl_3$) 1.76 (9H, s, C[C*H*$_3$]$_2$), 1.79 (9H, s, C[C*H*$_3$]$_2$), 4.81 (6H, d, *J* 7.2, H-1′/1″/1‴),, 5.44 (3H, t, *J* 7.2, H-2′/2″/2‴), 6.36 (1H, dd, *J* 2.1, 8.7, H-5), 6.40 (1H, d, *J* 2.1, H-3), 7.74 (1H, d, *J* 8.7, H-6); δ_c(75 MHz; $CDCl_3$) 18.1 (3C), 25.8 (3C), 62.0 (3C), 103.1, 106.2, 107.8, 118.1(3C), 132.0, 139.9 (3C), 161.9, 163.5, 170.0; HRMS (EI) *m/z* Found: $[M]^+$, 358.21440 Calc. For $C_{22}H_{30}O_4$: M, 358.21441.

3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranoside derivative 108a



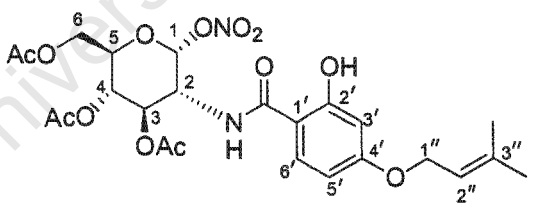
3,4,6-Tri-*O*-acetyl-*D*-glucal (2.00g, 0.0074 mmol) in acetonitrile (40 ml) was added to a mixture of sodium azide (0.72g, 0.11 mmol) and ammonium ceric sulphate (12.16g, 0.27 mmol). The resulting suspension was vigorously stirred at –15 °C for 14h and then cold diethyl ether (50 ml) and water (50 ml) were added. The organic layer was separated, washed with water, dried (Na_2SO_4) and concentrated to give a yellow syrup (2.60g, 94%). Trituration of the syrup with diethyl ether afforded **108a** as a white crystalline powder in (0.55g, 20%); mp = 131 -133 °C (from ethyl acetate-hexane); R_f = 0.70 (30% EtOAc/hexane);IRν_{max}(Nujol)/cm^{-1} 1052 (C-O), 1580 (C=C), 1664 (ONO_2), 1755 (C=O), 2120 (N_3); δ_H(400MHz; $CDCl_3$) 1.95 (3H, s, COC*H*$_3$) 1.98 (3H, s, COC*H*$_3$) 2.01

(3H, s, $COCH_3$) 3.75 (1H, dd, *J* 4.0, 10.8, H-2), 3.99 (1H, dd, *J* 2.4, 12.4, H-6a), 4.07 (1H, m, , H-5), 4.22 (1H, dd, *J* 2.4, 12.4, H-6b), 5.02 (1H, t, *J* 10.8, H-4), 5.29 (1H, t, *J* 10.8, H-3), 6.23 (1H, d, *J* 4.0, H-1); δ_c(100 MHz; $CDCl_3$) 23.3 (3C), 62.3, 63.9, 70.4, 73.3, 73.4, 99.2, 172.3, 172.4, 173.1; HRMS (EI) *m/z* Found: $[M]^+$, 376.08662 Calc. For $C_{12}H_{16}N_4O_{10}$: M, 376.08664.

General Method K for the preparation of compounds 106a-f and 110

Oxalyl chloride (4.05 mmol) and 4-drops of DMF were added to the solution of **97** (1.35 mmol) in 5ml of dichloromethane. The mixture was refluxed for 3h under nitrogen, cooled to room temperature and concentrated under reduced pressure. The residue was redissolved in dichloromethane (2.0 ml) and then added to a mixture of an amine (1.5 mmol) and polyvinyl pyridine (2.0g) in dichloromethane (3.0 ml). The resultant was stirred for 3h and then polymer-supported isocyanate (1.5 mmol/g) (3 equivalents to excess amine) was added and stirred a further 3h. Filtration of the reaction mixture followed by concentration under reduced pressure afforded the title compounds **106a-f** and **110**.

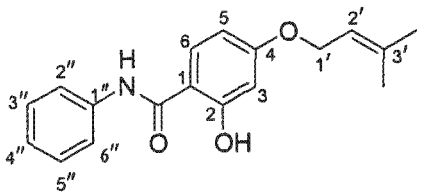
***3,4,6-Tri-O-acetyl-2-deoxy-α-D-glucopyranoside derivative* 110**



The conditions employed for the preparation of this compound were those described in General Method K, which gave **110** as a yellow oil (0.61g, 81%). R_f = 0.60 (10% EtOAc/hexane); IRν_{max}(Film)/cm^{-1} 1052 (C-O), 1600 (C=C), 1667 (ONO_2), 1747 (C=O); δ_H(400MHz; $CDCl_3$) 1.67 (3H, s, $C[CH_3]_2$), 1.70 (3H, s, $C[CH_3]_2$), 1.95 (3H, s, $COCH_3$) 1.98 (3H, s, $COCH_3$) 2.00 (3H, s, $COCH_3$) 3.76 (1H, dd, *J* 4.4, 10.0, H-2), 3.99 (1H, dd, *J* 4.0, 12.4, H-6a), 4.08 (1H, m, H-5), 4.22 (1H, dd, *J* 4.0, 12.4, H-6b), 4.71 (2H, d, *J* 6.8, H-1″), 5.02 (1H, t, *J* 10.0, H-4), 5.30 (1H, t, *J* 10.0, H-3), 5.35 (1H, t, *J* 6.8, H-2″), 6.22 (1H, d, *J* 4.4, H-1), 6.26 (1H, dd, *J* 2.4, 8.8, H-5′), 6.30 (1H, d, *J* 2.4, H-3′), 7.64

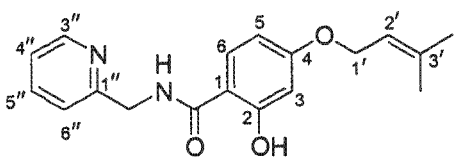
(1H, d, *J* 8.8, H-6′), 7.92 (1H, s, CON*H*); δ_c(100 MHz; $CDCl_3$) 20.4 (3C), 26.0, 29.6, 59.4, 61.5, 67.5, 68.5, 70.4, 70.5, 96.3, 103.0, 107.7, 115.5, 125.0, 127.9, 131.8, 157.0, 166.0, 169.4, 169.6, 170.2, 170.3; HRMS (EI) *m/z* Found: $[M]^+$, 554.17853 Calc. For $C_{24}H_{30}N_2O_{13}$: M, 554.17479.

2-Hydroxy-4-(3-methyl-but-2-enyloxy)-N-phenyl-benzamide **106c**



The conditions employed for the preparation of this compound were those described in General Method K, which gave 106c as a white solid (0.52g, 89%); mp = 116-117 °C (from ethylacetate-hexane); Rf = 0.62 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1600 (C=C), 1650 (C=O), δ_H(400MHz; $CDCl_3$) 1.77 (3H, s, C[C*H*$_3$]$_2$), 1.79 (3H, s, C[C*H*$_3$]$_2$), 4.60 (2H, d, *J* 7.2, H-1′), 5.64 (1H, t, *J* 7.2, H-2′), 6.56 (1H, d, *J* 2.4, H-3), 6.58 (1H, dd, *J* 2.4, 8.8, H-5), 7.10 (1H, m, H-4″), 7.34 (2H, d, *J* 8.8, H-2″/6″), 7.60 (1H, d, *J* 8.8, H-3″/5″), 8.01 (1H, d, *J* 8.8, H-6); δ_c(100 MHz; $CDCl_3$) 18.2, 25.7, 65.8, 100.4, 109.0, 113.5, 117.9, 120 (2C), 123.9, 129.0 (2C), 133.9, 138.6, 141.5, 158.6, 161.4, 163.9; HRMS (EI) *m/z* Found: $[M]^+$, 297.13651 Calc. For $C_{18}H_{19}NO_3$: M, 297.13649.

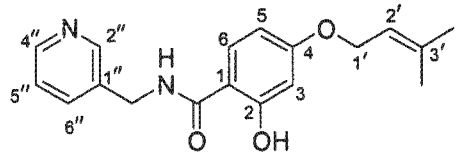
2-Hydroxy-4-(3-methyl-but-2-enyloxy)-N-pyridin-2-ylmethyl-benzamide **106d**



The conditions employed for the preparation of this compound were those described in General Method K, which gave **106d** as a yellow solid (0.60g, 99%); mp = 136-138 °C (from ethylacetate-hexane); Rf = 0.32 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1580 (C=N), 1600 (C=C), 1660 (C=O), δ_H(300MHz; $CDCl_3$) 1.74 (3H, s, C[C*H*$_3$]$_2$), 1.77 (3H, s, C[C*H*$_3$]$_2$), 4.43 (2H, s, PhC*H*$_2$N), 4.74 (2H, d, *J* 7.2, H-1′), 5.43 (1H, t, *J* 7.2, H-2′), 6.38 (1H, d, *J* 1.8, H-3), 6.50 (1H, dd, *J* 1.8, 8.7, H-5), 7.12 (1H, m, H-4″), 7.37

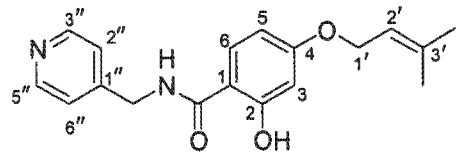
(1H, d, *J* 7.8, H-6″) 7.67 (1H, m, H-5″) 7.97 (1H, d, *J* 8.7, H-6), 8.84 (1H, t, *J* 5.1, H-3″); δ_c(75 MHz; $CDCl_3$) 18.2, 25.7, 45.3, 65.8, 100.2, 108.6, 112.6, 118.7, 122.4, 122.6, 133.5, 137.2, 138.8, 148.6, 157.4, 158.9, 161.8, 166.2; HRMS (EI) *m/z* Found: $[M]^+$, 312.14741 Calc. For $C_{18}H_{20}N_2O_3$: M, 312.14739.

2-Hydroxy-4-(3-methyl-but-2-enyloxy)-N-pyridin-3-ylmethyl-benzamide **106e**



The conditions employed for the preparation of this compound were those described in General Method K, which gave **106e** as a pale yellow solid (0.60g, 98%); mp = 132-133 °C (from ethylacetate-hexane); R_f = 0.33 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1040 (C-O), 1580 (C=N), 1600 (C=C), 1650 (C=O), δ_H(400MHz; $CDCl_3$) 1.73 (3H, s, C[C*H*$_3$]$_2$), 1.75 (3H, s, C[C*H*$_3$]$_2$), 4.47 (2H, d, *J* 6.4, PhC*H*$_2$N), 4.76 (2H, d, *J* 7.2, H-1′), 5.64 (1H, t, *J* 7.2, H-2′), 6.56 (1H, d, *J* 2.0, H-3), 6.35 (1H, dd, *J* 2.0, 8.8, H-5), 7.24 (1H, dd, *J* 1.6, 8.0, H-6″), 7.61 (1H, dd, *J* 4.8, 8.0, H-5″), 7.65 (1H, d, *J* 8.8, H-6), 8.20 (1H, t, *J* 6.4, N*H*CO) 8.49 (1H, dd, *J* 1.6, 4.8, H-4″) 8.52 (1H, d, *J* 1.6, H-2″); δ_c(100 MHz; $CDCl_3$) 18.0, 25.7, 29.6, 61.7, 102.9, 108.4, 118.3, 123.8, 132.9, 135.6, 135.9, 139.4, 148.8, 159.8, 163.6, 164.2 170.1; HRMS (EI) *m/z* Found: $[M]^+$, 312.14738 Calc. For $C_{19}H_{20}N_2O_3$: M, 312.14739.

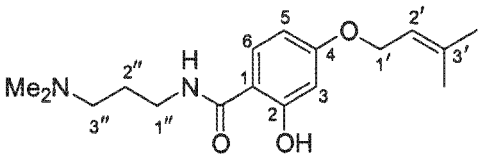
2-Hydroxy-4-(3-methyl-but-2-enyloxy)-N-pyridin-4-ylmethyl-benzamide **106f**



The conditions employed for the preparation of this compound were those described in General Method K, which gave **106f** as a pale yellow solid (0.38g, 62%); mp = 134-136 °C (from ethylacetate-hexane); R_f = 0.34 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1580 (C=N), 1600 (C=C), 1650 (C=O), δ_H(400MHz; $CDCl_3$) 1.75 (3H, s, C[C*H*$_3$]$_2$), 1.78 (3H, s, C[C*H*$_3$]$_2$), 4.54 (1H, d, *J* 6.4, PhC*H*$_2$N), 4.79 (2H, d, *J* 7.2, H-1′), 5.34 (1H, t, *J* 7.2, H-2′), 6.56 (1H, d, *J* 2.0, H-3), 6.58 (1H, dd, *J* 2.0, 8.8, H-5), 7.24 (2H,

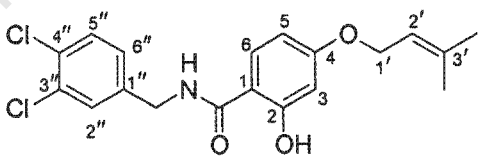
d, *J* 6.0, H-2″/6″), 7.70 (1H, d, *J* 8.8, H-6), 7.88 (1H, t, *J* 6.4, N*H*CO) 8.58 (2H, d, *J* 6.0, H-3″/5″); δ_c(100 MHz; $CDCl_3$) 18.1, 25.7, 29.7, 42.6, 61.7, 103.0, 108.2, 118.3, 121.3, 122.5, 131.8, 139.6, 146.2, 149.8, 159.8, 163.6, 170.1; HRMS (EI) *m/z* Found: $[M]^+$, 312.14743 Calc. For $C_{18}H_{20}N_2O_3$: M, 312.14739.

N-(3-Dimethylamino-propyl)-2-hydroxy-4-(3-methyl-but-2-enyloxy)-benzamide **106a**



The conditions employed for the preparation of this compound were those described in General Method K, which gave **106a** as a yellow oil (0.51g, 86%); R_f = 0.23 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1060 (C-O), 1660 (C=O); δ_H(300MHz; $CDCl_3$) 1.76 (3H, s, C[C*H*$_3$]$_2$), 1.79 (3H, s, C[C*H*$_3$]$_2$), 1.85 (2H, quint, *J* 6.6, H-2″), 2.44 (6H, s, N[Me]$_2$), 2.63 (2H, t, *J* 6.6, H-3″), 3.40 (2H, t, *J* 6.6, H-1″), 4.81 (2H, d, *J* 7.2, H-1′), 5.44 (1H, t, *J* 7.2, H-2′), 6.36 (1H, dd, *J* 2.1, 8.7, H-5), 6.40 (1H, d, *J* 2.1, H-3), 7.74 (1H, d, *J* 8.7, H-6); δ_c(75 MHz; $CDCl_3$) 18.1, 25.8, 27.6 (2C), 38 1, 44.8, 57.8, 62.0, 103.1, 106.2, 107.8, 118.1, 132.0, 139.9, 161.9, 163.5, 171.0; HRMS (EI) *m/z* Found: $[M]^+$, 306.19351 Calc. For $C_{17}H_{26}N_2O_3$: M, 306.19434.

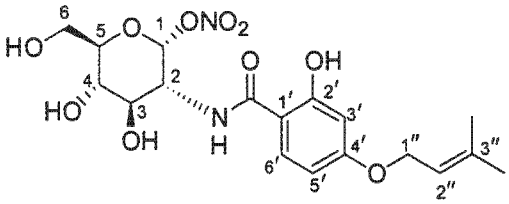
N-(3,4-Dichloro-benzyl)-2-hydroxy-4-(3-methyl-but-2-enyloxy)-benzamide **106b**



The conditions employed for the preparation of this compound were those described in General Method K, which gave **106b** as a pale yellow solid (0.30g, 41%); mp = 123-124 °C (from ethylacetate-hexane); R_f = 0.52 (30%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1660 (C=O); δ_H(300MHz; $CDCl_3$) 1.76 (3H, s, C[C*H*$_3$]$_2$), 1.79 (3H, s, C[C*H*$_3$]$_2$), 4.48 (2H, s, PhC*H*$_2$N), 4.81 (2H, d, *J* 7.2, H-1′), 5.44 (1H, t, *J* 7.2, H-2′), 6.36 (1H, dd, *J* 2.1, 8.7, H-5), 6.40 (1H, d, *J* 2.1, H-3), 6.81 (1H, dd, *J* 3.0, 9.0, H-6″), 7.05 (1H, d, *J* 3.0, H-2″), 7.12 (1H, d, *J* 9.0, H-5″) 7.74 (1H, d, *J* 8.7, H-6); δ_c(75 MHz; $CDCl_3$) 18.1, 25.8,

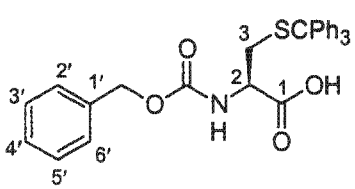
48.2, 62.0, 103.1, 106.2, 107.8, 118.1, 127.0, 128.5, 130.3 132.6, 135.1, 141,8, 132.0, 139.9, 161.9, 163.5, 170.0; HRMS (EI) *m/z* Found: $[M]^+$, 379.074211 Calc. For $C_{19}H_{19}Cl_2NO_3$: M, 379.07420.

***2-deoxy-α-D-glucopyranoside derivative* 111**



A solution of **110** (0.21g, 038 mmol) in 10% NaOMe-MeOH (5.0 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with Amberlite IRC-50 ion exchange resin. Filtration and evaporation of the solvent under reduced pressure furnished **111** as thick dark brown oil (0.14g, 84%). R_f = 0.13 (30%EtOAc/hexane); IRν_{max}(Film)/cm^{-1} 1061 (C-O), 1600 (C=C), 1650 (ONO_2), 3415 (br, OH/ CONH); δ_H(300MHz; MeOH): 1.79 (6H, s, C[CH_3]$_2$), 3.68 (1H, dd, *J* 4.2, 9.6, H-2), 3.86 (1H, dd, 4.2, 12.0, H-6a), 4.13 (1H, m, H-5), 4.30 (1H, 4.2, 12.0, H-6b), 4.82 (2H, d, *J* 7.2, H-1′), 5.02 (1H, t, *J* 9.6, H-4), 5.30 (1H, t, *J* 9.6, H-3), 5.46 (1H, t, *J* 7.2, H-2″), 6.28 (1H, d, *J* 4.2, H-1), 6.32 (1H, dd, *J* 2.1, 8.1, H-5′), 6.34 (1H, d, *J* 2.1, H-3′), 7.66 (1H, d, *J* 8.1, H-6′), 7.96 (1H, s, CONH); δ_c(75 MHz; MeOH) 25.8, 29.7, 57.4, 62.7, 68.2, 69.6, 71.6, 73.1, 97.3, 103.0, 109.0, 115.9, 125.9, 129.6, 131.8, 156.0, 165.0, 168.8; HRMS (EI) *m/z* Found: $[M]^+$, 428.14311 Calc. For $C_{18}H_{24}N_2O_{10}$: M, 428.14310.

2S-Benzyloxycarbonylamino-3-(methylene-triphenyl-sulfanyl)-propionic acid 149

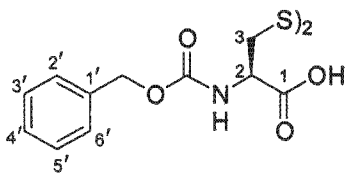


A vigorous stirred solution of S-trityl-L-cysteine (1.0g, 2.78 mmol) , in 1M sodium hydroxide (2.80 ml) and dry dioxane (5.0 ml) was cooled to 0 °C in an ice-salt bath, and two drops of phenolphthalein indicator were added. Benzyl chloroformate (0.69g, 0.56 ml, 3.89 mmol) and 1M sodium hydroxide (7.5 ml) were then added dropwise from

separate syringes, at such a rate that the solution remained alkaline. Towards the end of the addition a white solid precipitated which masked the colour of the indicator, but the addition of the reagents was maintained at approximately the same rate. The addition was completed within 10 min and, after stirring for an additional 15 min at room temperature , the mixture was diluted with water (7.0 ml). The solid did not dissolve, and dioxane (2.0 ml) was added. To the resulting solution (pH 7.5) was added 2M hydrochloric acid (2.0 ml). The dioxane was evaporated rapidly under reduced pressure, and the residual white gum was extracted into ethyl acetate (4 × 10 ml) . The organic extract was washed with dilute brine (2 × 10 ml), followed by saturated brine, and was then dried and evaporated to leave a light , golden syrup. This was dissolved in dichloromethane (10 ml) and the solvent reevaporated. Upon removal of the last traces of solvent, under high vacuum, the product became white foam. The crude product was dissolved in ether (15 ml) and treated with dicyclohexylamine (0.56 ml, 2.78 ml). A gelatinous precipitate formed rapidly, and the mixture soon became solid. Additional ether (10 ml) was added ,and the mixture was left for 3 h at 5°C with occasional scratching. The crystals were collected, washed with cold ether ,and dried under vacuum to give a white solid. A suspension of the dicyclohexylamine salt in ether was shaken with 20% aqueous citric acid (10 ml) until all the solid dissolved. The ether layer was then washed water (4 × 10 ml), followed by saturated brine (10 ml), dried, and evaporated to a leave white foam (0.99g, 72%).

mp = 112-114 °C (from ether) (Lit. 114-115 °C);[210] IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1530 (N-H), 1590 (C=C), 1700 (C=O), 2400-3500 (COOH), 3360 (NH); δ_H(300 MHz; $CDCl_3$) 2.67 (2H, br d ,H-3), 4.23 (1H, m, H-2), 5.07 (2H, s, PhC*H*$_2$), 5.22 (1H, br s, OCON*H*), 7.33 (20H, m, H-2/3/4/5/6′, $SCPh_3$).

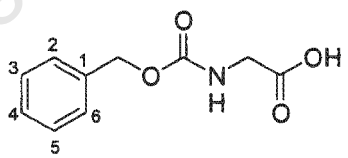
2S-Benzyloxycarbonylamino-3-(2-benzyloxycarbonyl-2-carboxy-ethyldisulfanyl)-propionic acid 120



A solution of *L*-cystine (4.00g, 16.67 mmol) in 1M sodium hydroxide (50 ml) was cooled to 5°C and benzyl chloroformate (5.69g, 4.78 ml, 33 mmol) and 1M sodium

hydroxide (25 ml) were added separately in portions, with vigorous stirring, at such a rate that the pH remained between 10 and 12. Addition of the reagents took 2 h, and a white precipitate was formed as the reaction progressed. The cooling bath was then removed and the mixture was allowed to warm to room temperature, with continued stirring. Most of the solid dissolved to give a slightly cloudy solution (pH 9.2), which was transferred to a separatory funnel and washed with ether (2 × 20ml). The aqueous layer was then quickly added to 4M hydrochloric acid (2 ml) and the resultant gummy precipitate extracted into ethyl acetate (3 × 50ml). The combined extracts were dried and evaporated to leave a pale amber syrup, which became a white foam upon removal of the last traces of the solvent under reduced pressure. The product was dried in vacuo and then triturated with (50 ml) chloroform. The foam dissolved initially, and the product rapidly crystallized out. The mixture was cooled at 5°C overnight and the crystals were the collected. The product was washed with cold chloroform and dried (5.45g, 65%).mp 116-118 °C (from chloroform-ethanol) (Lit. 113-114 °C);[210] IRν_{max}(Nujol)/cm^{-1} 1530 (N-H), 1680 (C=O), 1700 (C=O), 2200-3500 (COOH), 3335 (NH); δ_H(300 MHz; $CDCl_3$) 2.93 (4H, t, *J* 9.9 Hz, H-2), 4.29 (2H, d, *J* 9.9, H-3), 5.04 (4H, s, PhC*H*$_2$), 7.33 (10H, m, H-2/3/4/5/6′), 7.68 (2H, br s, OCON*H*).

benzyloxycarbonylamino-acetic acid 133

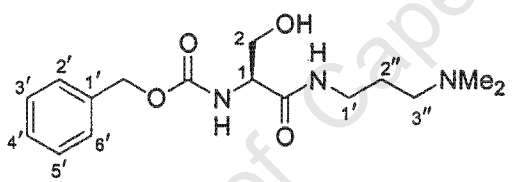


To a stirred solution of glycine (3.75g, 0.05 mmol) in 1M NaOH (50 ml) at 0°C was added a solution of benzyl chloroformate (9.38g, 7.85 ml, 0.055 mol) in toluene (50 ml) followed by 1M NaOH (25 ml). The reaction mixture was stirred vigorously for 1 hour. The toluene layer was separated and the aqueous solution was cooled to room temperature and then acidified to pH~2. The precipitate was filtered off, washed with cold water and dried to afford **133** as a white crystalline powder (8.46g, 81%). mp 119-120 °C (Lit mp 119 °C);[267] ; R_f = 0.70 (MeOH/DCM/17%NH_4OH 2:2:1); δ_H(300 MHz; $CDCl_3$) 3.86 (2H, s, NHC*H*$_2$COOH), 5.10 (2H, s, PhC*H*$_2$O), 7.33 (5H, m, H-2/3/4/5/6).

General Method L for the preparation of compounds 116, 134, 142 and 147

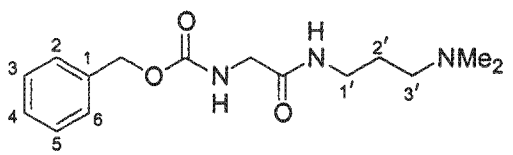
To a suspension of PS-EDC (20.0 mmol, 1.4 mmol/g) in chloroform (10 ml), acid (8.36 mmol), 1-hydroxybenzotriazole and amine (8.36 mmol) were added. The reaction mixture was shaken for 24 hours at room temperature and then filtered. The resin was washed with chloroform (3 × 5ml) and the combined filtrate was concentrated under reduced pressure to give a mixture of the product and 1-hydroxybenzotriazole. The mixture was re-dissolved in dichloromethane and then MP-carbonate (16.72 mmol, 3.0 mmol/g) was added. The resultant was shaken at room temperature for 2 h, filtered, washed with dichloromethane (3 × 5 ml), concentrated to give the title compounds **116**, **134**, **142** and **147**.

***[1S-(3-Dimethylamino-propylcarbamoyl)-2-hydroxy-ethyl]-carbamicacid benzyl ester* 142**



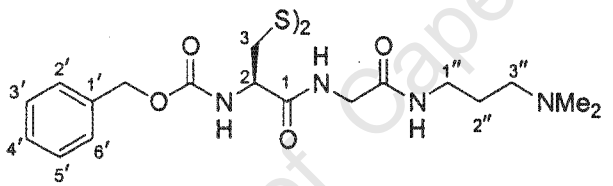
The conditions employed for the preparation of this compound were those described in General Method L, which afforded **142** as a cream solid (0.52g, 96%). mp = 89-90 °C (from chloroform-methanol); R_f = 0.43 (MeOH/DCM/17%NH_4OH 2:2:1); R_{ax}(Nujol)/cm^{-1} 1050 (C-O), 1540 (N-H), 1635 (C=C), 1685 (C=O), 3360 (OH); δ_H(300 MHz; $CDCl_3$) 1.65 (2H, quint. *J* 6.0, H-2″), 2.18 (6H, s, N[Me]]$_2$), 2.38 (2H, t, *J* 6.0, H-3″), 3.35 (2H, t, *J* 6.0, H-1″), 3.65 (1H, dd, *J* 3.5, 7.5, H-2a), 4.05 (1H, dd, *J* 3.5, 7.5, H-2b), 4.20 (1H, m, H-1); 5.12 (2H, PhCH_2), 5.82 (1H, br s, CHCON*H*CH_2), 7.34 (5H, m, 1/2/3/4/5′), 7.65 (1H, br s, N*H*CH);δ_c(75 MHz; $CDCl_3$) 25.7, 39.2, 45.1, 56.1, 58.2, 63.0, 67.1, 128.1 (2C), 128.2, 128.5 (2C), 136.1, 158.1, 170.1; LRMS (EI) *m/z* (%), 323 ($[M]^+$, 2), 239 ($[M\text{-}N(CH_2)_3N(CH_3)_2]^+$, 4), 91 $(CH_2Ph]^+$, 100).

[(3-Dimethylamino-proylcarbamoyl)-methyl]-carbamic acid benzyl ester 118



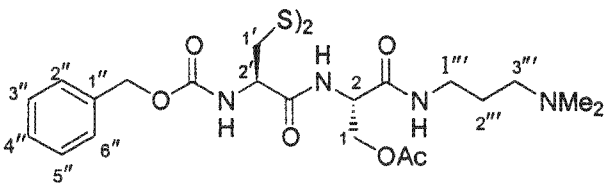
The conditions employed for the preparation of this compound were those described in General Method L, however, 1.40 mmol (PS-EDC, 1.4 mmol/g), 1.90 mmol (benzoyloxyglycine **133**), and 0.96 mmol (dimethylaminopropylamine) were used, which gave **134** as a white solid (0.59g, 91%). mp 45-47 °C (from ethyl acetate-hexane) (Lit. mp 46-48 °C);[198] δ_H(300 MHz; $CDCl_3$) 1.65 (2H, quint. *J* 6.0, H-2′), 2.17 (6H, s, N[Me]$_2$), 2.33 (2H, t, *J* 6.0, H-3′), 3.32 (2H, br s, CONHC*H*$_2$), 3.80 (2H, t, *J* 6.0, H-1′), 5.10 (2H, s, PhC*H*$_2$O), 5.60 (1H, br s, CHCON*H*CH$_2$), 7.33 (5H, m, 2/3/4/5/6), 7.70 (1H, br s, OCON*H*CH).

(2-(2-Benzyloxycarbonylamino-2-{[(3-dimethylamino-propylcarbamoyl)-methyl]-carbamoyl}-ethyldisulfanyl)-1-{[(3-dimethylamino-propylcarbomyl)-methyl] carbamoyl}-ethyl)-carbamic acid benzyl ester 116



The conditions employed for the preparation of this compound were those described in general method L, which afforded **116** as a white powder 125-128 °C (from chloroform-hexane) (Lit. mp 126-128 °C);[198] δ_H(300 MHz; $CDCl_3$) 1.62 (4H, quint. *J* 6.6, H-2″), 2.22 (12H, s, N[Me]$_2$), 2.32 (4H, t, *J* 6.6, H-3″), 3.02 (4H, m, H-3), 3.30 (4H, m, , H-1″), 3.87 (4H, m, NHC*H*$_2$CO), 4.80 (2H, m, H-2), 5.12 (4H, s, PhC*H*$_2$O), 6.15 (2H, br s,CH$_2$CON*H*CH$_2$), 7.33 (10H, m, H-2′/3′/4′/5′/6′), 7.90 (2H, br s, OCON*H*CH).

Acetic acid 2-(3-{2-[2-acetoxy-1-(3-dimethylamino-propylcarbonyl)-ethylcarbamoyl]-2-benzyloxycarbonylamino-ethyldisulfanyl}-2-benzyloxycarbonylamino-propionylamnio)-2-(3-dimethylamino-proylcarbamoyl)-ethylester **147**

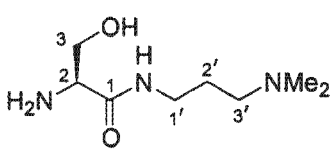


The conditions employed for the preparation of this compound were those described in general method L, which gave **147** as a yellow gum (0.16g, 74 % Yield; R_f = 0.17 (MeOH/DCM/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1550 (N-H amide II), 1600 (C=C), 1650 (C=O), 1710 (C=O) 1720 (C=O), 3080 (Ar-H), 3300 (NH); δ_H(300 MHz; $CDCl_3$) 1.89 (4H, quint. *J* 6.9, H-2‴), 2.00 (6H, s, CH_3CO) 2.66 (12H, s, N[Me]$_2$), 2.95 (4H, m, H-3‴), 3.02 (4H, m, H-1‴), 3.32 (4H, m, H-1′), 3.70 (2H, t, *J* 6.9, H-2′), 3.97 (2H, m, H-2), 4.43 (4H, m, H-1), 5.00 (4H, s, $PhCH_2O$), 6.15 (2H, br s, $CH_2CONHCH_2$), 7.33 (10H, m, H-2″/3″/4″/5″/6″), 7.90 (2H, br s, OCONHCH); δ_c(75 MHz; $CDCl_3$) 23.1 (2C), 24.4 (2C), 36.0 (2C), 40.0 (2C), 42.9 (4C), 55.0 (2C), 55.8 (2C), 62.7 (2C), 66.6 (2C), 69.0 (2C), 128.0 (4C), 128.2 (2C), 128.5 (4C), 141.0 (2C), 156.0 (2C), 170.1 (2C), 171.8 (2C), 175 (2C); HRMS (EI) *m/z* Found: M^+, 934.39279 Calc. For $C_{42}H_{62}N_8O_{12}S_2$: M, 934.39286.

General Method M for the preparation of compounds 109, 135, 143 and 145

To a stirred solution of azide or Cbz-protected amine (0.06 mmol) in MeOH (10 ml) at room temperature was added 10% Pd/C (0.050g). After being stirred for 2 hours under hydrogen, the mixture was filtered through celite and concentrated to give the title compounds **109**, **135**, **143** and **145**

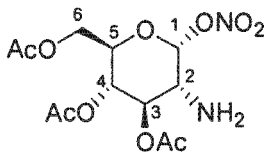
***2S-Amino-N-(3-dimethylamino-propyl)-3-hydroxy-propionamide* 143**



The conditions employed for the preparation of this compound were those described in general method M, which gave **143** as a yellow oil (0.26g, 100%). R_f = 0. 25 (MeOH/DCM/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1560 (N-H amide II), 1640 (C=O), 3320 (O-H/ NH); δ_H(300 MHz; $CDCl_3$) 1.64 (2H, quint. *J* 6.8, H-2′), 2.21 (6H, s, N[Me]$_2$), 2.40 (2H, t, *J* 6.8, H-3′), 3.16 (3H, m, H-2, H-1′), 4.84 (2H, m, H-3), 5.40 (1H, br s, NH_2), 7.92 (1H, br s, CONH); δ_c(75 MHz; $CDCl_3$) 27.3, 35.3, 45.1 (2C),

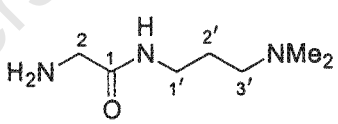
57.2, 61.0, 67.1, 176.1; LRMS (EI) *m/z* (%), 189 ($[M]^+$, 30), 173 ($[M\text{-}NH_2]^+$, 11), 129 ($[CON(CH_2)_3N(CH_3)_2]^+$, 51), 58 ($[C_3H_8N]^+$, 100).

3,4,6-Tri-O-acetyl-2-amino-2-deoxy-α-D-glucopyranoside derivative 109



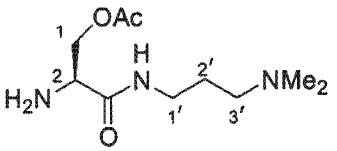
The conditions employed for the preparation of this compound were those described in general method M, which gave as a white crystalline powder (0.18g, 98%). mp = 99-102 °C (from methanol-hexane); R_f = 0.18 (10%EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1037 (C-O), 1664 (ONO_2), 1747 (C=O), 3369 (N-H), 3468 (N-H); δ_H(400MHz; $CDCl_3$) 1.95 (3H, s, $COCH_3$) 1.98 (3H, s, $COCH_3$) 2.01 (3H, s, $COCH_3$) 3.76 (1H, dd, *J* 4.4, 10.8, H-2), 3.98 (1H, dd, *J* 2.4, 12.4, H-6a), 4.07 (1H, m, H-5), 4.22 (1H, dd, *J* 2.4, 12.4, H-6b), 5.02 (1H, t, *J* 10.8, H-4), 5.29 (1H, t, *J* 10.8, H-3), 6.23 (1H, d, *J* 4.4, H-1); δ_c(100 MHz; $CDCl_3$) 20.4 (3C), 59.4, 61.5, 67.5, 70.4, 70.5, 96.3, 169.4, 169.6, 170.3; HRMS (EI) *m/z* Found: $[M+H]^+$,351.09623 Calc. For $C_{12}H_{18}N_2O_{10}$: M, 351.09615.

2-Amino-N-(3-dimethylamino-propyl)-acetamide 119



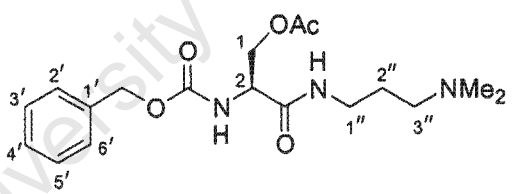
The conditions employed for the preparation of this compound were those described in general method M, which afforded **135** as a yellow oil (0.41g, 100%); R_f = 0.22 (MeOH/DCM/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1550 (N-H), 1660 (C=O), 3250 (NH), 3370 (NH); δ_H(400 MHz; $CDCl_3$) 1.64 (2H, quint. *J* 6.9, H-2′), 2.17 (6H, s, N[Me]$_2$), 2.29 (2H, t, *J* 6.9, H-3′), 3.18 (2H, s, H-2), 3.29 (2H, t, *J* 6.9, H-1′), 5.30 (1H, br s, NH_2CH), 7.70 (1H, br, $CHCONHCH_2$); δ_c(75 MHz; $CDCl_3$) 26.9, 39.3, 45.3 (2C), 46.4, 57.13, 172.8; LRMS (EI) *m/z* (%), 159 ($[M]^+$, 10), 129 ($[CON(CH_2)_3N(CH_3)_2]^+$, 10), 101 ($[M\text{-}NH_2CHCO]^+$, 84), 58 ($[C_3H_8N]^+$, 100).

Acetic acid –2S-amino-2-(3-dimethylamino-propylcarbamoyl)-ethyl ester **145**



The conditions employed for the preparation of this compound were those described in general method M, which afforded **145** as a yellow oil (0.34g, 100%). R_f = 0.20 (MeOH/DCM/17%NH_4OH 2:2:1); IRν_{max}(Film)/cm^{-1} 1050 (C-O), 1250 (C-O), 1550 (N-H amide II), 1660 (C=O), 3275 (NH), 3300 (NH); δ_H(300 MHz; $CDCl_3$) 1.68 (2H, quint. *J* 6.9, H-2′), 2.01 (3H, s, $COCH_3$), 2.31 (6H, s, $N[Me]_2$), 2.51 (2H, t, *J* 6.9, H-3′), 3.27 (2H, t, *J* 6.9, H-1′), 3.64 (1H, m, H-2), 3.89 (1H, dd, *J* 4.2, 11.1, H-1a), 4.39 (1H, dd, *J* 4.2, 11.1, H-1b), 5.87 (2H, br s, NH_2CH), 7.78 (1H, br s, CONHCH); δ_c(75 MHz; $CDCl_3$) 23.0, 30.6, 37.9, 44.5 (2C), 55.1, 56.9, 62.6, 169.0, 170.9; LRMS (EI) *m/z* (%), 231 ($[M]^+$, 6.8), 129 ($[CON(CH_2)_3N(CH_3)_2]^+$, 51), 58 ($[C_3H_8N]^+$, 100).

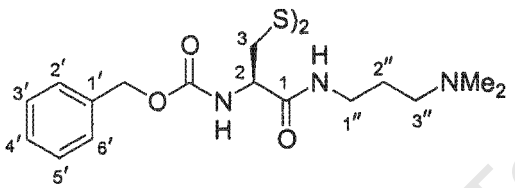
Acetic acid 2S-benzyloxycarbonylamino-2-amino-2-(3-dimethylamino-propylcarbamoyl)-ethyl ester **144**



A mixture of **142** (0.50g, 1.55 mmol), acetylchloride (0.25g, 0.22ml, 3.10 mmol), pyridine (0.25g, 0.25ml, 3.10 mmol) and PS-DMAP (0.31 mmol, 1.5 mmol/g) in toluene (5.0ml) was heated at reflux for 16 h. The reaction mixture was cooled to room temperature. Then, MP-carbonate (0.88g, 2.32 mmol, 3.5 mmol/g) was added and the resultant stirred for 2 h. The reaction mixture was filtered and concentrated under reduced pressure to furnish **144** as a yellow oil (0.55g, 97%). R_f = 0.47 (MeOH/DCM/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1530 (N-H), 1600 (C=C), 1660 (C=O), 1720 (C=O), 3080 (Ar-H), 3320 (CONH); δ_H(300 MHz; $CDCl_3$) 1.65 (2H, quint. *J* 6.6 , H-2″), 2.01 (3H, s, $COCH_3$), 2.26 (6H, s, $N[Me]_2$), 2.47 (2H, t, *J* 6.6, H-3″), 3.34 (2H, t, *J* 6.6, H-1″), 4.38 (3H, m, H-1a/1b/2), 5.11 (2H, s, $PhCH_2O$); 5.82

(1H, br s, CHCON*H*CH_2), 7.34 (5H, m, H-2/3/4/5/6′), 8.00 (1H, br s,OCON*H*CH); δ_c(75 MHz; $CDCl_3$) 20.7, 29.6, 39.2, 44.7 (2C), 54.3, 57.9, 64.4, 67.2, 128.1 (2C), 128.2, 128.5 (2C) 136.1, 156.0, 168.4, 170.7; LRMS (EI) *m/z* (%), 365 ($[M]^+$, 23.6), 129 ([M-$N(CH_2)_3N(CH_3)_2]^+$, 43.2), 91 ($[CH_2Ph]^+$, 67), 58 ($[C_3H_8N]^+$, 100).

(2-(2-Benzyloxycarbonylamino-2-{[(3-dimethylamino-propylcarbamoylcarbamoyl}-ethyldisulfanyl)]-1-{[(3-dimethylamino-propylcarbomyl) ethyl]- carbamic acid benzyl ester **151**

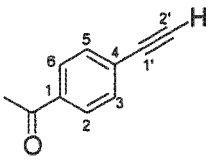


Compound **150** (0.050g, 0.086 mmol) and iodine (20mg, 0.086 mmol) were dissolved in methanol (1.0 ml) and the resultant stirred at room temperature for one hour. The reaction mixture was cooled in an ice-water bath and decolorized by dropwise addition of one molar solution of sodium thiosulphate in water. The mixture was diluted with water (5.0 ml) and the precipitated material filtered off. The filtrate was dried (magnesium sulphate) and concentrated to afford **151** a yellow oil. (0.033g, 57%) R_f = 0.17 (MeOH/DCM/17%NH_4OH 2:2:1); IRν_{max}(Nujol)/cm^{-1} 1050 (C-O), 1550 (N-H amide II), 1640 (C=O), 1720 (C=O); δ_H(300 MHz; $CDCl_3$) 1.65 (4H, quint. *J* 6.3, H-2″), 2.22 (12H, s, N([Me]$_2$), 2.37 (4H, t, *J* 6.3, H-3″), 3.19 (8H, m, , H-1″/3), 4.05 (2H, m, H-2), 5.20 (4H, br s, Ph*C*H_2O); 5.82 (2H, br s, CHCON*H*CH), 7.26 (10H, m, H-2′/3′/4′/5′/6′), 8.65 (2H, br s, OCON*H*CH); δ_c(75 MHz; $CDCl_3$) 27.0 (2C), 37.0 (2C), 39.6 (2C), 45.1 (4C), 57.3 (2C), 57.9 (2C), 69.0, (2C), 128.2 (2C), 128.5 (4C) 141.0 (2C), 156.5 (2C), 176.1 (2C); HRMS (EI) *m/z* Found: M^+, 676.30768 Calc. For $C_{32}H_{42}N_6O_6S_2$: M, 675.87681.

General Method N for the preparation of compounds 58 and 177

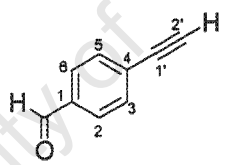
To a solution of trimethylsilyl protected acetophenone or benzaldehyde (4.50g, 20.83mmol) in methanol at room temperature was added 1M potassium hydroxide solution (20ml) and the mixture was stirred at room temperature for 1 hour and then the solvent removed under reduced pressure. The residue was extracted with ether (3×25ml). The combined ether extracts were concentrated to give the title compounds **58** and **177**.

1-(4-Ethynyl-phenyl)-ethanone 177



The conditions employed for the preparation of this compound were those described in General Method N, which gave **177** as a yellow solid (1.30g, 78%). m.p. 70-72°C (from ethylacetate-hexane) (Lit. mp 69-70 °C)[268]; R_f = 0.43 (30% EtOAc/ Hexane); δ_H(300MHz; $CDCl_3$) 2.59 (3H, s, $COCH_3$), 3.10 (1H, s, H-2′), 7.64 (1H, dd, *J* 1.5, 7.8, H-3/5), 8.03 (1H, dd, *J* 1.5, 7.8, H-2/6); δ_c(75MHz, $CDCl_3$) 27.3, 78.6, 83.5, 123.1, 128.4(2C), 132.3(2C), 136.1, 197.2.

4-Ethynyl-benzaldehyde 58

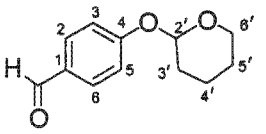


The conditions employed for the preparation of this compound were those described in General Method N, which gave **58** as a yellow solid (1.30g, 78%). m.p. 87-89°C (from ethylacetate-hexane) (Lit. mp 88-90 °C)[265]; R_f = 0.49 (30% EtOAc/ Hexane); δ_H(400MHz; $CDCl_3$) 3.29 (1H, s, H-2′), 7.62 (1H, d, *J* 6.8, H-3/5), 7.82 (1H, d, *J* 6.8, H-2/6); δ_c(75MHz, $CDCl_3$) 81.0, 82.6, 128.3, 129.4(2C), 132.7(2C), 136.0, 191.3.

General Method O for the preparation of compounds 45a-b

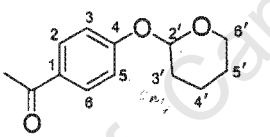
A solution of 4-hydroxyacetophenone or 4-hydroxybenzaldehyde (36.72 mmol), pyridinium-p-toluene sulfonate (1.47 mmol) and 3,4-dihydro-2H-pyran (58.76 mmol) in dry DCM (50 ml) was stirred for 16h at room temperature. The reaction mixture was washed with 1M sodium carbonate (50ml x 3), water (50ml), brine (50ml) and concentrated to give title compounds **45a-b**.

4-(Tetrahydro-pyran-2-yloxy)-benzaldehyde 45a



The conditions employed for the preparation of this compound were those described in General Method O, which gave **45a** as a yellowish oil (7.26g, 96%). R_f = 0.52 (30% EtOAc/ Hexane); δ_H(400 MHz; $CDCl_3$) 1.65 (3H, m, H-5a′/4′), 1.88 (2H, m, H-3a′/5b′), 2.01 (1H, m, H-3b′), 3.65 (1H, m, H-6a′), 3.87 (1H, m, H-6b′), 5.54 (1H, t, *J* 3.2, H-2′), 7.18 (2H, dd, *J* 8.8, H-3/5), 8.20 (2H, d, *J* 8.8, H-2/6), 10.3 (1H, s, C*H*O); δ_c(100 MHz; $CDCl_3$) 18.4, 25.0, 30.0, 62.0, 96.2, 116.9 (2C), 130.4, 131.6 (2C), 163.6, 188.4.

1-[4-(Tetrahydro-pyran-2-yloxy)-phenyl]-ethanone 45b



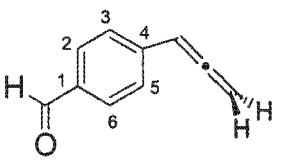
The conditions employed for the preparation of this compound were those described in General Method O, which gave **45b** as a yellow solid (8.05g, 83%). m.p. 85-87°C (from ethylacetate-hexane) (Lit. mp 84-86 °C)[269]; R_f = 0.55 (30% EtOAc/ Hexane); δ_H(400 MHz; $CDCl_3$) 1.68 (3H, m, H-5a′/4′), 1.87 (2H, m, H-3a′/5b′), 2.06 (1H, m, H-3b′), 2.54 (1H, s, COCH_3), 3.63 (1H, m, H-6a′), 3.87 (1H, m, H-6b′), 5.54 (1H, t, *J* 3.2, H-2′), 7.08 (2H, dd, *J* 2.0, 6.8, H-3/5), 7.92 (2H, d, *J* 2.0, 6.8, H-2/6); δ_c(100 MHz; $CDCl_3$) 18.5, 25.0, 26.3, 30.1, 62.0, 96.1, 115.9 (2C), 130.4 131.0 (2C), 131.6 (2C), 161.0, 196.8;

General Method P for the preparation of compounds 52 and 178a-d, 180a-c

A mixture of acetylenic chalcone (1.0 mmol), paraformaldehyde (2.5 mmol), amine (2.0 mmol) and anhydrous cuprous bromide or cupric chloride (0.5 mmol) in dry 1,4-dioxane (5.0 ml) was heated under reflux at 100°C for 3 h. The reaction mixture was cooled to room temperature and then filtered. The filtrate was poured into water and extracted with ether (3×10 ml). The combined ether extracts were concentrated to give a light brown

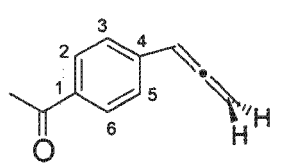
solid residue, which was subjected to column chromatography on silica gel (10% EtOAc: hexane and MeOH:$CHCl_3$: 17% NH_4OH 2: 2: 1) to afford the title compounds **52, 178a-d, 180a-c**.

4-Propa-1,2-dienyl-benzaldehyde **178a**



The conditions employed for the preparation of this compound were those described in General Method P, which gave **178a** as a brown oil (0.012g, 14%); IRν_{max}(Nujol)/cm^{-1} 1595 (C=C), 1680 (C=O), 1960 (C=C=C); δ_H(300 MHz; $CDCl_3$) δ 5.23 (2H, d, *J* 6.6, CH=C=CH_2), 6.22 (1H, t, *J* 6.6, C*H*=C=CH_2), 7.44 (2H, d, *J* 8.4, H-3/5), 7.81 (2H, d, *J* 8.4 , H-2/6), 9.96 (1H, s, C*H*O); δ_c(75 MHz; $CDCl_3$) 79.4, 93.7, 127.1 (2C), 130.1 (2C), 135.0, 140.8, 191.6, 211.0; HRMS (EI) *m/z* Found: M^+, 144.05753 Calc. For $C_{10}H_8O$: M, 144.05751.

1-(4-Propa-1,2-dienyl-phenyl)-ethanone **178b**



The conditions employed for the preparation of this compound were those described in General Method P, which gave **178b** as a light-yellow oil (0.016g, 35%); IRν_{max}(Nujol)/cm^{-1} 1600 (C=C), 1660 (C=O), 1955 (C=C=C); δ_H(400 MHz; $CDCl_3$) 2.60 (6H, s, CH_3) 5.21 (2H, d, *J* 6.8 , CH=C=CH_2), 6.20 (1H, t, *J* 6.6, C*H*=C=CH_2), 7.36 (2H, d, *J* 8.4, H-3/5), 7.89 (2H, d, *J* 8.4, H-2/6); δ_c(100 MHz; $CDCl_3$) 23.4, 79.2, 93.6, 126.7 (2C), 128.8 (2C), 135.6, 139.2, 197.4, 210.8; LRMS (EI) *m/z* (%), 158 ($[M]^+$, 35), 143 ($[M\text{-}CH_3]^+$, 41), 129 (([M-CH_3-CH_2]+, 2), 115 ([M-CH_3-2CH_2]+, 26)

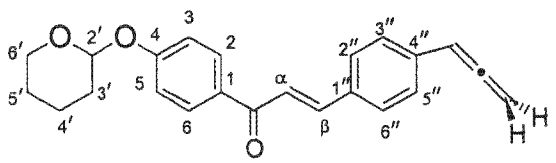
1-{4-[4-(4-Acetyl-phenyl)-buta-1,3-diynyl]- benzaldehyde **180b**

The conditions employed for the preparation of this compound were those described in general method P, which gave **180b** as a pale yellow solid (0.016g, 32%); mp 173-175°C (from ethylacetate-hexane); IRν_{max}(Nujol)/cm^{-1} 1590(C=C), 1680 (C=O) , 2120 (C≡C), δ_H(400 MHz; $CDCl_3$) 7.69 (4H, d, *J* 8.0, H-3/5), 7.87 (4H, d, *J* 8.0, H-2/6), 10.03 (2H, s, C*H*O); δ_c(100 MHz; $CDCl_3$) 82.4 (2C), 85.6 (2C), 123.2, 128.1, 129.0, 133.1, 136.7, 137.6, 197.0 (2C); HRMS (EI) *m/z* Found: M^+, 258.06908 Calc. For $C_{18}H_{10}O_2$: M, 258.06808.

1-{4-[4-(4-Acetyl-phenyl)-buta-1,3-diynyl]-phenyl}-ethanone **180a**

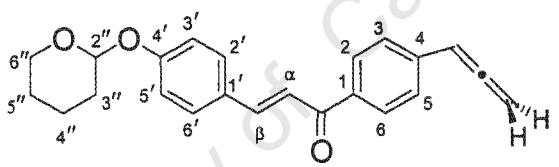
The conditions employed for the preparation of this compound were those described in general method P, which gave **180a** as a pale yellow solid (0.032g, 72%); mp 148-150 °C (from ethylacetate-hexane); IRν_{max}(Nujol)/cm^{-1} 1580 (C=C), 1670 (C=O), 2125 (C≡C), δ_H(300 MHz; $CDCl_3$) 2.61 (6H, s), 7.62 (4H, d, *J* 7.6, H-3/5), 7.94 (4H, d, *J* 7.6, H-2/6); δ_c(75 MHz; $CDCl_3$) 26.6 (2C), 82.0 (2C), 85.4 (2C), 126.3 (2C), 128.3 (4C), 133.1 (4C), 137.6 (2C), 197.0 (2C); LRMS (EI) *m/z* (%), 286 ([M]$^+$, 100), 271 ([M-CH_3]$^+$, 73), 228 ([M-2CH_3-CO]$^+$, 36).

3E-(4-Propa-1,2-dienyl-phenyl)-1-[4-(tetrahydro-pyran-2-ylmethyl)-phenyl]-propenone **178c**



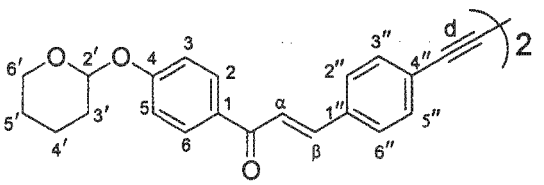
The conditions employed for the preparation of this compound were those described in General Method P, which gave **178c** as a brown solid (0.019g, 34%); mp 154-156°C (from ethylacetate-hexane); IRν_{max}(Nujol)/cm^{-1} 1025 (C-O), 1594 (C=C), 1660 (C=O); 1950 (C=C=C); δ_H(300 MHz; $CDCl_3$) 1.66 (3H, m, H-4′/5a′), 1.88 (2H, m, H-3a′/5b′), 1.99 (1H, m, H-3b′), 3.62 (1H, m, H-6a′), 3.88 (1H, m, H-6b′), 5.20 (2H, d, *J* 6.9 , CH=C=CH_2), 5.54 (1H, t, *J* 3.0, H-2′), 6.18 ((1H, t, *J* 6.9, C*H*=C=CH_2), 7.13 (2H, d, *J* 9.0, H-3/5), 7.33 (2H, d, *J* 8.4 , H-2″/6″), 7.51 (1H, d, *J* 15.6, H-α), 7.58 (2H, d, *J* 8.4, H-3″/5″), 7.77 (1H, d, *J* 15.6, H-β), 8.01 (2H, d, *J* 9.0, 2/6); δ_c(75 MHz; $CDCl_3$) 18.5, 25.1, 30.1, 62.0, 79.1, 93.8, 96.1, 116.1(2C), 121.4, 127.1 (2C), 128.7 (2C), 130.6, 131.8 (2C), 133.7, 136.3, 143.6, 160.9, 188.8, 210.5; HRMS (EI) *m/z* Found: M$^+$, 346.15754 Calc. For $C_{23}H_{22}O$: M,346.15689.

1-(4-Propa-1,2-dienyl-phenyl)-3E-[4-(tetrahydro-pyran-2-ylmethyl)-phenyl]-propenone **178d**



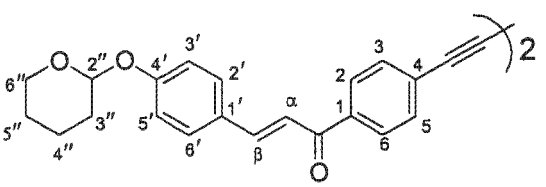
The conditions employed for the preparation of this compound were those described in General Method P, which gave **178d** as a brown solid (0.012g, 20%); mp 163-165 °C (from ethylacetate-hexane); IRν_{max}(Nujol)/cm^{-1} 1030 (C-O), 1590 (C=C), 1660 (C=O), 1950 (C=C=C); δ_H(300 MHz; $CDCl_3$) 1.66 (3H, m, H-4″/5a″), 1.88 (2H, m, H-3a″/5b″), 2.00 (1H, m, H-3b″), 3.63 (1H, m, H-6a″), 3.88 (1H, m, H-6b″), 5.22 (2H, d, *J* 6.6, CH=C=CH_2), 5.48 (1H, t, *J* 3.0, H-2″), 6.22 (1H, t, *J* 6.6, C*H*=C=CH_2), 7.09 (2H, d, *J* 8.7, H-3′/5′), 7.40 (2H, d, *J* 8.7, H-2′/6′), 7.44 (1H, d, *J* 15.6, H-α), 7.59 (2H, d, *J* 8.4, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.97 (2H, d, *J* 8.4, 2/6); δ_c(75 MHz; $CDCl_3$) 18.5, 25.1, 30.2, 62.0, 79.1, 93.8, 96.1, 116.1 (2C), 121.4, 127.1, 128.7 (2C), 130.6 (2C), 131.8 (2C), 133.7, 136.4, 144.4, 159.1, 189.7, 210.8; HRMS (EI) *m/z* Found: M$^+$, 346.15564 Calc. For $C_{23}H_{22}O_3$: M,346.15689.

1-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-3E-{4-[4-(4-{3E-[4-(tetrahydro-pyran-2-yloxy)-phenyl]- acryloyl}-phenyl)-buta-1,3-divinyl]-phenyl}-propenone **180c**



The conditions employed for the preparation of this compound were those described in General Method P, which gave **180c** as a pale Orange (0.008g, 13%); mp 121-123 °C (from ethylacetate-hexane); IRν_{max}(Nujol)/cm^{-1}), 1030 (C-O),1660 (C=O), 2250 (C≡C); δ_H(400 MHz; $CDCl_3$) 1.56-1.73 (6H, m, H-4′/5a′), 1.84-1.89 (4H, m, H-3a′/5b′), 1.96-2.03 (2H, m, H-3b′), 3.63 (2H, m, H-6a′), 3.85-3.90 (2H, m, H-6b′), 5.49 (2H, br s, H-2′), 7.09 (4H, d, *J* 8.4, H-3/5) 7.38 (2H, d, *J* 15.6, H-α), 7.59 (4H, d, *J* 8.4, H-2″/6″), 7.65 (4H, d, *J* 8.4, H-3″/5″), 7.79 (2H, d, *J* 15.6, H-β), 7.98 (4H, d, *J* 8.4, H-2/6); δ_c(100 MHz; $CDCl_3$) 18.5 (2C), 25.0 (2C), 30.1 (2C), 62.0 (2C), 82.1 (2C), 89.0 (2C), 96.2 (2C), 116.8 (4C), 119.5 (2C), 125.6 (2C), 128.1 (4C), 128.4 (2C), 130.1 (4C), 132.6 (4C), 138.6 (2C), 145.3 (2C), 159.3 (2C), 189.3 (2C); LRMS (EI) *m/z* (%), 663 ([M+ H]$^+$, 2), 579 ([M-(THP+H)]$^+$, 10), 85 ([THP+H]$^+$, 100).

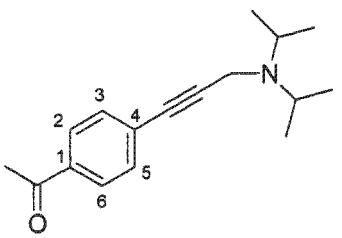
3E-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-1-{4-[4-(4-{3E-[4-(tetrahydro-pyran-2-yloxy)-phenyl]- acryloyl}-phenyl)-buta-1,3-divinyl]-phenyl}-propenone **52**



The conditions employed for the preparation of this compound were those described in General Method P, which afforded **52** as a pale Orange (0.011g, 30 %); mp 182-184 °C (from ethylacetate-hexane); IRν_{max}(Nujol)/cm^{-1} 1040 (C-O), 1659 (C=O), 2254 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.57-1.74 (6H, m, H-4″/5a″), 1.86-190 (4H, m, H-3a″/5b″), 1.90-2.06 (2H, m, H-3b″), 3.63 (2H, m, H-6a″), 3.85-3.90 (2H, m, H-6b″), 5.49 (2H, br s, H-

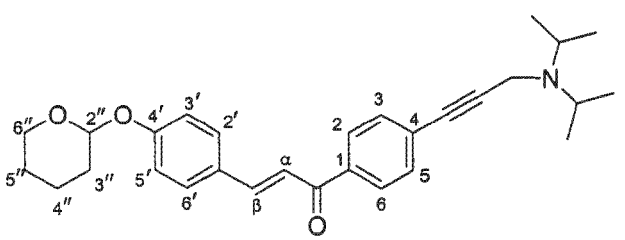
2″), 7.09 (4H, d, *J* 8.8, H-3′/5′) 7.39 (2H, d, *J* 15.2, H-α), 7.59 (4H, d, *J* 8.8, H-2′/6′), 7.65 (4H, d, *J* 8.4, H-2/6), 7.79 (4H, d, *J* 15.2, H-β), 7.99 (4H, d, *J* 8.4, H-3/5); δ_c(75 MHz; $CDCl_3$) {18.5, 25.0, 30.1, 62.0, 82.1, 89.0, 96.2, 116.8 (2C), 119.5, 125.6, 128.1 (2C), 128.4, 130.1 (2C), 132.6 (2C), 138.6, 145.3, 159.3, 189.4} x 2; LRMS (EI) *m/z* (%), 662 ($[M]^+$, 6), 579 ($[M-(THP+H)]^+$, 15), 85 ($[THP+H]^+$, 100).

1-[4-(3-Diisopropylamino-prop-1-ynyl)-phenyl]-ethanone **179a**



The conditions employed for the preparation of this compound were those described in General Method C, which afforded **179a** as a brownish oil (0.066g, 36%). R_f = 0.22 (30% EtOAc/hexane); IRν_{max}(Nujol)/cm^{-1} 1053 (C-O), 1594 (C=C), 1680 (C=O), 2253 (C≡C); δ_H(300 MHz; $CDCl_3$) 1.20 (12H, d, *J* 6.6, N[CH(CH_3)$_2$]$_2$), 2.61 (3H, s, COCH_3), 3.23 (2H, sep, *J* 6.6, N[CH(CH$_3$)$_2$]$_2$), 3.78 (2H, s, C≡CCH_2), 7.45 (2H, d, *J* 8.1, H-3/5a, 7.86 (2H, d, *J* 8.1, H-2/6); δ_c(75 MHz; $CDCl_3$) 20.2 (4C), 26.5, 34.8, 49.2 (2C), 83.7, 91.4, 128.3, 130.1 (2C), 131.6 (2C), 136.1, 197.2; HRMS (EI) *m/z* Found: M^+, 257.17793 Calc. For $C_{17}H_{23}NO$: M, 257.17796.

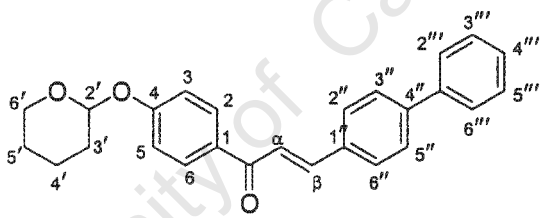
1-[4-(3-Diisopropylamino-prop-1-ynyl)-phenyl]-3E-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone **179b**



The conditions employed for the preparation of this compound were those described in

General Method P, which gave **179b** as a yellow solid (0.014g, 36%); mp 81-83 °C (from ethylacetate-hexane); R_f = 0.17 (30%EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1593 (C=C), 1680 (C=O), 2245 (C≡C); δ_H(400 MHz; $CDCl_3$) 1.17 (12H, d, *J* 6.4 Hz, NCH[C*H*$_3$]$_2$), 1.66 (3H, m, H-5a″/4″), 1.88 (2H, m, H-3a″/5b″), 2.01 (1H, m, H-3b″), 3.29 (2H, sep, *J* 6.4 Hz, N[C*H*(CH$_3$)$_2$]), 3.64 (1H, m, H-6a″), 3.68 (1H, s, C≡CC*H*$_2$), 3.88 (1H, m, H-6b″), 5.49 (1H, t, *J* 3.2, H-2″), 7.09 (2H, d, *J* 8.8, H-3′/5′) 7.38 (1H, d, *J* 15.6, H-α), 7.59 (2H, d, *J* 8.8, H-2′/6′), 7.60 (2H, d, *J* 8.8, H-3/5), 7.78 (1H, d, *J* 15.6, H-β), 7.96 (2H, d, *J* 8.8, H-2/6); δ_c(100 MHz; $CDCl_3$) 18.5, 20.7 (4C), 25.2, 30.1, 35.2 (2C), 48.9, 62.5, 80.3, 83.6, 96.2, 116.7 (2C), 123.5, 126.2, 128.5 (2C), 131.1, 131.8 (2C), 133.0 (2C), 136.5, 143.2, 162.2, 188.7; HRMS (EI) *m/z* Found: M$^+$, 445.26073 Calc. For $C_{29}H_{35}O_2N$: M,445.26169.

***3E-Biphenyl-4-yl-1-[4-(tetrahydro-pyran-2-yloxy)-phenyl]-propenone* 167**



Compound **165** (0.1g, 0.26 mmol) and boronic acid (0.05g, 0.39 mmol) were dissolved in a mixture of toluene (5 ml) and ethanol (2 ml) and 12 mg of $Pd_2(dba)_3$ were added. After addition of a saturated solution of potassium carbonate (5 ml), the mixture was heated under reflux for 5h. Water was added and the mixture was extracted with dichloromethane. The organic layers were combined, dried ($MgSO_4$) and the solvent removed. The crude product was columned on silica gel (10% EtOAc/ Hexane) to afford **167** as a pale yellow solid (0.038g, 38%); mp 146-148 °C (from ethyl acetate-hexane); R_f = 0.65 (30%EtOAc/hexane); IR ν_{max}(Nujol)/cm^{-1} 1590 (C=C), 1660 (C=O), ; δ_H(300 MHz; $CDCl_3$) 1.68 (3H, m, H-5a′/4′), 1.90 (2H, m, H-3a′/5b′), 2.00 (1H, m, H-3b′), 3.64 (1H, m, H-6a′), 3.88 (1H, m, H-6b′), 5.55 (1H, t, *J* 3.0, H-2′), 7.15 (2H, d, *J* 9.0, H-3/5), 7.38 (1H, t, *J* 8.0, H-4″), 7.47 (2H, t, *J* 8.0, H-3‴/5‴), 7.58 (1H, d, *J* 15.3, H-α), 7.63 (2H, d, *J* 8.4, H-2″/6″), 7.65 (2H, d, *J* 8.4, H-3″/5″), 7.72 (2H, d, *J* 8.0, H-2‴/6‴),

7.84 (1H, d, *J* 15.3, H-β), 8.04 (2H, d, *J* 9.0, H-2/6); δ_c(75 MHz; $CDCl_3$) 18.7, 25.3, 30.4, 62.3, 96.4, 116.4, 122.1, 127.2 (2C), 127.8 (2C), 128.1, 129.7 (2C), 129.9 (2C), 130.9, 131.4 (2C), 134.8, 140.0, 142.7, 143.7, 162.1, 187.8; HRMS (EI) *m/z* Found: M^+, 384.17249 Calc. For $C_{26}H_{24}O_3$: M, 384.17255.

PROCEDURES FOR BIOLOGICAL ASSAYS

Assay of enzyme inhibition: Falcipain-2

IC_{50}s against falcipain-2 were determined as described by Rosenthal et al.[42] Briefly, an equal amount of recombinant falcipain-2 were incubated with different concentrations of Mannich base derivatives (added from 100x stocks in dimethyl sulfoxide [DMSO]) in 100 mM sodium acetate (pH 5.5)-10 mM dithiothreitol for 30min at room temperature before addition of the substrate benzoxycarbonyl-Leu-Arg-7-amino-4-methyl- coumarin. Fluorescence was continuously monitored for 30 min at room temperature in a labsystem Fluoroskan II spectrofluorometer. IC_{50}s were determined from plots of activity over enzyme concentration with GraphPad Prism software.

Assay of parasite development: W2

Effects of inhibitors on the parasite development were determined as described earlier.[42] Synchronized W2-strain *P. falciparum* parasites[40] were cultured with compound derivatives (added from 1000x stocks in DMSO) for 48 h beginning at the ring stage. The medium was changed after 24 h, while maintaining appropriate inhibitor concentration. Giemsa-stained smears were made after 48 h, when control cultures contained nearly all ring-stage parasites. The number of new ring forms per 500 erythrocytes was counted, and compared with those of controls cultured in 0.1 % DMSO. IC_{50}s for growth inhibition were determined with GraphPad Prism software from plots of percentages of the level of parasitemia of the control relative to inhibitor concentration.

Assay of hemoglobin degradation

Cultured *P. falciparum* parasites were incubated with the Mannich base derivatives that were potent inhibitors of falcipain-2. To evaluate hemoglobin degradation, smears were prepared after 24 h of growth and the morphologies of treated and control (0.1% DMSO) parasites were inspected after staining with Giema. The presence or absence of a typical food vacuole abnormality in the majority of the parasites was noted.

Assay of Enzyme inhibition: Cruzain and Rhodesain

Recombinant cruzain and rhodesain were recombinantly determined as described previously.[45,56] Cruzain (2 nM) or rhodesain (3 nM) was incubated with 0.5 to 10 μM inhibitor in 100 mM sodium acetate, pH 5.5 containing 5 mM DTT (buffer A), for 5 minutes at room temperature. Then buffer A containing Z-Phe-Arg-AMC (Bachem, Km=1 μM) was added to enzyme inhibitor to give 20 μM substrate in 200 μl, and the increase in fluorescence (excitation at 355 nM and emission at 460 nM) was followed with an automated microtiter plate spectrofluorimeter (molecular Devices, Flex station). Inhibitor stock solutions were prepared at 20 nM in DMSO and serial dilutions were made in (0.7 % DMSO assay). Controls were performed using enzyme alone, and enzyme with DMSO. Percentage residual enzyme activity was determined. Those that were near or lower than 50 % residual enzyme activity were further screened at concentration of inhibitor ranging from 10 down to 0.1 μM. IC_{50} values were determined graphically using inhibitor concentrations in the linear portion of a plot inhibitor versus log [I].

Assay for parasite development: D10 and K1

All samples were tested in duplicate on a single occasion against a chloroquine sensitive strain of *P. falciparum* (D10). Compounds active against *P. falciparum* D10 were also tested against the chloroquine resistant K1 strain of the parasite. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[270] Quantitative assessment of antiplasmodial activity *in vitro* will be determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[271] The average percentage parasite viability is determined at three different concentrations.

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

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