2. REVIEW OF LITERATURE

Malaria parasite is mutating and various resistance strains are evolving with time. Older drugs that are being currently used prove to be inadequate and hence there is constancy for the development a new and effective entity that could constant P. falciparum.

New treatments for malaria are urgently needed due to the increasing problem of drug-resistance in malaria parasites. The long-established use of quinine and the more recent introduction of artemisinin and its derivatives as highly effective antimalarials demonstrates that plant species are an important resource for the discovery of new antimalarial agents. Furthermore, many plant species continue to be used in traditional medicines for the treatment of malaria and many people depend on such remedies as they cannot afford and/or do not have access to effective antimalarial drugs.

There is currently increasing interest in the use and development of traditional herbal remedies for the treatment of malaria as these may have the potential to provide affordable antimalarial treatment for many who cannot afford the drugs needed to treat chloroquine-resistant P. falciparum infections. However, little is known with respect to the efficacy and safety of traditional antimalarials and clinical studies are urgently needed to establish their value.

It is estimated that malaria is directly responsible for the deaths of 1–2 million people each year and in addition, the disease contributes to an unknown number of other deaths as a result of malaria-related anaemia. The majority of malaria deaths are due to cerebral malaria and other complications following infection with P. falciparum that is transmitted by female mosquitoes of the genus Anopheles.

Undoubtedly, the situation has become steadily worse in the last 30 years, and a major factor responsible has been the increasing prevalence of P. falciparum resistant to
chloroquine and to other antimalarial agents. Studies in a number of African countries have shown that the emergence of chloroquine-resistant malaria parasites is associated with a two-fold increase in malaria deaths but in one study in Mlomp, Senegal it was shown that malaria mortality in children under the age of 4 years increased 11-fold within 6 years of the emergence of chloroquine-resistance.73

Artemisinin derivatives the problem of recrudescence where drug treatment initially appears to clear all the parasites from the blood but, after a few weeks parasites re-appear and the disease recurs. Recrudescence is not due to drug resistance or re-infection of the patient but occurs because the drug has failed to kill all of the parasites and those that survive continue to multiply, so that after a few weeks the patient again experiences malaria symptoms. This problem may be related to the relatively short half lives of the commonly used artemisinin derivatives and the insensitivity of the early blood stage forms of the malaria parasite to these drugs.74 The second and perhaps more important limitation is that compared to chloroquine, the artemisinin derivatives are expensive and out of the reach of many of those who suffer from malaria.

The rise in malaria mortality and morbidity as a result of chloroquine-resistant malaria parasites coupled with poverty in means that there continues to be an increasingly urgent need for effective and affordable antimalarial therapies. Even if the artemisinin derivatives could be made available to all those that need them, the possibility of the future development of malaria parasites resistant to these drugs must be borne in mind.

Two different approaches to the development of new medicines plant derived for malaria will be considered: In the first an example of the potential of plants to yield novel compounds that can be investigated as leads to new drugs will be discussed, while the second will explore some of the issues with respect to the use of traditional herbal medicines for the treatment of malaria.

In some cases, the constituent(s) responsible for their activities have been isolated and their structures elucidated but relatively few have been studied.
2.1(a) Optimization of therapy with existing agents

A first approach is to optimize therapy with existing agents. New dosing regimens or formulations may optimize activity. Combination therapies, including newer agents (e.g. artemisinin derivatives, atovaquone) and new combinations of older agents (e.g. amodiaquine/sulfadoxine/pyrimethamine, chlorproguanil/dapsone), are under study as first-line therapies for Africa and other areas with widespread drug resistance.

The use of combination antimalarial therapy offers two important potential advantages.

1. The combination should improve antimalarial efficacy, providing additive or, ideally, synergistic antiparasitic activity. In the case of both the artemisinin derivatives and atovaquone, the new agents have had unacceptable failure rates when used as single agents to treat falciparum malaria but they have been highly effective in combination with other established antimalarials.

2. Probably most important, the use of combination therapy should slow the progression of parasite resistance to the new agents. Ideally, a combination regimen that prevents resistance development should include at least two agents against which parasite resistance has not yet developed and which have similar pharmacokinetics, so that low blood levels of a single agent will not be present.

2.1(b) Development of analogs of existing agents

Another approach to antimalarial therapy is to improve upon existing antimalarials by chemical modifications of these compounds. This approach does not require knowledge of the mechanism of action or the biological target of the parent compound. Indeed, this approach was responsible for the development of many existing antimalarials. For example, chloroquine, primaquine and mefloquine were discovered through chemical strategies to improve upon quinine. More recently, 4-aminoquinolines that are closely related to chloroquine appear to offer the antimalarial potency of the parent drug, even against chloroquine-resistant parasites.
2.1(c) Natural products

Natural products are the sources of the two most important drugs currently available to treat severe falciparum malaria, quinine and derivatives of artemisinin. In the case of artemisinin, relatively simple chemical modifications of the natural product parent compound have led to a series of highly potent antimalarials that are playing an increasingly important role in the treatment of malaria.78

Figure 30: Semisynthetic Artemisinin Analogs

Figure 31: Structure of Artemisinin-Quinoline Hybrids (1 – 3)
2.1(d) Compounds active against other diseases

A fourth approach to antimalarial chemotherapy is to identify agents that are developed or marketed as treatments for other diseases. These compounds might act against orthologs of their targets in other systems or by different mechanisms against malaria parasites. Considering the difficulties of funding antimalarial drug discovery, the advantage of these compounds is that, whatever their mechanism, they have already been developed for a human indication, so will be quite inexpensive to develop as antimalarials.

Figure 32: Representation of an intra-erythrocytic *P. falciparum* trophozoite, highlighting key parasite intracellular compartments and the site of action of some of the major classes of antimalarial drugs.
<table>
<thead>
<tr>
<th>Approach</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimize therapy with existing agents</td>
<td>Amodiaquine/sulfadoxine/pyrimethamine</td>
<td>79-89</td>
</tr>
<tr>
<td></td>
<td>Amodiaquine/artesunate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Artesunate/sulfadoxine/pyrimethamine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Artesunate/mefloquine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Artemether/lumefantrine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorproguanil/dapsone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorproguanil/dapsone/artesunate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atovaquone/proguanil</td>
<td></td>
</tr>
<tr>
<td>Develop analogs of existing agents</td>
<td>New aminoquinolines</td>
<td>90-94</td>
</tr>
<tr>
<td></td>
<td>New endoperoxides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New folate antagonists</td>
<td></td>
</tr>
<tr>
<td>Natural products</td>
<td>New natural products</td>
<td>95</td>
</tr>
<tr>
<td>Compounds active against other diseases</td>
<td>Folate antagonists</td>
<td>96-98</td>
</tr>
<tr>
<td></td>
<td>Antibiotics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atovaquone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iron chelators</td>
<td></td>
</tr>
<tr>
<td>Drug resistance reversers</td>
<td>Verapamil, desipramine, trifluoperazine</td>
<td>99-100</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine</td>
<td></td>
</tr>
</tbody>
</table>

Table 8: Development of new drugs against an even wider range of sub-cellular compartments and parasite targets
Figure 33: Principal enzymes & substrates of folate pathway involved in formation of (THF) and its utilisation in the thymidylate cycle in *P. falciparum*
2.2 FALCIPAIN-2 INHIBITORS\textsuperscript{101-103}

Malaria, the most prevalent and most pernicious parasitic disease of humans, is estimated to kill about two million people, mainly children, each year. Several new drugs along with chloroquine, the first synthetically developed antimalarial drug proved to be a successful cure in the past 50 years. The emergence and spread of resistant parasites to some of the drugs and their combination has made it virtually ineffective in most parts of the world. Few newer drugs like lumefantrine, Maloprim, Primaquine retain efficacy, have limitations and have a high cost. And thus, it is indispensable to discover, design, and develop new drugs with different mechanisms, with diversity in chemical structure and be efficacious against drug resistant strain. In addition to this it should be safe for small children and pregnant women, and be affordable. The ability to treat and control \textit{P. falciparum} infection through chemotherapy has been compromised by the advent and spread of resistance to antimalarial drugs. Resistance has emerged to all classes of antimalarial drugs. Except the artemisinin and is responsible for a recent increase in malaria-related mortality. Generally, resistance is the mutations in or changes in the copy of number of genes encoding or relating to the drug’s parasite target or influx/efflux pumps that affect intraparasitic concentrations of the drug.

Among promising new targets for antimalarial chemotherapy are the enzymes cysteine protease hemoglobinases falcipain-2 and falcipain-3. Falcipain-2 (FP2) is a papain family cysteine protease and important hemoglobinase of erythrocytic \textit{P. falciparum} parasites.

\textbf{Figure 34: Crystal structure of \textit{P. falciparum} enoyl-ACP reductase (PfENR) complexed with NAD+ cofactor}
Inhibitors of FP2 block hemoglobin hydrolysis and parasite development, suggesting that this enzyme is a promising target for antimalarial chemotherapy. FP2 and related plasmodial cysteine proteases have an unusual 14-aa motif near the C terminus of the catalytic domain. Recent solution of the structure of FP2 showed this motif to form a β-hairpin that is distant from the enzyme active site and protrudes out from the protein.

*Bis*-Schiff Bases form an important class of organic compounds with a wide variety of biological properties. Development of a new chemotherapeutic Schiff bases is now attracting the attention of medicinal Chemist. Many studies have reported regarding the biological activities of *bis*-Schiff Bases, including their anticancer, antibacterial, antifungal, and herbicidal activities. *Bis*-Schiff Bases, derived from various heterocycles, were reported to possess cytotoxic, anticonvulsant, antiproliferative, anticancer and antifungal activities. A number of Schiff bases have been tested for antibacterial, antifungal, anticancer and herbicidal activities.

### 2.3 Isatin & *Bis*-Schiff Bases

An Insight into Structural Requirement

Isatin and its analoges are versatile substrates, which can be used for the synthesis of numerous heterocyclic compounds. Isatins also have important pharmacological and biological activities.

A variety of biological activities are associated with *bis*-Schiff Bases of isatin including CNS activities as potentiation of pentobarbitone induce nercosis, analgesic, anticonvulsant, antidepressant, antiinflammatory, antimicrobial and effects on the central nervous system. Isatins are capable of crossing the blood-brain-barrier.

Isatin (*1H-indole-2,3-dione*, Figure 17) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids.

The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis.
The synthetic versatility of isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives.

![Image of isatin structure]

**Figure 35: Isatin (1H-indole-2,3-dione.)**

In nature, isatin is found in plants of the genus *Isatis*\textsuperscript{138}, in *Calanthe discolor* LINDL.\textsuperscript{139} and in *Couroupita guianensis* Aubl.\textsuperscript{140}, and has also been found as a component of the secretion from the parotid gland of *Bufo* frogs\textsuperscript{141}, and in humans as it is a metabolic derivative of adrenaline\textsuperscript{142-144}. Substituted isatins are also found in plants, for example the melosatin alkaloids (methoxy phenylpentyl isatins) obtained from the Caribbean tumorigenic plant *Melochia tomentosa*\textsuperscript{145-147} as well as from fungi: 6-(3’-methylbuten-2’-yl)isatin was isolated from *Streptomyces albus*\textsuperscript{148} and 5-(3’-methylbuten-2’-yl)isatin from *Chaetomium globosum*\textsuperscript{149}. Isatin has also been found to be a component of coal tar\textsuperscript{150}.

### 2.3.1 Synthesis of Isatins

The Sandmeyer methodology

The method developed by Sandmeyer is the oldest and the most frequently used for the synthesis of isatin. It consists in the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulphate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulphuric acid, furnishes isatin in >75% overall yield\textsuperscript{151}. The method applies well to anilines with electron-withdrawing substituents, such as 2-fluoroaniline\textsuperscript{152}, and to some heterocyclic amines, such as 2-aminophenoxathine\textsuperscript{153}. *(Scheme 2.1)*
Application of Isatins in Organic Synthesis

Many synthetic methodologies have been described for the conversion of isatins to other heterocyclic systems. This chemistry can be generalized as one of the following strategies:

a) Partial or total reduction of the heterocyclic ring, leading to indoles and derivatives;

b) Oxidation of the heterocyclic ring, (Ex.) Isatin conversion to isatoic anhydride, with subsequent conversion to other heterocyclic systems. (Scheme 2.2)
c) Nucleophilic addition at position C-3, which may be further followed by a cyclization process, with or without N1-C2 bond cleavage or by a spiro-annelation at position C-3. (Scheme 2.3, 2.4)

\[ \text{Diagram of Scheme 2.3} \]

\[ \text{Diagram of Scheme 2.4} \]

d) Nucleophilic substitution at position C-2, leading to the opening of the heterocyclic ring. This process may be followed by an intra molecular or by an intermolecular exo-trig cyclization. (Scheme 2.5)
Isatins and derivatives can suffer nucleophilic attack at positions C-2 and/or C-3. The chemoselectivity of these reactions depends on the nature of the nucleophile, on the nature of the substituents attached to the isatin nucleus, and especially of those bonded to the nitrogen atom, as well as upon the solvent and temperature employed. The initial products obtained can suffer further reaction in the presence of a second nucleophilic group to give cyclization products. For didactic reasons, these reactions have been sorted by the nature of the nucleophile.

Isatin and 1-alkylisatins furnish condensation products at the C-3 position when reacted with: hydrazine\textsuperscript{154}, alkyl and arylhydrazines \textsuperscript{155-157}, hetero aryl hydrazines derived from pyrimidine\textsuperscript{158}, pyrazine\textsuperscript{159}, thiazole\textsuperscript{160}, 1,2,4-triazine\textsuperscript{161}, quinazoline\textsuperscript{162}, benzimidazole\textsuperscript{163}, benzothiazole\textsuperscript{164}, phthalazine\textsuperscript{165}, triazines\textsuperscript{166,167}, as well as acylhydrazides of oxalic\textsuperscript{168}, benzoic\textsuperscript{169}, phenoxyacetic\textsuperscript{170} and oxanilic acids\textsuperscript{171}, Arylsulfonyl hydrazides\textsuperscript{172}, guanylhydrazones\textsuperscript{173}, semicarbazines\textsuperscript{174} and thiosemicarbazides\textsuperscript{175-177}.
Isatin-3-imines also react with hydrazine derivatives such as heteroarylhydrazines$^{178}$, thiosemicarbazides$^{179}$ and acylhydrazides$^{180}$, resulting in a substitution reaction at the C-3 position. Substitution reactions are also described to occur when O-methylisatin is treated with thiosemicarbazines, furnishing isatin-2-thiosemicarbazones. The stereochemistry of isatin-3-thiosemicarbazone-5- sulfonate was studied in aqueous solution, and in acidic pH the Z isomer was determined to be the most stable, but after deprotonation, the corresponding anion slowly converted to the E isomeric anion.$^{181}$ (Scheme 2.6)

(Scheme 2.6)

Isatin hydrazones and thiosemicarbazones can also be used as substrates for the Mannich reaction, leading to functionalization at N-1.$^{182, 183}$ Isatin-3-hydrazone reacts with 1,1-dimethylamino-2-nitroethene to give a transamination product.$^{184}$ (Scheme 2.7)
Isatin, due to its cis α-dicarbonyl moiety, is a potentially good substrate for the synthesis of metal complexes, either alone or with other ligands. Their derivatives, mostly those substituted at C-3, such as isatin-3-hyrazones and isatin-3-imines bearing an extra heteroaromatic ring are also generally employed as ligands. In this manner, Schiff bases formed from isatin and amino silica gel are useful sorbents for divalent cations and for Fe(III).185

2.3.2 Crystallographic and Spectral Analyses

Crystallographic data

The crystallographic data for isatin reveals that it is almost planar, with a bond length between the two carbonyls of 1.55 Å. This large value was attributed to lone pair electron repulsion between the two oxygen atoms. 186, 187 This interpretation was however,
subsequently refuted by comparison of bond lengths of cis and trans 1,2-diketones where no systematic or substantial difference between the bond lengths was observed\textsuperscript{188}. A similar bond length was observed for 1-acetylisatin\textsuperscript{189}, 1-\textalpha-chloroacetylisatin\textsuperscript{190}, diethyl (2,3-dihydro-2-oxo-3-indolylidene) propanedioate\textsuperscript{191}, 1,1’ oxalylbisatin \textsuperscript{192} and 1-methylisatin\textsuperscript{193}. Further, similar bond lengths were also observed in derivatives where C-3 is tetrahedral, such as 3,3-dichloro-1H-indol-2(3H)-one\textsuperscript{194} and 5’-bromospiro-[1,3-dioxolano-2,3-indolin]-2’-one\textsuperscript{195}, as well as for 3-methyleneoxindoles\textsuperscript{196} (Table 9). Ring opened products, obtained by nucleophilic attack upon 1-acetylisatin, possess a 1,2-dicarbonyl system that assumes a s-trans conformation\textsuperscript{197} that also reveals a similar bond length.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
X     & R\textsubscript{1} & R\textsubscript{2} & C2-C3 (Å) \\
\hline
O     & H    & H    & 1.55 \\
\hline
\textbullet    & A\texttextsuperscript{c} & H    & 1.538 \\
\hline
\textbullet    & M\texttextsuperscript{e} & H    & 1.545 \\
\hline
Cl, Cl     & H    & H    & 1.556 \\
\hline
OCH\textsubscript{2}CH\textsubscript{2}O & H    & Br   & 1.539 \\
\hline
CH\textsubscript{2}=C(CH\textsubscript{2})\textsubscript{2} & H    & H    & 1.508 \\
\hline
\end{tabular}
\caption{Bond lengths between C-2 and C-3 in Isatin and derivatives}
\end{table}

2.3.3 Infrared spectroscopy

The infrared spectrum of isatin shows two strong bands at 1740 and 1620 cm\textsuperscript{-1} corresponding to the carbonyl stretching vibrations. A broad band occurs at 3190 cm\textsuperscript{-1} due to the N-H stretching, and it is accompanied by many sub-bands, all of which are moved to lower frequency on deuteration. This also affects several bands in the region 1400-1100 cm\textsuperscript{-1} which are associated with N-H in-plane bending\textsuperscript{198,199}. Although the
\( \nu_{C=O} \) values are not modified by N-alkylation, N-acetylation leads to a hypsochromic shift of the lactam absorption of about 50-70 cm\(^{-1}\), while the ketone band shifts to 1750 cm\(^{-1}\), as a consequence of the extension of conjugation of the nitrogen lone pair with the acetyl group. On the other hand, 3-methyleneoxindoles show a bathochromic shift for the lactam band of around 20 to 30 cm\(^{-1}\), this shift being greater when there are groups at the C-3 position, such as OH, which can form a hydrogen bond with the lactam carbonyl. In this case, \( \nu_{C=O} \) appears at 1660 cm\(^{-1}\). 3,3-Difluorooxindoles reveal a hypsochromic shift of about 20 cm\(^{-1}\) in comparison to the respective isatin.

### 2.3.4 \(^1\)H NMR spectroscopy

The \(^1\)H NMR spectrum of isatin shows the signals of the aromatic nucleus at \( \delta \) 6.86 (d), 7.00 (t), 7.47 (d) and 7.53 (t) (DMSO-d\(_6\)), corresponding to H-7, H-5, H-4 and H-6 respectively. While N-alkylation does not alter this pattern, N-acetylation leads to a downfield shift of all the signals, but most significantly of H-7 due to the anisotropic effect of the carbonyl group. In a similar fashion, 3-methyleneoxindoles bearing cyano groups reveal a high frequency shift of H-4 by about 0.6-1.0 ppm, with no significant effect over the other signals.\(^{200,201}\)

![Isatin NMR Spectrum](image)

<table>
<thead>
<tr>
<th>X</th>
<th>R</th>
<th>H-4</th>
<th>H-5</th>
<th>H-6</th>
<th>H-7</th>
<th>CH(_3)CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>H</td>
<td>7.50d</td>
<td>7.07t</td>
<td>7.60t</td>
<td>6.92d</td>
<td>-</td>
</tr>
<tr>
<td>O</td>
<td>Me</td>
<td>7.59d</td>
<td>7.12t</td>
<td>7.61t</td>
<td>6.91t</td>
<td>-</td>
</tr>
<tr>
<td>O</td>
<td>Ac</td>
<td>7.27d</td>
<td>7.33t</td>
<td>7.70t</td>
<td>8.38d</td>
<td>2.73s</td>
</tr>
<tr>
<td>C(CN)(_2)</td>
<td>H</td>
<td>7.87d</td>
<td>7.12t</td>
<td>7.59t</td>
<td>6.94d</td>
<td>-</td>
</tr>
</tbody>
</table>

Solvent: DMSO-d\(_6\)

**Table 10: Influence of N-1 and C-3 substituents on \(^1\)H NMR chemical shifts of Isatins**
2.3.5 Mass spectrometry

The electron-impact mass spectra of isatin, 1-alkylisatins\textsuperscript{202} and derivatives, such as hydrazones\textsuperscript{203}, usually show an intense molecular ion peak. In the case of 3,3-dissubstituted oxindoles\textsuperscript{204}, the base peak corresponds to the loss of the substituents at C-3. A peak corresponding to the loss of CO (ion a) can also be observed, whose intensity decreases with the increase in size of the alkyl chain of 1-alkylisatins\textsuperscript{205}. Ion a usually loses HCN, leading to a fulvene ion (ion b). An arene aziridine is also observed (ion c), which arises from a second loss of CO\textsuperscript{206-208}. The ions b and c are also observed in the gas-phase pyrolysis of isatin\textsuperscript{209}. In a general manner, the mass spectra of 3-substituted Isatins show a sequential loss of neutral molecules.\textsuperscript{210} (Scheme 2.9)
2.4 Biological activities of Schiff bases

Schiff bases, named after Hugo Schiff $^{211}$, are formed when any primary amine reacts with an aldehyde or a ketone under specific conditions. Structurally, a Schiff base (also known as imine or azomethine) is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group (C=O) has been replaced by an imine or azomethine group. Schiff bases are some of the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilisers $^{212}$. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, & antipyretic properties $^{213}$. Imine or azomethine groups are present in various natural, natural-derived, and non-natural compounds. (Fig. 18) The imine group present in such compounds has been shown to be critical to their biological activities $^{214-216}$.

![Diagram of Schiff bases and their biological activities]

Figure 36: Natural, natural-derived, and non-natural compounds of Schiff bases

A large number of Schiff bases and their complexes have been studied for their interesting and important properties, e.g., their ability to reversibly bind oxygen $^{217}$, catalytic activity in hydrogenation of olefins $^{218}$ and transfer of an amino group $^{219}$, photochromic properties $^{220}$, and complexing ability towards some toxic metals $^{221}$. The high affinity for the chelation of the Schiff bases towards the transition metal ions is utilized in preparing their solid complexes.
In recent years, Schiff and Mannich bases of isatin are reported to exhibit broad-spectrum chemotherapeutic properties such as antiviral\textsuperscript{222} anti-TB\textsuperscript{223}, antifungal and antibacterial activities\textsuperscript{224}. Recently it has been reported that a bis-imine of isatin has antimicrobial properties\textsuperscript{225} and affects cell viability\textsuperscript{226}.

Several Bis-Schiff Bases of Isatins of varied chemical structures with antimalarial activity have been investigated to inhibit falcipain. The mechanism of action of Isatins derivatives appears to be based on the competitive inhibition of malarial cysteine protease (falcipain). This is a key parasitic enzyme responsible for the degradation of hemoglobin, which generates the essential amino acids needed for the \textit{P. falciparum} to grow.

2.4.1 Bis-Schiff Bases of Isatins

\textit{Bis}-Schiff Bases of Isatin are group of compounds with various substitution patterns on the one aromatic rings of (\textit{Z})-3-((\textit{E})-ethylidenehydrazono)indolin-2-one. Recently, there has been strong interest in the potential antimalarial activity of \textit{bis}-Schiff Bases of Isatins. Among many promising substances, \textit{bis}-Schiff Bases of Isatins of varied chemical structures with antimalarial activity have been investigated.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure37.png}
\caption{\textit{Bis}-Schiff Bases of Isatin}
\end{figure}

2.4.2 Mechanism of \textit{Bis}-Schiff Bases of Isatins as Falcipain Inhibitors:

At the time of the first QSAR study involving \textit{Bis}-Schiff Bases of Isatins derivatives only one parasitic papain like cysteine protease was known. Falcipain–1 was extracted, isolated and identified by Salas and co–workers\textsuperscript{227} who thought it was the main enzyme involved in the haemoglobin degradation.
Partial charge Studies involving falcipain–1 have proved not to be promising due to its low abundance. More recently, two other cysteine proteases have been identified. It was shown that the highest concentration of cysteine protease in the parasitic food vacuole is due to Falcipain–2\textsuperscript{228}. The last member of this family, falcipain–3\textsuperscript{229} was shown to be approximately 1.8 times less concentrated and twice as active as falcipain–2, which gives these enzymes approximately the same importance in the parasitic haemoglobin degradation process.

2.4.3 Development of Different Falcipain Inhibitors

1. In 1994, some peptide analogs as potent inhibitors of cysteine protease enzyme for \textit{in vivo} use were granted a US Patent Wide Patent Number 5374623\textsuperscript{230}.

2. In 1994, \textbf{Puran and co-workers}\textsuperscript{231} published critical role for the cysteine protease falcipain-2 in haemoglobin hydrolysis by plasmodium falciparum, based on the gene disruption.

3. In 1997, MALTIDO .S and co-workers\textsuperscript{232} studied the \textbf{hemoglobin} metabolism in the malaria parasite \textit{P. falciparum}. Hemoglobin degradation in intra erythrocytic malaria parasites is a vast process that occurs in an acidic digestive vacuole. Proteases that participate in this catabolic pathway have been defined. Studies of protease biosynthesis have revealed unusual targeting and activation mechanisms. Oxygen radicals and heme

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![Figure 38: Initial mechanism of the cleavage of a peptide bond by cysteine protease.](image-url)
are released during proteolysis and must be detoxified by dismutation and polymerization, respectively. The quinoline as antimalarials appears to act by preventing sequestration of this toxic heme.

4. In 1997, US Patent 5663380[^233] was granted to an investigation that cysteine protease inhibitors containing heterocyclic leaving groups specifies a class of cysteine protease inhibitors which inactivate a cysteine protease by covalently bonding to the protease and releasing a heterocyclic leaving group. The cysteine protease inhibitors used in this invention comprise a first portion which targets a desired cysteine protease and positions the inhibitor near the thiolate anion portion of the active site of the protease, and a second portion which covalently bonds to the cysteine protease and irreversibly deactivates that protease by providing a carbonyl or carbonyl-equivalent which is attacked by the thiolate anion of the active site of the cysteine protease to sequentially cleave a heterocyclic leaving group.


![Structure-based design of parasitic protease inhibitors](image)

7. In 2004, Rosenthal PJ[^235] reviewed antimalarial enzyme and summary of review is as follows:

A number of cysteine proteases of malaria parasites have been described, and many more putative cysteine proteases are suggested by analysis of the Plasmodium falciparum genome sequence. Studies with protease inhibitors have suggested roles for cysteine
proteases in hemoglobin hydrolysis, erythrocyte rupture, and erythrocyte invasion by erythrocytic malaria parasites. The best characterised Plasmodium cysteine proteases are the falcipains, a family of papain-family (clan CA) enzymes. Falcipain-2 and falcipain-3 are hemoglobinases that appear to hydrolyse host erythrocyte hemoglobin in the parasite food vacuole. This function was recently confirmed for falcipain-2, with the demonstration that disruption of the falcipain-2 gene led to a transient block in hemoglobin hydrolysis.

A role for falcipain-1 in erythrocyte invasion was recently suggested, but disruption of the falcipain-1 gene did not alter parasite development. Other papain-family proteases predicted by the genome sequence include dipeptidyl peptidases, a calpain homolog, and serine-repeat antigens. The serine-repeat antigens have cysteine protease motifs, but in some the active site Cys is replaced by a Ser. One of these proteins, SERA-5, was recently shown to have serine protease activity. As SERA-5 and some other serine-repeat antigens localise to the parasitophorous vacuole in mature parasites, they may play a role in erythrocyte rupture. The P. falciparum genome sequence also predicts more distantly related (clan CD and CE) cysteine proteases, but biochemical characterisation of these proteins has not been done. New drugs for malaria are greatly needed, and cysteine proteases may provide useful new drug targets. Cysteine protease inhibitors have demonstrated potent antimalarial effects, and the optimisation and testing of falcipain inhibitor antimalarials is underway.

8. In 2007 Snehashis and co-workers\textsuperscript{236} published novel molecular targets for antimalarial chemotherapy.

9. Recently, in 2008 Awasthi SK and co-workers\textsuperscript{237} had synthesized novel 1,3-diaryl propenone derivatives based on structural requirement data and performed antimalarial evaluation in vitro against asexual blood stages of the human malaria parasite, P. falciparum.
It can be concluded from their study that there is requirement of hydrogen bond acceptor at C4 of ring B in a particular orientation to provide stronger and effective to provide stronger and effective hydrogen bonding with His 67 of cysteine protease.

![Figure 39: Peptide bond cleavage by the enzyme, cysteine protease](image)

2.4.4 SAR of Bis-Schiff Bases of Isatin Nucleus:

![Figure 40: Structure of bis-Schiff Bases of Isatin](image)

(Z)-3-((E)-ethylidenehydrazono)indolin-2-one

1. The structures of a large number of falcipain-2 inhibitors have in common two aryl units separated by a central linker. Frequently, at least one of these aryl moieties must contain 1,2-dihydroxy substituent’s in order to exhibit high inhibitory potency.

2. The influence of the B ring on the activity is related to size considerations, need bulky substitution, while the A ring may be more important in influencing hydrophobicity.

3. Relatively bulky substituent near the C3, C4 position of the ring A is favorable for activity.
4. Presence of chloro and fluoro substitution on ring A does not necessarily increase the antimalarial activity. The influence of these atoms on ring A seems to be dependent on the kind of substitution on ring B. Steric and hydrophobic factors, particularly substituent A to be width–limited so that the molecular width along x axis of bis-Schiff Bases of Isatins derivatives be small.

5. The association of the 3-quinoliny1 ring with good activity is an interesting recurring feature among all alkoxylated Bis-Schiff Bases of Isatins.

2.5 RESEARCH ENVISAGED

AIMS AND OBJECTIVES:
The current interest in the creation of large searchable libraries of organic compounds has captured an imagination of organic chemist and the drug discovery community, considering the necessity for new and novel chemical inhibitors of biological functions. Here the basic objective to introduce chemical diversity in the molecular frame work of some heterocyclic compounds, in order to synthesize pharmacologically interesting heterocyclic compounds. Synthesis, Characterization and looking to the application of heterocyclic compounds.

There is an urgent need to discover new antimalarials, due to the spread of chloroquine resistance and the limited number of available drugs.

Bis-Schiff bases of Isatins are one of the classes of natural products that are known to possess antiplasmodial properties.

The mechanism of action of bis-Schiff Bases of Isatins derivatives appears to be based on the competitive inhibition of malarial cysteine protease (falcipain). This is a key parasitic enzyme responsible for the degradation of hemoglobin, which generates the essential amino acids needed for the P. falciparum to growth.

The current work is based on the design, synthesis, biological evaluation and molecular modeling of some bis-Schiff Bases of Isatins based falcipain-2 inhibitors as antimalarial agents as new entities for the antagonism the enzyme.
2.6 PLAN OF WORK

The project was carried out in the following steps:

- Literature Review
- Designing of compounds
- Docking studies of the designed compounds along with existing compounds
- Identification of best scoring compounds according to docking score
- Selection of scheme for synthesis
- Synthesis of designed compounds
- Determination of Physicochemical Properties & Characterization of synthesized derivatives at appropriate steps which includes
  - Thin layer chromatography
  - Melting point determination
  - Solubility determination
- Confirmation of structure:
  - IR
  - NMR
  - MASS
- Biological evaluation of synthesized compound for antimalarial activity.
- Compilation of Results
  The Literature review was done from different laboratories and institutes as mentioned below –
  - National Institute of Pharmaceutical Education and Research (NIPER), Mohali
  - Indian Institute of Chemical Technology (IICT), Hyderabad
  - National Institute of Science Communication and Information Resources (NISCAIR), New Delhi
  - National Chemical Laboratory (NCL), Pune
  - International Centre for Genetic Engineering and Biology (ICGEB), New Delhi
  - M.P. Council of Science & Technology, Bhopal
Design of compounds to be synthesized was done based on the literature as mentioned in Section 2.3 & 2.4.
In order to avoid repetition of research, the designed compounds were reviewed using the Chemical Abstracts 2010 & SciFinder®. The review was also used to prepare feasible synthetic schemes shown in Section 3.2.
Procurement of chemicals & reagents of different companies mentioned in Appendix 1 were made from Scientific Systems & Chemicals (P) Ltd. Bhopal (MP) INDIA.
All the compounds were synthesized and purified in Medicinal Chemistry Laboratory, of SIRTS Pharmacy Sagar Group of Institutions Ayodhoya Bypass Bhopal (MP).
The compounds were characterized for purity and structure at appropriate steps, using different techniques mentioned in Section 3.4.
The **UV** and **IR spectrophotometric** analysis was carried out at SIRT Pharmacy Sagar Group of Institutions Ayodhoya Bypass Bhopal (MP) INDIA.

The **$^1$H-NMR spectra** were recorded at Punjab University (PU), Chandigarh INDIA.

**C logP** calculations performed by using CS ChemOffice Ultra 6 at Molecular Modeling Group, CADD laboratory, School of pharmaceutical sciences (SOPS) RGPV Bhopal.

**Mass spectral** analysis carried out at “Sophisticated Analytical Instrument Laboratory” (SAIL) School of pharmaceutical sciences (SOPS) RGPV Bhopal.

Research Envisaged & Plan of Work SIRTS Pharmacy Sagar Group of Institutions Ayodhoya Bypass Bhopal (MP).

For antimalarial evaluation, following laboratories and institutes were approached.

- Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore(Karnataka)
- Haffkine Institute, Mumbai (MS)
- Indian Institute of Science, Bangalore (Karnataka)
- International Centre for Genetic Engineering and Biology, New Delhi (NCT)
- Central Drug Research Institute (CDRI), Lukhnow (UP)
- Tata Institute of Fundamental Research (TIFR), Mumbai (MS)
Finally, the antimalarial studies were being performed in **Central Drug Research Institute (CDRI), Lucknow (UP) INDIA.**

Compilation of results was done in Computer Laboratory of **SIRTS Pharmacy, Sagar Group of Institutions Ayodhoya Bypass Bhopal (MP).**