2.1 BRIEF REVIEW ON HETEROCYCLIC COMPOUNDS AS ANTIMALARIAL

Heterocyclic ring system is ubiquitously present in majority of bioactive molecules ranging from natural to synthetic compounds (Khokra and Choudhary, 2011). The useful pharmacological actions of bioactive compounds are attributed to the presence of heterocyclic ring(s) in their structure. Heterocyclic compounds are well recognized pharmacophore of a large number of medicinal products (Sperry and Wright, 2005). Different families of nitrogen and oxygen containing heterocycles, especially furanone, pyrrolone and their derivatives have also been extensively used by medicinal chemists as scaffolds in drug design and discovery (Shindo et al., 2007). Furanone and its bioisosteric derivative, pyrrolone are simple, unsaturated, five membered heterocyclic lactone and lactam rings, respectively, representing important and interesting classes of bioactive compounds (Jaimes et al., 2015).

Furanone, also called as butenolide or butyrolactone, is an important structural feature of several natural products, known to exhibit a wide range of useful and significant biological actions (Mao, 2011). Butenolides contain four carbon unsaturated γ-lactone ring and depending upon the relative positions of the carbonyl group and the double bond in the hetero ring occurs in three isomeric forms such as furan-2(3H)-ones, furan-2(5H)-ones and furan-3(2H)-ones. But, 2(3H)-furanones or \( \Delta^{\beta,\gamma}\)-butenolides and \( \Delta^{\alpha,\beta}\)-butenolides or 2(5H)-furanones (Jones et al., 1949) are very commonly found in nature formed as a result of polyketide biochemical synthesis. Steroids, which contain \( \alpha,\beta \) unsaturated butenolide ring in the side chain are known as cardenolides. The butenolide ring is an important structural component of many biologically active products as shown in fig 8.

Along with natural derivatives, synthetic ones based on butenolides nucleus had received much attention during the last decades due to outstanding biological activities such as anti-inflammatory, analgesic, antipyretic, potent COX-II inhibition (Husain et al., 2002a; 2005b; 2013c), antifungal (Husain et al. 2010; Pour et al. 2003), anti-HIV (Tyvorskii et al., 1998), antitumor (Chimchi et al., 2002), anticonvulsant (Azam et al., 2009) and antioxidant (Weber et al., 2002). Because of such versatile biological properties and therapeutic potentials of butenolide ring, a lot of work has been carried out worldwide.
Although furan derivatives are known to possess a broad spectrum of biological activities such as antimalarial, antifungal, antibacterial, antiinflammatory, analgesic, and antiviral properties. In addition, antioxidant and cardiotonic activities have also been reported. Several natural compounds containing furan ring have been reported as effective plasmodial inhibitors such as gersemolide alkaloids (IC$_{50}$ = 21.3µg/mL) (Marrero et al., 2006), neurolenin B (IC$_{50}$ = 0.62 µM), hirsutinolides (IC$_{50}$ = 1,800 and 2,600ng/mL, respectively), and bulaquine (BQ). The 2(5H)-furanone substructure
Literature Review

present in mucochloric acid and mucobromic acid (Pillay et al., 2007) is reported to be active against the malaria parasite. The antiplasmodial activities of mucochloric acid and mucobromic acid (IC$_{50}$ = 137 and 359ng/mL, respectively) suggest that the 2(5H)-furanone unit is the key pharmacophore for antiplasmodial activity.

As there is high level of resistance to all the classes of antimalarial compounds, including artemisinin derivatives, there is an urgent need for the design and development of novel and potent antimalarial drugs particularly against the *P. falciparum* which causes severe malaria. Considering the immediate need of effective antimalarial drugs, various scientist and their co-workers have been contributing their efforts in the perspective of research and development of antimalarial drug discovery.

Quinoline containing compounds have long been used for the treatment of malaria, beginning with quinine, which was 4,6-substituted quinoline. Systematic modification of quinine led to diverse quinoline antimalarial drugs (Kumar et al., 2003; Sharma S., 1990) with diverse substitutions around the quinoline ring. One of the first drugs to be prepared was the potent and inexpensive chloroquine (Chauhan et al., 2001; Jefford et al., 2001; Sweeney, 1981), which was 7-chloroquinoline with an amino substituent at position 4. The other known drugs from this family include: amodiaquine, piperaquine, primaquine, and mefloquine. The the mode of action of quinoline based antimalarials has been explored widely in the recent years, but remains incomplete (Ginsburg et al., 1992; Foeley et al., 1997; 1998; Fitch et al., 2004).

A short history of hybrid molecules based on heterocyclic moieties such as quinoline, pyrazole and some other hetero atom (furan, pyrimidine etc.) gave interesting and important information useful for organic and medicinal chemistry, which are deeply involved in design and development of new antimalarial agents (Akhter et al., 2014; Paliwal et al., 2014). Nowadays, double-drug development and/or multi therapeutic strategies, which utilize new chemical entities with two (or more than two) different heterocyclic skeletons (pharmacophores), are valid and perspective to create new antimalarial drugs. These strategies have the potential to overcome drug resistant parasites’ problem, for example that design and synthesis of aminoquinoline-containing dual inhibitors or ‘‘double drugs’’ that would potentially inhibit hemozoin formation and another target within *P. falciparum*, and will not be recognized by the
proteins involved in drug efflux, are very productive in the generation of new chemical entities that are effective against drug resistant parasites in the long term studied. The success of this hybridization approach, having a wonderful example of trioxaquines or artemisinin–quinine hybrid (Walsh et al., 2007), stimulates further organic, medicinal and biochemical activities to struggle malaria, the world’s most widespread and devastating infectious disease. The hybridization approach will represent more and more a new challenge for medicinal chemists, pharmacologists and biochemists (Sparatore et al., 2008) because it benefits not only to drug-design efforts, but also to better understand drug resistant problem.

It has been reported that the PfLDH is measured as one of the potential bio targets due to its major role in the survival of the parasites (Dunn et al., 1996; Granchi et al., 2010). It is a core enzyme for energy generation in malarial parasites. Although several isoforms of LDH are also present in the mammalian host (man), a Plasmodium parasite appears to be especially susceptible to agents acting against LDH, at least in their blood-borne stages. Therefore, inhibitors of PfLDH almost certainly kill the P. falciparum, therefore potentially provide a route to new antimalarial drugs directed against a novel molecular target.

It has been shown that the chloroquine interacts specifically with PfLDH in the NADH binding site, occupying a position analogous to that of the adenyl ring cofactor and therefore acts as a competitive inhibitor for this critical glycolytic enzyme PfLDH (Menting et al., 1997; Read et al., 1999). A series of heterocyclic, azole-based compounds were described that preferentially inhibit P. falciparum LDH at sub-micromolar concentrations, typically at concentrations about 100-fold lower than required for human lactate dehydrogenase inhibition (Cameron et al., 2004). Two potential binding sites for each ligand have been identified through docking studies. Promising vital structural requirements of quinoline-based derivatives have been identified by adopting molecular docking and QSAR studies to enhance their antimalarial activity (Sharma et al., 2014). The binding of a few quinoline-based drugs to the glycolytic enzyme lactate dehydrogenase have been carried out by employing molecular docking studies (Waingeh et al., 2010). In context to the above discussed strategies in the drug discovery of newer and effective antimalarials, relevant research
work and findings of various scientist and their team, has been discussed in this chapter.

2.2 RECENT RESEARCH CONDUCTED IN ANTIMALARIALS

The molecular docking and 3D-QSAR CoMFA were carried out to analyse a set of novel quinolinyl chalcone derivatives (1, 2). The studies are performed to analyse the structural requirements for the design and development of highly potent inhibitors against PfLDH. The outcome of the docking study suggests that the hydrogen bonding interactions are the major interaction which could be considered as vital in the modification of inhibitory activities of quinolinyl chalcone derivatives. These models could be used as a tool in the design of effective anti-malarial compounds to combat malaria. (Thillainayagam et al, 2015)

Quantitative structure–activity relationship (QSAR) and Pharmacophore studies were performed on a series of 35 azaaurones derivatives (3) to find out the structural requirements of their antimalarial activities
The results revealed some important SAR features for antimalarial activity such as the presence of chloro or fluoro substituents would increase the anti-malarial activity, and the presence of bulky withdrawing groups at R₃, R₄ and R₅ positions of the aurone ring would increase/decrease the anti-malarial activity. The presence of bulky substituents at the R₁ and R₂ positions of the aurone nucleus is important for designing new potent molecules with increased potency (Sharma et al., 2013).

Pyrazole derivatives (4) have been reported as novel *P. falciparum* inhibitor. The dibenzylideneacetone derivatives were converted to pyrazolines using a reported method The compound (1E, 4E)-1,5-bis(3,4-dimethoxyphenyl)penta-1,4-dien-3-one was found to be the most active with IC₅₀ 1.97 mM against chloroquine sensitive strain and 1.69 mM against chloroquine-resistant field isolate (Aher et al., 2011).

Securinine, (5) an alkaloid from the plant *Securinega suffructicosa* was extracted and evaluated for antimalarial activity. Securinine containing α-β unsaturated carbonyl moiety showed potential antimalarial activity with IC₅₀ 5.4µg/mL (Zhang et al., 2011).

A series of substituted 3-[(substituted-2-chloroquinolin-3-yl)methylene]-5-(substituted-phenyl)-furan-2(3H)-ones have been synthesized and evaluated for their
in vitro antimalarial activity against *P. falciparum*. The title compounds (6) were synthesized by condensing 3-(substituted-benzoyl)propionic acids with substituted 2-chloroquinoline-3-carbaldehydes following modified Perkin’s reaction. Compounds 3-[2-chloro-6-methylquinolin-3-yl)methylene]-5-(2,4-dimethyl-phenyl)-furan-2(3H)-one and 3-[2-chloro-6-methoxyquinolin-3-yl)methylene]-5-(2,4-dimethyl-phenyl)-furan-2(3H)-one showed promising antimalarial activity with MIC of 10 µg/mL. (Husain *et al*., 2011)

ADMET prediction studies were conducted on a library of new molecules based on the 4-aminoquinolone-related structure (7, 8) of CQ. A combination of *in silico*, *in vitro*, and *in vivo* ADMET assays in mice helped to identify a few lead molecules with promising therapeutic efficacy, improved ADMET properties that will be essential for further lead optimization efforts. (Ray *et al*., 2010)

α-pyranochalcones and pyrazoline analogs (9, 10) were synthesized to discover chemically diverse antimalarial leads. The (E)-3-(3-(2,3,4-trimethoxyphenyl)-acryloyl)-2H-chromen-2-one turned out to be the most potent analog of the series, showing IC₅₀ 3.1mg/ml against chloroquine sensitive (3D7) strain and IC₅₀ 1.1mg/ml.
against chloroquine resistant field isolate (RKL9) of *P. falciparum*. Furthermore, docking of compounds into active site of *falcipain* enzyme revealed its predicted interactions with active site residues (Wanare *et al*., 2010).

![Chemical structures](image1)

A series of 4-aminoquinoline–triazine conjugates (11) with different substitution pattern have been synthesized and evaluated for their in vitro antimalarial activity against chloroquine-sensitive and resistant strains of *Plasmodium falciparum*. (Manohar *et al*., 2010)

![Chemical structure](image2)

11

X= alkylamine  R= aryl group

A series of 1,3,5-trisubstituted pyrazolines (12) was synthesized and evaluated *in vitro* antimalarial efficacy against chloroquine sensitive (MRC-02) as well as chloroquine resistant (RKL9) strains of *Plasmodium falciparum*.

![Chemical structure](image3)

12
The activity was at nano molar concentration. β-hematin formation inhibition activity (BHIA50) of the pyrazolines were determined and correlated with antimalarial activity. A reasonably good correlation was observed between antimalarial activity (IC$_{50}$) and BHIA50. This suggests that antimalarial mode of action of this class of compounds appears to be similar to that of chloroquine and involves the inhibition of hemozoin formation. Some of the compounds were showing better antimalarial activity than chloroquine against resistant strain of *P. falciparum* and were also found active in the in vivo experiment (Kaushik *et al.*, 2010).

In the search for new pharmacophore for antimalarial activity, analogs (13) developed were screened for their in vitro antimalarial activity against drug-sensitive *P. falciparum* strain and in vivo assay was performed against *P. yoleii nigeriensis*. Two of the synthesized compounds were found more active than CQ when tested against the drug-sensitive strains whereas, none of them displayed significant activity when screened in-vivo (Madapa *et al.*, 2009).

Some new 6-ureido-4-anilinoquinazolines (14) were synthesized and evaluated against the chloroquine-sensitive *P. falciparum* strain. Several analogs elicited the antimalarial effect and have IC$_{50}$ value better than the standard chloroquine (Batra *et al.*, 2009)
In order to preserve artemisinin effectiveness, artemisinin chemistry was explored and a report on the recent developments on various kinds of artemisinin derivatives including artemisinin dimers, trimers and tetramers was given to compare their efficacy towards malaria parasite with that of artemisinins and various other anti-malarials (Chaturvedi et al., 2009).

The organocatalyzed highly economic one pot synthesis of tetrahydropyridines (15) as antimalarials was reported and the synthesized compounds were screened against *Plasmodium falciparum* in vitro and one of them showed antimalarial activity with MIC as low as 0.09μg/mL (Misra, et al., 2008).

![Chemical structure of compound 15](image)

The condensation reaction between chalcone and nicotinic acid hydrazide was carried out in methanol at 65°C to yield the target compound (16) as shown below.

![Chemical structure of compound 16](image)
The compounds were evaluated against chloroquine sensitive (MRC-02) and resistant (RKL9) strains of *P. falciparum* which exhibited antimalarial activity in nanomolar range, comparable with Chloroquine (Acharya et al., 2008; 2010).

A series of quinoline derivatives (17), substituted with halogen at 4-position have been synthesized. Several pharmacophoric elements were taken into consideration while developing the racemic synthesis of these compounds (Konda et al., 2008).

![Chemical structure of 17](image)

17 \( X = \text{Cl, Br, F} \)

A series of 4-anilinoquinolines bearing an amino side chain linked to the aromatic ring with a carbamate or an amide bond (18) were synthesized which exhibited good antimalarial activity. (Cochin et al., 2008).

![Chemical structure of 18](image)

18

The synthesis of novel 1,3-diaryl propenone derivatives (19) was carried out and their antimalarial activity in vitro against asexual blood stages of human malaria parasite, *Plasmodium falciparum*, are described (Mishra et al., 2007).
It was reported that *Plasmodium falciparum* lactate dehydrogenase (pfLDH) is a key enzyme for energy generation of malarial parasites and is a potential antimalarial chemotherapeutic target. It is known that the oxamate moiety, a pyruvate analog, alone shows higher inhibition against pfLDH than human LDHs, suggesting that it can be used for the development of selective inhibitors. Oxamic acid derivatives were designed and synthesized. The synthesized derivatives exhibited activities against pfLDH with IC$_{50}$ values of and 1.75 µM-3.13 µM, and have 59- and 7-fold selectivity over mammalian LDH, respectively. They also have micromolar range activities against *Plasmodium falciparum* malate dehydrogenase (pfMDH), which may fill the role of pfLDH when the activity of pfLDH is reduced. Thus, certain members of these oxamic acid derivatives may have dual inhibitory activities against both pfLDH and pfMDH. (Choi et al., 2007).

1,3-and 1,5-diarylsubstituted pyrazole derivatives (20, 21) have been synthesized and evaluated their antimalarial activity. The compounds analyzed inhibited the
plasmodium parasite with IC50 values ranged 30-50 mM. Furthermore, the mode of ligand binding was also investigated by docking the synthetic inhibitors at the active site of the crystal structure of the receptor protein. (Kumar et al., 2006)

A series of chloroquine-pyrazole analogs have been synthesized from the reaction of 1,1,1-trifluoro-4-methoxy-3-alken-2-ones with 4-hydrazino-7-chloroquinoline (22). The antimalarial activity of, synthesized derivatives have been evaluated in vitro against a chloroquine resistant P. falciparum clone. SAR studies showed that the aromatic functionality of the pyrazole ring was critical (Cunico et al. 2006)

\[
\text{R= H, OH, Cl, F, Br, NO}_2
\]

A series of 8-quinolinamine analogs (23) were synthesized, which showed potent antimalarial compounds. The synthesized compounds were tested for their in vitro antimalarial activity against chloroquine sensitive and resistant P. falciparum strains whereas; in vivo screening was performed against Plasmodium berghei (Jain et al., 2005).
A series of 2,4,5-triamino-8-chloropyrimido-[4,5-b]quinoline have been designed, synthesized and evaluated for antimalarial activity. The synthesized compounds were screened using Rane's test for blood schizonticidal activity in mice infected by *P. berghei*. The antimalarial activity of compound (24) was found comparable with chloroquine. (Joshi et al., 2005)

![Image of compound 24](image)

The synthesis of 2,4-substituted-6-[5-methylsulfanyl-3-(substitutedphenyl)-pyrazol-1-yl]-[1,3,5]-triazine derivatives (25) was carried out, which were subjected to nucleophilic substitution with different amines to afford the final targeted compounds. The compounds having substitution on R and R₁ as methoxy and piperizine, were 32 times more potent than the cycloguanil (standard drug) (Katiyar et al., 2005).

![Image of compound 25](image)

Some tetranortriterpenoids, domesticulide from seeds of *Lansium domesticum Corr* have been isolated. Some compounds (26) exhibited antimalarial activity against *Plasmodium falciparum*. Their structure elucidation state that they were analogues to butenolides derivatives. (Saewan et al., 2005)
1,3-Diphenylpyrazole-4-carboxaldehyde and 1-(4-nitrophenyl)-3-phenylpyrazole-4 carboxaldehyde (27) were obtained from the appropriated phenylhydrazones via the Vilsmeiere Haack reaction. These aldehydes were functionalized by various substituted anilines or substituted benzylamines to yield the targeted compounds (28). Antiparasitic activities of the corresponding azomethines were assessed. In the most cases, nitrated compounds were found to be more efficient than non-nitrated ones against *P. falciparum, Trichomonas vaginalis* and *Leishmania infantum* (Rathelot *et al.*, 2002).

A large number of antimalarial compounds with a wide variety of structures have been isolated from plants and can play a role in the development of new antimalarial drugs. Ethnopharmacological approaches appear to be a promising way to find plant metabolites that could be used as templates for designing new derivatives with improved properties (Taki *et al.*, 1988; Zhang *et al.*, 1999).
The Antimalarial activity of extracts of this Malaysian medicinal plants *Andrographis paniculata* was studied (Misra P, *et al.*, 1992; Najib Nik *et al.*, 1999). SAR studies indicated that the γ-alkyldene butenolide moiety of andrographolide (29) derivatives along with other groups were responsible for important biological properties (Dai *et al.*, 2006; Mishra *et al.*, 1992). The constituent of andrographolide are used extensively in traditional Chinese medicine (Abeyskera *et al.*, 1988; Sabu *et al.*, 2001).

Various hybrid molecules of artemisinin and 4-aminoquinoline (30) in the treatment of malaria and the evolution of trioxaquine hybrid were reported as a promising antimalarial drug candidate (Chauhan *et al.*, 1999).

The antimalarial activity of Sergeolide, (31) a chemical constituent from *Picrolemma pseudocoffea* was investigated. Sergeolide showed a very strong antiplasmodial activity in vitro as well as in vivo, even a very low conc. (0.006µg/mL) against the CQ-resistant and sensitive strains of *P. falciparum*. (Frandeur *et al.*, 1985)
The above reported research findings elucidated some important initiative ideas, for our aim to search for new antimalarial compounds, such as the derivatives based on the heterocyclic moieties (furanone, quinoline and azoles) may result in the promising antimalarial drug candidates. Beside that the strategies to explore new pharmacophore, new drug targets and computational approaches may add benefit in the discovery and development of novel antimalarials. Consequently, these overall observations prompted us to design and explore libraries of ligands based on hybrids of furanone-Quinoline and furanone-pyrazole moieties as potential antimalarial agents.
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AIM AND OBJECTIVES OF STUDY

The urgent need for the discovery highly effective antimalarial, with the importance of computational tools in designing of hybrid molecules, having amalgamation of two different chemical entities together in a single compound, prompted us to carry out this study. This is based on the literature survey that the merging of heterocyclic moieties such as furanone, quinoline and pyrazole can lead to compounds with improved antimalarial activity, particularly against the resistant strains of the plasmodium falciparum.

In accordance to the above, the primary aim of this study was to design and synthesize some furanone based derivatives, hybridized with quinoline and pyrazole moieties, which will be evaluated further for the antimalarial activity using suitable in vitro methods.

The process of achieving this aim involved the following steps:

1. Designing the library of hypothetical ligands having furanone fused with quinoline and pyrazole moieties.

2. Optimization of the ligands, using computational approaches such as Molecular docking and in silico ADME-T prediction, to screen out the best potent hits having “drug-likeliness” and nontoxic profile having good binding affinity with the receptor, better physiochemical (“drug like”) properties and less toxic profile.

3. Synthesis, purification, structural elucidation and confirmation of the new intermediate and final compounds using IR, $^1$H and $^{13}$C NMR, mass spectroscopy and elemental analyses.

4. Evaluation for in vitro antimalarial activity of the final compounds, in comparison to that of Chloroquine (CQ) as standard drug.