Summary of Project

MOLECULAR MODELLING BASED DESIGNING, SYNTHESIS AND EVALUATION OF BUTENOLIDE DERIVATIVES AS NOVEL ANTIMALARIAL EFFECTIVE TO TREATRESISTANT FORM OF PARASITE AND CEREBRAL MALARIA

U.G.C. Reference Number- F. No. 42-381/2013(SR)

Name of the Principal Investigator- DR. SUKHBIR LAL KHOKRA

Period of Report- 31-03-2013 to 30-03-2017

SUMMARY OF THE PROJECT WORK

Title of Research project: MOLECULAR MODELLING BASED DESIGNING, SYNTHESIS AND EVALUATION OF BUTENOLIDE DERIVATIVES AS NOVEL ANTIMALARIAL EFFECTIVE TO TREATRESISTANT FORM OF PARASITE AND CEREBRAL MALARIA

Plan of work:

Year wise Plan of work	Status of work
FIRST YEAR • To study the current chemotherapy of malaria and scope of development in existing antimalarial • To decide the hypothetical compounds on the basis of literature explored • Computational analysis of designed compounds using *in silico* approaches of ADME-T predictions including determination of lipophilic profile of compounds, Pharmacophore Modelling and Docking Studies.	**Completed** **Completed** **Completed**
SECOND YEAR • Synthesis of selected potent hits in laboratory • Spectroscopic Characterisation of synthesized compounds	**Completed** **Completed**
THIRD YEAR • Evaluation of compounds for *in vitro* as well as *in vivo* antimalarial activity • Compilation of data	**Completed** **Completed**

Current chemotherapy of malaria

As malaria had become the most serious health problem worldwide. Above all due to availability of counterfeit ACTs (drugs that contain fake artemisinin derivatives) and substandard ACTs (drugs of poor quality), the probabilities of mutation and resistance in parasite are increasing.

Various antimalarial drugs, prescribed in current drug regimen for malaria along with their chemical class are shown in table below.

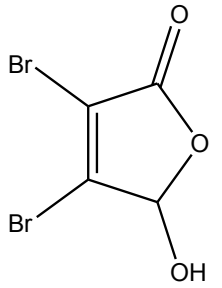
Table- Chemical classification of Antimalarial drugs

Chemical class	Examples
4-aminoquinolines	Chloroquine(CQ), hydroxychloroquine etc.
8-aminoquinolines	Pamaquine, Primaquine, Pentaquine etc.
Arylamino alcohols 4-quinolinemethanol 9-phenanthrene methanol	Quinine & quinidine Mefloquine Halofantrine, Lumefantrine
9-aminoacridines	Quinacrine, acriquine, aminoacrichin etc.
Biguanides	Proguanil, chloroproguanil, bromoguanil etc.
Diaminopyrimidines	Pyrimethamine, trimethoprim
Hydroxynaphthoquinone	Atovaquone
Benzonaphthyridine	Pyronaridine
Sulfonamides	Sulfadoxine, Sulfamethoxazole
Sulfones	Dapsone
Sesquiterpenelactone endoperoxides	Atemisinin, artemether arteether, artesaunate etc.
Antibiotics	Tetracycline, doxycycline, clindamycin etc.

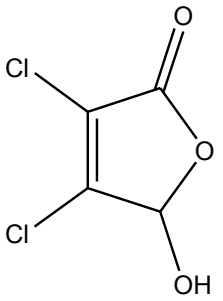
Almost all currently available antimalarial drugs except artemisinin and its derivatives were found associated with the problem of drug resistance. Recently the failure of artesunate-mefloquine combination therapy for uncomplicated *Plasmodium falciparum* malaria has been reported in Southern Cambodia.

All these situations shows that there is an urgent need to discover newer, better and safe antimalarials. Therefore research findings of some scientists, regarding development of newer antimalarial based on both plants as well as synthetic origin is discussed here as under:

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 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.

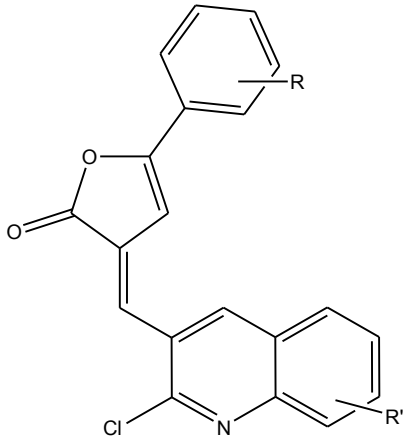


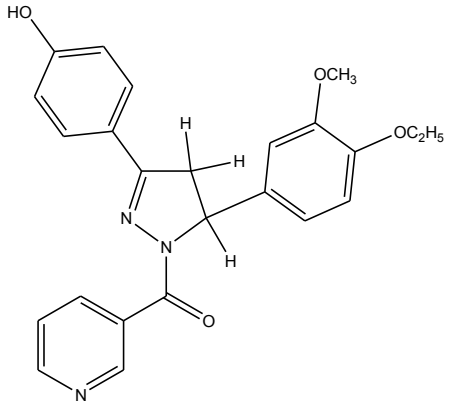
Mucobromic acid



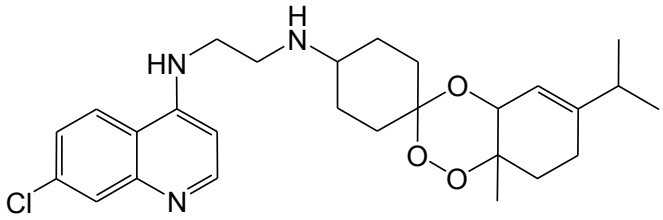
Mucochloric acid

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





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 *Pf*LDH 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 *Pf*LDH 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 *Pf*LDH 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 *Pf*LDH (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).

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.

STRUCTURAL DESIGNING OF HYPOTHETICAL COMPOUNDS, COMPUTATIONAL ANALYSIS AND. TARGET IDENTIFICATION

The development of resistance by the parasite against first line and second line antimalarial drugs, has underscored the importance to develop new drug targets and pharmacophores to treat the disease. With the development of computational chemistry and molecular docking studies, Structure Activity Relationship or SAR- and pharmacophore-based drug design have been modified to target based drug discovery using sophisticated computational tools (Soumendranath Bhakat, 2012). In structure-based drug design, the three-dimensional structure of a drug target interacting with small molecules is used to guide drug discovery. Within many of the rational drug design projects in the group, computer-aided methods such as virtual screening and de novo design techniques, play an important role (Kore *et al*., 2012). Generally the *in silico* drug designing process comprising mainly 3 stages:

Stage 1 It involves Identification of a therapeutic target and building a heterogeneous small molecule library to be tested against it.

Stage 2 These selected hits are checked for specificity by docking at binding sites of the targets.

Stage 3 These selected hits are subjected to detail *in silico* ADMET profiling studies and those molecules that pass these studies are termed as leads.

The same is valid for the drug designing and discovery of new antimalarials. *In silico* studies of new pharmacophores attacking different targets in the parasite can bring in new strategies to

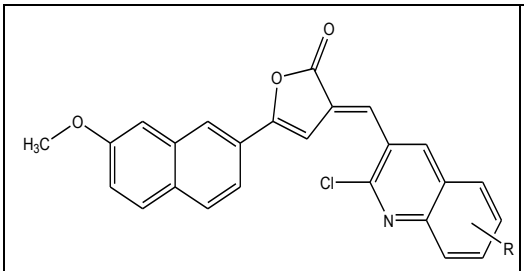
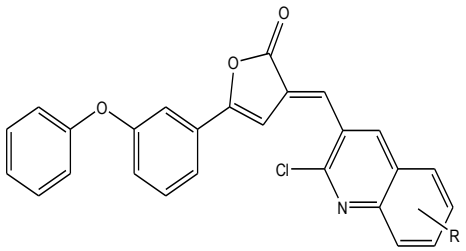
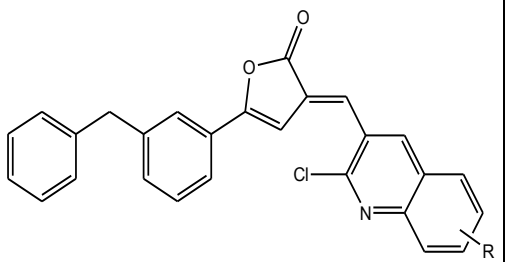
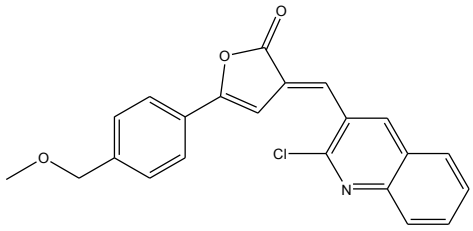
combat malaria (Padmanaban *et al*., 2007). The strategies such as exploring the vital enzymes which are essential for life of parasite, having significant different properties than human host may serve as the potential drug targets and this kind of strategy may help in designing of better therapeutic agents with more specific action and lesser side effects (Makler *et al*., 1998; Winter *et al*., 2003). L-lactate dehydrogenase from *Plasmodium falciparum* (*Pf*LDH) is one among such enzymes, which have been classified as L-lactate dehydrogenase from *Plasmodium falciparum.*

The crystal structure of *pf*LDH reveal that insersion of five distinctive amino acid residues forms a cleft adjacent to the active site, responsible for binding with inhibitors (Dunn *et al*., 1996). All these unique features made this a potential and specific antimalarial target. *Pf*LDH is being utilized by various researchers as potential antimalarial target (Razakantoanina *et al*., 2000; Conners *et al*., 2005; Franca & Krettli *et al*., 2011).

Further the role of computational tools in drug discovery and designing (Gehlhaar et al., 1995; Khokra *et al*., 2013), prompted us to design libraries of ligands based on furanone-quinoline and furanone-pyrazole derivatives. In this chapter we discussed the designing of some furanone based hypothetical ligands, screened them on the basis of their ADME-Toxicity profile and employing molecular docking studies with *Pf*LDH, to identify the best hits with antimalarial potential.

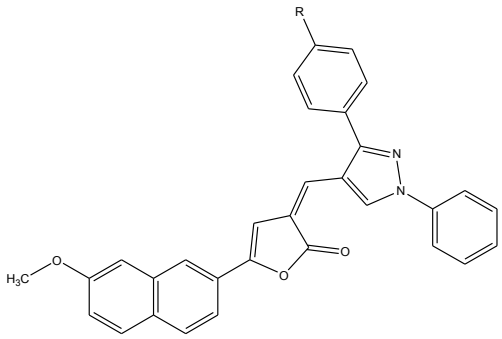
General structures of hypothetical compounds are shown in table below:

Table -General structures of hypothetical ligands based on furanone bearing quinoline

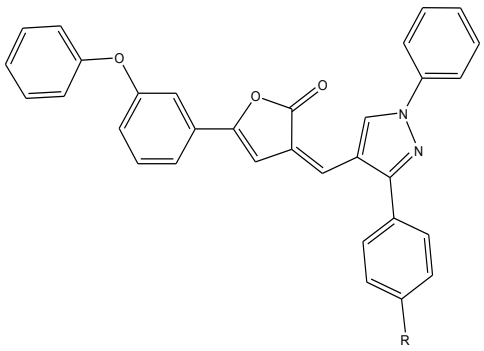
 **7a-h**	 **8a-h**
	 **10a-h**

9a-h									
		a	b	c	d	e	f	g	h
where	R=	H	CH_3	OH	OMe	Cl	F	Br	NO_2

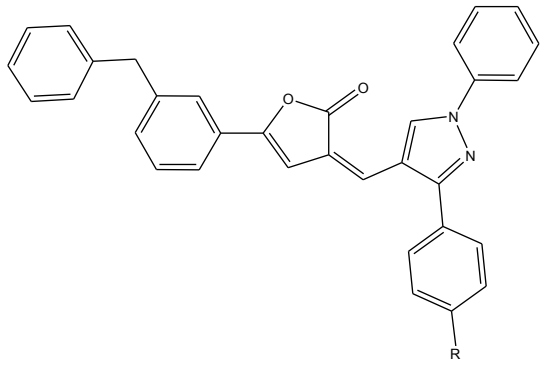
Table - General structures of hypothetical ligands based on furanone bearing pyrazole



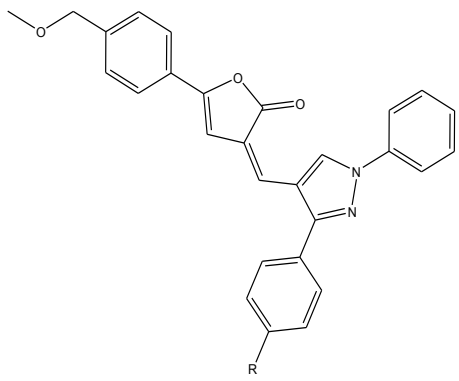
11a -h



12a-h



13a-h



14a-h

		a	b	c	d	e	f	g	h
where	R=	H	CH_3	OH	OMe	Cl	F	Br	NO_2

The protocol used for virtual screening is shown in fig below. The designed libraries of ligands were subjected to ADME-T prediction. Toxicity analysis employing carcinogenicity and mutagenicity (rat model) filter were carried out using TOPKAT (Toxicity Prediction by Komputer-Assisted Technology) module in discovery studio 2.5. After then ADME property descriptors were calculated for these hits using QikProp 3.6 from Schrodinger. Thereafter these

31 “druglike” non toxic hits were finally screened by docking analysis. Docking is considered as a virtual idea of bonding between ligands and protein receptors, we have to validate it using more than just one tool before coming to any conclusion. Therefore preliminary docking was done using software Molegro Virtual Docker (MVD), (Thompson *et al*., 2006) to calculate energies between ligands and macromolecules, which were compared with NADH (Wishart *et al*., 2008) and Chloroquine (CQ) (Menting *et al*., 1997). The docking results were then validated using other software Autodock 4.2. A total 31 molecules were docked within the active site of the enzyme protein *Pf*LDH (PDB- 1LDG). Docking poses were visually analyzed in detail for the following criteria: (i) energetically reasonable ligand geometry; (ii) H-bond interactions with the key residues of active site such as Gly 29, Ile31, Asp53, Thr97, Try85, Gly99 and Asn140; (iii) binding mode and superimposition with the NADH and substrate. Hence on the basis of these criteria, final 29hits were obtained.

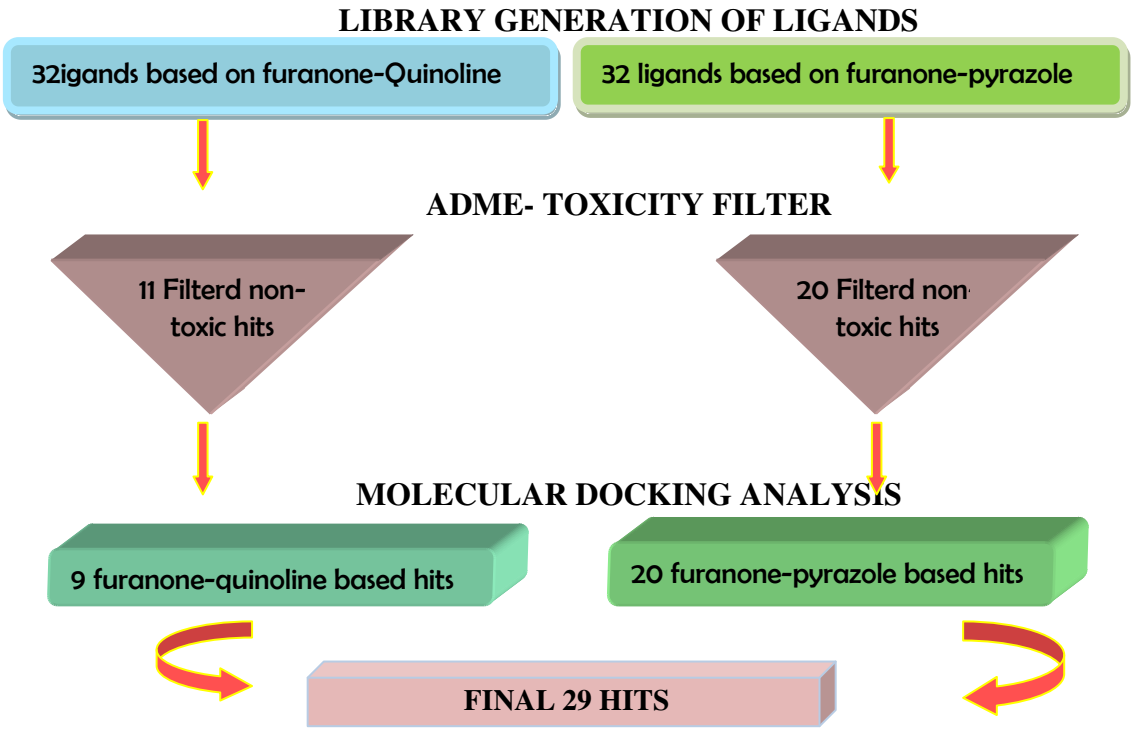


Figure - Flow of virtual screening

In this study, a combined approach of virtual screening including, ADME and Toxicity prediction and molecular docking simulation was applied effectively to evaluate and screen a set of designed hypothetical furanone (butenolides) for most potent drug like hits, which may inhibit

*Pf*LDH as new antimalarial potential agents. Primary screening of designed library of 64 hits was based on ADME and toxicity prediction using QikProp 3.6 and TOPKAT respectively. This effort leads to 31filtered non toxic-druglike hits from designed library. Final screening was based on molecular docking analysis. Best docked conformations were selected based on energy score of ligand-protein complexes, H-bond interactions with the key residues of active site and binding mode. Finally total 29 hits were obtained, 9 compounds (**7e**, **7f**, **7g**, **8c**, **8e**, **8f**, **8h**, **9a** and **9h)** from fuanone-quinoline library and 20 hits (**11a**, **11b**, **11c**, **11d**, **11f**, **11h**, **12a**, **12b, 12c, 13a, 13b, 13c, 13f, 14a, 14b, 14c, 14d, 14e, 14f** and **14g**) from furanone-pyrazole library were selected which exhibited good binding affinities and strong H-bond interactions with *Pf*LDH. The selected ligands formed consistent H-bond interaction with the main key amino acid residues such as Met30, Gly29, Ile31, Arg109, Asn140, Arg171, Ser245, Ala236 and Thr232. The docking conformations of these poses were either having superimposition with NADH or showed dual binding with both NADH and substrate binding site in the same binding pocket. It shows the consistency in docking results. In addition to these, Crystal structure of ICAM-1 was employed to perform structure based designing of these butenolide derivatives as potential agents against cerebral malaria. The results revealed that desighned hits exhibited comparative to good anticytoadher-ent properties with receptor ICAM-1, considered as main receptorinvolved in pathogenesis of CM. The compounds with highest score function, best binding conformation and lowest conformational energy can be will be explored further in future for designing the potent inhibitors of *Pf*LDH as new potent antimalarial agents, which in addition may be effective against cerebral malaria.

SYNTHESIS AND CHARACTERIZATION:

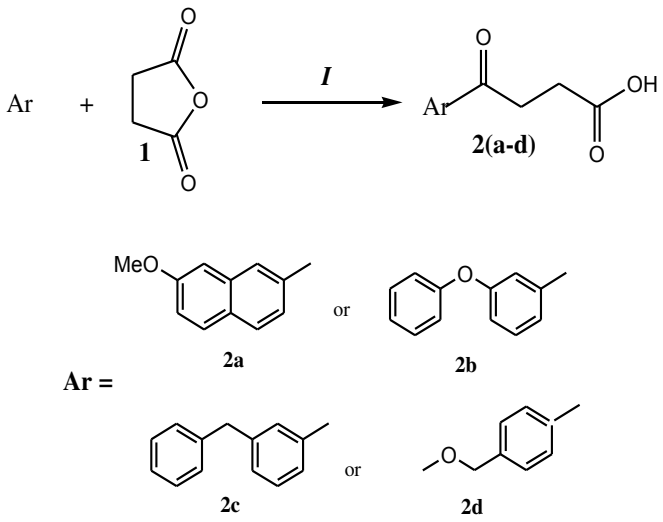
Melting points (mps) were taken on slides in an electrical apparatus Labindia visual melting range apparatus and are uncorrected. Calibration of melting point apparatus was done using benzoic acid as reference. IR spectra were recorded on a Perkin-Elmer 1800 FT-IR spectrophotometer. 1H NMR spectra were recorded on a Bruker 300 & 400 MHz instrument using tetramethylsilane as an internal standard. Mass spectra were recorded on 2500 eV (ESI Source) using a water's Q-TOF microinstrument and elemental analysis on Perkin-Elmer 2400 instrument. All the reagents were purchased from the commercial sources and were used without further purification.Progress of the chemical reaction and the purity of the synthesized compound was checked on silica gel G coated thin-layer chromatography plates in either of the followinng

solvent systems; Toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v) or Petroleum ether: Toluene: Ethyl acetate (5:4:1, v/v/v) or Ethyl acetate: Hexane (3:7, v/v). The visualization of spots on TLC was carried out in iodine chamber and UV cabinet at long wavelength under UV lamp.

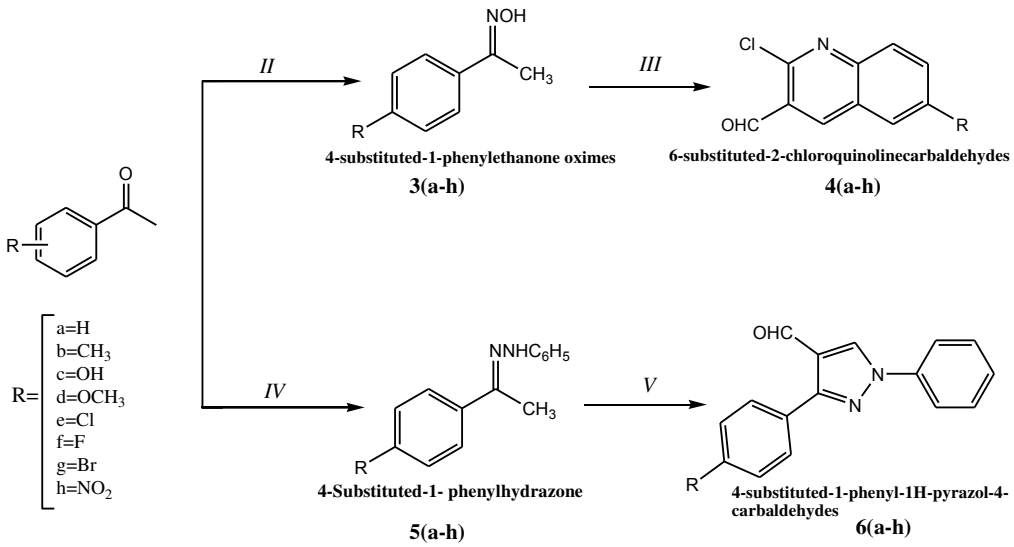
On the basis of *in silico* ADME-T prediction and molecular docking analysis, the virtual screening process left us with 31 best hits from the total 64 hypothetical ligands. The synthetic route for the preparation of potent screened derivatives, 3-{(2-chloro-6-substituted-quinolin-3-yl)methylene}-5-(aryl-2-yl)-furan-2(3*H*)-one and 3-{(4-Substitutedphenyl-1-phenyl-1*H*-pyrazol-4-yl)methylene}-5-(aryl-2-yl)-furan-2(3*H*)-one has been illustrated in Synthetic Scheme 1 under different steps.

SYNTHETIC SCHEME: The whole synthetic scheme can be studied under following steps

STEP-1 Synthesis of β-aroyl propionic acids (2a-d)

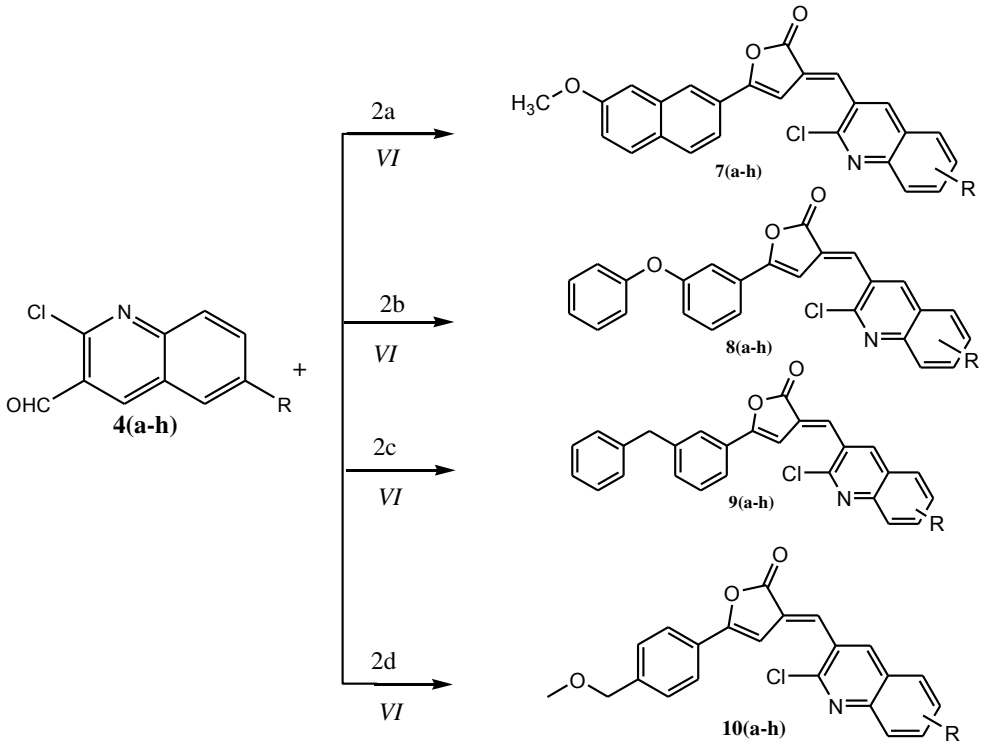


STEP-2 Synthesis of 6-substituted-2-chloroquinoline carbaldehyde (4a-h) and 4-substituted-1-phenyl-1H-pyrazol-4-carbaldehyde (6a-h)

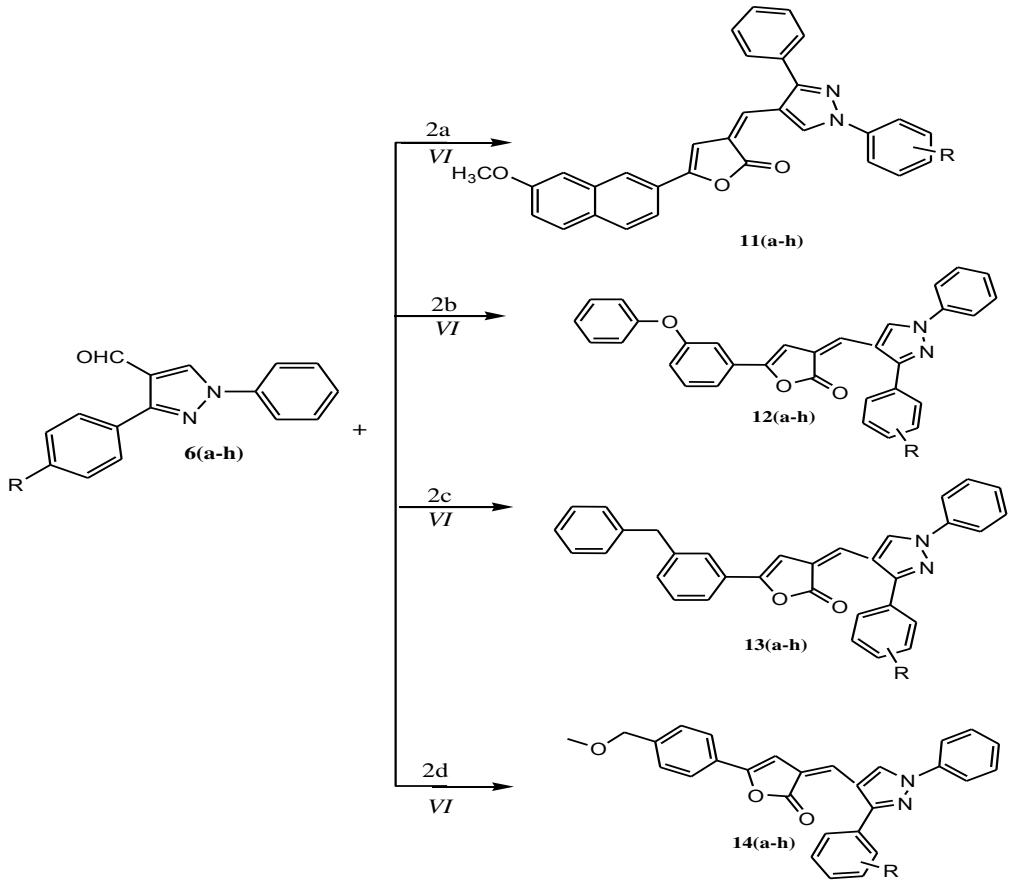


Synthetic scheme-Reagents and conditions: (I) anhydrous $AlCl_3$, reflux, 2-4 h; (II) $NH_2OH.HCl$, CH_3COONa, EtOH, water, reflux; (III) DMF, $POCl_3$, anhydrous condition, 50-60°C, 16h with stirring; (IV) phenyl hydrazine, HCl, EtOH, stirring, rt, 30 min; (V) DMF, $POCl_3$, anhydrous condition, 50-60°C, 4-6h with stirring

STEP-3 Synthesis of furanone bearing quinoline



STEP-4 Synthesis of furanone bearing pyrazole



Synthetic scheme-Reagents and conditions: (VI) acetic anhydride, triethylamine, heat, 5min., anhydrous condition

EVALUATION OF ANTIMALARIAL ACTIVITY

Mainly two techniques were employed:

- ***In vitro* estimation of antiplasmodial activity**
- ***In vivo* estimation of antimalarial activity.**

In vitro estimation of antiplasmodial activity: two assay methods were used

1) **Schizont maturation inhibition assay (SMI)**
2) **Lactate dehydrogenase inhibition (LDH) assay**

The *in vitro* antimalarial activity of the new compounds was assessed using the chloroquine-resistant (K_1) strains of *P. falciparum*. The strain was procured from Parasite bank of National Institute of Malaria Research (NIMR), New Delhi. All the *in vitro* tests were carried out in the

laboratory of NIMR, with some modifications in the known techniques Schizont Maturation inhibition Assay (Rieckmann *et al*., 1968; Trager & Jensen, 1976; WHO standard microtest) and *Plasmodium* Lactate Dehydrogenase Inhibition Assay (Makler and Hinrich, 1993; Stephan Karl *et al*., 2009).

The general methodology for *in vitro* antimalarial activity can be divided into following steps:

IN VITRO CULTIVATION OF _PLASMODIUM FALCIPARUM_

(A) Preparation of culture medium for cultivation of *Plasmodium falciparum*

-Preparation of RPMI medium

-Preparation of Washing medium (Incomplete medium)

-Preparation of Serum

-Preparation of Complete medium

-Preparation of erythrocytes (RBCs) for culture

-Initiation of culture

MAINTENANCE OF _PLASMODIUM FALCIPARUM_ CULTURE

(A) Monitoring culture growth

(B) Smear preparation and Estimation of the percentage of erythrocytes infected (% parasitaemia) with *Plasmodium falciparum*

(C) Subculturing (Passaging)

ESTIMATION OF ANTIMALARIAL ACTIVITY

(A)Synchronization of *Plasmodium falciparum*

(B)Preparation of Drug solution

(C)The harvesting of parasite and assay for the antimalarial activity by:

-Schizont maturation assay

- Microscopic analysis of erythrocytes from thin smear of culture
- Counting of infected erythrocytes (no. of schizonts)
- Calculation of IC_{50} and data analysis

-*Plasmodium* lactate dehydrogenase (*Pf*LDH) inhibition assay

- Measurement of LDH activity (absorbance of each well)
- Calculation of IC_{50} and data analysis

Almost all the synthesized hybrids were having antimalarial activity but in series of quinoline-furanone hybrids, compounds **7g** and **8e** were found to exhibit maximum activity with IC_{50} value

almost equal to the CQ (the standard used), followed by compounds **7e**, **7f**, **8a**, **8b**, **9a** and **9f**, having IC_{50} value comparable to CQ(<5). Beside these derivatives, the compounds such as 8f and 9h were found less potent comparatively and two derivatives (8c and 9h) were considered to have poor antimalarial activity (IC_{50}>10) as shown in table 8. In the similar manner if we talk about the antimalarial potential of furanone- pyrazole series, the more number of compounds were found active in comparison to quinoline based series. The compounds such as **11a**, **11f**, **13f**, **14a** and **14e** were found best having IC_{50} valve (<5) comparable to that of CQ, followed by compound **11b**, **12a**, **13a**, **14d**, **14f** and **14g** having IC_{50} value <10. But compounds such as 11c, 11h, 13b, 13c and 14c were exhibited very low antimalarial activity. The whole *in vitro* analysis data revealed some important points regarding the SAR of these newly synthesized derivatives such as the no. of active compounds were more in pyrazole based furanone, but on the same place the best compounds with minimum IC_{50} were found in quinoline based furanone series, this observation leads to the conclusion that the both quinoline as well as pyrazole were important pharmacophore responsible for the antimalarial potential of targeted butenolide derivatives, but due to the R- substitution variation in activity results. *In vitro* activity data revealed that, derivatives having halogen as R substituent will result in increase in activity but if halogens were replaced with either the strong electronic withdrawing such as $–NO_2$ or strong electronic donating -OH group will lead to almost very less active derivatives. Besides these points the derivatives with substituent like H, OMe and CH_3 have varied type of effect on antimalarial activity in different series, as shown in table 1, for example compound 11a having H as R sustituent were found to have very good activity (IC_{50} 2.247), but compound 12a was having moderate antimalarial activity (IC_{50} 8.271), same was reported in case of methoxy as sustituent. The compounds 11d and 12d exhibited excellent antimalarial activity but compound 13d were moderately active.

IN VIVO ESTIMATION OF ANTIMALARIAL ACTIVITY

Swiss albino mice (18-25 g) of either sex were acclimatized to laboratory conditions in the animal house of Kurukshetra University, Kurukshetra. The mice were housed in plastic cages in a well ventilated room (25 ± 5°C), fed with standard rodent feed and allowed free access to drinking water. Chloroquine-sensitive *Plasmodium berghei* (NK 65 strain) was obtained from the National Institute for Malaria Research (NIMR), New Delhi. Parasite viability was maintained

by continuous re-infection in mice, via intraperitoneal injection. All experiments were carried out after ethical clearance was obtained from the ethics committee of the institute.

Parasite inoculation

Swiss albino mice were parasitized with *Plasmodium berghei (NK65)* from National Institute of malaria research, New Delhi and were maintained in the laboratory by serial passage of blood from infected mice to non infected mice. The parasitized albino mice having parasitemia level of 20–30 % were used as donors. Their parasitaemia levels were first determined and their blood diluted with normal saline. Then 0.2mL of the diluted blood (contained 1 x 10^7 *P. berghei* infected red blood cells) was administered intra-peritoneally to each test mouse.

Drugs used

Both chloroquine phosphate and the test compounds were dissolved in 70% Tween 80 and 30% ethanol. These solutions were further diluted tenfold with distilled water to result in stock solutions containing 7% Tween 80 and 3% ethanol.

***In vivo* antimalarial activity test method:**

The test protocol is based on the 4 day suppressive test as described by Peters (1965). Mice were then weighed and randomly divided into 16 groups of five mice per cage. After 2 hr of infection, the first 13 groups received the synthesized compounds suspended in a vehicle containing 7% Tween 80 and 3% ethanol in water orally, at 48.46 µmol/kg dose level. Group 14 received the vehicle only and acted as a negative control. The standard drug, chloroquine phosphate dissolved in the same solvent, was administered orally at a dose of 48.46µmol/kg to mice in group 15 and served as positive control (Domingues *et al*., 2009). On days 1 to 3 (24 hr time interval on successive dosing), animals in test groups were treated again with the same dose of the synthesized compounds through the same route as in day 0. On day 4 that is 24 hr after the last dose or 96 hr post-infection, the mice were weighed and blood smear was prepared on slides. The blood was then fixed with absolute methanol and stained with Giemsa stain. Level of parasitemia was determined microscopically by counting 4 fields of approximately 100 erythrocytes per field. The difference between the mean 35 parasitemia level of the negative control group (taken as 100%) and that of test compound treated group was calculated and expressed as percent suppression. The survival time for each test mouse was recorded except for chloroquine treated ones which were completely cured of the parasite (Peter *et al.*, 1999).

The standard 4-day suppressive test, which mainly evaluates the antimalarial activity of candidate drugs on early infections were compared against the control groups, as shown in Table 12. The percentage parasitemia determined for all test compounds were significantly low relative to the negative control ($p < 0.05$), showing that the compounds are active. The test compounds, **7e**, **7f, 7g, 8e**, **9a** and **14a** displayed mean percentage suppression of greater than 50 %. On the other hand, compounds **8a, 8b, 9f, 13f** and **14e** had more than 40 % mean percent suppression compared to the untreated group (as shown below in Table). Compound **7e** was the most active of the tested compounds with mean percentage suppression of 72.86 %. The mean parasitemia level in mice treated with **7e** (17.71 ± 0.45) was found to be approximately four times lower than the negative control (65.25 ± 0.73), showing the compound has greatly reduced the parasite load. This significant activity was further supported by better mean survival time (9.2 ± 0.43) of mice compared with other test compounds but less than those of positive control (chloroquine-treated) group that did not show any death during the experimental period. Compound **8e** displayed the next significant ($p < 0.05$) antimalarial activity with percentage suppression of 67.60 %, which was further confirmed by mean survival time (8.3 ± 0.55) (as shown in table). The highest suppression effect of **7e** and **8e** may be attributed to the presence of chloro groups (–Cl) in both. The same was also reported in case of *in vitro* studies, this finding revealed that presence of halogen as a substituent (more in case of –Cl) leads to increase in antimalarial activity of the resultant compounds. The compounds with good antimalarial potential may be explored further to get better antimalarial agents in future. Although we have conducted antimalarial studies at a single dose level, due to availability of limited number of mice, but these test compounds can be tested at different dose levels to analyze dose-response relationship.

Table: Data for *in vivo* antimalarial activity of synthesized (dose 48.46µmol/kg equivalent) compounds using 4-day suppression test

Treatment	Dose (µmol/kg)	*Percentage parasitemia	Percentage suppression	Mean survival time (days)*
7e	48.46	17.71±0.45	**72.86**	9.2±0.43
7f	48.46	27.14±0.91	55.41	8.3±0.68
7g	48.46	28.45±0.78	56.34	7.8±0.87
8a	48.46	34.70±1.15	46.87	6.9±0.91
8b	48.46	36.73±1.11	43.71	7.1±0.99

8e	48.46	21.14±0.43	**67.60**	8.3±0.55
9a	48.46	30.29±0.48	53.58	7.6±0.47
9f	48.46	46.71±0.25	6.78	5.9±0.21
11a	48.46	31.57±0.49	36.99	7.6±0.13
11f	48.46	40.16±0.18	19.86	6.7±0.83
13f	48.46	29.96±0.73	40.21	6.9±0.54
14a	48.46	18.34±0.56	**63.40**	10.2±0.46
14e	48.46	27.30±0.35	45.52	9.2±0.54
Control (NC)**	-	65.25±0.73	0.0	6.3±0.86
CQ	25	0.0	100	ND***

*Values are Mean ± SD, P<0.05, **NC: Negative control, **ND*****No death recorded over the experimental period.

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PUBLICATIONS

- Deepika Choudhary, Girish Kumar Gupta, Sukhbir Lal Khokra, Nisha. Designing and ADME-T Studies of Butenolide Derivatives as ICAM-1 receptor inhibitors: A Drug Target for Cerebral Malaria, Journal of Computational Sciences. Elsevier, 10 (2015) 156–165. (PUBLISHED)
- Deepika Choudhary, Sukhbir Lal Khokra. The Synthesis of 6-sustituted-2-chloroquinoline-3-carbaldehyde using Vilsmeier-Haack Reaction, International Journal of Science and Research, 2016, Volume 5 Issue 6, 2552-2555.

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- Deepika Choudhary, Sukhbir Lal Khokra, Rajender Kumar, Pramila Thakur, Prabha Garg. Designing, Molecular Docking and ADME-T studies of Furanone bearing Pyrazole Moieties as Potential Antimalarial Agents. Mini Reviews in Organic Chemistry.

  (ACCEPTED)
- Deepika Choudhary, Sukhbir Lal Khokra. Synthesis and Biological evaluation of Quinoline based 2(3h)-Furanones as *Plasmodium falciparum* Lactate Dehydrogenase Inhibitors. (COMMUNICATED)
- Deepika Choudhary, Sukhbir Lal Khokra, Asif Hussain. Pyrazole based furanone hybrids as novel antimalarial and selective lactate dehydrogenase inhibitors: design, synthesis and antimalarial activity. (COMMUNICATED)

PAPER PRESENTATION

- A paper had been presented on "Designing and Virtual Screening of Furanone Derivatives as Receptor ICAM-1 Inhibitor: A Drug Target for Cerebral Malaria" at the second international conference held at Mahatma Gandhi University, Kottayam, Kerala, India.
- A poster presentation on "*In silico* analysis of furanones and their derivatives having anti-malarial activity" at National conference on Breaking new Grounds in Pharmaceutical Research, organized by Department of Pharmacy, Manav Bharti University, Solan, H.P.

- A poster presentation on "Molecular modelling and ADMET prediction studies of novel butenolide as potential inhibitor of *Pf*-Lactate Dehydrogenase" at national seminar on Role of community pharmacist in handling of antibiotics, sponsored by IPGA, organized by Department of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana on 12 April 2014 and awarded with the Best Poster Award.
- A poster presentation on "Design, Molecular Docking and ADME-T studies of some Furanone bearing Pyrazole moieties as Potential antimalarial agents" at 29th Annual Conference of Indian Pharmacy Graduates' Association at Lovely Professional University, Phagwara, Punjab on 22-23 Nov.2014.
- A poster presentation on "Pathophysiology, clinical presentation and treatment of cerebral malaria" at 2nd one day national symposium on New Horizons in Drug Discovery & Development-2, organized by Department of Pharmaceutical Chemistry, Jamia Hamdard, Hamdard Nagar, New Delhi on 16 Nov. 2015.
- A poster presentation on "Structure based designing and virtual screening of Novel Butenolides as Potential Inhibitor of *Plasmodium falciparum* L-Lactate Dehydrogenase" at national seminar in MMU, Mullana, Ambala (Haryana).