Journal of Computer-Aided Molecular Design
https://doi.org/10.1007/s10822-019-00210-2

Anti-malarial, cytotoxicity and molecular docking studies of quinolinyl chalcones as potential anti-malarial agent

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Received: 9 January 2019 / Accepted: 31 May 2019
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Abstract

The quinolinyl chalcones series (A_1–A_{14}) were screened for antimalarial activity. According to in vitro antimalarial studies, many quinolinyl chalcones are potentially active against CQ-sensitive and resistance *P. falciparum* strains with no toxicity against Vero cell lines. The most active quinolinyl chalcones A_4 (with IC_{50} 0.031 μM) made a stable A_4–heme complex with − 25 kcal/mole binding energy and also showed strong π–π interaction at 3.5 Å. Thus, the stable A_4–heme complex formation suggested that these quinolinyl chalcones act as a blocker for heme polymerization. The docking results of quinolinyl chalcones with Pf-DHFR showed that the halogenated benzene part of quinolinyl chalcones made strong interaction with Pf-DHFR as compared to quinoline part. A strong A_4–Pf-DHFR complex was formed with low binding energy (− 11.04 kcal/mole). The ADMET properties of quinolinyl chalcones were also studied. The in vivo antimalarial studies also confirmed the A_4 as an active antimalarial agent.

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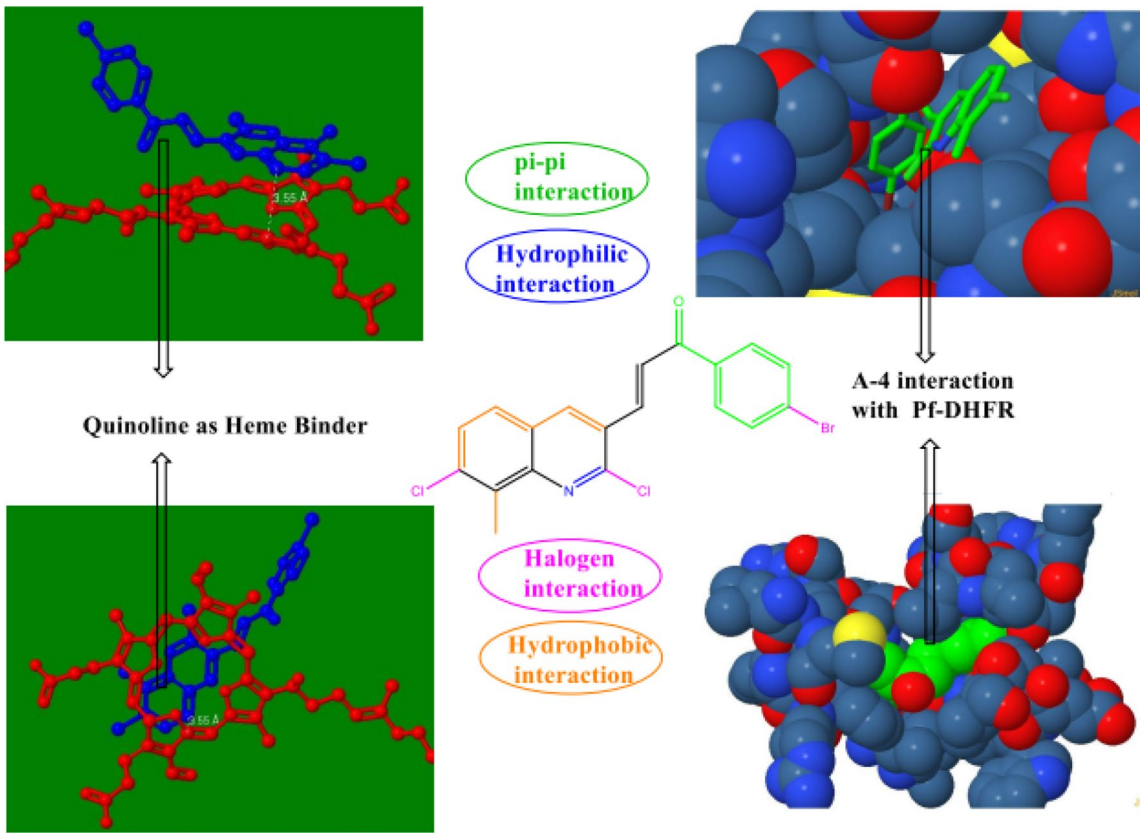
Published online: 03 July 2019

Graphical abstract



Keywords Quinolinyl chalcones · Heme binder · Acetophenone · Antimalarial · Pf-DHFR

Introduction

The tropical and subtropical countries of the world have a foremost health problem is malaria. Malaria affected 400–900 million people each year in the world and also becomes the cause of death of about one to three million people annually [1]. The malaria caused in humans by the parasites such as *Plasmodium falciparum, P. malariae, P. vivax* and *P. ovale*. However, one of the most dangerous parasite is Plasmodium *falciparum,* the 80% of infection and 90% of deaths caused by Plasmodium *falciparum* parasite [2]. For the treatment of malaria, researcher have been developed many drugs, the chloroquine is one of most commonly used drug against malaria. The chloroquine is not the effective drug because *Plasmodium falciparum* has become resistant to chloroquine and other conventional anti-malarial drugs, so there is search for effective anti-malarial drug is still under process [3, 4]. Domínguez et al. reported potent phenylurenyl substituted chalcones with (IC50 = 1.76–10 μM) as a growth inhibitors for in vitro cultured P. falciparum. They did variation of the substituents in the B-ring of the chalcones and reported that the activity is largely depends on the kind of substituent on ring B of the chalcones. The chloro group on the para-position of the 4-phenylurenyl chalcones plays a very important role in the anti-malarial activity and the chloro-substituted phenylurenyl chalcones showed good anti-malarial activity [5].

Moreover, after a lot of research work, there is no available vaccine made against malaria for commercial uses. The GlaxoSmithKline pharmaceuticals recently developed a RTS, S/AS01 vaccine candidate, which is in Phase lll clinical trials. This vaccine provided only mild protection in young infant against both severe and clinical malaria [6, 7]. Thus, the treatment of malaria through chemotherapy remains the main procedure to deal with malarial problems.

The drug resistance development against antimalarial medicines such as amodioquine, chloroquine, artemisinin and anti-folates is turn out to be a severe health issue that stimulates the researcher to synthesize novel antimalarial compounds [8]. There is a need to develop new safe and affordable anti-malarial agents to overcome the growing malarial resistance against drugs. The twelve novel

quinolinyl chalcones synthesized by Domínguez et al. and screened these chalcones against chloroquine resistant strain of *P. falciparum*. Only one compound out of twelve showed promising activity against chloroquine resistant strain with $IC_{50} = 19.0$ μM [9].

In search of new drugs for malarial parasites, the most important target for drugs are to bind with Pf-DHFR (Plasmodium falciparum dihydrofolate reductase) or heme. During the life cycle of malarial parasite at pathogenic blood stage, the malarial parasite catabolizes the hemoglobin as a key reserve of amino acids, dismissing free heme that is supposed to be poisonous to the malarial parasite [10, 11]. The parasite has developed a detoxification path to confiscate free heme into inert and insoluble compound recognized as hemozoin or malaria pigment. Quinoline based antimalarial agents, for example quinacrine (QA), quinine (Q), mefloquine (MQ) and chloroquine (CQ) are backbones of chemotherapy against malarial parasite and are thought to be employed their effect through disturbing the formation of malarial pigment, therefore producing parasite poisonousness from the accumulation of free heme [10, 12, 13]. The quinolinyl chalcones were also reported as anti-HIV, antibacterial, and inhibitors of bacterial DNA gyrase and viral reverse transcriptase enzyme [14, 15]. Herein, we screened proficient quinolinyl chalcones as antimalarial, Plasmodium falciparum dihydrofolate reductase (Pf-DHFR) inhibitors and heme binder. We introduced the substituents such as Br (bromo) and Cl chloro at different position of quinolinyl chalcones, which are highly hydrophobic in nature and enhance the drug alike features. Thus, these substituents eases the drug to readily pass cell boundary. The effect of substituent and its position on antimalarial activity is also screened by changing the substituent and its location. Nowadays, to overawed drug resistance issues the idea of hybrid molecules have been accessible, in which more than one pharmacophores are connected together and it is assumed that these molecules act by impeding instantaneously two conservative targets. So in our case we linked the quinoline with benzene (containing various type of substituent such as –Cl, –Br, $–OCH_3$ and CH_3) via chalcone bridge as depicted in Fig. 1.

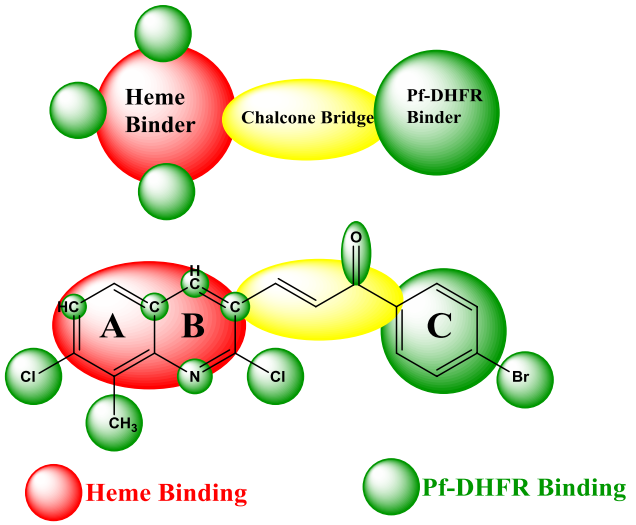


Fig. 1 Systematic illustration of quinolinyl chalcones interaction with Heme and Pf-DHFR

Results and discussion

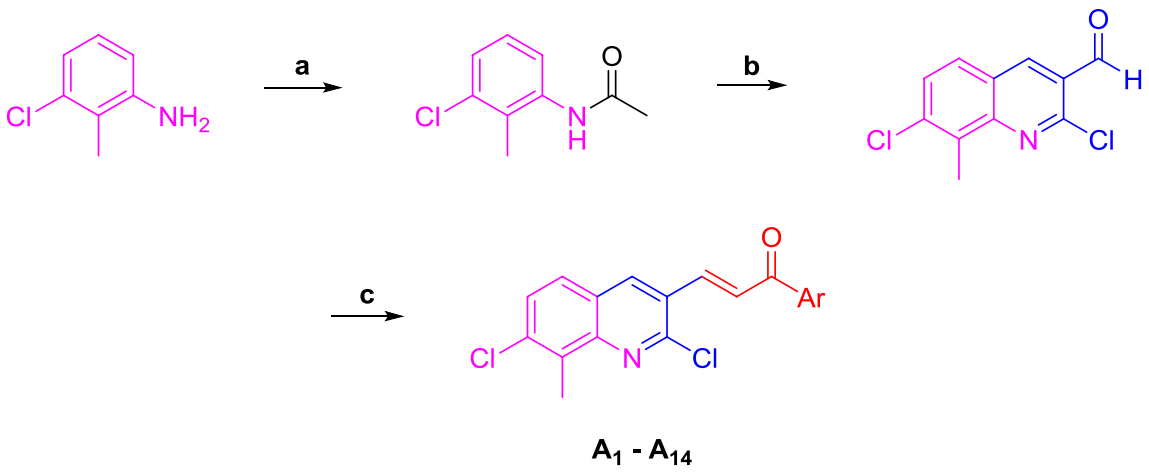
Chemistry

The condensing partner formyl quinoline (2,7-dichloro-8-methyl-3-formyl quinoline) for aryl ketone was synthesized via meth-chon method [15, 16]. The series of quinolinyl chalcones A_1–A_{14} were synthesized via Claisen–Schmidt condensation reaction according to previously reported methods as shown in Scheme 1 [15]. In this reaction the different aryl ketones were condensed with formyl quinoline in the presence of NaOH as shown in Chart 1 [17, 18].

Antimalarial activity

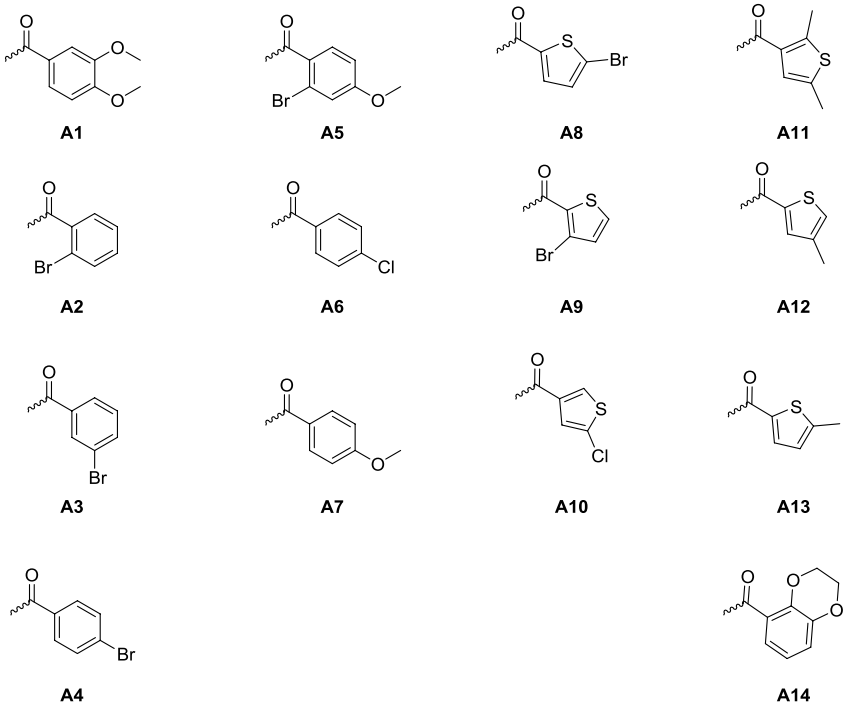
The quinolinyl chalcones were evaluated in vitro for their antimalarial activity against chloroquine (CQ) sensitive and resistance (CQ- resistance) strain of *P. falciparum* using chloroquine as reference drug (Table 1). The cytotoxicity of quinolinyl chalcones drug was also determined against PBM cell (uninfected PHA stimulated Human), CEM (T-lympho-blastoid cell line) obtained from American Type Culture Collection and Vero cells line (obtained from kidney of green monkey of Africa) as shown in Table 2. A potent anti-malarial activity was shown by most of the compounds. Five compounds (A_1, A_2, A_3, A_5 and A_7) out of fourteen exhibited ant-malarial activities with an IC_{50} value less than 0.05 μM, While A_8 and A_{10} showed anti-malarial almost equal to reference drug chloroquine against chloroquine sensitive strain of *P. falciparum*. The compound A_4 and A_6 exhibited better antimalarial activity against chloroquine sensitive strain of *P. falciparum* than chloroquine. The entire compounds displayed better antimalarial activity against chloroquine-resistance strain except A_{11}, A_{12}, A_{13} and A_{14}, while the compound A_4 and A_6 showed very good activity as compared to other compounds against chloroquine resistance strain.

According to antimalarial studies the phenyl derivative of quinolinyl chalcones that is A_4 is more active than A_2 and A_3. The main difference among A_2, A_3 and A_4 is the different location of Br group on the ring C of quinolinyl chalcones. The antimalarial effect of Br group is less effective at 2 (A_2) and 3 (A_3) position of ring C as compared to position 4



Scheme 1 Reaction protocol for the synthesis of chalcones (A_1–A_{14}). **a** AcOH, H_3PO_4, reflux, 4–6 h, **b** $POCl_3$, DMF, 80 °C, **c** Methyl Aryl Ketones, NaOH, rt, 2 h [15]

Chart 1 Different aryl ketones used in Claisen–Schmidt condensation reaction A_1–A_{14}



(A_4). Conversely, the 4 position of ring C with –Cl, –OCH_3 (methoxy) that is A_6 and A_7 are also less active as compared to A_4, which is due to the greater electronic and lipophilic effect of Br group at 4 position of ring C (Fig. 1, Table 1).

The thiophene derivatives of quinolinyl chalcones, the A_8 and A_{10} displayed better antimalarial activity against chloroquine sensitive and resistance strain as compared to other thiophene derivatives such as A_{11}, A_{12}, A_{13} and A_{14} as illustrated in Table 1. The methyl substituted thiophene A_{11}, A_{12} and A_{13} in quinolinyl chalcones decreases the antimalarial activity while Cl or Br group at five position (A_8 and A_{10}) of thiophene ring enhances the antimalarial activity to a good extent. Conversely, the dimethylated thiophene A_{11} is also less active as compared to monomethylated thiophene derivatives. Generally, the EDG (electron donating group) on thiophene or benzene ring decreased the activity whereas EWD (electron withdrawing groups) enhances the antimalarial activity. The theoretical and bioassay studies are also supported the above conclusions (Tables 1, 2, and 3).

Table 1 Antimalarial activity of quinolinyl chalcones in vitro

Compound	*P. falciparum* CQ-sensitive IC_{50} (μM)	Substituents	*P. falciparum* CQ-resistant IC_{50} (μM)
A_1	0.047	3,4-OCH_3	0.058
A_2	**0.040**	2-Br	**0.050**
A_3	**0.042**	3-Br	**0.049**
A_4	**0.031**	4-Br	**0.038**
A_5	0.048	2-Br, 4-OCH_3	0.060
A_6	**0.034**	4-Cl	**0.041**
A_7	0.049	4-OCH_3	0.068
A_8	**0.036**	3-Br	**0.043**
A_9	0.065	2-Br	0.069
A_{10}	**0.038**	5-Cl	**0.045**
A_{11}	0.090	2,5-Dimethyl	0.391
A_{12}	0.089	4-Methyl	0.372
A_{13}	0.087	3-Methyl	0.370
A_{14}	0.089	1,4-Benzodioxne	0.363
CQ	0.035	CQ	0.359

The bold values indicate the compound are more active than others

Table 2 Cytotoxicity of (A_1-A_{14}) in PBM, CEM, VERO IC_{50}

Comp	R	PMB	CEM	VERO
Cytotoxicity (IC_{50}, μM)				
A_1	3,4-OCH_3	40.01	39.7	27.31
A_2	2,-Br	20.01	30.4	52.01
A_3	3-Br	34.01	47.01	34.01
A_4	4-Br	32.09	34.08	28.07
A_5	2-Br, 4-OCH_3	47.08	43.09	39.99
A_6	4-Cl	22.99	31.09	24.73
A_7	4-OCH_3	50.5	33.98	31.90
A_8	3-Br	27.01	33.02	29.01
A_9	2-Br	47.90	87.09	45.04
A_{10}	5-Cl	24.8	28.90	26.50
A_{11}	2,5-CH_3	87.00	70.09	72.09
A_{12}	4-CH_3	55.99	62.04	46.78
A_{13}	3-CH_3	51.89	63.03	39.90

Computational detail

The density functional theory (DFT) was used for all calculations. All the compounds structures (A_1–A_{14}) were optimized using B3LYP functional with 6–311 + G (d,p) basis set [19, 20]. The single crystal structure of heme was obtained from Cambridge Crystallographic Data Centre [21]. The calculations were performed in order to calculate the binding energy of heme with the most active compound A_4. The structure of heme was optimized at B3LYP/TZVP level of theory [22]. All calculations were performed using Gaussian 09 package [23]. Field based similarity of the most active A_4 compound was evaluated relating to the reference drugs chloroquine using Field Templator 2.1.1of FieldAlign 2.1.1 software package (Cresset BioMolecular Discovery Ltd., UK) [24].

SAR studies of chalcones

The theoretical evaluation of theoretically studied ADME properties confirmed that quinolinyl chalcones A_1–A_{14} do not violates Lipinski's rule of five, so making the quinolinyl chalcones as a promising drug candidates [25]. The polar surface area (PSA) termed as a predictive indicator of a drug's capability for membrane diffusion. The PSA values are well-matched with hydrophobic and hydrophilic nature of all studies compounds, for example halogenated and methylated quinolinyl chalcones are hydrophobic in nature with low value of PSA [26]. Molecular similarity concepts is one of the best extensively employed models in the computer-assisted strategies to design molecular drugs. According to principle of "molecular similarity" compounds with alike molecular structures are more possibly to have alike biological activities and physicochemical properties [27–31]. Similar activities of different molecules for the identical molecular targets could be described by deliberating the molecules' fields rather than their molecular structure since the field pattern is an outlying superior explanation for molecule's binding possessions than its molecular structure. Molecules that are structurally different but display analogous activity, have alike fields and, henceforth, comparable binding properties so that these molecules can attach to the identical target place and provoke the similar biological results [24]. A 3D standard pattern was produced taking chloroquine and A_4 molecules at a time (loaded as single 2D structures) employing FieldTemplator 2.1 that hunts for mutual field patterns through the studied conformational space of molecules observing for similarity. The best pattern was preferred based on their field likeness, shape resemblance and whole similarity scores. The A_4 structure features well-matching with chloroquine are also consistent with field template results as shown in Fig. 2.

For the understanding the binding between a receptor ligand and a comprehensive analysis of (SAR) in structure activity relationships along with three dimensional interaction of ligand is important in drug development and synthesis. Numerous chemical factors are reported to be accountable to determine the molecular interactions. Although many studies has been reports to relate the biological activities with structural features [32, 33]. We used the ACD labs for SAR calculation in order to relate the structural properties of quinolinyl chalcones with anti-malarial activity as shown in Table 3.

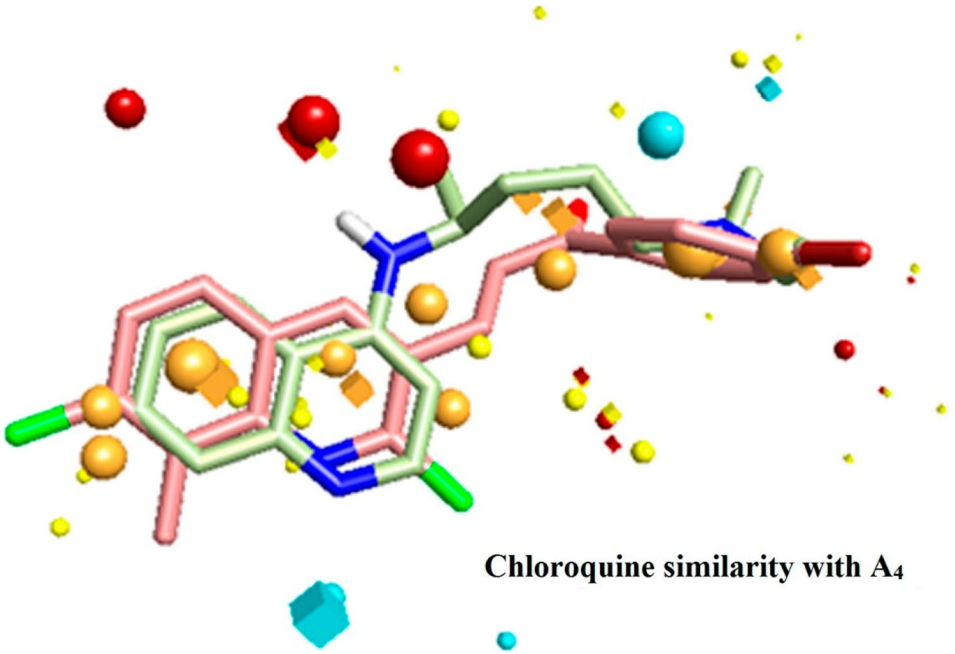


Fig. 2 Chloroquine similarity with A_4 calculated by field templater maroon, blue, brown and yellow colors show positive field, negative field, hydrophobic field and surface field points

Table 3 Calculated molecular descriptors

Compounds	R	(LogP)	LogKoc (Koc)	LogBCF (BCF)	No. of H-Donor	No.of H-acceptor	PSA
A_1	3,4-OCH_3	5.72	4.1 (30,712.00)	4.1 (13,040.45)	0	4	48.40
A_2	2,-Br	5.83	4.5 (35,322.38)	4.2 (15,854.16)	0	2	29.94
A_3	3-Br	6.14	4.6 (44,596.77)	4.7 (47,165.41)	0	2	29.95
A_4	4-Br	6.42	4.9 (77,083.59)	4.9 (74,054.95)	0	2	29.96
A_5	2-Br, 4-OCH_3	6.12	4.7 (32,737.98)	4.4 (26,168.79)	0	3	39.19
A_6	4-Cl	6.24	4.5 (20,229.20)	4.8 (59,355.09)	0	2	29.96
A_7	4-OCH_3	5.65	4.4 (28,074.36)	4.1 (11,502.72)	0	3	39.19
A_8	3-Br	6.14	4.7 (51,823.04)	4.4 (27,084.16)	0	2	58.20
A_9	2-Br	5.38	4.3 (20,229.20)	3.9 (7277.05)	0	2	58.20
A_{10}	5-Cl	6.00	4.6 (43,889.70)	4.3 (21,473.55)	0	2	58.20
A_{11}	2,5-CH_3	5.64	4.5 (48,218.08)	4.4 (24,448.88)	0	2	29.09
A_{12}	4-CH_3	5.89	4.4 (24,667.63)	4.0 (9600.87)	0	2	29.08
A_{13}	3-CH_3	5.54	4.4 (24,667.63)	4.0 (9600.87)	0	2	58.20
A_{14}	1,4-benzodioxane	5.65	4.4 (26,078.77)	4.1 (11,505.26)	0	4	48.22

Molecular descriptors

We have used polar surface area (PSA), bioconcentration factor (BCF) and Octanol–water partition coefficient (Log P) as molecular descriptors. The lipophilicity (Koc) of a molecule is normally described as a partition coefficient, stated as the comparative distribution of solute between 1-octanol (a copycat of hydrophobic lipid bilayer) and water.

For expediency, Koc values are more usually described as logKoc, where a high value of logKoc specifies higher lipophilicity while a low value of logKoc describes higher hydrophilicity. The halogenated quinolinyl chalcones have high value of logKoc as compared to other quinolinyl chalcones. Thus, the halogenated quinolinyl chalcones are more hydrophobic in nature and can easily cross the lipid bilayer. The LogP (Octanol–water partition coefficient) is descriptive of steric features and interactions. In the current work, LogP displayed an excellent correlation with the antimalarial activity of chalcones. The quinolinyl chalcones with larger LogP are predicted to have good antimalarial activity as shown by halogenated quinolinyl chalcones (Table 1). Among all the series the quinolinyl halogenated chalcones

(with –Br and –Cl groups) A_4, A_6, A_8 and A_{10} have higher LogP 6.42, 6.24, 614, and 6.00, values respectively and are also more active as compared to the others compounds as shown in Table 1, 2.

The substituents position on the benzene and thiophene ring of quinolinyl chalcones effect the antimalarial activity. For example: in A_2, the two position for bromo group in benzene ring C is less effective (LogP = 5.83) as compared to four position as in A_4 (LogP = 6.42). Both theoretical and experimental studies showed that halogenated quinolinyl chalcones are more active against malarial parasite than methoxy and methyl group, which is may be the greater electronic and lipophilic effect of halogen group.

In vivo antimalarial activity

In vitro A_4 compound have significant antimalarial activity. Thus, for in vivo antimalarial activity evaluation the compound A_4 was selected. The *P. berghei* infected mouse model was used to determine antimalarial activity of A_4 in vivo via oral administrative route. The A_4 compound was given to infected mice by the use of oral gavage on daily basis after infection and observed for specious signs of toxicity, survival and parasitemia after 28 days of infection as illustrated in Table 4. The parasite suppression was 21.75, 40.5, and 97.5% at 10, 32.5 and 100 mg.kg^{-1} on 4.5 day, CQ showed 100% suppression on 7 day. At highest dose level, the effect was almost disappeared, whereas the mean survival time for A_4 was 11.8 days, which was lower than CQ (26.2 days) treated animals.

Heme binding studies

The quinoline-based drug as chloroquine and amino Quinolines is believed to exert anti-malarial activity by binding with heme to stop the formation of hemozoin, the hemozoin formation is stopped by π–π interaction of quinoline ring with heme. The hemozoin formation is necessary for the growth of parasite [34, 35]. The capability of quinoline ring containing drug as an inhibitor of hemozoin formation can be demonstrated in vitro by inhibiting the formation of β-hematin. The β-hematin formation process is similar to formation of hemozoin in the food vacuole of parasite [36]. The complex formation of quinoline ring of CQ with ferriprotoporphrin IX (FPIX) was demonstrated by Cohen at, el. in aqueous solution, the λ-max of heme (FPIX) is red shifted in the presence of drug [37]. Hence many studies have explained the formation of (FPIX) complex both by computational as well as spectroscopic methods [38, 39]. Therefore, we decided to evaluate the binding of the most active compound A_4 with heme through computational and experimental studies. In order to understand the heme binding studies, we performed UV–visible spectroscopy using the most active compound A_4. A solution of Heme (Hematin) in 40% DMSO/water shown λ-max peak at 402 nm under condition used (0.02 M HEPES buffer of pH 7.4). When A_4 (0–20 μL) was added, the solution shows λ-max peak at 450 nm. The red shift in wavelength confirmed the formation of heme (Hematin)–quinoline complex as shown in Fig. 3.

More ever in order to confirm the association of A_4 to heme, we also performed theoretical studies of heme binding with the most active compound A_4. According to computational results, the A_4 binds the heme at distance of 3.55 Å with binding energy $E_B = -25$ kcal/mole as shown in Fig. 4.

Table 4 In vivo antimalarial activity of A_4 compound

Treatment	Dose (mg kg^{-1})	% Parasitemia suppression[a]		MST[b]	Toxicity
		Day 4.5	Day 7		
Vehicle	NA × 3	–	–	7.6	NA
CQ	100 × 3	100	100	26.2	NC
A_4	10 × 3	21.75	10.5	7.3	NC
	32.5	40.5	15.75	7.1	NC
	100	97.5	2.05	11.8	NC

[a]% Parasitemia suppression was measured by viewing the vehicle parasitemia control as a 100% b mean survival time (MST)

NA not active; *NC* not toxic up to high Con.c test

Molecular docking of Pf-DHFR

The molecular docking studies were carried out using Pf-DHFR enzyme with newly prepared quinolinyl chalcones. For comparison the molecular docking studies of reference inhibitor Pyrimethamine was also performed. We have obtained Pf-DHFR and its inhibitor (Pyrimethamine) structure from RCSB database of Protein (PDB ID 3QG2). The co-ordinates (×2.25), (Y -35.45) and (Z 23.94) for Pf-DHFR active site was obtained from its X-ray structure. The Pf-DHFR is a distinctive enzyme, which is responsible for the formation of folic acid in parasite. We used the Auto Dock to calculate the docking energy, the binding energy (ΔG) and the inhibition constant Ki related with antimalarial activity (Tables 1, 2). The calculated docking and binding energies of A_1-A_{14}) are well-matched with experimental data, which illustrated that quinolinyl chalcones A_4, A_5, A_6, A_8 and A_{10} are powerful inhibitor of Pf-DHFR as compared to reference drug and other quinolinyl chalcones (Tables 1, 4). The part of the chain of Pf-DHFR to which the most active inhibitors that is A_4, A_6, A_8 and A_{10} interacts are following amino acids LEU46, LEU164, PHE58, ASP54, TRY170, VAL195, ASN108, CYS15 and SER111 as shown in Fig. 5. The close interaction of A_4 quinolinyl ring with LEU46, VAL195 and SER111 amino acid (at distance 3.15, 3.10 and

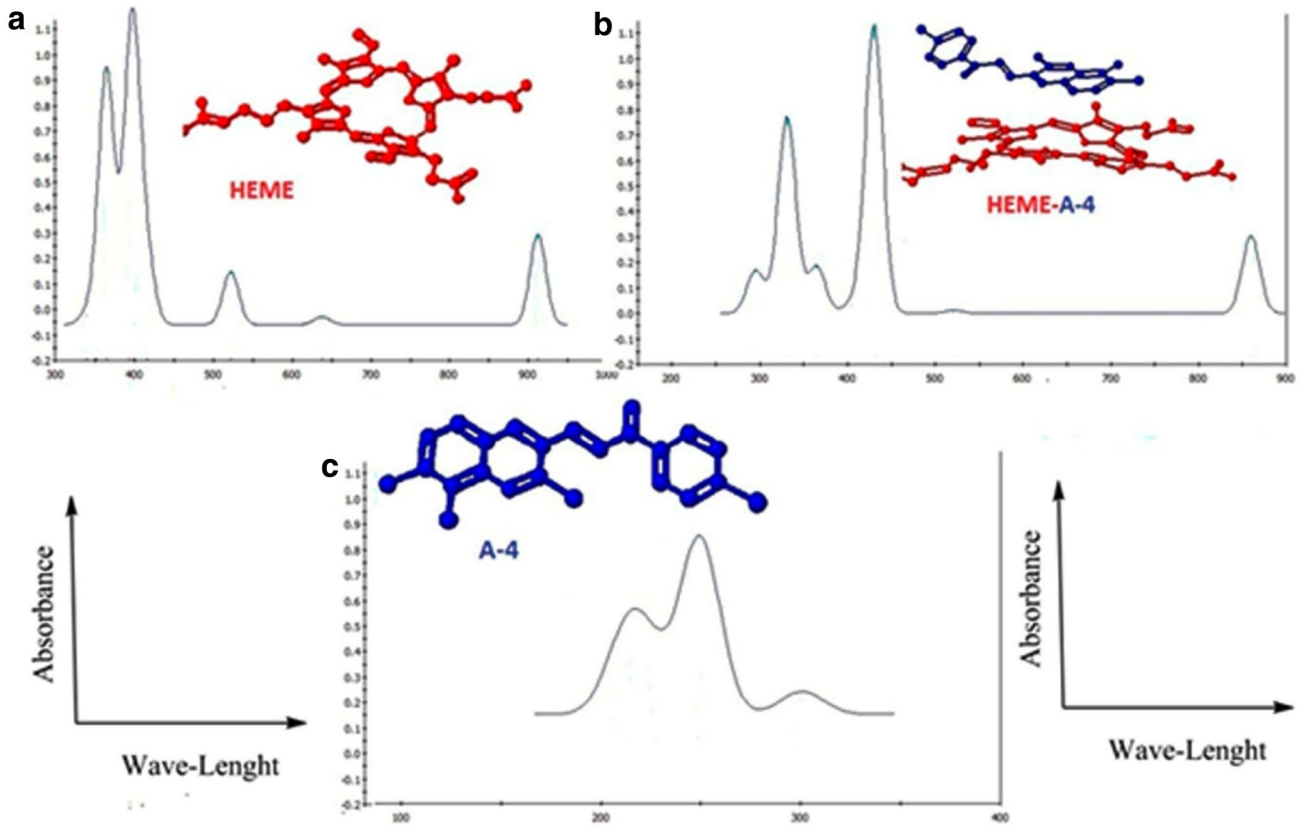
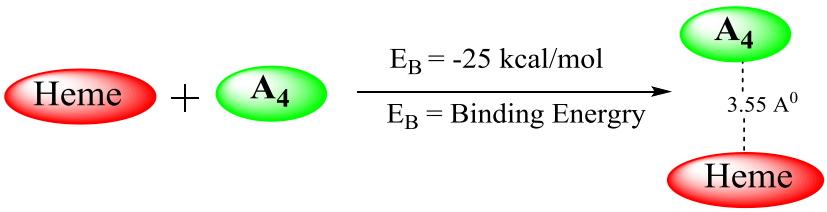


Fig. 3 UV–visible spectra of **a** heme (hematin), **b** (hematin)–A_4 complex **c** A_4



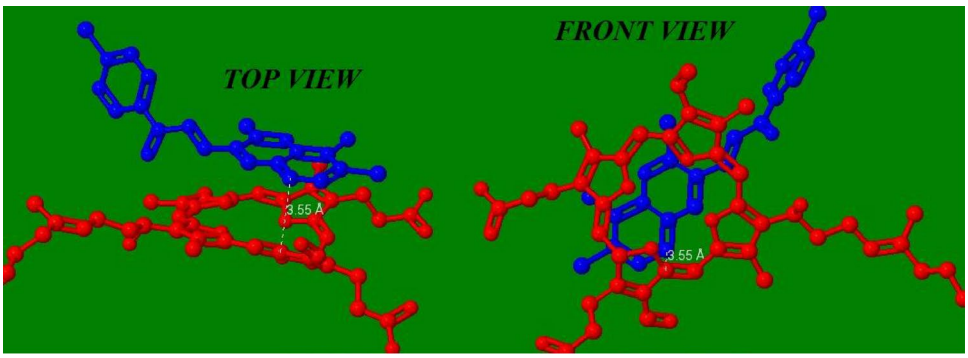


Fig. 4 Top and Front views of optimized structures of Heme and A_4 overlapped on each other, hydrogen atoms are removed for clarity

3.76 Å, respectively) proposed hydrophobic type of interaction. The quinolinyl nitrogen make hydrogen bonding with ASN108 (at distance 2.34 Å). Conversely, quinolinyl chloro group has halogen interaction with TRY170 (at distance 3.46 Å) as depicted in Fig. 5.

The Br group on four position of A_4 interact with CYS15 and ASP54 (at distance 3.15 Å and 3.15 Å respectively) while the Cl group on quinoline benzene ring A interacts with LEU164 (3.75 Å). The double bond of chalcone moiety makes very strong interactions with PHE58 (at distance

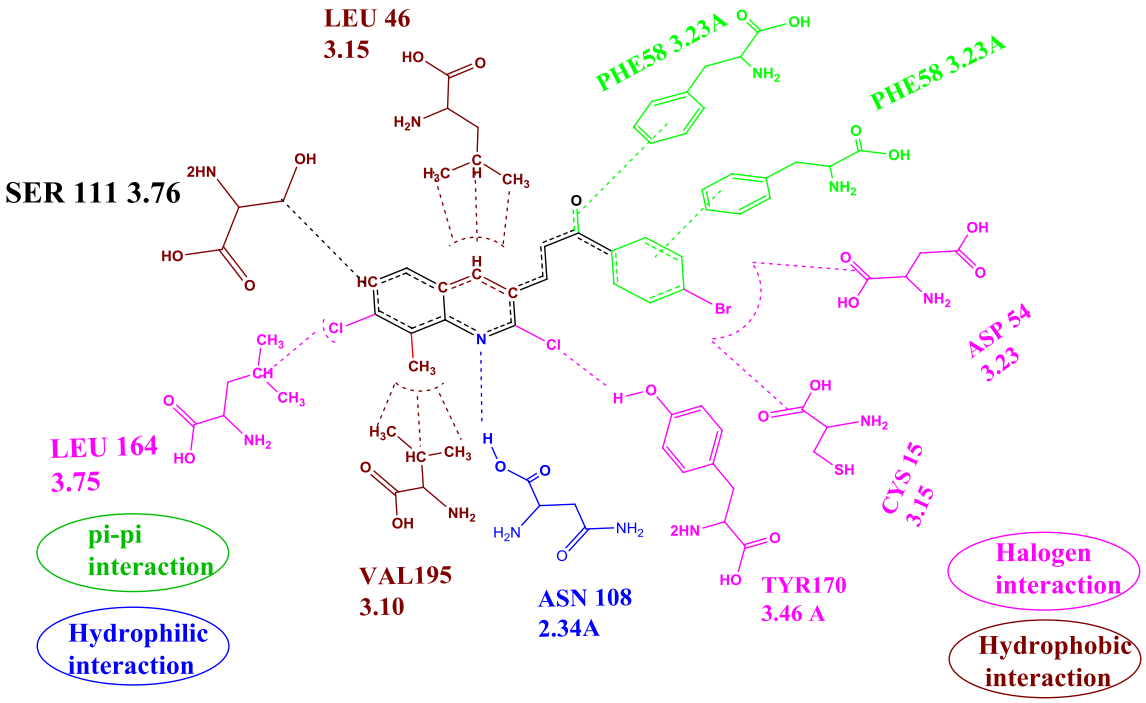


Fig. 5 Binding interaction of A_4 with amino acid of Pf-DHFR binding pocket

3.23 Å). The benzene ring and chalcone moiety C=C=O bond interact with PHE58 (at distance 3.23 Å) via π–π interaction.

All the above binding interactions of A_4 with active site of Pf-DHFR stabilized and lowered the A_4 binding and docking energy (ΔG = – 11.01 kcal/mole) for A_4–Pf-DHFR complex formation as shown in Table 5. Moreover the inhibition constant Ki for A_4–Pf-DHFR complex formation is also higher than the other quinolinyl chalcones, which also indicated that high stability of A_4–Pf-DHFR complex. The A_4–Pf-DHFR complex 2D and 3D model were predicted in Figs. 6 and 7, which showed that the halogenated benzene ring C of quinolinyl chalcones made strong interaction with Pf-DHFR as compared to quinoline part while the –C=C=O chalcone moiety acts as bridge between these two interacting quinoline and benzene ring C as shown in Figs. 1, 6 and 7. The experiment, theoretical and docking results showed that A_4, A_6, A_8 and A_{10} compounds act as potential inhibitor of Pf-DHFR (Tables 1 and 2).

Table 5 Summary of calculated binding parameters of chalcones (A_1–A_{14}) docked with Pf-DFHR

Compounds	Free energy of binding (ΔG) Kcal/mole	Docking energy Kcal/mole	Ki (inhibition constant) nM
A_1	– 8.64	– 9.01	140.79
A_2	– 9.03	– 9.50	220.60
A_3	– 9.82	– 10.57	344.60
A_4	**– 11.01**	**– 12.80**	**440.39**
A_5	**– 8.56**	**– 8.88**	**162.62**
A_6	**– 10.64**	**– 11.00**	**430.35**
A_7	– 8.80	– 9.57	100.01
A_8	– 9.50	– 10.61	290.66
A_9	– 9.46	– 10.80	117.03
A_{10}	**– 9.85**	**– 10.40**	**300.58**
A_{11}	– 7.69	– 8.97	150.36
A_{12}	– 7.84	– 9.04	160.20
A_{13}	– 8.90	– 9.01	170.63
A_{14}	– 8.98	– 9.29	154.15

The bold values indicate the compound are more active than others

Experimental section

Cytotoxicity assay

We have used the reported method for performing the cytotoxicity activity on human PBM cell (i.e. uninfected PHA stimulated Human PBM cell), Vero (African green monkey kidney) cells Log phase and CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD) [40, 41]. A density of 5×10^3 2.5×10^3, and 5×10^4 cells/well were used to seed all of the cell lines respectively. The 96-well was used to plate the

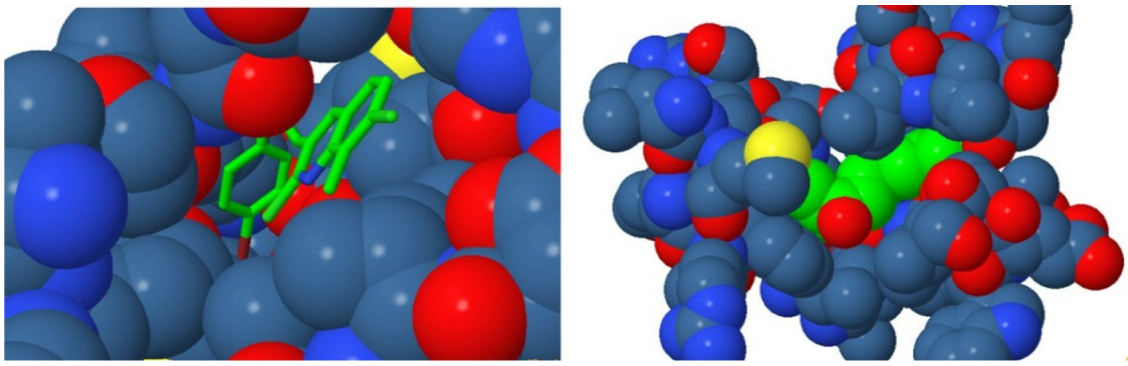


Fig. 6 3D Docking model derived for A_4 with Pf-DHFR binding pockets

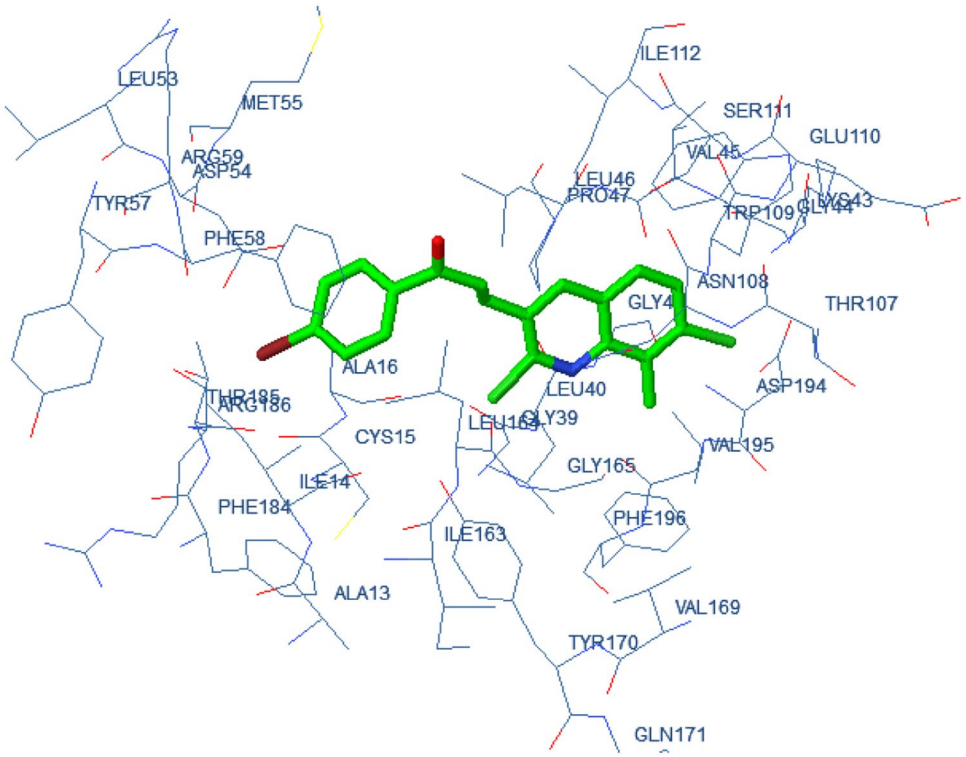


Fig. 7 A_4 2D docking model with Pf-DHFR binding pockets, describing the interaction of different amino acid with A_4

all cell line culture. All cell lines cultures were incubated in an environment of moistened 5% CO_2–air at 37 °C for 2–4 days. Then all the wells incubated for overnight and MTT dye was added in each well. The plates were read at 570 nm with the help of ELISA plate reader. The median effect method and concentration-responsive curve were used to calculate the IC_{50} values. The results are précised in Table 2. The (EC50 s) median effective concentrations and (IC_{50}) inhibitory concentrations were resulting from the computer simulated median effect plot of the dose effect data, as defined earlier [41, 42].

Docking protocol

The ligand protein docking were performed by using Auto dock software package. The drawing software ChemBio Ultra 11.0 were used to draw the structures of quinolinyl chalcones. The energies of all studied compounds were optimized at B3LYP functional and 6-311 + G (d,p) by using Gaussian 09 software package before doing the docking experiment. The charges were assigned to ligand by Gasteiger-Huckel method. We have obtained Pf-DHFR and its inhibitor (Pyrimethamine) structure from RCSB database of Protein (with PDB ID 3QG2). In order to prepare the

enzymes, the hydrogen atoms were added at a pH range of (pH 6.5–8.1). By the help of Auto Dock tools the solvation factors and kollman united atom type chargers were also added. The auto grid software package was used to produce the affinity (gird) maps of 20×20×20 Å at 0.375 Å spacing.

Anti-malarial activity

According to literature, the antimalarial activity was performed by analyzing plasmodial LDH activity [43]. A suspension of RBCs treated with D6 or W2 strain of *P. falciparum* (200 µL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 µg/mL amikacin) was mixed to the wells of a 96-well plate containing 10 µL of successively diluted test samples. A gas mixture of 90% N2, 5% CO_2, and 5% O_2 was flushed from the plate. Then this plate was incubated for 72 h at 37 °C in a modular incubation chamber (Billups-Rothenberg, CA). According to Makler and Hinrichs method, parasitic LDH activity was determined [44, 45]. Shortly, in this procedure, 100 µL of the Malstat TM reagent (Flow Inc., Portland, OR) was added to the 20 µL of the incubation mixture and incubated it further for 30 min at room temperature. After that added 20 µL of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO). Then the plate is incubated for an hour in the dark. Finally 100 µL of a 5% acetic acid solution is prepared to stop the reaction of the plate. At 650 nm, the plate was read. As antimalarial drug controls, pyrimethamine and Chloroquine were included in each assay. The dose response curves give the IC_{50} values.

Conclusions

In conclusion, we reported the quinoline based chalcones (A_1–A_{14}) as a potential antimalarial agent. The in vitro antimalarial activity of quinolinyl chalcones were performed against W2 and D6 strain of *P. falciparum*. The A_4 compound is found potent antimalarial agent as compared to the others quinolinyl chalcones. The antimalarial activity of quinolinyl chalcones influenced by the position and type of substituents, the hydrophobic effect of bromo group is greater at 4-position of benzene ring C as compared to the other substituents. The quinoline chalcones showed very low toxicity against all the studied cell lines. We have also performed the heme binding studies of the most active A_4 compound in order to find its mode of action. The A_4 strongly bind the heme at a distance of 3.5 Å, confirmed by both theoretical studies and UV–visible spectroscopy. The most active compound A_4 also showed a strong interaction with Pf-DHFR and made a strong A_4–Pf-DHFR complex with low binding and docking energy. The experimental and theoretical studies tell us that compounds A_4 are the most potent antimalarial agent. The low toxicity and promising antimalarial activity of quinolinyl chalcones publicize their potential as an antimalarial drug for further development.

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