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Experimental and computational evaluation of new quinolinyl chalcones as potent antiplasmodial agents

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In a search for new antiplasmodial agents, a series of thirty five diversely substituted chalcones derived from a quinoline-chalcone scaffold e.g. (*E*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one / (*E*)-(2-chloro-6-ethoxyquinolin-3-yl) (2-hydroxyphenyl) prop-2-en-1-one and (*2Z*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl)-3-iodoprop-2-en-1-one are synthesized and studied. Compounds are prepared *via* Claisen–Schmidt condensations of 2-chloro-3-formyl quinoline / 2-chloro-6-ethoxy-3-formyl quinoline with appropriately substituted 2-hydroxy acetophenones. All compounds are assayed for their binding in the active sites of *Plasmodium falciparum lactate dehydrogenase* (pfLDH) enzyme. The quinoline chalcone derivatives showed negative binding energies promising potent pfLDH inhibitory activity. Compounds showing the highest negative binding scores have been studied for their *in vitro* antimalarial activity against cultured *Plasmodium falciparum 3D7 strain*. The compounds **2c** and **2u** have completely inhibited the maturation of parasites at MIC 10 µg/mL and above whereas **3b** inhibited 95% maturation of parasites at MIC 50 µg/mL. Additional efforts are being directed towards elaborating these leads towards the discovery and development of new quinolinyl heterocycles as anti-malarial agents.

Keywords: (*E*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one, (*2Z*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl)-3-iodoprop-2-en-1-one;(*E*)-(2-chloro-6-ethoxyquinolin-3-yl) (2-hydroxyphenyl) prop-2-en-1-one, docking, anti-malarial activity

Malaria, caused by protozoa of the genus *Plasmodium*, is the most serious and widespread of the parasitic diseases encountered by mankind because of its prevalence, virulence and drug resistance. Out of the four species that affect humans, *Plasmodium falciparum* is the most prevalent and pathogenic. Resistance of Plasmodia to anti malarial drugs is now recognized as one of the significant problems in the eradication of malaria. Rapidly increasing resistance of *Plasmodium falciparum* malaria parasites to commonly used drugs such as chloroquine renders them ineffective[1]. Control of malaria is further hampered by emergence of resistance to new and more expensive chemotherapeutic agents, such as mefloquine and halofantrine, mosquitoes resistant to insecticides and by restriction in the use of chemical sprays. An inadequate arsenal of drugs for the treatment of malaria allied with the lack of affordability of new pharmaceuticals severely limit fight against malaria. This underscores the need for ongoing research in this arena.

Quinolines and their derivatives have been extensively explored for their biological[2-4], anti-filarial[5], anti-bacterial[6,7] and anti-malarial[8,9] activities and additionally, for their cardiovascular, anti-neoplastic and receptor agonist activities. Also many chalcones are of potential therapeutic relevance as anti-bacterial, antifungal, antiviral, anti-parasitic, anti-cancer, antileishmanial and anti-tubercular agents[10-12].

Even though chloroquine (CQ) and related quinoline compounds have been extensively used throughout the world as prophylactics to prevent the development of malaria, the molecular mechanism by which CQ exerts its effects on the malarial parasite *Plasmodium falciparum* remains unclear. CQ is presumed to exert its inhibitory effects by interfering with the function of the food vacuole in the mature stages of the erythrocytic parasite. CQ is a weak base and accumulates at high concentrations within the acidic food vacuole, where hemoglobin is degraded by proteases to fulfill the amino acid needs of the parasite: the overall process results in production of toxic heme moieties known as hematin[13]. Previously, CQ was thought to exert its antimalarial effect by complexation with hematin that is toxic to parasite

cells. This hypothesis is no longer favoured: various other reports suggest that CQ targets unspecified proteins involved in the digestion or disposal of haemoglobin. Photo-reactive analogues of chloroquine interacted specifically with two proteins in the infected red blood cells, one of which was recognized as pfLDH[14]. Examination of the crystal structure of the complex between CQ and pfLDH revealed that CQ occupies a position similar to that of adenyl ring of the cofactor. Thus, CQ competes with NADH for binding to the enzyme, acting as a competitive inhibitor for this critical glycolytic enzyme. Additionally, CQ acts as mild inhibitor of pfLDH. Accumulation of CQ in millimolar concentrations within the food vacuole in the guts of parasite renders it potent as an anti-malarial even with low inhibition. Thus, the crystal structure of enzyme-inhibitor complex provides a stencil that can be utilized to develop more efficient antiplasmodial quinoline analogues.

The recent renewed interest in various quinoline analogues prompted the present study to demonstrate the successful use of synthetic and docking techniques to inhibit the pfLDH enzyme and identify new *anti-plasmodium* quinoline containing pharmacophores.

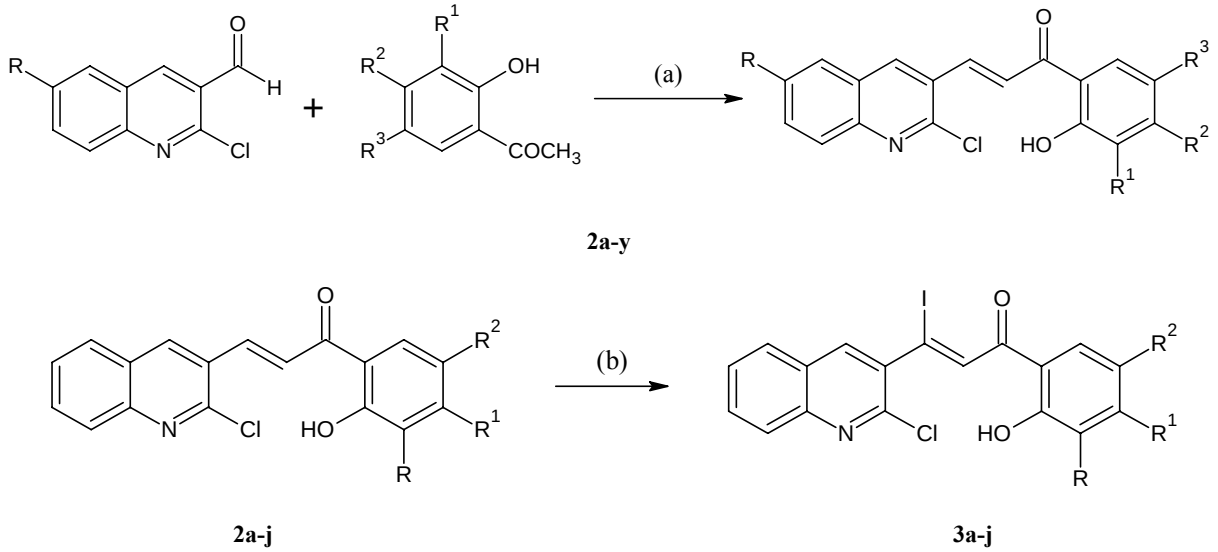
Results and Discussion

Synthesis

A series of thirty five compounds *viz*. (*E*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one/(*E*)-(2-chloro-6-ethoxyquinolin-3-yl) (2-hydroxyphenyl) prop-2-en-1-one and (*2Z*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl)-3-iodoprop-2-en-1-one has been prepared *via* Claisen–Schmidt condensations, in ethanolic sodium hydroxide, of 2-chloro-3-formyl quinoline or 2-chloro-6-ethoxy-3-formyl quinoline with diversely substituted 2-hydroxy acetophenones in presence of ethanolic sodium hydroxide and its further treatment with DMSO/I_2. (**Scheme I**).

In the ^{1}H NMR spectra of **2a**, the protons of *α,β* unsaturated carbonyl moieties appear as two doublets in the range of δ 7.48 for Hα and 7.89 for $H_β$ with *J* values 2.52 and 6.44 Hz. A singlet at δ 12.55 accounts for the presence of –OH functional group. The IR bands at 1646 (C=O), 3033 (-OH), 976 cm^{-1} (CH=CH wagging) lend credence to the proposed structure of (*E*)-1-(5-chloro-2-hydroxyphenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one **2a-j**. The IR spectra of compounds **2k-y** showed peaks at 3450 cm^{-1} due the –OH function. Strong, sharp absorption bands observed at 1640-1670 cm^{-1} were attributable to the carbonyl (–C=O), bands at 1564-1575 cm^{-1} suggested the presence of C=C group. In addition, bands were observed at 2880-3070 cm^{-1} corresponding to the –OC_2H_5 group.

The ^{1}H NMR spectra of compounds **2k-y** displayed signals at δ 1.39-1.55 and δ 4.05-4.21 due to the –CH_3 and –CH_2 of an ethoxy group; the signal due to –OH group appeared at δ 12.47-12.51. For compounds **2l, n, o** and **p** the singlet at δ 2.3-2.4 integrated for the –CH_3 protons. The signal attributable to –CHO group



Reagents and conditions: a) Ethanol, 40% NaOH, R.T.; b) Dimethyl sulphoxide, one iodine crystal, two drops of Conc. H_2SO_4, reflux for 30 min.

Scheme I

of quinoline aldehyde structure was not evident. The ^{1}H NMR spectra of compounds **2k-y** revealed peaks at δ 7.78-8.30 indicating the presence of –CH=CH– group. In addition, -OCH_3 group of compounds **2 w-y** resonated as singlets at δ 3.8 integrating for three protons. An additional δ 5.3 singlet corresponding to two protons indicated the presence of an –NH_2 group in compound **2q**. The elemental analysis and molecular ion peaks of compounds **2a-y** are consistent with the assigned structure.

When differently substituted (*E*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl) prop-2-en-1-ones **2a-j** were subjected to flavone cyclization reaction, a clear, amber coloured reaction-mixture was obtained. Instead of the desired product, a different product was obtained and characterized by IR, ^{1}H and ^{13}C NMR, mass and CHN analysis.

DMSO/I_2, normally an oxidizing agent also functions as an iodinating agent for substrates of poor solubility in alcohol, DMSO, ethyl acetate and acetic acid. The ^{1}H NMR spectra of **3a** revealed a singlet at δ 12.30 for the -OH functional group; another singlet at δ 8.84 was assigned to the single hydrogen attached to the ethylenic linkage of **3a**. IR spectra of the compound showed absorption at 1668 cm^{-1} representing unsaturation, another peak at 3350 also supported the presence of –OH group. Molecular peaks at *m/z* 469 suggested the tentative assignment of structure **3a** to the product.

All assigned structures were also supported by ^{13}C NMR spectroscopic data, which showed carbon signals near δ 127, 145 and 189 corresponding to a chalcone linkage while compounds **3a-j** showed peaks near δ 102, 136 and 189. Physical and analytical data of the newly synthesized compounds **2a-y**, and **3a-j** are discussed in **Table I**.

Pharmacology

Antimalarial activity

The *in-vitro* antimalarial activity of the compounds was carried out in 96 well microtiter plates as per the method of Madapa *et al*.[15] The culture of CQ-sensitive *3D7* strain of *P. falciparum* is routinely maintained in medium RPNI[16] supplemented with gentamycin at 40 µg/mL; (Sigma), Fungizone at 0.25 µg/mL; (GIBCO) and 10% fetal bovine serum

Table I — Physical and analytical data of the newly synthesized compounds **2a-y** and **3a-j**

Compd	R	R^1	R^2	R^3	m.p. (°C)	Yield (%)	Mol. formula	(Calcd) (%) Found		
								C	H	N
2a	H	Cl	H	H	182	80	$C_{18}H_{11}Cl_2NO_2$	61.98 (62.81	3.08 3.22	3.87 4.07)
2b	H	CH_3	H	H	162	74	$C_{19}H_{14}ClNO_2$	69.98 (70.48	4.32 4.36	4.28 4.33)
2c	H	Cl	H	Br	252	72	$C_{18}H_{10}BrCl_2NO_2$	50.98 (51.10	2.42 2.38	3.28 3.31)
2d	H	CH_3	H	Br	229	70	$C_{19}H_{13}BrClNO_2$	55.54 (56.67	3.15 3.25	3.32 3.48)
2e	H	Cl	H	I	175	78	$C_{18}H_{10}Cl_2INO_2$	45.74 (45.99	2.15 2.14	3.02 2.98)
2f	H	CH_3	H	I	177	74	$C_{19}H_{13}ClINO_2$	51.32 (50.75	2.85 2.91	3.09 3.11)
2g	H	Cl	H	NO_2	162	68	$C_{18}H_{10}Cl_2N_2O_4$	54.25 (55.55	2.75 2.59	7.21 7.20)
2h	H	CH_3	H	NO_2	155	72	$C_{19}H_{13}ClN_2O_4$	61.42 (61.88	3.46 3.55	7.27 7.60)
2i	H	Br	OCH_3	H	186	78	$C_{19}H_{13}BrClNO_3$	55.26 (54.51	3.14 3.13	3.67 3.35)
2j	H	I	OCH_3	H	177	83	$C_{19}H_{13}ClINO_3$	50.06 (49.01	2.48 2.81	3.13 3.01)
2k	OC_2H_5	H	H	Cl	196	78	$C_{20}H_{15}Cl_2NO_3$	61.93 (61.87	3.82 3.83	3.29 3.61)
2l	OC_2H_5	H	H	CH3	209	78	$C_{.21}H_{18}ClNO_3$	68.11 (68.57	4.31 4.93	3.52 3.80)

—Contd

Table I — Physical and analytical data of the newly synthesized compounds **2a-y** and **3a-j—** *Contd*

Compd	R	R^1	R^2	R^3	m.p. (°C)	Yield (%)	Mol. formula	(Calcd) (%) Found		
								C	H	N
2m	OC_2H_5	I	H	Cl	288	83	$C_{20}H_{14}Cl_2NO_3$	46.51 (46.72	2.50 2.74	2.32 2.72)
2n	OC_2H_5	I	H	CH3	240	79	$C_{21}H_{17}ClNO_3$	51.22 (51.09	3.82 3.47	2.72 2.84)
2o	OC_2H_5	NO_2	H	CH_3	159	84	$C_{21}H_{17}ClN_2O_5$	61.68 (61.10	4.52 4.15	6.40 6.79)
2p	OC_2H_5	Br	H	CH_3	196	88	$C_{21}H_{17}ClBrNO_3$	56.07 (56.46	3.33 3.86	3.65 3.14)
2q	OC_2H_5	H	H	NH_2	198	78	$C_{20}H_{17}ClN_2O_3$	65.86 (65.13	4.86 4.65	7.52 7.60)
2r	OC_2H_5	Cl	H	H	179	77	$C_{20}H_{15}Cl_2NO_3$	61.82 (61.87	3.52 3.89	3.89 3.61)
2s	OC_2H_5	NO_2	H	Cl	201	72	$C_{20}H_{14}Cl_2N_2O_5$	55.22 (55.45	3.94 3.26	6.38 6.47)
2t	OC_2H_5	Br	H	Cl	222	74	$C_{20}H_{14}Cl_2BrNO_3$	51.19 (51.42	3.07 3.02	3.40 3.00)
2u	OC_2H_5	Cl	H	Br	198	80	$C_{20}H_{14}Cl_2BrNO_3$	51.86 (51.42	3.13 3.02	3.78 3.00)
2v	OC_2H_5	Cl	H	NO_2	210	73	$C_{20}H_{14}Cl_2N_2O_5$	55.92 (55.45	3.33 3.26	6.52 6.47)
2w	OC_2H_5	H	OCH_3	H	118	76	$C_{21}H_{18}ClNO_4$	65.56 (65.71	4.43 4.73	3.40 3.65)
2x	OC_2H_5	H	OCH_3	NO_2	257	79	$C_{21}H_{17}ClN_2O_6$	58.19 (58.82	4.06 4.00	6.22 6.53)
2y	OC_2H_5	H	OCH_3	Br	268	81	$C_{21}H_{17}ClBrNO_4$	54.92 (54.51	3.43 3.70	3.07 3.03)
3a	H	Cl	H	H	>290	85	$C_{18}H_{10}Cl_2INO_2$	45.86 (45.99	2.18 2.14	2.96 2.98)
3b	H	CH_3	H	H	215	79	$C_{19}H_{13}ClINO_2$	50.86 (50.75	2.98 2.91	3.18 3.11)
3c	H	Cl	H	Br	235	82	$C_{18}H_9BrCl_2INO_2$	38.26 (39.38	1.77 1.65	2.54 2.55)
3d	H	CH_3	H	Br	228	78	$C_{19}H_{12}BrClINO_2$	43.16 (43.17	2.27 2.29	2.68 2.65)
3e	H	Cl	H	I	292	86	$C_{18}H_9Cl_2I_2NO_2$	35.16 (36.27	1.46 1.52	2.38 2.35)
3f	H	CH_3	H	I	>290	77	$C_{19}H_{12}ClI_2NO_2$	38.16 (39.65	2.54 2.10	2.36 2.43)
3g	H	Cl	H	NO_2	>290	77	$C_{18}H_9Cl_2IN_2O_4$	42.04 (41.97	1.23 1.76	4.99 5.44)
3h	H	CH_3	H	NO_2	>290	76	$C_{19}H_{12}ClIN_2O_4$	46.14 (46.13	2.41 2.45	5.69 5.66)
3i	H	Br	OCH_3	H	218	72	$C_{19}H_{12}BrClINO_3$	41.07 (41.91	2.02 2.22	2.38 2.57)
3j	H	I	OCH_3	H	260	78	$C_{19}H_{12}ClI_2NO_3$	37.87 (38.58	2.00 2.04	2.38 2.37)

(*p*H 7.2). For evaluation of schizontocidal activity, the asynchronous parasite culture was synchronized after 5% D-sorbitol treatment to obtain only ring stage of parasite.

The compounds were dissolved in DMSO at 5 mg/mL and desired dilutions were prepared in a template plate in RPMI-1640 medium. 20 µL of final concentrations ranging from 1.0 µg/mL to 50 µg/mL were transferred in duplicate wells in the test plate and two wells receiving 20 µL of vehicle were kept as untreated control. Chloroquine was used as standard drug. Finally 180 µL of 3% erythrocyte suspension

Table II — Antimalarial activity profile of synthetic compounds **2c**, **3b** and **2u**

Compd	Concentration evaluated	Number of parasites/100 infected RBCs			Percent Schizont Maturation inhibition	MIC µg / mL
		Rings	Trophozoites	Schizonts		
2c	50	100	00	00	100	10
	10	100	00	00	100	
	2.0	00	20	80	20	
	1.0	00	00	100	00	
3b	50	100	00	00	100	50
	10	40	55	05	95	
	2.0	00	20	100	00	
	1.0	00	00	100	00	
2u	50	100	00	00	100	10
	10	100	00	00	100	
	2.0	00	50	50	50	
	1.0	00	00	100	00	
Chloroquine	0.062	100	0	0	100	0.032
	0.032	83	17	0	100	
	0.015	45	52	03	97	
	0.007	20	50	30	70	
	0.0035	00	20	90	20	
Control	----	0	00	100	----	--

containing 1% parasitized cells were added to each well containing test compounds. The plates were incubated at 37°C for more than 40 hr in a CO_2 incubator. After which thin smears were prepared from each well on grease-free glass slides. The smears were fixed in methanol, stained with Giemsa's stain and examined under light microscope (100X; oil immersion). Maturation of ring stage parasites into trophozoites and schizonts were recorded in each smear. The minimum inhibitory concentration (MIC) of test compounds is designated as the minimum concentration required producing 100% inhibition of schizont maturation and calculated as-

Percent inhibition of maturation = (CS -TS / CS) × 100
CS - No. of schizonts in untreated culture
TS - No. of schizonts in treated culture

The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 hr, and percent maturation inhibition with respect to untreated control group are indicated in **Table II**.

Molecular modelling

Docking of known antimalarials in the active site of pfLDH enzyme

A comparative study involving the interaction of known antimalarials *viz*. Chloroquine, mefloquine, atovaquone, pyrimethamine and artimisinin in the active site pocket of pfLDH was made for better understanding of their antimalarial action. Docking scores of these drugs are depicted in (**Table III)**.

Table III — Docking score (kcal/mole) of various antimalarials with pfLDH (pdb ID:1CET)

S.No	Ligand	Docking score (Kcal/mole)
1	Atovaquone	-11.1610
2	Chloroquine	-7.72472
3	Mefloquine	-8.08456
4	Pyrimethamine	-8.25773
5	Artemisinin	-8.62421

In case of pfLDH selective Atovaquone **(Figure 1)**, the negative binding energies are in agreement with its pfLDH selectivity as reported in several literatures. Binding of Atovaquone in the binding pocket of pfLDH resulted from the conformational placement of amino acid residues in the active site and through hydrophobic interactions. The fused bicyclic ring with two carbonyl linkages at 1,2 position of atovaquone is enveloped by F100, I 119, A98 while the remaining portion of the drug is encircled with other amino acid residues like G27, F52, Y85, I 54, E 122, D53, V26, and Y 85.

Complexation of the docked ligands **2a-y** and **3a-j** with pfLDH enzyme was interpreted by looking at the

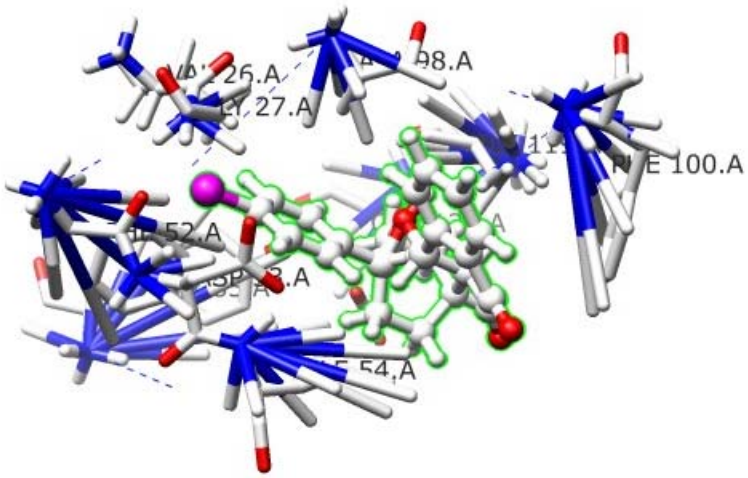


Figure 1 — Docked structure of Atovaquone into the active site of pfLDH enzyme

Table IV— Docking score (kcal/mole) of different quinoline analogue ligands **2a-y** and **3a-j** with pfLDH (pdb ID:1 CET)

S.No	Ligand	Docking score (Kcal/mole)	S.No	Ligand	Docking score (Kcal/mole)	S.No	Ligand	Docking score (Kcal/mole)
1	**2a**	-10.1523	13	**2m**	-7.8671	25	**2y**	-9.3349
2	**2b**	-9.5259	14	**2n**	-7.9126	26	**3a**	-10.1965
3	**2c**	-10.8542	15	**2o**	-7.9927	27	**3b**	-10.4993
4	**2d**	-9.6149	16	**2p**	-7.2988	28	**3c**	-10.3002
5	**2e**	-9.6231	17	**2q**	-8.2304	29	**3d**	-10.2964
6	**2f**	-9.6562	18	**2r**	-9.6745	30	**3e**	-8.9384
7	**2g**	-9.2419	1	**2s**	-8.8018	31	**3f**	-8.3574
8	**2h**	-9.2448	20	**2t**	-8.2911	32	**3g**	-10.4113
9	**2i**	-9.7892	21	**2u**	-8.8395	33	**3h**	-9.0827
10	**2j**	-9.3815	22	**2v**	-8.1910	34	**3i**	-10.3286
11	**2k**	-10.9698	23	**2w**	-8.2919	35	**3j**	-8.6395
12	**2l**	-8.8793	24	**2x**	-7.1082			

H-bonding or hydrophobic interactions of the ligand with the amino acid residues in the active site. The same procedure was adapted for docking different quinoline analogues into the active site of pfLDH: The results are summarized in the **Table IV**.

All compounds can be generalized into a single quinoline framework, which has all the carbon atoms and a single nitrogen atom of the central core (quinoline skeleton) as sp^2 hybridized while the C-2 carry a chlorine atom; C-3 linkage is used to link quinoline to differently substituted phenyl ring bridged by a characteristic α,β unsaturated system. All the synthesized derivatives incorporate promising quinoline and chalcone linkages within their structure; these molecules are expected to show their potency as synthetic inhibitor. All the ligands showed difference in their binding energies pointing towards the significant role of various substituents in modulating binding abilities.

Docking of synthesized ligands into pfLDH active site:

All the thirty five synthesized quinoline analogues showed binding in the pfLDH active site with binding scores between -7.1082 and -10.9698 kcal/mole, as shown in **Table IV**. These compelling data can be utilized further to develop potent anti-plasmodium heterocycles.

Structure activity relationships

Depending upon the structural features essential for binding in the cavity of 1CET, all newly synthesized molecules **2a-y** could be divided into three segments

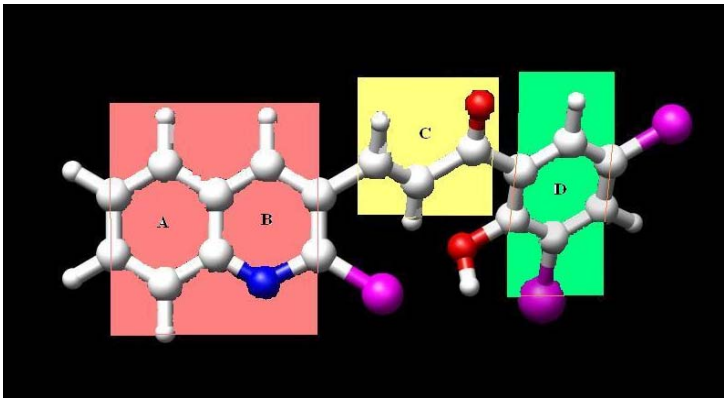


Figure 2 General Structure of Quinoline chalcones

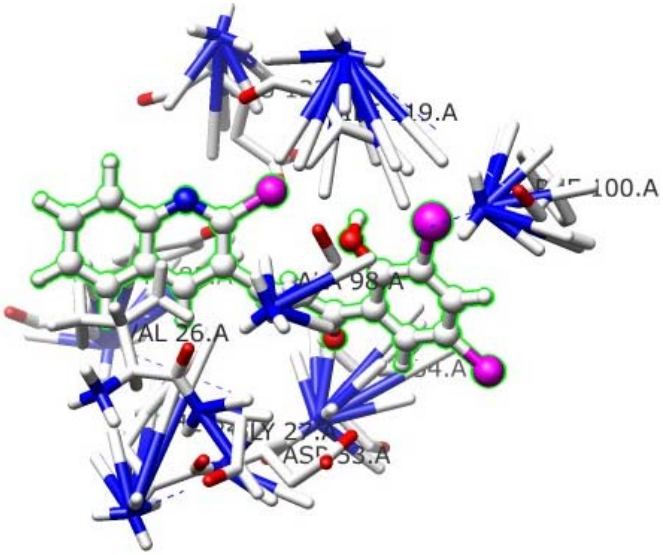


Figure 3 — Docked structure of **2c** into the active site of pfLDH enzyme

viz. quinoline central core structure (Ring A and B); characteristic chalcone linkage (Unit C); and differently substituted phenyl ring (Ring D) **(Figure 2)**.

Compounds **2c, 2k** and **3b** showed the highest binding score with pfLDH enzyme active site cavity with comparison to other ligands, including some well-known anti-malarials. The highest binding score of ligand-pfLDH complex prompted us further for *in vitro* antimalarial evaluation of the respective compounds **2c, 2u** and **3b.** To validate the computational evaluation, the compound **2u** was selected randomly though it possesses less binding energy score. It was observed that **2c** and **2u** completely inhibited the maturation of parasites at minimum inhibition concentration (MIC) 10 µg/mL and above whereas **3b** inhibited 95% maturation of parasites at MIC 50 µg/mL. No marked inhibition was recorded at 2 µg/mL concentration.

Further rationalization of mode of binding of these quinoline molecules in active site of 1CET is based upon the amino acid residues present around the ligand. For ligand **2c (Figure 3),** the central quinoline skeleton is being enveloped by amino acid residues like TYR85, GLU122, PHE52 and VAL26 while phenyl ring is surrounded by the amino acid residues like ALA98, PHE100, ILE119, and ILE54. The chalcone linkage attached to the C-3 of the central quinoline structure was found to have very characteristic orientation within the active site, encircled by GLY27 and ASP53. The binding score of **2c** indicated potential pfLDH inhibition. Amino acid residues around all segment of quinoline derivatives when docked into the pfLDH (pdb ID: 1CET) active site are depicted in (**Table V)**.

Conclusion

We report herein, facile syntheses of 35 appropriately substituted quinoline chalcone derivatives:

Table V — Amino acid residue around all segment of quinoline derivative when docked into the pfLDH (pdb ID:1CET) active site

S.No	Ligand	Central Quinoline core	Chalcone linkage	2-Phenyl
1	**2a**	ALA98, ASP53, GLU122, GLY27, PHE52, TYR85, VAL26	ILE54, ILE119	PHE100
2	**2b**	GLU122, PHE52, TYR85, VAL26	ASP53, GLY27	ALA98, ILE54, ILE119, PHE100
3	**2c**	TYR85, GLU122, PHE52, VAL26	GLY27, ASP53	ALA98, PHE100, ILE119, ILE54
4	**2d**	PHE100, ALA98, GLY27	VAL26, ILE119	ASP53, GLU122, ILE54, PHE52, TYP35
5	**2e**	GLU122, TYR85, ASP53, PHE52	ILE54, GLY27, VAL26	ALA98, ILE119, PHE100
6	**2f**	ILE54, ILE119, PHE100	ASP53, GLY27, PHE52	ALA98, GLU122, TYR85, VAL26
7	**2g**	ASP53, PHE52, ILE54, TYR85	GLY27, VAL26, GLU122	PHE100, ILE119, ALA98
8	**2h**	ALA98, ILE119, PHE100	ASP53, GLY27, VAL26	GLU122, ILE54, PHE52, TYR85
9	**2i**	ALA98, ILE119, ILE54, PHE100	ASP53, GLU122, GLY27	PHE52, TYR85, VAL26
10	**2j**	ALA98, ILE119, PHE100	ASP53, GLY27, VAL26	GLU122, ILE54, PHE52, TYR85
11	**2k**	ASP53, GLU122, PHE52, TYR85, VAL26	ALA98, GLY27, ILE119, ILE54	PHE100
12	**2l**	ALA98, GLY27, ILE119, PHE100	ASP53, GLU122, ILE54, VAL26	PHE52, TYR85
13	**2m**	GLU122, ILE54, PHE52, TYR85	ASP53, VAL26	ALA98, GLY27, ILE119, PHE100
14	**2n**	GLU122, ILE54, PHE52, TYR85	ASP53, ILE119, VAL26	ALA98, GLY27, PHE100
15	**2o**	ALA98, GLY27, ILE119, PHE100, VAL26	ASP53, ILE54, PHE52	GLU122, TYR85
16	**2p**	GLU122, ILE119, TYR85	ILE54, PHE100, VAL26	ALA98, ASP53, GLY27, PHE52
17	**2q**	ALA98, GLY27, ILE119, PHE100	ASP53, GLU122, ILE54, VAL26	PHE52, TYR85
18	**2r**	ASP53, GLU122, ILE54, PHE52, TYR85	GLY27, VAL26	ALA98, ILE119, PHE100
19	**2s**	GLU122, PHE52, TYR85, VAL26	GLY27, ILE119	ALA98, ASP53, ILE54, PHE100
20	**2t**	ALA98, GLY27, ILE119, PHE100, VAL26	ASP53, GLU122, ILE54	PHE52, TYR85
21	**2u**	ALA98, ASP53, GLY27, ILE119, PHE100	GLU122, ILE54, PHE52, VAL26	TYR85
22	**2v**	ALA98, GLY27, ILE119, PHE100, VAL26	ASP53, GLU122, ILE54	PHE52, TYR85
23	**2w**	ALA98, GLY27, ILE119, PHE100, VAL26	ASP53, GLU122, ILE54	PHE52, TYR85
24	**2x**	GLU122, ILE119, TYR85	ILE54, PHE100	ALA98, ASP53, GLY27, PHE52, VAL26
25	**2y**	ASP53, GLU122, GLY27, ILE119, PHE52, TYR85, VAL26	ALA98, ILE54	PHE100
26	**3a**	ALA98, GLY27, ILE119, PHE100	ASP53, GLU122, ILE54, VAL26	PHE52, TYR85
27	**3b**	GLU122, GLY27, PHE52, TYR85, VAL26	ALA98, ASP53, ILE119, ILE54	PHE100
28	**3c**	GLU122, PHE52, TYR85	ASP53, ILE119, ILE54, VAL26	ALA98, GLY27, PHE100
29	**3d**	GLU122, ILE54, PHE52, TYR85	ASP53, ILE119, VAL26	ALA98, GLY27, PHE100
30	**3e**	ASP53, GLY27, ILE119, ILE54, PHE100	ALA98, GLU122, PHE52, TYR85	VAL26
31	**3f**	ALA98, GLY27, ILE119, PHE100	ASP53, ILE54, PHE52, VAL26	GLU122, TYR85
32	**3g**	ALA98, GLY27, ILE119, PHE100	ASP53, GLU122, ILE54, VAL26	PHE52, TYR85
33	**3h**	GLU122, ILE54, PHE52, TYR85	ASP53, ILE119, VAL26	ALA98, GLY27, PHE100
34	**3i**	GLU122, PHE52, TYR85	ASP53, GLY27, ILE119, ILE54, VAL26	ALA98, PHE100
35	**3j**	GLU122, TYR85	ASP53, ILE119, ILE54, PHE52, VAL26	ALA98, GLY27, PHE100

docking studies and experimental observations suggest that they could constitute a unique class of pfLDH inhibitors. Diversity of substitution at various points on the skeleton of quinoline chalcones leads to significantly modulated selectivity in the pfLDH active site cavity. The exact overlapping shown in **Figure 3** defines the best choice. These data may pave the way for definition of new potential anti-plasmodium agents. The negative binding scores, relatively short and easy synthesis of these molecules make them attractive candidates for further exploration. Further, computational evaluation was validated by experimental study. Two samples **2c** and **2u** completely inhibited the maturation of parasites at MIC 10 µg/mL and above concentrations while **3b** inhibited 95% maturation of parasite at MIC 50 µg/mL of concentration. No marked inhibition was recorded at 2 µg/mL concentration and hence samples are not recommended for in *vivo* evaluation.

Experimental Section

Melting points were determined by open capillary method and are uncorrected. All solvents were distilled prior to use. TLC was performed on silica gel G. ^{1}H NMR spectra were recorded from $CDCl_3$ or DMSO-d_6 solutions on a Brucker AC 400 (MHz)-NMR instrument. Chemical shifts are reported in ppm using TMS as internal standard. IR spectra were obtained on a Perkin-Elmer 1800 spectrophotometer using KBr discs and mass spectra were measured with a GC-MS (70*ev*).

General methodology for the synthesis of differently substituted (*E*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl)prop-2-en-1-ones/substituted (*E*)-3-(2-Chloro-6-ethoxyquinolin-3-yl)-1-(2-hydroxyphenyl) prop-2-en-1-ones, 2a-y

In a clean beaker, 2-chloro-3-formyl-quinoline/2-chloro-6-ethoxy-3-formyl-quinoline (0.01 M) was added to an ethanolic (15 mL) solution of substituted 2-hydroxy acetophenones (0.01 M). To the above reaction-mixture, aqueous NaOH (40%, 0.03 M, 3 mL) was added drop-wise with constant stirring. The reaction-mixture was kept overnight. It was decomposed by cold 1:1 HCl, filtered, washed, and dried. Further it was recrystallized by using appropriate solvents.

(*E*)-1-(5-Chloro-2-hydroxyphenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one, 2a

IR (KBr): 1646, 3033, 976 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.02-7.00 (d, 1H), 7.61-7.69 (m, 2H), 7.78-7.82 (m, 1H), 7.48-7.46 (d, 1H, Hα, J = 6.44), 7.89-7.89 (d, 1H, Hβ, J = 2.52), 7.92-7.94 (d, 1H), 8.02-8.04 (d, 1H), 8.33-8.36 (d, 1H), 8.54 (s, 1H), 12.55 (s, 1H) 7.48 (dd,1H, H_α, J = 2.48), 7.89 (d, 1H, H_β, J = 2.52), 12.55 (s, 1H), 7.0-8.54 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 117.8, 124.1, 127.8, 130.5, 135.6, 145.2, 147.0, 149.4, 159.9, 189; MS: *m/z* 343.

(*E*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-5-methylphenyl) prop-2-en-1-one, 2b

IR (KBr): 1666, 3000, 972 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H), 7.42-7.44 (d, 1H, H_α, J = 6.40), 7.0-7.03 (d, 1H), 7.61-7.69 (m, 2H), 7.78-7.82 (m, 1H), 7.85-7.85 (d, 1H, H_β, J = 2.48), 7.92-7.94 (d, 1H), 8.04-8.02 (d, 1H), 8.27-8.23 (d, 1H), 8.54 (s, 1H), 12.56 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 116.3, 122.6, 126.4, 127.5, 130.5, 135.6, 145.2, 147.0, 149.4, 158.8, 189.7; MS: *m/z* 323.

(*E*)-1-(3-Bromo-5-chloro-2-hydroxyphenyl)-3-(2-chloroquinolin-3-yl)prop-2-en-1-one, 2c

IR (KBr): 1648, 3032, 978 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.46-7.48 (d, 1H, H_α, J = 6.48), 7.61-7.69 (m, 2H), 7.78-7.82 (m, 1H), 7.89-7.89 (d, 1H, H_β, J = 2.52), 7.92-7.94 (s, 1H), 8.02-8.04 (d, 1H), 8.33-8.36 (d, 1H), 8.54 (s, 1H), 12.57 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 115.7, 126.3, 127.4, 130.4, 135.6, 137.6, 145.2, 149.4, 160.0, 189.3; MS: *m/z* 421.

(*E*)-1-(3-Bromo-2-hydroxy-5-methylphenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one, 2d

IR (KBr): 1666, 3000, 972 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 3H), 7.42-7.45 (d, 1H, H_α, J = 6.40), 7.61-7.69 (m, 2H), 7.78-7.82 (m, 1H), 7.82-7.82 (d, 1H, H_β, J = 1.28), 8.04-8.02 (d, 1H), 8.27-8.23 (d, 1H), 8.54 (s, 1H), 12.58 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 23.6, 114.2, 124.8, 127.4, 130.6, 141.0, 145.2, 147.0, 149.4, 158.9, 189.7; MS: *m/z* 401.

(*E*)-1-(5-Chloro-2-hydroxy-3-iodophenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one, 2e

IR (KBr): 1640, 3132, 987 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.49-7.46 (d, 1H, H_α, J = 6.40), 7.78-7.61 (m, 2H), 7.79-7.82 (m, 1H), 7.89 (d, 1H, H_β, J = 2.48),

7.94-7.92 (s, 1H), 8.02-8.04 (d, 1H), 8.36-8.33 (d, 1H), 8.54(s, 1H), 12.53 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 89.4, 125.7, 127.6, 130.8, 135.6, 145.2, 147.0, 149.0, 159.3, 189.3; MS: *m/z* 467.

(*E*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-3-iodo-5-methylphenyl) prop-2-en-1-one, 2f

IR (KBr): 1660 (C=O), 3210 (-OH), 979 cm^{-1} (CH=CH wagging); ^{1}H NMR (DMSO-d_6): δ 2.59(s, 3H); 7.42-7.44 (d, 1H, H_α, J = 6.4), 7.61-7.69 (m, 2H), 7.78-7.82 (m, 1H), 7.85 (d, 1H, H_β, J = 2.52), 7.92-7.94 (s, 1H), 8.02-8.04 (d, 1H), 8.23-8.27 (d, 1H), 8.54 (s, 1H), 12.60 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 23.4, 87.9, 124.2, 127.4, 130.4, 133.1, 135.6, 145.2, 147.0, 149.4, 158.0, 189.8; MS: *m/z* 447.

(*E*)-1-(5-Chloro-2-hydroxy-3-nitrophenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one, 2g

IR (KBr): 1644, 3032, 977 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.44-7.47 (d, 1H, H_α, J = 6.44), 7.61-7.65 (m, 2H), 7.88 (d, 1H, H_β, J = 2.44), 7.69-7.82 (m, 1H), 7.89-7.92 (s,1H), 8.02-8.04 (d, 1H), 8.36-8.33 (d, 1H), 8.54 (s, 1H), 12.63 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 126.1, 127.4, 130.5, 135.6, 137.5, 145.4, 147.0, 149.4, 151.7, 188.0; MS: *m/z* 388.

(*E*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-5-methyl-3-nitrophenyl) prop-2-en-1-one, 2h

IR (KBr): 1627, 3215, 979 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.8(s, 3H); 7.42-7.44 (d, 1H, H_α, J = 6.44), 7.84-7.85 (d, 1H, H_β, J = 2.52), 7.68-7.61 (m, 2H), 7.78-7.82 (m, 1H), 7.94-7.92 (s, 1H), 8.02-8.04 (d, 1H), 8.23-8.26 (d, 1H), 8.54 (s, 1H), 12.69 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 23.3, 123.5, 126.3, 127.2, 130.5, 131.5, 135.6, 137.7, 145.2, 147.0, 149.4, 150.6, 189.5; MS: *m/z* 368.

(*E*)-1-(5-Bromo-2-hydroxy-4-methoxyphenyl)-3-(2-chloroquinolin-3-yl) prop-2-en-1-one, 2i

IR (KBr): 1630, 3110, 982 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.02(s, 3H), 7.56-7.58 (d, 1H, H_α, J = 6.48), 7.89 (d, 1H, H, β, J = 2.52), 7.69-7.61 (m, 2H), 7.78-7.82 (m, 1H), 7.94-7.92 (s, 1H), 8.02-8.04 (d, 1H), 8.36-8.33 (d, 1H), 8.54 (s, 1H), 12.58 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 55.2, 104.7, 117.2, 126.4, 127.2, 130.7, 135.8, 145.2, 161.8, 164.0. 189.7; MS: *m/z* 417.

(*E*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-5-iodo-4-methoxyphenyl) prop-2-en-1-one, 2j

IR (KBr): 1630, 3110, 982 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 4.04(s, 3H), 7.56-7.58 (d, 1H, H_α, J = 6.48), 7.89 (d, 1H, H_β, J = 2.52), 7.69-7.61 (m, 2H), 7.78-7.82 (m, 1H), 7.94-7.92 (s, 1H), 8.02-8.04 (d, 1H), 8.36-8.33 (d, 1H), 8.54 (s, 1H), 12.64 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 55.0, 79.0, 104.1, 116.6, 126.6, 127.4, 130.6, 135.6, 139.8, 145.2, 147.0, 161.5, 166.7, 189.2; MS: *m/z* 463.

(*E*)-1-(5-Chloro-2-hydroxyphenyl)-3-(2-chloro-6-ethoxyquinolin-3-yl) prop-2-en-1-one, 2k

IR (KBr): 1575, 1645, 1670, 2930, 3065, 3450 cm^{-1} ; ^{1}H NMR (DMSO-d_6): δ 1.39-1.43 (3H, t), 4.05-4.10 (2H, q), 6.90-6.92 (1H, d, J = 8.92), 7.08-7.09 (1H, d, J = 2.68), 7.31-7.39 (2H, d,d), 7.65-7.69 (1H, d, J = 15.4), 7.78-7.80 (1H, d, J = 9.2), 7.88-7.88 (1H, d, J = 2.52), 8.21-8.25 (1H, d, J = 15.44), 8.46 (1H, s), 12.47 (1H, s); ^{13}C NMR (DMSO-d_6): δ 13.9, 63.2, 103.2, 117.8, 122.3, 124.1, 127.4, 128.9, 130.0, 131.4,132.0, 134.0, 136.1, 144.3, 147.0, 148.2, 159.2, 159.9, 189.7; MS: *m/z* 389.

(*E*)-3-(2-Chloro-6-ethoxyquinolin-3-yl)-1-(2-hydroxy-5-methylphenyl) prop-2-en-1-one, 2l

IR (KBr): 1600, 1645, 2900, 3000, 3400 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.51-1.55 (3H, t), 2.38 (3H, s), 4.16-4.21 (2H, q), 6.96-6.98 (1H, d, J = 8.48), 7.14-7.15 (1H, d, J = 2.68), 7.28 (1H, s), 7.35-7.37 (1H, dd), 7.42-7.44 (1H, dd), 7.71-7.75 (2H, d, J = 15.56), 7.91-7.94 (1H, d, J = 9.2), 8.27-8.30 (1H, d, J = 15.48), 8.39 (1H, s), 12.51 (1H, s); ^{13}C NMR (DMSO-d_6): δ 14.8, 23.9, 62.6, 104.3, 115.5, 121.3, 122.5, 128.3, 129.2, 129.8, 130.5, 131.3, 134.0, 135.3, 142.7, 144.0, 145.1, 156.6, 157.6, 189.7; MS: *m/z* 367.

(*E*)-1-(5-Chloro-2-hydroxy-3-iodophenyl)-3-(2-chloro-6-ethoxyquinolin-3-yl) prop-2-en-1-one, 2m

IR (KBr): 689.3, 1690, 2910 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.42-1.45 (3H, t), 4.12-4.16 (2H, q), 7.01-7.03 (1H, d, J = 8.8), 7.2-7.3 (1H, dd), 7.5-7.6 (1H, dd), 7.85-7.86 (1H, d, J = 6.04), 8.01-8.04 (1H, d, J = 11.92), 8.12-8.15 (1H, d, J = 8.56), 8.27 (1H, s), 11.5 (1H, s); ^{13}C NMR (DMSO-d_6): δ 15.7, 65.8, 88.3, 104.3, 122.1, 125.6, 126.2, 128.5, 128.9, 129.2, 131.7, 133.3, 141.6, 143.4, 144.1, 146.1, 158.7, 160.3, 187.6; MS: *m/z* 515.

(*E*)-3-(2-Chloro-6-ethoxyquinolin-3-yl)-1-(2-hydroxy-3-iodo-5-methylphenyl)prop-2-en-1-one, 2n

IR (KBr): 1645, 1700, 2940, 3035, 3467 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.35-1.37 (3H, t), 2.35 (3H, s), 3.96-4.10 (2H, q), 7.00-7.02 (1H, d, *J* = 7.4), 7.25-7.38 (1H, dd), 7.58-7.64 (1H, dd), 7.85-7.86 (1H, d, 4.76), 8.01-8.04 (1H, d, *J* = 9.8), 8.12-8.15 (1H, d, *J* = 11.12), 8.27 (1H, s), 12.21 (1H, s); ^{13}C NMR (DMSO-d_6): δ 14.8, 23.4, 64.7, 87.9, 105.0, 123.0, 124.2, 127.4, 129.0, 130.5, 131.0, 133.1, 134.0, 142.7, 145.2, 147.1, 147.2, 157.0, 158.0, 189.7; MS: *m/z* 493.

(*E*)-3-(2-Chloro-6-ethoxyquinolin-3-yl)-1-(2-hydroxy-5-methyl-iodo-3-nitrophenyl)prop-2-en-1-one, 2o

IR (KBr): 735, 1610, 1680, 2980, 3030, 3530 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.49-1.52 (3H, t), 2.16 (3H, s), 4.15-4.21 (2H, q), 6.90-6.92 (1H, d, *J* = 8.36), 7.09-7.09 (1H, d, *J* = 2.6),7.31-7.34 (1H, dd), 7.62-7.64 (1H, d, *J* = 8.36), 7.75-7.76 (1H, d, *J* = 6.4), 7.87-7.88 (1H, d, *J* = 2.96), 8.21-8.25 (1H, d, *J* = 15.52), 8.46 (1H, s), 12.31 (1H, s); ^{13}C NMR (DMSO-d_6): δ 13.6, 21.2, 63.7, 106.5, 123.0, 123.6, 126.2, 129.8, 130.0, 130.6, 131.5, 133.2, 137.6, 138.8, 141.5, 145.1, 145.4, 149.8, 157.0, 189.7; MS: *m/z* 412.

(*E*)-1-(3-Bromo-2-hydroxy-5-methylphenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl)prop-2-en-1-one, 2p

IR (KBr): 695, 1590, 1680, 3490 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.49-1.52 (3H, t), 2.16 (3H, s), 4.15-4.21 (2H, q), 6.95-6.96 (1H, d, *J* = 2.76), 7.20-7.20 (1H, d, *J* = 2.98), 7.38-7.39 (1H, dd), 7.73-7.77 (1H, d, *J* = 15.52), 7.85-7.87 (1H, d, *J* = 9.2), 7.9-8.03 (1H, m), 8.09-8.13 (1H, d, *J* = 15.68), 8.57 (1H, s), 11.96 (1H, s); ^{13}C NMR (DMSO-d_6): δ 14.8, 23.6, 64.7, 104.1, 113.7, 124.1, 125.8, 127.7, 128.9, 129.9, 129.6, 132.5, 134.8, 140.9, 143.7, 144.7, 146.1, 156.8, 157.3, 188.9; MS: *m/z* 447.

(*E*)-1-(5-Amino-2-hydroxyphenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl)prop-2-en-1-one, 2q

IR (KBr): 725, 1610, 1710, 2924, 3550 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.44-1.50 (3H, t), 4.17-4.23 (2H, q), 5.3 (2H, s), 7.39-7.39 (1H, d, *J* = 14.32), 7.50-7.53 (1H, dd), 7.67-7.69 (1H, d, *J* = 8.88), 7.78-7.81 (1H, dd), 7.92-7.94 (1H, d, *J* = 9.04), 8.09-8.09 (1H, d, *J* = 2.4), 8.63 (1H, s), 9.6 (1H, s); ^{13}C NMR (DMSO-d_6): δ 13.9, 64.8, 105.2, 115.8, 117.4, 123.6, 123.7, 127.5, 129.8, 131.7, 134.5, 141.4, 142.8, 145.7, 147.1, 151.7, 157.6, 189.6; MS: *m/z* 368.

(*E*)-1-(3-Chloro-2-hydroxyphenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl) prop-2-en-1-one, 2r

IR (KBr): 650, 1645, 1705, 2905, 3430 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.47-1.50 (3H, t), 4.16-4.22 (2H, q), 6.29-6.34 (2H, dd), 7.13-7.15 (2H, d, *J* = 8.64), 7.26-7.27 (1H, d, *J* = 2.48), 7.39-7.41 (1H, dd), 7.75-7.76 (1H, d, *J* = 2.08), 7.84-7.86 (1H, d, *J* = 9.24), 8.24 (1H, s), 10.09 (1H, s); ^{13}C NMR (DMSO-d_6): δ 14.7, 65.6, 104.8, 122.8, 124.8, 124.3, 123.7, 125.3, 128.8, 129.8, 133.8, 135.0, 138.1, 141.7, 146.2, 148.2, 157.2, 161.5, 189.7; MS: *m/z* 389.

(*E*)-1-(5-Chloro-2-hydroxy-3-nitrophenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl)prop-2-en-1-one, 2s

IR (KBr): 699, 1675, 1715, 2960, 3565 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.58-1.59 (3H, t), 3.98 (2H, s), 6.96-6.99 (1H, d, *J* = 8.88), 7.44-7.49 (1H, dd), 7.57-7.58 (1H, d, *J* = 2.12), 7.68-7.72 (1H, d, *J* = 15.16), 7.98-7.98 (1H, d, *J* = 2.56), 8.04-8.05 (1H, d, *J* = 1.24), 8.59-8.6 (1H, d, *J* = 17.6), 8.67 (1H, s), 12.75 (1H, s); ^{13}C NMR (DMSO-d_6): δ 15.9, 63.9, 104.7, 121.2, 123.9, 127.4, 127.3, 128.7, 131.4, 131.7, 135.8, 137.3, 137.8, 142.8, 144.9, 147.2, 152.8, 158.9, 188.8; MS: *m/z* 434.

(*E*)-1-(3-Bromo-5-chloro-2-hydroxyphenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl)prop-2-en-1-one, 2t

IR (KBr): 1585, 1655, 1675, 2945, 3495 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.49-1.51 (3H, t), 4.37-4.40 (2H, q), 6.77-6.78 (1H, d, *J* = 4.88), 7.31-7.34 (1H, dd), 7.41-7.42 (1H, d, *J* = 4.08), 7.72-7.75 (1H, d, *J* = 13.9), 7.78-7.79 (1H, d, *J* = 4.08), 7.87-7.88 (1H, d, *J* = 4.66), 8.18-8.19 (1H, d, *J* 2.64), 8.42 (1H, s), 11.83 (1H, s); ^{13}C NMR (DMSO-d_6): δ 13.9, 65.8, 106.3, 116.8, 121.9, 125.1, 126.2, 128.5, 129.7, 131.5, 132.9, 133.3, 138.8, 141.5, 146.7, 146.8, 156.8, 161.3, 187.7; MS: *m/z* 467.

(*E*)-1-(5-Bromo-3-chloro-2-hydroxyphenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl)prop-2-en-1-one, 2u

IR (KBr): 1635, 1665, 2915, 3035 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.42-1.45 (3H, t), 4.12-4.16 (2H, q), 7.01-7.03 (1H, d, *J* = 8.32), 7.29-7.33 (1H, dd), 7.58-7.62 (1H, dd), 7.85-7.86 (1H, d, *J* = 6.04), 8.01-8.04 (1H, d, *J* = 11.92), 8.12-8.15 (1H, d, *J* = 12.08), 8.27 (1H, s), 11.50 (1H, s); ^{13}C NMR (DMSO-d_6): δ 12.9, 65.6, 103.2, 117.8, 121.5, 126.7, 127.2, 127.7, 128.0, 130.8, 133.2, 133.3, 138.5, 146.6, 147.4, 148.8, 158.2, 160.3, 188.7; MS: *m/z* 467.

(*E*)-1-(3-Chloro-2-hydroxy-5-nitrophenyl)-3-(2-chloro-6-ethoxyquinoln-3-yl)prop-2-en-1-one, 2v

IR (KBr): 1650, 1715, 2955, 3050, 3487 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.46-1.49 (3H, t), 4.17-4.28 (2H, q), 6.90-6.92 (1H, d, *J* = 8.04), 7.09-7.09 (1H, d, *J* = 2.44), 7.31-7.34 (1H, dd), 7.62-7.64 (1H, d, *J* = 8.36), 7.75-7.76 (1H, d, *J* = 3.00), 7.87-7.88 (1H, d, *J* = 4.48), 8.21-8.25 (1H, d, *J* = 16.4), 8.46 (1H, s), 12.14 (1H, s); ^{13}C NMR (DMSO-d_6): δ 11.6, 64.7, 105.7, 122.1, 123.8, 125.1, 125.2, 128.2, 130.9, 131.8, 132.4, 133.4, 141.9, 143.2, 146.1, 148.4, 158.9, 167.2, 190.2; MS: *m/z* 434.

(*E*)-3-(2-Chloro-6-ethoxyquinoln-3-yl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one, 2w

IR (KBr): 760, 1635, 1675, 2995, 3045 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.54-1.56 (3H, t), 3.68 (1H, s), 4.16-4.27 (2H, q), 6.96-6.98 (1H, d, *J* = 8.4), 7.14-7.15 (1H, d, *J* = 2.8), 7.35-7.37 (1H, dd), 7.42-7.44 (1H, dd), 7.71-7.75 (1H, d, *J* = 15.72), 7.91-7.94 (1H, d, *J* = 9.2), 8.27-8.30 (1H, d, *J* = 15.2), 8.39 (1H, s), 12.51 (1H, s); ^{13}C NMR (DMSO-d_6): δ 13.7, 57.7, 63.9, 101.8, 103.4, 107.4, 116.2, 125.8, 126.2, 128.9, 132.7, 135.6, 135.7, 143.7, 145.5, 148.3, 156.9, 161.9, 166.7, 187.9; MS: *m/z* 383.

(*E*)-3-(2-Chloro-6-ethoxyquinoln-3-yl)-1-(2-hydroxy-4-methoxy-5-nitrophenyl)prop-2-en-1-one, 2x

IR (KBr): 695, 1675, 1890, 3500 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.49-1.52 (3H, t), 3.86 (3H, s), 4.22-4.28 (2H, q), 6.80-6.81 (1H, d, *J* = 1.56), 7.05-7.09 (1H, d, *J* = 3.88), 7.24-7.29 (1H, dd), 7.63-7.68 (1H, d, *J* = 18.16), 7.76-7.78 (1H, d, *J* = 10.6), 7.89-7.90 (1H, dd), 7.99-8.02 (1H, d, *J* = 10.6), 8.47 (1H, s), 11.30 (1H, s); ^{13}C NMR (DMSO- d_6): δ 12.7, 53.9, 62.7, 101.1, 106.0, 117.8, 123.0, 127.2, 127.5, 128.3, 129.6, 133.7, 135.2, 144.1, 146.2, 147.5, 155.6, 165.4, 169.6, 188.8; MS: *m/z* 428.

(*E*)-1-(5-Bromo-2-hydroxy-4-methoxyphenyl)-3-(2-chloro-6-ethoxyquinolin-3-yl)prop-2-en-1-one, 2y

IR (KBr): 685, 1702, 2935, 3012 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.35-1.37 (3H, t), 3.2 (3H, s), 3.96-4.01 (2H, q), 7.00-7.02 (1H, d, *J* = 9.0), 7.25-7.38 (1H, dd), 7.58-7.64 (1H, dd), 7.85-7.86 (1H, d, *J* = 5.0), 8.01-8.04 (1H, d, *J* = 9.8), 8.10-8.11 (1H, d, *J* = 3.16), 8.21 (1H, s), 12.01 (1H, s); ^{13}C NMR (DMSO-d_6): δ 14.8, 55.2, 64.7, 104.7, 105.0, 105.3, 117.2, 123.0, 127.4, 129.0, 131.0, 134.0, 135.8, 142.7, 145.2, 147.2, 157.0, 161.8, 164.3, 189.7; MS: *m/z* 463.

General procedure for differently substituted (2*Z*)-3-(2-chloroquinolin-3-yl)-1-(2-hydroxyphenyl)-3-iodoprop-2-en-1-one: 3a-j

To a solution (0.01 M) of α,β-unsaturated chalcone was added 12 mL of DMSO. To the above reaction-mixture 1-2 crystal of iodine was added and further, the reaction was acidified by adding 1-2 drops of H_2SO_4. The solution was refluxed for 1 hr. The reaction was then quenched with water, and the organic compound obtained was then successively washed with 10 mL 5% solution of sodium thiosulphate and finally with 25 mL of water. The organic compound was filtered, washed, dried and recrystallized to furnish the corresponding iodochalcones.

(2*Z*)-1-(5-Chloro-2-hydroxyphenyl)-3-(2-chloroquinolin-3-yl)-3-iodoprop-2-en-1-one, 3a

IR (KBr): 1668, 3460, 1493 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.30 (s, 1H), 8.83 (s, 1H, H_α), 8.04-7.26 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d6): δ 117.8, 124.1, 102.7, 130.5, 135.6, 136.3, 147.0, 149.4, 159.9, 189.7; MS: *m/z* 469.

(2*Z*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-5-methylphenyl)-3-iodoprop-2-en-1-one, 3b

IR (KBr): 1668, 3455, 1474 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H); 8.94 (s, 1H, H_α), 11.96 (s, 1H), 7.15-8.66 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 116.3, 122.6, 126.4, 102.5, 130.5, 135.6, 136.2, 147.0, 149.4, 158.8, 189.7; MS: *m/z* 449.

(2*Z*)-1-(3-Bromo-5-chloro-2-hydroxyphenyl)-3-(2-chloroquinolin-3-yl)-3-iodoprop-2-en-1-one, 3c

IR (KBr): 1670, 3466, 1498 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.73 (s, 1H), 8.85 (s, 1H, H_α), 7.27-8.06 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 115.7, 126.3, 102.7, 130.4, 135.6, 137.6, 136.2, 149.4, 160.0, 189.3; MS: *m/z* 549.

(2*Z*)-1-(3-Bromo-2-hydroxy-5-methylphenyl)-3-(2-chloroquinolin-3-yl)-3-iodoprop-2-en-1-one, 3d

IR (KBr): 1672, 3466, 1505 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.55 (s, 3H); 8.74 (s, 1H, H_α), 12.13 (s, 1H), 7.25-8.01 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 23.6, 114.2, 124.8, 102.8, 130.6, 141.0, 144.1, 147.0, 149.4, 158.9, 189.7; MS: *m/z* 529.

(2*Z*)-1-(5-Chloro-2-hydroxy-3-iodophenyl)-3-(2-chloroquinolin-3-yl)-3-iodoprop-2-en-1-one, 3e

IR (KBr): 1668, 3466, 1498 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.31 (s, 1H), 8.84 (s, 1H, H_α), 7.27-8.04 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 89.4, 125.7, 101.8, 130.8, 135.6, 145.3, 147.0, 149.0, 159.3, 189.3; MS: *m/z* 595.

(2*Z*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-3-iodo-5-methylphenyl)-3-iodoprop-2-en-1-one, 3f

IR (KBr): 1670, 3466, 1505 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H); 8.66 (s, 1H, H_α), 11.98 (s, 1H), 7.23-8.08 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 23.4, 87.9, 124.2, 102.7, 130.4, 133.1, 135.6, 143.2, 147.0, 149.4, 158.0, 189.8; MS: *m/z* 575.

(2*Z*)-1-(5-Chloro-2-hydroxy-3-nitrophenyl)-3-(2-chloroquinolin-3-yl)-3-iodoprop-2-en-1-one, 3g

IR (KBr): 1660, 3455, 1488 cm^{-1}; ^1H NMR (DMSO-d_6): δ 12.68 (s, 1H), 8.85 (s, 1H, Hα), 7.30-8.81 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 126.1, 101.8, 130.5, 135.6, 137.5, 146.0, 147.0, 149.4, 151.7, 188.0; MS: *m/z* 514.

(2*Z*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-5-methyl-3-nitrophenyl)-3-iodoprop-2-en-1-one, 3h

IR (KBr): 1662, 3458, 1489 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.55 (s, 3H); 8.96 (s, 1H, H_α), 12.03 (s, 1H), 7.24-8.70 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 23.3, 123.5, 126.3, 100.9, 130.5, 131.5, 135.6, 137.7, 143.8, 147.0, 149.4, 150.6, 189.5; MS: *m/z* 494.

(2*Z*)-1-(5-Bromo-2-hydroxy-4-methoxyphenyl)-3-(2-chloroquinolin-3-yl)-3-iodoprop-2-en-1-one, 3i

IR (KBr): 1660, 3402, 1490 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.97 (s, 3H); 8.57 (s, 1H, H_α), 12.01 (s, 1H), 7.00-7.78 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 55.2, 104.7, 117.2, 126.4, 101.7, 130.7, 135.8, 146.0, 161.8, 164.0. 189.7; MS: *m/z* 545.

(2*Z*)-3-(2-Chloroquinolin-3-yl)-1-(2-hydroxy-5-iodo-4-methoxyphenyl)-3-iodoprop-2-en-1-one, 3j

IR (KBr): 1666, 3410, 1480 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.94 (s, 3H); 8.50 (s, 1H, H_α), 11.74 (s, 1H), 6.93-7.71 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 55.0, 79.0, 104.1, 116.6, 126.6, 104.4, 130.6, 135.6, 139.8, 146.1, 147.0, 161.5, 166.7, 189.2; MS: *m/z* 591.

Computational details

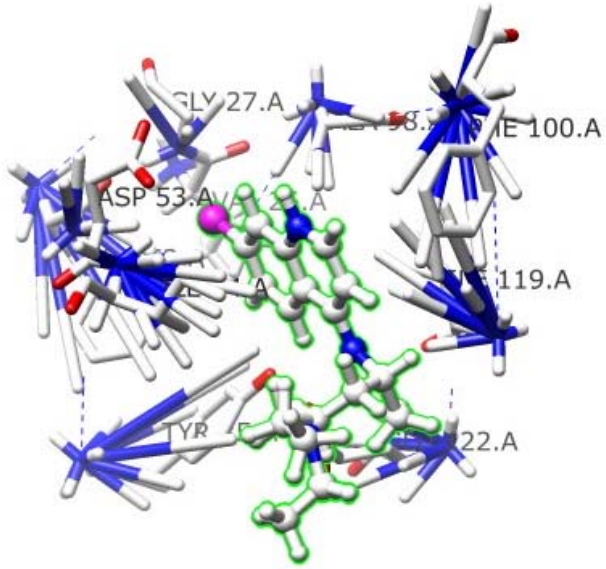


Figure 4 — Active site of pfLDH with Chloroquine

All the ligands used were made using Chemdraw 3D Ultra 8.0 (Ref.17). Before the docking calculation of the ligands, the structures were fully optimized. Details of the calculations used are available in the literature Argus Lab 4.0 (Ref. 18) was used to perform all the docking techniques. The crystal structure for the complex with inhibitor chloroquine was downloaded from Protein Data Bank (http://www.rscb.org/) as PDB files.

Plasmodium falciparum lactate dehydrogenase: The downloaded file containing the crystal structure of pfLDH with its selective inhibitor chloroquine in the active site (**PDB entry 1CET**) shows a monomeric structure with a single chain A consisting of 299 residues. The chain has one N4 (7 Chloro Quinolin 4 Yl) N1 N1 Diethyl Pentane 1, 4 Diamine.

The chain A with the residues, water and hetero groups within a radius of 3 Å was refined and further cleaned by ascertaining the hybridization and the valence of each atom of Chloroquine and introducing H-atoms to the protein residues. The cleaned structure of *Plasmodium falciparum lactate dehydrogenase* (pfLDH) carried no charge and 2327 atoms. The active site residues of pfLDH are shown in **Figure 4**.

Docking and Binding Evaluation

In the automated Argus Lab 4.0 system, using a generic algorithm with a fast-simplified Potential of Mean Force (PMF) carried docking of synthesized ligands into active site of pfLDH. It was assumed that the protein and the ligand docked non-covalently. The

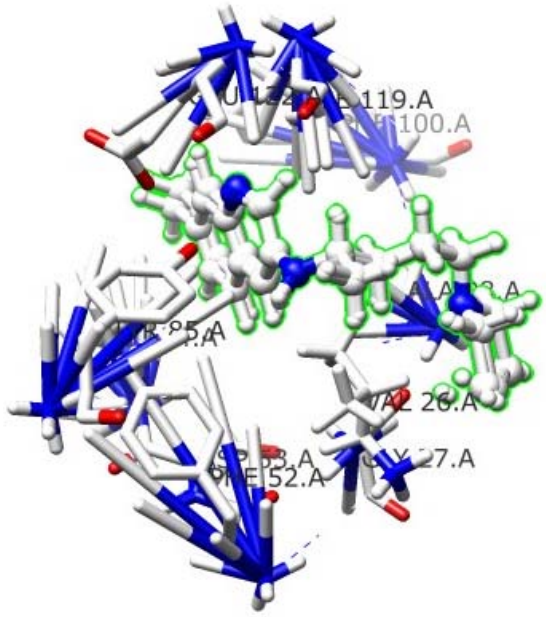


Figure 5 — The close overlapping shown by the docked structure of Chloroquine with its crystal structure with the rms value 0.25

standard PMF implementation used UFF potential for this purpose. The docking was carried with both flexible and rigid ligand into a rigid protein active site. As many ligands failed to undergo docking procedure when they were considered to be flexible in nature; on the other hand while considering their rigid nature they underwent the whole procedure with ease. The general procedure for the docking process started with the addition of energy minimized target ligand on the enzyme. The active site and the ligands were specified in the programme. Using 15×15×15 Å box located at the centre of the target active site optimized the different starting parameters. The whole procedure of docking was repeated until a constant value of docking score was achieved. Concluding docking results were parameterized in terms of docking score in kcal/mole.

Validation of PMF method: To validate the programme involved in current study, before docking the test ligands (synthesized quinoline derivatives **2a-y** and **3a-j**), the docking of chloroquine into the active site of pfLDH was performed. This selective inhibitor binds into the active site cavity with a binding score of -7.91224 kcal/mole and rms deviation of 0.25 was observed. The docked structure of chloroquine in the active site of pfLDH enzyme is shown in **Figure 5**. The close overlapping of a docked structure with the native ligand (X-ray crystal structure) demonstrates the validity of the Programme.

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References

1 Tran C V & Saier M H, *Microbiology Comment*, **2004**, DOI 10.1099/mic.0.26818-0.
2 Gupta R, Gupta, A K & Paul S, *Indian J Chem,* 37B, **1998**, 1211.
3 Dube D, Blowin M & Brideau C, *Bioorg Med Chem Lett*, 8, **1998**, 1255.
4 Gupta R, Gupta A K & Paul S, *Indian J Chem*, 39B, **2000**, 847.
5 Tiwari S, Chauhan P M S & Bhaduri D P, *Bioorg Med Chem Lett*, 10, **2000**, 1409.
6 Kidwai M, Bhushan K R & Sapra, *Bioorg Med Chem*, 8, **2000**, 69.
7 Fujita M, Ciba K & Tominaga Y, *Chem Pharm Bull*, 46, **1998**, 787.
8 Ziegler J, Linck R & Wright D W, *Curr Med Chem*, 8, **2001**, 171.
9 Chauhan P M S & Srivastava S K, *Curr Med Chem*, 8, **2001**, 1535.
10 Peters F N & Musher S, *Indian Eng Chem*, 24, **1937**, 146.
11 Bratt K, Sunnerheim K, Breyngelssons Fagerlund A, Engman L, Andersson R E & Dimberg L H, *J Agri Food Chem*, 51, **2003**, 594.
12 Crambie L & Mistry J, *Tetrahedron Lett*, 31, **1990**, 2647.
13 Yagon A & Cabantchick Z I & Ginsburg H, *EMBO J*, 3, **1984**, 2695.
14 Foley M, Deady L W, Ng K, Cowman A F & Tilley L, *J Biol Chem*, 269, **1994**, 6955.
15 Madapa S, Tusi Z, Sridhar D, Kumar A, Siddiqi M I, Srivastava K, Rizvi A, Tripathi R, Puri S K, Shiva Keshava G B, Shukla P K & Batra S, *Bioorg Med Chem*, 17, **2009**, 203.
16 Srivastava K & Puri S K, *Experimental Parasitology*, 108, **2004**, 74.
17 Chemdraw 3D Ultra software; www.cambridgesoft.com
18 Argus Lab 4.0.1 docking software; http://www.arguslab.com