# Accepted Manuscript


Design, *in silico* and *in vitro* evaluation of curcumin analogues against *Plasmodium falciparum*

Chandrajit Dohutia, Dipak Chetia, Kabita Gogoi, Kishore Sarma

PII: S0014-4894(17)30070-X

DOI: 10.1016/j.exppara.2017.02.006

Reference: YEXPR 7367

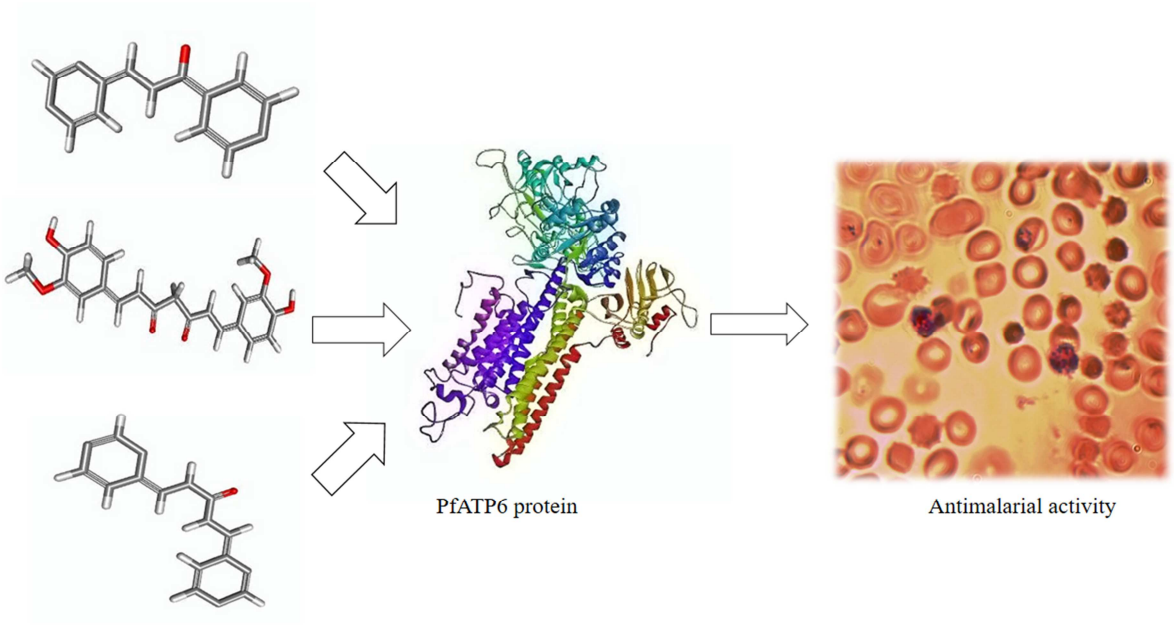
To appear in: *Experimental Parasitology*

Received Date: 17 August 2016

Revised Date: 27 December 2016

Accepted Date: 3 February 2017

Please cite this article as: Dohutia, C., Chetia, D., Gogoi, K., Sarma, K., Design, *in silico* and *in vitro* evaluation of curcumin analogues against *Plasmodium falciparum*, *Experimental Parasitology* (2017), doi: 10.1016/j.exppara.2017.02.006.




Curcumin and its analogues

# Design, *in silico* and *in vitro* evaluation of curcumin analogues against *Plasmodium falciparum*

Keywords: Curcumin, synthesis, PfATP6, *Plasmodium falciparum*, SAR.

**Name, affiliation and contact address of authors:**

Chandrajit Dohutia[1*], Dipak Chetia[1], Kabita Gogoi[2], Kishore Sarma[2]

[1]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004

[2]Regional Medical Research Centre NE (Indian Council of Medical Research), Dibrugarh-786001

*corresponding author

Chandrajit Dohutia

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004

Contact No: +91-8011713589

Email: chnadrajit@gmail.com

**Abstract**

The polyphenolic compound curcumin has been reported for its antimalarial properties in various scientific studies. *Plasmodium falciparum* ATP6, the parasite orthologue of mammalian sarcoplasmic $Ca^{2+}$ ATPase (SERCA) has been identified as a key molecular target of both artemisinin and curcumin. The work was thereby undertaken to study the anti-malarial properties of two different series of curcumin analogues based on their docking interactions with PfATP6 and correlating the results with their anti-malarial activity. The compounds were designed retaining similar functional groups as that of the parent curcumin nucleus while incorporating changes in the carbon chain length, unsaturated groups and the number of ketone groups. The compounds (1E,4E)-1,5-bis(4-methylphenyl)penta-1,4-dien-3-one (CD-9), (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (CD-8) and (E)-1,3-bis(4-hydroxylphenyl)prop-2-en-1-one (CD-1) showed $IC_{50}$ values of 1.642 µM, 1.764 µM and 2.59 µM in 3D7 strain and 3.039 µM, 7.40 µM and 11.3 µM in RKL-2 strain respectively. Detailed structure-activity relationship studies of the compounds showed that CD-9 and CD-8 had a common hydrophobic interaction with the residue Leu268 of the PfATP6 protein and has been postulated through our study to be the reason for their antimalarial activity as seen after corroborating the results with the *in vitro* study. The study provided valuable insight about the ligand-protein interaction of the various functional groups of curcumin and its analogues against the PfATP6 protein and their importance in imparting antimalarial action.

## 1. Introduction

Globally malaria is beginning to show signs of abatement yet it still afflicts millions across the globe. Its worldwide figures border on two hundred million clinical cases and half a million deaths [1]. Artemisinin has long been the backbone of anti-malarial studies but of late concerns over its resistance in Southeast Asian countries and Greater Mekong areas have led to newer combinations of anti-malarial agents being employed [2]. Thereby, newer, more potent and less toxic anti-malarial leads are the need of the hour. Despite the requirement of a novel anti-malarial agent, drug discovery for malaria is a time consuming and expensive process [3, 4]. The continuous evolution of the drug discovery methods and high-quality lead generation process is likely to deliver potential compounds with better therapeutic activity [5]. The PfATP6 protein, an orthologue of the mammalian sarcoplasmic endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA) is considered as a target for artemisinin and its derivatives [6]. Curcumin possesses incredible properties to combat a variety of ailments plaguing people [7]. Studies have shown that curcumin exhibits a considerable amount of activity against both human and rodent strains of malaria parasite in *in-vitro* and *in vivo* [8-11]. The antiparasitic action of curcumin could be attributed to its binding to a hydrophobic pocket in the transmembrane region of the PfATP6 (modelled) protein in the parasite and thereby interfering with its calcium transport [12]. A detailed structural elucidation of the curcumin molecule had earlier revealed that the phenolic groups in the curcumin nucleus play a crucial role in its anti-malarial activity. Any change in these groups apart from esterification has been reported to have decreased the anti-malarial efficacy of the compound [13]. The ß-diketone moiety has been associated with the instability of curcumin and has undergone considerable modifications of late [14]. However, some researchers consider the diketo group to be essential for antimalarial activity [15]. Ring closure of the ß-diketone moiety by condensation reactions involving hydrazines has led to considerable improvement in the antimalarial properties [16]. Aher et al. (2011) synthesized and screened a series of dibenzalacetone analogues against *P. falciparum* using different substituents and obtained promising results [17]. Various synthetic and natural chalcones have been reported to have proficient antimalarial property, some, of which also showed good activity against CQ-resistant *P. falciparum* strains [18-22]. Encouraging results were earlier obtained through docking studies of different chalcone derivatives against the cysteine protease (falcipain) enzyme [23]. Studies using the homology models of falcipains 1, 2 and 3 and their screening against the W2 and D6 strains showed promising results [24-26]. Based on these studies we

designed a library of fifteen dibenzalacetones and chalcones respectively which were structurally similar to curcumin and docked them against the PfATP6 protein. Based on their binding scores we synthesized and evaluated a total of ten compounds and tested against the CQ sensitive 3D-7 and mutant RKL-2 strain of *P. falciparum*. Though studies based on some chalcones and dibenzalacetones as anti-malarial agents have been earlier performed [19-23], molecular docking studies against PfATP6 protein, impact of the different functional groups, particularly that of the hydroxyl groups, difference in the carbon chain lengths, unsaturated carbons, ketone groups and the presence/absence of electron donating/withdrawing groups on the *in vitro* anti-malarial efficacy have been reported for the first time. The ideology behind the study was to the synthesize compounds based on their binding energies to PfATP6, corroborating the results with their *in vitro* activity and formulate an SAR to identify the residues and groups responsible for increase/decrease in antimalarial activity of the compounds. The work is likely to help in developing potential leads for the development of newer drugs/hybrid molecules against malaria.

## 2. Materials and methods

### *2.1. Target identification*

The protein PfATP6, also known as PfSERCA is a 139 kDa protein composed of 1228 amino acids which share 51% identity with mammalian SERCA protein and has been proved to be a major molecular drug target of artemisinin antimalarials [27]. PfATP6 has been identified as the common binding site of both artemisinin and curcumin. Hong-Fang Ji and Liang Shen used PfATP6 as the drug target for curcumin binding which indicated its interactions with PfATP6 through hydrophobic and hydrogen bonds and its subsequent inhibition [28]. As a consequence, in the present study a series of curcumin analogues were designed and targeted against PfATP6 to check for their binding affinity and pattern considering curcumin as a secondary control and chloroquine as a primary control. This, in turn, unveils their anti-malarial potential. In absence of crystallographic structure of PfATP6, the modelled structure of PfATP6, (PDB ID: 1U5N) designed by Krishna et al. and Salas-Burgos et al. was used [29, 30].

### *2.2. Ligand dataset preparation and optimization*

A ligand library of two different series of curcumin analogues each containing 15 compounds was designed using ChemDraw Professional (PerkinElmer Informatics 2016). Selection of the ligands was based on structural similarity to the parent compound curcumin.

The carbon chain was decreased and modification was made in the aryl groups using substituents found in the curcumin molecule in the hope of increasing its anti-malarial efficacy. Table 1 shows the positions of different substituents on the synthesized analogues. The "Prepare ligand" protocol of DS 4.5 was used to prepare the ligands which remove duplicate structures, standardizes the charges of common groups, calculates the ions and ionization of the ligand's functional groups, generates isomers and tautomers, 2D-3D conversion, verifying and optimizing the structures, and other tasks established by user-defined parameters. Energy minimizations of all the ligands were done by applying CHARMM force field.

*2.3. Docking of receptor with ligand*

*PfATP6* was used as receptor molecule for the docking study to probe the binding free energy between the ligand library and receptor using AutoDock 4.2 [31]. Autodock Tools (ADT) was used to optimize the receptor and ligand molecules. For the preparation of the receptor molecule, polar hydrogen's, Kollman charges and AD4 type of atoms were added, while Gasteiger charges were added on the ligands and maximum numbers of active torsions were given. AutoGrid4 was used to prepare a grid map of interaction energies around Leu263, Phe264, Gln267, Ile977, Ile981, Ala985, Asn1039, Leu1040, Ile1041 and Asn1042 with a grid box of 90 X 90 X 90 Å3 centred on X, Y, Z = 52.27, 16.45, 11.48 with a grid spacing of 0.375Å. The residues in the current study have been used with reference to earlier reports of their interactions with artemisinin and curcumin[32-34]. Molecular docking was performed using Lamarckian Genetic Algorithm (LGA), keeping the receptor molecule rigid throughout the docking simulation and rest of the docking parameters was set to default values. Ten different poses were generated for each ligand and scored using AutoDock 4.2 scoring functions and were ranked according to their docked energy. AutoDock Tools, PyMOL [35, 36] and Discovery studio 4.5 client (BIOVIA Discovery Studio Client 4.5) were used for post-docking analysis.

*2.4. Chemistry*

Melting points were measured with a Buchi B-540 melting point apparatus and are uncorrected. IR spectra were recorded on Bruker ALPHA FT-IR spectrometer on a thin film using chloroform. $^{13}C$ and $^{1}H$ NMR spectra were recorded on Bruker Avance II 400-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Waters, Q-TOF micromass (ESI-MS) spectrometer. All the commercially

available reagents were used as received. All experiments were monitored by thin layer chromatography (TLC). TLC was performed on prepared silica glass plates. Column chromatography was performed on silica gel (60-120 mesh, Merck Chemicals). In this study, one step facile synthesis of dibenzalacetone and chalcones based on their structural homology with curcumin and binding energy scores were carried out via the pathways illustrated in Scheme 1, 2 (Fig. 2, Fig. 3). Dibenzalacetones (CD 8, 9, 10) were prepared with substituted benzaldehydes and acetone (2:1) in a base catalyzed reaction via aldol condensation [37, 38]. Synthesis of various benzylideneacetophenones (chalcones) was carried out using Claisen condensation [39]. Hydroxyllated dibenzalacetones (CD 5, 12) were synthesized under protic conditions using an alternate route [40].

*2.4.1 General procedure for synthesis of chalcone derivatives (CD-1, 2, 13, 14, 15):*

Equimolar amounts of benzaldehydes and acetophenones were dissolved and stirred in a beaker to which 50% sodium hydroxide was added dropwise with moderate heating. Stirring was continued till the mixture solidified. The residue was washed with ice cold water and recrystallized with hot ethanol.

*2.4.2 (E)-1,3-bis(4-hydroxylphenyl)prop-2-en-1-one (CD-1):* Yield 71%; Rf = 0.78 (Toluene: Ethyl acetate: Formic acid = 5:4:1); Light brown crystals; m.p. 203-205°C. IR ($cm^{-1}$): 3147, 1646, 1654. $^{1}H$ NMR (DMSO, 400 Mhz): δ 8.1206 (s. 2H), 7.4904-7.5143 (m, 3H), 6.8060-6.9429 (m, 2H), 6.6034-6.6336 (m, 2H), 7.2725-7.3155 (m, 3H). $^{13}C$ NMR (DMSO, 400 Mhz): δ 191.72, 168.49, 159.70, 120.80, 143.22, 130.22, 123.22, 120.80, 113.94, 135.45. MS (EI, m/z) = 224 (M-60+).

*2.4.3 (E)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (CD-2):* Yield 87%; Rf = 0.2 (EtOAc/Hexane = 1:4); Pale yellow crystals; m.p. 71-73°C. IR ($cm^{-1}$): 3721, 1664, 1651. $^{1}H$ NMR ($CDCl_3$, 400 MHz): δ 3.84 (s, 3H), 6.9210-6.9429 (m, 2H), 7.9949-8.0165 (m, 2H), 7.3919-7.6083 (m, 6H), 15.68 (d, 1H) $^{13}C$ NMR ($CDCl_3$, 400 MHz): δ 190.57, 161.71, 144.72, 119.76, 138.51, 114.45, 128.44, 128.59, 127.61, 132.60, 130.27 and 55.42. MS (EI, m/z) = 239 (M-60+).

*2.4.4 (2E)-1,3-diphenylprop-2-en-1-one (CD-13):* Yield 93%; Rf = 0.3 ($CHCl_3/CH_3OH/C_6H_6$ = 6:3:1); Lemon yellow coloured crystals; m.p. 57-59°C. IR ($cm^{-1}$): 1680, 1645. $^{1}H$ NMR ($CdCl_3$, 400 Mhz): δ 7.7933-7.8326 (m, 2H), 7.5895-7.6549 (m, 2H), 7.4878-7.5051 (m, 2H), 7.4130-7.4816 (m, 2H), 7.5124-7.5686 (m, 2H), 7.5784-7.5838 (m, 2H). $^{13}C$ NMR ($CDCl_3$): δ 190.61, 145.2, 138.22, 134.89, 132.85, 130.60, 129.00, 128.67, 128.55, 128.50, 122.10. MS (EI, m/z) = 209 (M-60+).

*2.4.5 (E)-3-(4-hydroxylphenyl)-1-phenylprop-2-en-1-one (CD-14):* Yield 71%; Rf = 0.4 ($CHCl_3/CH_3OH/C_6H_6$ = 5:2:1); Pale brown coloured crystals; m.p. 83-85°C. IR ($cm^{-1}$): 3640, 1670, 1644. $^{1}H$ NMR (DMSO, 400 Mhz): δ 9.3494 (s, 1H), 6.336-6.6705 (t, 2H), 7.3919-7.6036 (m, 3H), 7.6083-7.6152 (m, 2H), 8.3765-8.3783 (m, 2H). $^{13}C$ NMR (DMSO): δ

193.45, 173.09, 138.22, 134.03, 133.85, 128.66, 128.43, 114.46, 144.90, 123.99, 118.76. MS (EI, m/z) = 224 (M-60+).

*2.4.6 (E)-1-phenyl-3-(p-tolyl)prop-2-en-1-one (CD-15):* Yield 89%; Rf = 0.7 (CHCl3/CH3OH/C6H6 = 7:2:1); Yellow coloured crystals; m.p. 99-101°C. IR ($cm^{-1}$): 1680, 1260, 1650. $^{1}H$ NMR ($CdCl_3$, 400 Mhz): δ 2.3865 (s, 3H), 8 (t, 2H), 7.4686-7.5738 (m, 6H), 15.72 (d, 1H), 8.68 (t, 2H). $^{13}C$ NMR ($CdCl_3$, 400 Mhz): 190.67, 144.98, 141.13, 138.38, 132.71, 132.17, 129.74, 128.62, 128.52, 128.50, 121.10, 21.58. MS (EI, m/z) = 222 (M-60+).

*2.5.1 General procedure for synthesis of dibenzalacetone derivatives (CD-8, 9, 10):*

Substituted benzaldehydes and acetone were mixed together (2:1) in 99% ethanol and 10% sodium hydroxide was added drop wise till the contents solidified. The reaction was monitored by TLC using a solvent mixture of ethyl acetate and hexane (3:7). The product was washed repeatedly with cold water, dried and recrystallized with ethyl acetate to obtain pure crystals.

*2.5.3 (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (CD-8):* Yield 87%; Rf = 0.4 (EtOAc/Hexane = 1:8); lemon yellow coloured crystals; m.p. 79°C. IR ($cm^{-1}$): 3280, 1250, 1710, 1652. $^{1}H$ NMR ($CdCl_3$, 400 Mhz): δ 3.8187 (s, 3H), 6.9176-6.9691 (m, 2H), 7.5730-7.5800 (d, 1H), 7.4512-7.5011 (m, 1H), 7.5442-7.5683 (m, 2H). $^{13}C$ NMR ($CDCl_3$): δ 188.81, 161.60, 161.53, 55.37, 130.08, 129.97, 114.46, 127.02, 127.58. MS (EI, m/z) = 294 (M-60+).

*2.5.4 (1E,4E)-1,5-di-p-tolylpenta-1,4-dien-3-one (CD-9):* Yield 83%; Rf = 0.3 (EtOAc/Hexane = 2:7); Light Yellow coloured crystals; m.p. 87-89°C. IR ($cm^{-1}$): 1711, 1260, 1650. $^{1}H$ NMR ($CdCl_3$, 400 Mhz): δ 2.3836 (s, 3H), 7.0149-7.0548 (d, 1H), 7.2033-7.2532 (t, 2H), 7.4990-7.5193 (d, 2H), 7.6893-7.7721 (d, 1H). $^{13}C$ NMR ($CDCl_3$): δ 21.56, 189.07, 143.18, 140.98, 132.13, 129.73, 128.43, 124.60. MS (EI, m/z) = 263 (M-60+).

*2.5.5 (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (CD-10):* Yield 91%; Rf = 0.5 (EtOAc/Hexane = 3:7); Yellow coloured crystals; m.p. 95-97°C. IR ($cm^{-1}$): 1650, 1255, 1645. $^{1}H$ NMR ($CdCl_3$, 400 Mhz): δ 7.0566-7.0964 (d, 2H), 7.3799-7.4145 (m, 3H), 7.5911-7.6512 (m, 2H), 7.7142-7.7541 (d, 1H). $^{13}C$ NMR ($CDCl_3$): δ 188.95, 143.35, 134.81, 130.55, 129.01, 128.45, 125.45. MS (EI, m/z) = 235 (M-60+).

*2.6.1 General procedure for synthesis of dibenzalacetone derivatives (CD-5, 12):*

Substituted aldehyde and ketone (2:1) were dissolved in glacial acetic acid saturated with anhydrous HCl in an ice bath. The mixture was then kept at room temperature for an hour and subsequently heated in a water bath at 25-35°C for 2 hours. The resultant mixture was kept at room temperature for two days after which it was treated with cold water and filtered. The solid obtained was washed, dried and purified using column chromatography.

*2.5.2 (1E,4E)-1,5-bis(2-hydroxylphenyl)penta-1,4-dien-3-one (CD-5):* Yield 70%; Rf = 0.6 (EtOAc/Hexane = 1:8); cream coloured flaky crystals; m.p. >300°C. IR ($cm^{-1}$): 3227, 1672, 1644. $^{1}H$ NMR ($CdCl_3$, 400 Mhz): δ 9.9402 (s, 1H), 8.3773 (m, 1H), 7.3604-7.4039 (m, 2H),

7.4933-7.5176 (m, 1H), 6.6198-6.7226 (m, 2H). $^{13}$C NMR (CdCl3): δ 195.25, 170.53, 137.93, 129.62, 123.57, 121.87, 119.76, 115.83, 113.94. MS (EI, m/z) = 266 (M-60+).

*2.6.2 (1E,4E)-1,5-bis(4-hydroxylphenyl)penta-1,4-dien-3-one (CD-12):* Yield 77%; Rf = 0.4 (EtOAc/Hexane = 4:6); Green coloured crystals; m.p. 239-241°C. IR (cm$^{-1}$): 3227, 1672, 1644. $^{1}$H NMR (DMSO, 400 Mhz): δ 9.4700 (s, 1H), 8.3773-8.3783 (d, 1H), 7.6848-7.7066 (m, 2H), 7.0149-7.0548 (d, 1H), 6.7183-6.7500 (t, 2H). $^{13}$C NMR (DMSO): δ 193.63, 160.40, 133.75, 124. 58, 118. 40, 158.40. MS (EI, m/z) = 266 (M-60+).

## 3. Antimalarial activity

### *3.1 Preparation of parasites*

The chloroquine sensitive 3D7 (West Africa) and chloroquine resistant RKL-2 strain (Raurkela, Orissa, India) of *P. falciparum* were routinely maintained in stock cultures in medium RPMI-1640 supplemented with 25 mmol HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human AB +ve serum in a $CO_2$ incubator maintained at 37°C and 5% $CO_2$ level [41]. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage of the parasite. For carrying out the assay, the initial ring stage parasitemia was maintained at 1% and 3% hematocrit.

### *3.2 In vitro anti-malarial screening*

The *in-vitro* antimalarial screening was carried out following the microassay methods of Reickmann and Desjardins with minor modifications [42 - 44]. We used two different dosages 5ug/ml and 50 µg/ml for preliminary screening of the compounds and the compounds with good antimalarial activity were further tested for determination $IC_{50}$ values. The compounds were dissolved in 1:200 dimethyl sulfoxide (DMSO) to get a stock solution of 5 mg/ml concentration. The further required dilutions of the stocks were prepared with incomplete media (without serum). To a 96-well flat bottom microtiter plate required amount of the test compounds of the secondary standard curcumin and different test dosages were charged per well in triplicates and accordingly the required volume of synchronized parasites in 3% hematocrit containing 1% parasitemia was added to get the final test dose. Chloroquine (Sigma chemicals) as a primary standard and curcumin (Sigma chemicals) was used as a secondary standard for this test to validate the integrity of the assay. The negative control wells inoculated with 20 µl of CRPMI to which 180 µl of 3% hematocrit with 1% parasitaemia were added while another set of negative control wells received 20 µl of

incomplete culture medium and 0.5 % DMSO (Fig. 5). The plates were incubated for 40 hrs in a water jacketed incubator at 37°C and 5% $CO_2$ environment, after which thin blood smears were prepared from each well, fixed with methanol, stained with 3% Giemsa and observed under microscope. The level of parasitemia in terms of % dead rings and trophozoites was determined by counting a total of 100 asexual parasites (both live and dead) microscopically.

*3.3 Determination of $IC_{50}$ values of the screened compounds*

The compounds which showed a minimal antimalarial activity of 20% (at 5 ug/ml) to 80% (at 50ug/ml) of inhibition were selected for determination of their $IC_{50}$ values. Multiple doses were taken based on their parasite inhibiting properties as determined microscopically. The compounds and the parasite concentrations were adjusted in the same way as that of the screening method. Percentage reductions were used to plot percentage inhibition of growth as a function of drug concentrations. $IC_{50}$ values are determined by log-concentration–response probit analysis program [45].

## 4. Results and Discussion

*4.1. Molecular docking study*

The docking studies of all the synthetized compounds were performed into the binding pocket of PfATP6 (1U5N.pdb) protein (Leu263, Phe264, Gln267, Ile977, Ile981, Ala985, Asn1039, Leu1040, Ile1041). Selection of the ten compounds for synthesis was based on the binding energies obtained by using AutoDock 4.2 against the PfATP6 protein (Table 1) Compounds CD-1, 8 and 9 had slightly higher binding energies (-5.44, -6.36 and -6.37 Kcal/mol respectively) then the secondary standard curcumin (-4.95 Kcal/mol) while the rest of the compounds (CD-2, 5, 10, 12, 13, 14, 15) displayed binding energies closer to that of curcumin (-4.58, -4.64, -4.79, -4.95, -4.44, -4.86 and -4.99 Kcal/mol respectively). 2D interactions using discovery studio client 4.5 show that the major binding source of the designed compounds is through Van der Waals forces, Pi-Sigma and other hydrophobic interactions (Fig. 4).

*4.2. Chemistry*

As stated earlier the compounds were designed based on the structural features of curcumin. In various synthesis the substituents used consisted of electron donating groups such as hydroxyl, methoxy and methyl groups. The chalcones were prepared using the facile one step Claisen-Schmidt condensation reaction while the dibenzalacetone analogues not containing hydroxyl groups were prepared using Aldol condensation. Dibenzalacetone analogues containing hydroxyl groups were prepared using an alternative route under anhydrous conditions using hydrogen chloride gas produced simultaneously using sodium chloride and sulphuric acid and passing it through the reaction mixture. All the spectroscopic data have been presented in the experimental section. For compound CD-8 IR stretching bonds at 3294, 1259, 1625 were observed signalling the formation of aromatic rings, methoxy groups and ketones. A strong band at 3721 in compound CD-1 expressed the stretching pattern of the hydroxyl group while a band at 1625 indicated the presence of a ketone group. The stretching bands from 2945-3295 show the presence of methyl groups while the band at 1625 denotes the keto group in the compound CD-9.

*4.2 Antimalarial evaluation and Structure-Activity Relationship*

All ten compounds passed the screening conditions and were further evaluated for the determination of their $IC_{50}$ values. Compounds (1E,4E)-1,5-bis(4-methylphenyl)penta-1,4-dien-3-one (CD-9) with methyl substitution and (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (CD-8) with methoxy substitution showed the best activity with an $IC_{50}$ of 1.64 µM and 1.764 µM against the 3D-7 strain and 3.03 µM, 7.40 µM against the RKL-2 strain respectively. (E)-3-(2-hydroxylphenyl)-1-phenylprop-2-en-1-one (CD-1) with an hydroxyl substitution showed an $IC_{50}$ value of 2.59 µM against the 3D-7 strain and 11.3 µM against the RKL-2 strain respectively. CD-2, CD-14, CD-15 were chalcone derivatives containing methoxy, hydroxyl and methyl groups respectively while CD-13 was devoid of any functional group substituents. CD-14 had a higher binding energy than CD-2 and CD-13 (-4.86, -4.58, -4.44 Kcal/mol respectively) due to the interaction of its hydroxyl group with Asn 1042. This can be observed from their $IC_{50}$ values (14.03, 29.68, 41.372 against 3D-7 and 25.26, 44.55, and more than 100 higher than 100 <mu>g/ml against RKL-2 strains). Dibenzalacetone analogues CD-5 and CD-12 containing hydroxyl groups showed binding energies of -4.64 and -4.95 Kcal/mol but higher $IC_{50}$ values than that of CD-9 and CD-8. This showed that hydrogen bond interactions between the hydroxyl groups CD-5 and CD-12 with residues Leu 257 of the PfATP6 protein do not play an active part in altering the antimalarial properties of these compounds. In view of the hydrophobic nature of dibenzalacetones and

chalcones, it can be inferred that the hydrophobic interactions play more important roles in the binding of these compounds with PfATP6. Curcumin has also been reported to have significant number of hydrophobic interactions with PfATP6 [25]. Two residues are involved in the hydrogen bond formation between Asn267 with one of the phenolic groups and Arg 1034 with a ketonic group of curcumin respectively. Arg1048 showed an unfavourable linkage with the second phenolic group of curcumin. Compounds CD-9, CD-8, CD-1 were observed to fit in the same binding pocket as that of curcumin and interact with the PfATP6 protein through a series of hydrophilic and hydrophobic interactions. Compound CD-9 was showed to exhibit π-alkyl interaction between the methyl groups and Lys260, Leu1040, Ile981 and Ile272. The aromatic ring of CD-9 was found to interact with Leu268 via π-σ bonds. No hydrogen bond formation was seen in the CD-9 compound. Compound CD-8 showed π-alkyl interactions in between the aromatic rings and the residues Ile1041, Ile977, Ile271. Interaction via π-σ bonds was observed in-between the aromatic ring of CD-8 and Leu 268. A common site of interaction was seen in case of CD-9 and curcumin with the Leu1040 residue though its role in antimalarial activity remains to be seen. *In-vitro* antimalarial activity results showed that the most active compounds CD-9 and CD-8 showed higher antimalarial activity against both strains of *P. falciparum*, which could be confirmed by the interactions with Leu268. Both the compounds belonged to the class of dibenzalacetones. Presence of electron donating groups (methyl, methoxy) in CD-9 and CD-8 contributed significantly to their anti-malarial activity as can been seen from table 1. All chalcone based compounds had different binding interactions with the PfATP6 molecule, making it difficult to isolate the specific interaction responsible for their antimalarial properties. CD-1 showed good anti-malarial activity comparable to that of curcumin. This could be attributed to the hydrogen bonding interactions between the hydroxyl groups of the acetophenone and benzaldehydic segments of the chalcones with Arg1048 and Asn1042. Presence of an unfavourable linkage in the curcumin molecule was observed which might hinder the attachment of the molecule to the receptor and therefore lead to lesser duration of anti-malarial action (Fig. 4). In earlier studies it was reported that the antimalarial action of curcumin was mainly due to the interaction of its phenolic groups with the PfATP6 molecule via hydrogen bonding with Leu1040 and Ala985 residues respectively [13, 28]. However the dibenzalacetone compounds CD-9 and CD-8 did not contain hydroxyl groups as substituents even though they showed higher binding energies and antimalarial activity then curcumin. This showed that hydrophobic interactions between the PfATP6 residues and dibenzalacetones is mainly responsible for its antimalarial

activity and though sharing a close structural resemblance to curcumin, their ways of protein interaction with PfATP6 are not the same.

## 5. Conclusion

In the present work we synthesized ten curcumin analogues based on their binding energies to the modelled structure of the PfATP6 protein. Visualization studies showed favourable hydrophobic interactions (π-σ, π-alkyl) between the aromatic rings of CD-9 and CD-8 with the residue Leu268 which we have postulated as being the reason for their better *in vitro* antimalarial activity against the 3D7 and RKL-2 strains when compared to curcumin. Electron donating groups increased the antimalarial activity of the synthesized analogues. Further *in vivo* studies would be required to determine the efficacy of the compounds, their toxicities and the effect of biotransformation on the compounds. The study is likely to provide valuable insight and in-depth assessment to researchers in the design and development of curcumin derivatives/analogues as promising drug candidates in the battle against malaria. The decreased molecular weight of the compounds can be useful in the formation of synthetic hybrids with piperine which is well known to augment the activity and bioavailability of curcumin [46, 47].

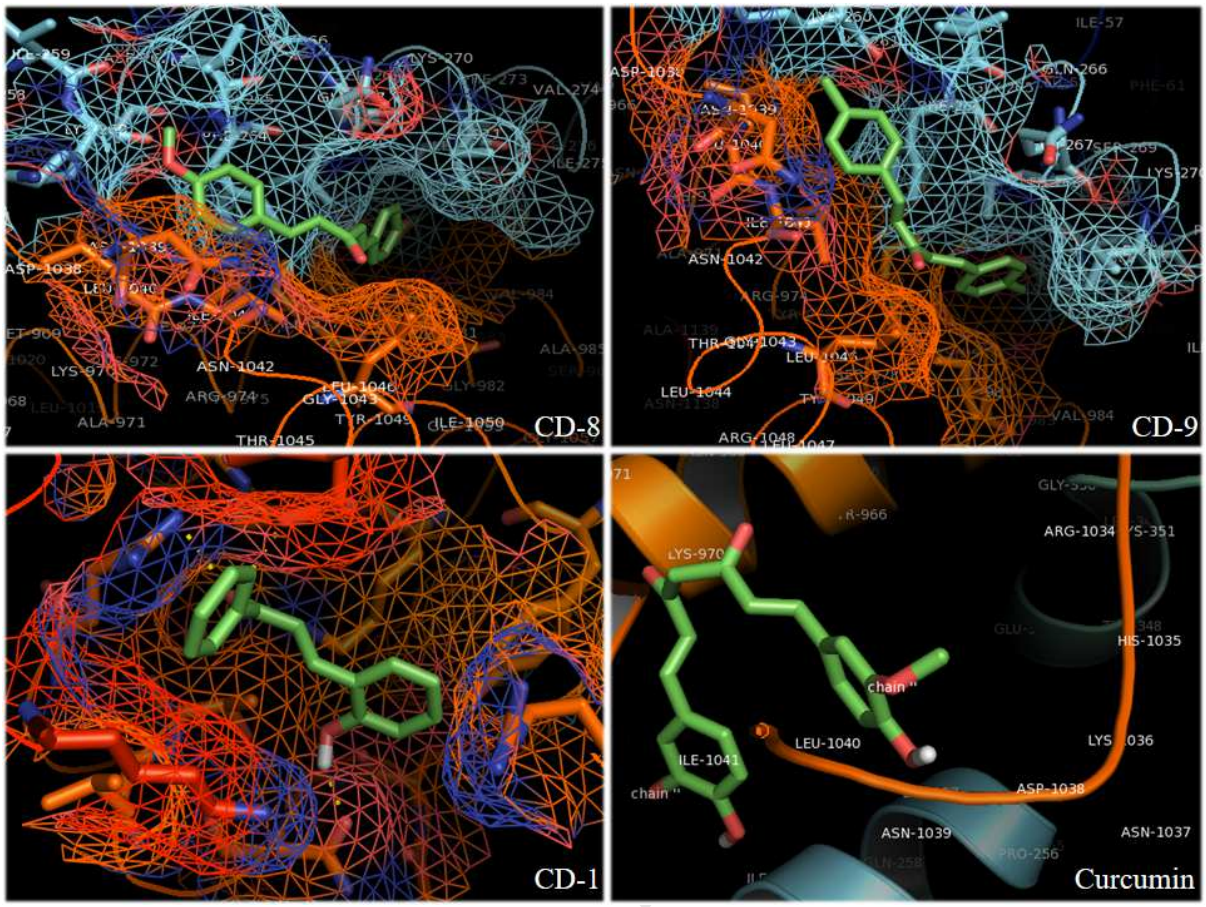
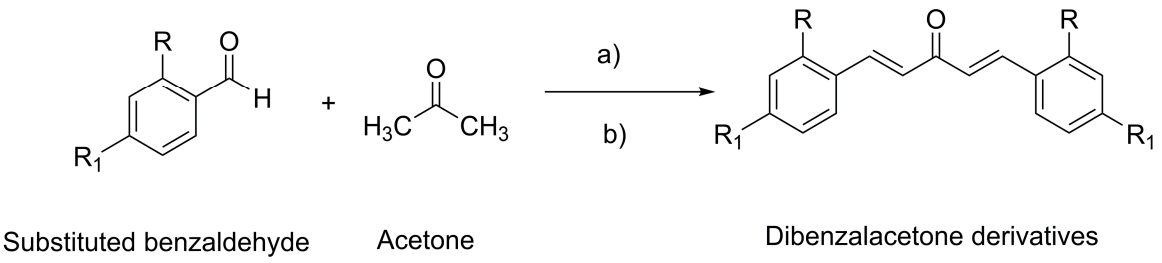



Fig 1. Docked orientation showing the fitting of compounds CD-8, CD-9, CD-1 and curcumin into the binding pockets of PfATP6 protein.




Substituted benzaldehyde Acetone Dibenzalacetone derivatives

Fig 2. Reaction scheme: Synthesis of dibenzalacetone derivatives

a) 99% Ethanol, 10% Sodium hydroxide, stir, 24 hours, rt.

b) Glacial acetic acid, Hydrogen chloride gas, stir, 2-3 hrs 25-35°C, kept for two days.

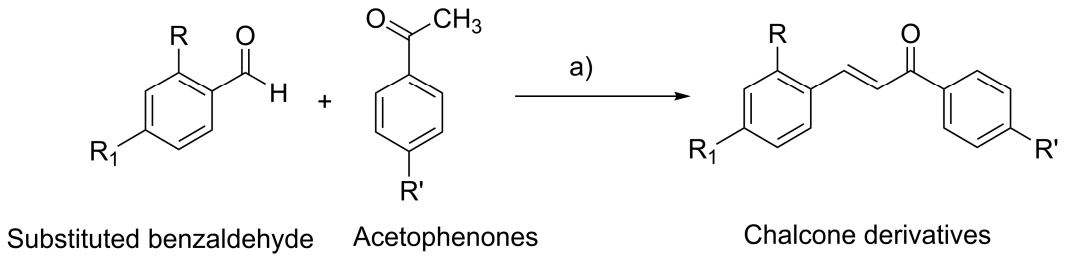



Fig 3. Reaction scheme: Synthesis of chalcones

a) 95% Ethanol, 50% Sodium hydroxide added dropwise.

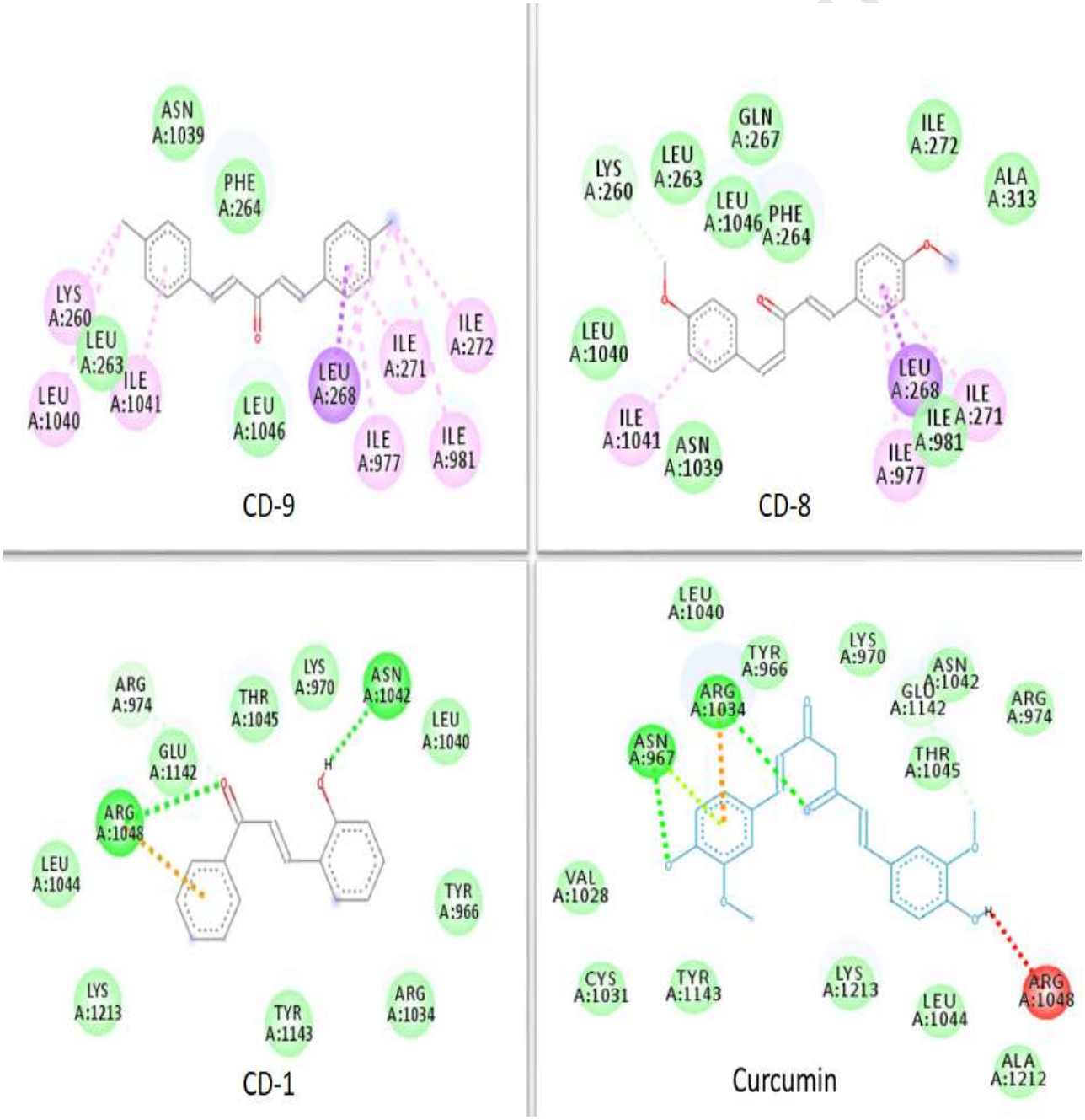



Fig 4. CD-9, CD-8, CD-1 and curcumin ligand protein interactions. All interacting amino acids exhibiting Cation- π and π- π interactions with the ligands are shown as dotted violet

lines, while hydrogen bonds are shown as dotted green lines. The pink coloured lines indicate π- Alkyl interactions while the red dotted lines indicate unfavourable bumps. Orange dotted lines indicated π- cation linkage. The green spheres represent Van der Waals forces.

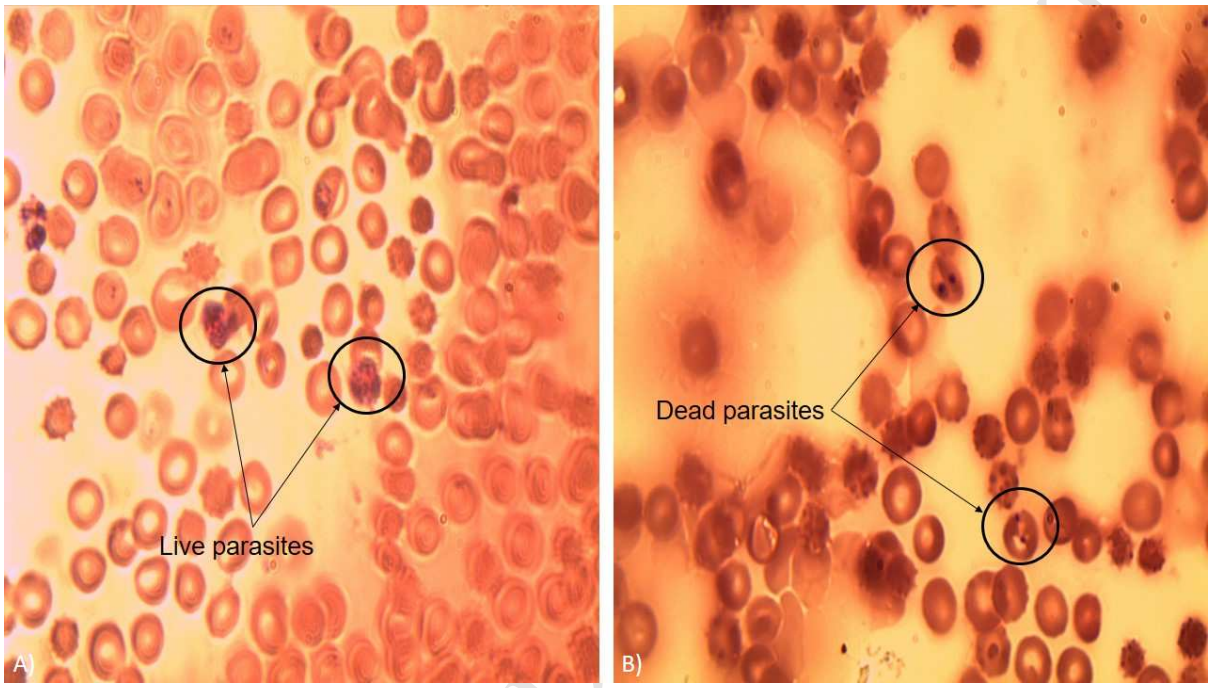



Fig 5.A) Negative control (3D7 strain) and B) CD-9 treated slide (3D7 strain)

Table 1: Binding energies, substituents and $IC_{50}$ values of the synthesized compounds

| Compound code | Substituents | | | Binding energy (Kcal/mol) | 3D7 | | | RKL-2 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | R | $R_1$ | R' | | $IC_{50}$ (ug/ml) | $*R^{2=}$ | #Y= | $IC_{50}$ (ug/ml) | $R^2$= | Y= |
| CD1 | H | OH | H | -5.44 | 2.59 | 0.809 | 6.741 X +32.5 | 11.3 | 0.851 | 0.977X + 38.96 |
| CD2 | H | $OCH_3$ | OH | -4.58 | 29.68 | 0.945 | 0.828X + 25.42 | 44.55 | 0.957 | 0.606X + 23 |
| CD5 | O H | OH | - | -4.64 | 21.32 | 0.860 | 0.507X + 31.66 | 40.584 | 0.908 | 0.650X + 23.62 |
| CD8 | H | $OCH_3$ | - | -6.36 | 1.764 | 0.886 | 7.182X +37.33 | 7.40 | 0.843 | 1.116X + 41.74 |
| CD9 | H | $CH_3$ | - | -6.37 | 1.642 | 0.924 | 13.17X + 28.37 | 3.039 | 0.935 | 15.3X + 3.5 |
| CD10 | H | H | - | -4.79 | 18.66 | 0.876 | 1.885X + 14.82 | 35.6 | 0.900 | 0.68X + 25.79 |
| CD12 | H | OH | - | -4.95 | 12.67 | 0.844 | 1.901X + 25.91 | 21.86 | 0.865 | 1.293X + 21.73 |
| CD13 | H | H | H | -4.44 | 41.372 | 0.900 | 0.561X + 26.79 | Higher than 100ug/ml | | |
| CD14 | H | OH | H | -4.86 | 14.03 | 0.867 | 2.073X + 20.91 | 25.26 | 0.892 | 1.191X 19.91 |
| Curcumin | - | - | - | -4.95 | 2.13 | 0.903 | 13.11X + 26.33 | 5.59 | 0.893 | 14.79X + 4.1 |
| Chloroquine | - | - | - | - | 0.7 | - | - | 1.44 | - | - |

$*R^2$= Chi-square value

#Y= mx + c

**Acknowledgements**

We would like to thank the Department of Pharmaceutical Sciences, Dibrugarh University for providing the facilities to carry out the synthetic work and Regional Medical Research Centre, Dibrugarh for providing the *Plasmodium falciparum* strains for antimalarial assay. We would also like to thank SAIF, Panjab University for carrying out the NMR and MS analysis of the compounds. In this regard, we would like to appreciate the efforts of Partha Pratim Kaishap of CSIR, NEIST in providing assistance while analyzing spectral data.

**References and notes**

1. World Health Organization, 2015. World Malaria Report 2015. WHO, Geneva, Switzerland.
http://apps.who.int/iris/bitstream/10665/205224/1/WHO_HTM_GMP_2016.2_eng.pdf ;
2. World Health Organization, 2016. Artemisinin and artemisinin-based combination therapy resistance.
http://apps.who.int/iris/bitstream/10665/208820/1/WHO_HTM_GMP_2016.5_eng.pdf ;
3. P. Olliaro, Pharmacol. Therapeut. 89 (2001) 207-19;
4. M.H. Gelb, Curr. Opin. Chem. Biol. 11 (2007) 440-445;
5. E. Ratti, D. Trist, Pure Appl. Chem. 73 (2001) 67-75;
6. M. Jung, H. Kim, K.Y. Nam, K.T. No, Bioorg. Med. Chem. Lett. 15 (2005) 2994–2997;
7. B.B. Aggarwal, C. Sundaram, Chitra; N. Malani, H. Ichikawa, Adv. Exp. Med. Biol. 595 (2007) 1-75;
8. R.C Reddy, G. Palakkodu, V.G. Vatsala, G.P. Keshamouni, P.N. Rangarajan, Biochem. Biophys. Res. Commun. 326 (2005) 472–74;
9. R. Chakrabarti, P.S. Rawat, B.M. Cooke, R.L. Coppel, S. Patankar. PLoS One. 8 (2013) 57302;
10. P.B. Memvanga, R. Coco, V. Préat, J. Control. Release 172 (2013) 904–913;
11. S. Kunwittaya, L. Treeratanapiboon, A. Srisarin, C. Isarankura-Na-Ayudhya, V. Prachayasittikul. EXCLI. J. 13 (2014) 287-299;
12. P.K. Naik, M. Srivastava, P. Bajaj, S. Jain, A. Dubey, P. Ranjan, R. Kumar, H. Singh, J Mol Model, 17 (2011) 333–357;
13. S. Mishra, K. Karmodiya, N. Suroliab, A. Surolia. Bioorg. Med. Chem. 16 (2008) 2894–2902;
14. G.D. Straganz, B. Nidetzky, J. Am. Chem. Soc. 127 (2005) 12306-12314;
15. A. Simon, D.P. Allais, J.L. Duroux, J.P. Basly, S. Durand-Fontanier, C. Delage, Cancer Lett. 129 (1998) 111-116;
16. S.N. Balaji, M.J. Ahsan, S.S. Jadav, V. Trivedi, Arabian Journal of Chemistry (2015), http://dx.doi.org/10.1016/j.arabjc.2015.04.011;

17. R.B. Aher, G. Wanare, N. Kawathekar, R.R. Kumar, N.K. Kaushik, D. Sahal, V.S. Chauhan. Bioorg. Med. Chem. Lett. 21 (2011) 3034–36;

18. J.N. Domíngueza, J.E. Charrisa, G Loboa, N.G. Domínguezb, M.M. Morenob, F. Riggionec, E. Sanchezd, J. Olsone, P.J. Rosenthale, Eur. J. Med. Chem. 36 (2001) 555–560;

19. N. Tadigoppula, V. Korthikunta, S. Gupta, P. Kancharla, T. Khaliq, A. Soni, R.K. Srivastava, K. Srivastava, S.K. Puri, K.S. Raju, S.P.S. Wahajuddin, V Kumar, I.S. Mohammad, J. Med. Chem. 56 (2013) 31-45;

20. S.K. Awasthi, N. Mishra, B. Kumar, M. Sharma, A. Bhattacharya, L.C. Mishra, V.K. Bhasin, Med. Chem. Res. 8 (2009) 407-420;

21. M. Liu, P. Wilairat, M.L. Go, J. Med. Chem. 44 (2001), 4443–4452;

22. S.S. Lim, H.S. Kim, D.U. Lee. Bull. Korean Chem. Soc. 28 (2007) 2495-2497;

23. L.F. Motta, A.C. Gaudio, Y. Takahata, Internet. Electron. J. Mol. Des. 5 (2006) 555–569;

24. Y. Sabnis, P.J. Rosenthal, P. Desai, M.A. Avery, J. Biomol. Struct. Dynamics 19 (2002) 765–774;

25. Y.A. Sabnis, P.V. Desai, P.J. Rosenthal, M.A. Avery, Protein Sci. 12 (2003) 501–509;

26. R.S. Li, G.L. Kenyon, F.E. Cohen, X.W. Chen, B.Q. Gong, J.N. Dominguez, E. Davidson, G. Kurzban, R.E.Miller, E.O. Nuzum, P.J. Rosenthal, J.H. McKerrow, J. Med. Chem. 38 (1995) 5031–5037;

27. L.U. Eckstein, R.J. Webb, I.D. Van Goethem, J.M. East, A.G. Lee, M. Kimura, et al. Nature. 424 (2003) 957-61;

28. J. Hong-Fang, S. Liang, Bioorg. Med. Chem. Lett. 19 (2009) 2453-2455;

29. PDB ID: 1U5N, S. Krishna, A.C. Uhlemann, A. Cameron, U, Eckstein, W.Y. Ho, S. Croft, J. Fischbarg, P. Iserovich, F. Zuniga, M. East, A. Lee, L. Brady, R. Haynes, Homology model of *PfATP6*. (2009) www.rcsb.org;

30. A. Salas-Burgos, P. Iserovich, F. Zuniga, J.C. Vera, J. Fischbarg, Biophys. J. 87 (2004) 2990-99;

31. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, J. Comput. Chem. 30 (2009) 2785-2791;

32. M. Jung, H. Kim, K.Y. Nam, K.T. No, Bioorg. Med. Chem. Lett. 15 (2005) 2994-97;

33. F.B. Garah, J.L. Stigliani, F. Cosledan, B. Meunier, A. Robert, Chem. Med. Chem. 4 (2009) 1469-79;

34. A. Shandilya, S. Chacko, B. Jayaram, I. Ghosh, Sci. Rep. 3 (2013) 2513-19;

35. W.L. DeLano, (2002), The PyMol molecular graphics system. http://www.pymol.org ;

36. M.G. Lerner, H.A. Carlson, (2008), Apbs plugin for pymol. University of Michigan, Ann Arbor;

37. C. R. Conard, M.A. Dolliver, Org. Synth. 2 (1943) 167-168;

38. A.J. Kiran, K. Chandrasekharan, S.R. Nooji, H.D. Shashikala, G. Umesh, B. Kalluraya, Chem. Phys. 32 (2006) 699-704;

39. L. Claisen, A. Claparede, Ber. Dtsch. Chem. Ges.14 (1881) 2460–2468;

40. L. Lin, Q. Shi, A.K. Nyarko, K.F. Bastow, C.C. Wu, C.Y. Su, C.C. Shih, K.H. Lee, J. Med. Chem. 49 (2006) 3963-72;

41. W. Trager, J.B. Jensen, Science 193 (1976) 673-75;

42. In vitro Micro-test (Mark-III) for the assessment of the response of Plasmodium falciparum to chloroquine, mefloquine, quinine, amodiaquine, sulfadoxine/pyrimethamine and artemisinin. WHO, Geneva, Switzerland, (2001);

43. K.H. Rieckmann, L.J. Sax, G.H. Campbell, J.E. Mrema, Lancet 1 (1978), 22-23;

44. R.E. Desjardins, C.J. Canfield, J.D. Haynes, J.D. Chulay, Antimicrob. Agents. Chemother. 16 (1979) 710-718;

45. H. Chi, Computer program for the probit analysis. (1997) National Chung Hsing University, Taichung, Taiwan.

46. M.K. Bhutani, M. Bishnoi, S.K. Kulkarni, Pharmacol. Biochem. Behav. 92 (2009) 39–43;

47. G. Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran, P.S. Srinivas, Planta. Med. 64 (1998) 353–356;

# Highlights

- The work involves design of dibenzalacetones and chalcones based on their structural similarities to curcumin and their subsequent docking against the *Plasmodium falciparum* ATP6 protein (PfATP6). Based on their binding energies ten compounds were synthesized, screened and evaluated against the sensitive 3D-7 and mutant RKL-2 strains of *Plasmodium falciparum*.
- The compounds (1E,4E)-1,5-bis(4-methylphenyl)penta-1,4-dien-3-one (CD-9), (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (CD-8) and (E)-1,3-bis(4-hydroxylphenyl)prop-2-en-1-one (CD-1) showed better binding energies (-6.37, -6.36, -5.44 Kcal/mol respectively) and *in vitro* antimalarial activity ($IC_{50}$ 1.642 µM, 1.764 µM and 2.59 µM in 3D7 and 3.039 µM, 7.40 µM and 11.3 µM in RKL-2) than the secondary standard curcumin (-4.95 Kcal/mol; $IC_{50}$ 2.13 µM in 3D7, 5.59 µM in RKL-2).
- The study is likely to provide valuable insight and in-depth assessment to researchers in the design and development of newer curcumin analogues and synthetic hybrid molecules which could serve as promising drug candidates in the battle against malaria.