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

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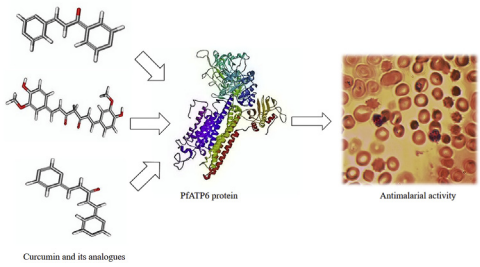
HIGHLIGHTS

- We designed dibenzalacetones and chalcones based on their analogy to curcumin and their subsequent docking against the PfATP6 protein.
- The selected compounds were synthesized, screened and evaluated against the sensitive 3D-7 and mutant RKL-2 strains of *Plasmodium falciparum.*
- The compounds coded CD-9, CD-8 andCD-1showed better binding energies and *in vitro* antimalarial activity against both 3D-7 and RKL-2 strains than curcumin.
- The study would provide valuable insight to researchers in the design and development of newer curcumin analogues which could serve as promising drug candidates against malaria.

GRAPHICAL ABSTRACT



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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 antimalarial 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

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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 (World Health Organization, 2015). 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 (World Health Organization, 2016). 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 (Olliaro, 2001; Gelb, 2007). The continuous evolution of the drug discovery methods and high-quality lead generation process is likely to deliver potential compounds with better therapeutic activity (Ratti and Trist, 2001). 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 (Jung et al., 2005a,b). Curcumin possesses incredible properties to combat a variety of ailments plaguing people (Aggarwal et al., 2007). Curcumin exhibits a considerable amount of *in vitro* and *in vivo* antiplasmodial activity against both human and rodent strains of malaria parasite (Reddy et al., 2005; Chakrabarti et al., 2013; Memvanga et al., 2013; Kunwittaya et al., 2014). 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 (Naik et al., 2011). 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 (Mishra et al., 2008). The β-diketone moiety has been associated with the instability of curcumin and has undergone considerable modifications of late (Straganz and Nidetzky, 2005). However, some researchers consider the diketo group to be essential for antimalarial activity (Simon et al., 1998). Ring closure of the β-diketone moiety by condensation reactions involving hydrazines has led to considerable improvement in the antimalarial properties (Balaji et al., 2015). Aher et al. (2011) synthesized and screened a series of dibenzalacetone analogues against *P. falciparum* using different substituents and obtained promising results (Aher et al., 2011). 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 (Domíngueza et al., 2001; Tadigoppula et al., 2013; Awasthi et al., 2009; Liu et al., 2001; Lim et al., 2007). Encouraging results were earlier obtained through docking studies of different chalcone derivatives against the cysteine protease (falcipain) enzyme (Motta et al., 2006). Studies using the homology models of falcipains 1, 2 and 3 and their screening against the W2 and D6 strains showed good results (Sabnis et al., 2002, 2003; Li et al., 1995). 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 (Tadigoppula et al., 2013; Awasthi et al., 2009; Liu et al., 2001; Lim et al., 2007; Motta et al., 2006), 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 (Eckstein et al., 2003). 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 (Hong-Fang and Liang, 2009). As a consequence, in the present study a series of curcumin analogues were designed and targeted against PfATP6 to check 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 (Krishna et al., 2009; Salas-Burgos et al., 2004).

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.