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INHIBITION OF *P. falciparum* PFATP6 BY CURCUMIN AND ITS DERIVATIVES: A BIOINFORMATIC STUDY

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Abstract

Curcumin, a yellow spice has been shown to have many pathological uses including cancer and malaria. Recent experimental data have shown the inhibitory effect of curcumin and its two derivatives on the growth of *Plasmodium falciparum* in cell culture at low micromolar concentrations. Previous studies have suggested that Ca^{2+}-ATPase (PfATP6) of *P. falciparum* is the target of many antimalarial drugs. However, the mechanism of inhibition of Ca^{2+}-ATPase (PfATP6) is not known. In addition, it is not clear which specific isomeric form of curcumin is the most potent inhibitor of *P. falciparum*. Here we address this issue using bioinformatics tools. We generated a molecular model of Ca^{2+}-ATPase (PfATP6) of *P. falciparum* and carried out molecular docking of all curcumin analogues of Zinc database of compounds (zinc.docking.org). Two molecular docking programs Glide and FlexX were used to determine binding feasibility of 351 analogues of curcumin. The comparison of docking parameters showed, more than 20 analogues are better ligands of PfATP6 than curcumin itself. . The binding of curcumin and its analogues to PFATP6 is mediated by both hydrophobic and polar interactions. Our results suggest that curcumin analogues are promising lead compounds for the development of antimalarial drugs.

Key words: *P. Falcipurum*, Zinc database, curcumin analogues, docking, molecular modeling, binding energy.

Article information

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INTRODUCTION

Curcumin is popularly used as a spice for flavor and yellow color of curry in many South Asian countries. It is found in the roots of *Curcuma longa.* Curcumin is a β-diketone compound and has been used as medicine to cure diseases such as jaundice, indigestion, urinary tract diseases, rheumatoid arthritis, and insect bites for ancient Indian times. Curcumin has also been demonstrated to act as an anti-cancer agent (15,13,1,2,14). Recently, curcumin has been shown to possess synergistic effects with artemisinin against *Plasmodium berghei* (3). The data show that curcumin can serve as a potent inhibitor against chloroquine-resistant (CQ-R) *Plasmodium falciparum* strains (12). The apparent anti-malarial activity of curcumin is partially due to the generation of reactive oxygen species (ROS), and down-regulation of the PfGCN5 HAT (*P. falciparum* histone acetyltransferase) activity (10).

Malaria is caused by protozoa *Plasmodium falciparum*. Due to its prevalence and frequent emergence of drug resistant strains, malaria is one of the most deadly parasitic diseases, especially in tropical countries. Despite the attempts to control mosquito vector and widespread use of anti-malarial drugs, nearly 3 million individuals die every year from malaria (http://www.who.int/mediacentre/factsheets/fs094/en/).

The control of mosquitoes is one of the major methods to curb malaria. However, economic constraints limit the availability of such measures mainly in developing countries. Therefore, the urgent need for new and cost-effective anti-malarial compounds exists more than ever. Recently 12 curcumin derivative compounds were investigated for the inhibition of *P. falciparum* in cell culture assays (5). Three of these compounds exhibited EC_{50} in micromolar concentration promising to be effective anti-malarial drug candidates.

The most common drugs for treatment of malaria is artemisinin and its derivatives, which targets PfATP6, a parasite orthologue of mammalian sarcoplasmic–endoplasmic reticulum Ca^{2+}–ATPase (SERCA) (8). This enzyme has been suggested as the target of curcumin. To examine the possibility of curcumin analogues as ligands for PfATP6, we carried out docking of curcumin derivatives available in Zinc database (www.zinc.org) (10) in the homology derived model structure of PfATP6 using two docking programs. The comparison of docking parameters showed that more than 50 curcumin analogues can bind PfATP6 with similar (or better affinity) than curcumin suggesting the possibility of these analoguesas lead compounds.

MATERIALS AND METHODS

Preparation of compounds for docking

The chemical structures of curcumin analogues in Structure Data Format (sdf) were downloaded from the Zinc database of compounds (zinc.docking.org). We used 'LigPrep' (Schrodinger Inc., NY), a ligand preparation tool interfaced with Maestro (Schrodinger Inc. NY) to generate three-dimensional models of the all compounds. The protonation state and tautomer search of the compounds was carried out by the 'Epik' (16) (Schrodinger Inc. NY).

Homology modeling of P. falciparum PfATP6

The structure of PfATP6 was generated by homology-based molecular modeling protocol using the crystal structure of sarco/endoplasmic reticulum Ca^{2+}–ATPase (SERCA) (PDB file 2o9j) (17) as the template structure. SERCA and PfATP6 share ~44% amino acid sequence homology. The molecular model of PfATP6 was generated by 'Prime' software integrated in Maestro (Schrodinger Inc., NY). The 'Protein Preparation Wizard' (Schrodinger Inc. NY) workflow of Schrodinger suit was used to generate the structure of PfATP6 suitable for docking of curcumin analogues. The 'Protein Preparation Wizard' automatically adds missing hydrogen atoms, fixes metal ionization states, and assigns proper formal charges, bond orders and force field. The possible binding sites for curcumin and its analogues were searched by the Q-site finder program (11).

Flexible docking curcumin analogues

The structures of curcumin analogues generated by 'LigPrep' were first docked into a pocket of PfATP6 formed by of residues L357, K2260, I261, F264, Q267, L268, I271, I275, L309, P315, L318, I973, I981, V984, F988, L1040 and L1049. This site was searched using the Q-site finder program (11). The docking at this site was selected since the volume of pocket wascomparable to the volume of most curcumin analogues. We used 'Glide' software with extra precision (XP) (6,7) followed by Induced Fit Docking (7) workflow incorporated in Maestro (Schrodinger Inc. NY). We also used FlexX (9)flexible docking. These two independent docking programs allowed us to determine the binding affinity of curcumin analogues in terms of Glide and FlexX scores.

RESULTS AND DISCUSSION

Predicted binding pocket for docking of curcumin analogues

The Q-site finder program predicted more than six binding pockets in the molecular model of PfATP6. Some predicted ligand binding sites located at the surface is shallow crevices. These binding pockets were not considered for the docking of the compounds. We selected the binding pocket constituted by amino acid residues L357, K2260, I261, F264, Q267, L268, I271, I275, L309, P315, L318, I973, I981, V984, F988, L1040 and L1049 as a possible binding site for curcumin and its analogues since (i) size of the pocket (~365 Å^2) is comparable to most of the analogues (350 Å^2) and (ii) the pocket contains distribution of hydrophobic and hydrophilic residues suitable for

Table 1. Glide and FlexX docking scores of top six compounds and comparison with docking scores of curcumin.

Glide docking		FlexX Docking	
Compound Zinc ID	**Glide Score**	**Compound Zinc ID**	**FlexX Score**
Zinc13781298	-7.890	Zinc49111530	-19.8003
Zinc49881409	-7.878	Zinc05606394	-19.2761
Zinc44281717	-7.796	Zinc28955244	-18.2869
Zinc05606394	-7.775	Zinc35050563	-15.8370
Zinc13781298	-7.755	Zinc00899824	-15.2578
Zinc49124982	-7.669	Zinc28955244	-13.9284
Curcumin	-6.753	Curcumin	-13.9685

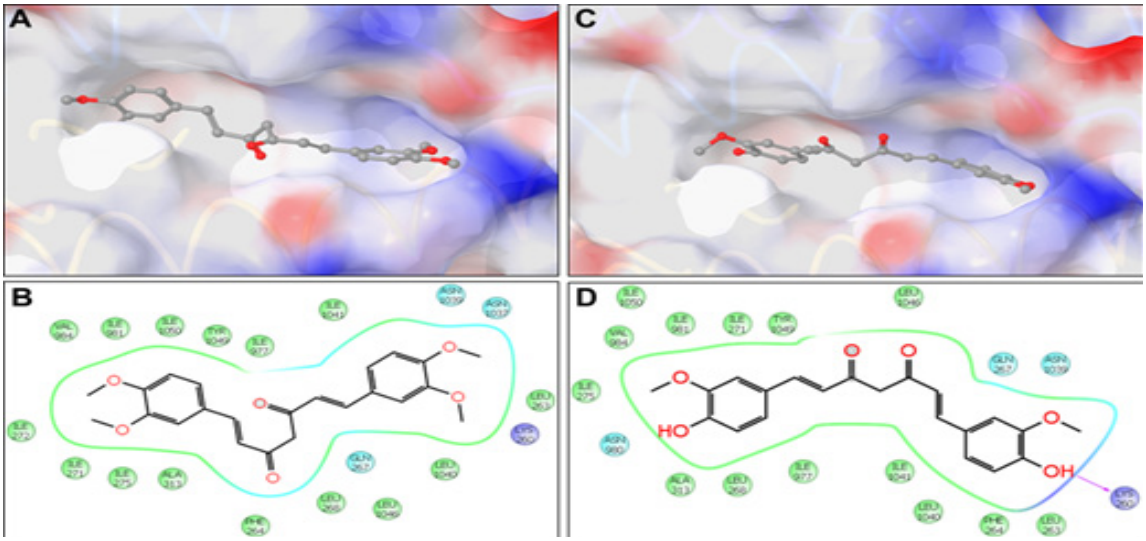

Figure 1. Docked poses of Zinc13781298 and curcumin in the modeled structure of PfATP6 – Panels A and B show the docking of Zinc13781298 and panels C and D show the docking of curcumin. The surface representation corresponds to the electrostatic potential of PfATP6. The positive potential is colored blue and the negative potential, red. The gray surface represents hydrophobic region. The carbon atoms are colored gray and the oxygen atoms, red. Panels B and D show the positions of interacting amino acidresidues of PfATP6 with Zinc13781298 and curcumin, respectively. The hydrophobic residue are colored green, polar residues are colored cyan and positive residues are colored blue. The hydrogen bond is shown by the red arrow.

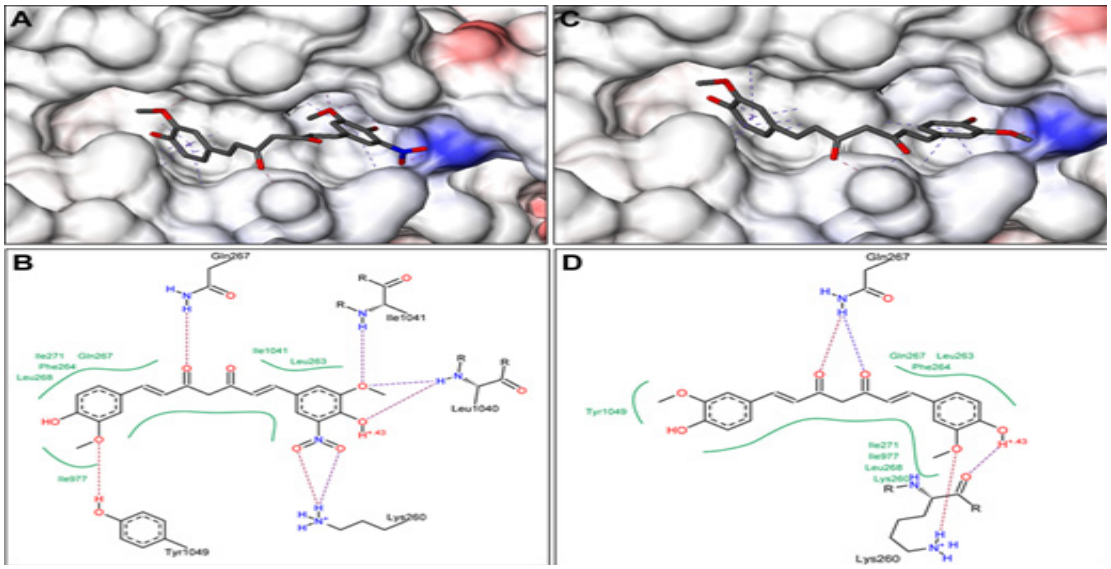

Figure 2. Docked poses of Zinc13781298 and curcumin in the modeled structure of PfATP6 – Panels A and B show the docking of Zinc49111530 and panels C and D show the docking of curcumin. The surface representation corresponds to the electrostatic potential of PfATP6. The carbon atoms are colored gray and the oxygen atoms, red. The positive potential corresponds to blue and the negative potential, to red. The gray surface represents hydrophobic region. Dotted blue lines represent the hydrophobic interactions. Panels B and D show the positions of interacting residues of PfATP6 with Zinc49111530 and curcumin, respectively. The hydrophobic interactions are shown by green lines and the hydrogen bonds are shown with dotted lines.

hydrophobic interactions between aromatic residues protein and phenolic rings of curcumin. In addition, the pocket contains polar residues, which can form H-bond with polar groups of curcumin and its analogues.

Binding affinity of curcumin and its analogues

The Glide and FlexX scores for six compounds curcumin-related compounds together with the same scores of curcumin are collected in Table 1. The Glide docking predicts that the Zinc database compound Zinc13781298 has best binding probability, whereas FlexX docking predicts that Zinc49111530 has the best binding possibility with PfATP6. Both compounds have better binding probability than curcumin (diketo form).

The comparison of docking poses of Zinc13781298, the compound with best Glide score and curcumin is shown in Figure 1. Panels A and B show the docking of Zinc13781298 in PfATP6 and panels C and D show the docked pose of curcumin.

It is clear from figures 1A and 1B that both compounds bind in the same cavity in the structure of PfATP6. Both compounds interact with the same amino acid residues. The hydrophobic interactions (represented by green colored residues) dominate polar interaction (represented by cyan and red colors). The reason for better binding of Zinc13781298 appears due to O-CH_3 group on both phenolic rings, which is occupied by a hydroxyl (OH) group in curcumin. It is well known that the methyl group (-CH3) has hydrophobic nature and hydroxyl (OH) group is polar in nature. It is also worth mentioning here that the two compounds differ only in the presence of O-CH_3 group in Zinc13781298 and OH group in curcumin.

The docking poses of the compound with best FlexX score and its interaction with PfATP6 amino acids is shown in Figs. 2A and 2B. The docking pose of curcumin and the details of interaction with PfATP6 are shown in Figures 2C and D. Both polar and hydrophobic interactions mediate the binding of compounds with protein. It is clear from the Fig. 2 that Zinc49111530 has more interactions with protein compared to curcumin and hence binds with better score.

One curcumin analogue Zinc05606394 was predicted to bind better than curcumin by both Glide and FlexX docking program. The structure of Zinc05606394 together with the structure of curcumin is shown in Figure 3. At this point, it is not clear why Zinc05606394 has better affinity for binding to PfATP6 than natural compound. A more detailed analysis is currently underway and the results will be reported in future. In summary, our docking results provide a basis for synthesis and *in vivo* testing of curcumin analogues for the development of antimalarial compounds.

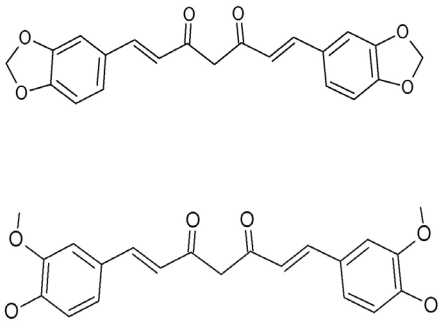

Figure 3. Chemical structures of Zinc05606394 and curcumin.

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Other articles in this theme issue include references (19-46).

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