

In-silico Antimalarial Study of Monocarbonyl Curcumin Analogs and Their 2,4-Dinitro Phenylhydrazones Using the Inhibition of Plasmepsin II as Test Model

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Abstract A well-known component of the Indian spice turmeric, curcumin, has received a lot of attention in recent years as a potential antimalarial agent but the inherent problems associated with low bioavailability tends to limit its applicability. The bioavailability is linked to its low solubility in water and its rapid break down in the blood plasma. In this study, we have proposed the use of synthetic analogs of curcumin and their derivatives which are expected to be less prone to degradation in the blood plasma as possible antimalarials. The binding affinity of monocarbonyl analogs of curcumin and their 2,4-dinitrophenylhydrazone derivatives for the chain A domain of plasmepsin II, one of the key enzymes involved in hemoglobin digestion in the food vacuole of the malaria parasite was determined by computational docking analysis, performed using Auto Dock Vina 1.1.2, pymol and Chem 3D ultra 12.0. The binding energies of the 20 compounds studied was compared with that of pepstatin A (a known inhibitor of plasmepsin II), curcumin and chloroquine. The 3D structure of the protein was obtained from the protein data bank (PDB ID:1M43), the compounds' 3D structure was generated with the Chem 3D ultra 12.0 and visualization done with pymol. Out of the 20 compounds docked with plasmepsin II, 17 had binding energies higher than that of pep A (-32.6, kJ/mol) and 19 of the compounds had binding energies higher than that of curcumin (30.96, kJ/mol). The docked compounds, 5b, 6b and 7b had the highest binding energies (-44.73 kJ/mol, -42.64 kJ/mol and -41.80 kJ/mol respectively). It is expected that the compounds with binding energies higher than that of pep A may be considered for further antimalarial studies *in-vitro* and *in-vivo*.

Keywords: monocarbonyl curcumin analog, 2,4-dinitrophenyl hydrazone, low bioavailability, plasmepsin II, pepstatin A

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1. Introduction

Resistance to the most widely used antimalarial drug, chloroquine, has increased continuously in recent years; it is now estimated that failure rates for chloroquine are as high as 50-60 % in East and Central Africa. Failure rates for treatment with other prevalent drugs, sulfadoxine-pyremethamine and mefloquine, are also increasing [1]. The malaria causing organism, plasmodium's ability to adapt in the face of selective pressure brought on by drug treatment has thus helped establish an environment in which novel drugs against additional targets are in constant need. The continued search for new molecular targets for drug design is expected to broaden the therapeutic arsenal and strategies to fight drug resistance in human malaria. Curcuminoids have been reported to have a prominent antimalarial activity both *in-vitro* (against chloroquine resistant and

sensitive plasmodium falciparum laboratory strains) and *in-vivo* (against plasmodium berghei) studies [2,3,4,5]. In 2005, it was first observed that curcumin has antimalarial activity [5], Nandakumar and co-researchers [4], also discovered in 2006 that curcumin has a synergistic effect when used in combination with artemisinin, specifically, the combination was effective in preventing parasite recrudescence. In a study involving plasmodium berghei. It was also observed that curcumin was a potent inhibitor of chloroquine resistant P. falciparum. Curcumin itself has low bioavailability especially when administered orally which is an intrinsic problem of the curcumin that may prevent it from becoming a first line antimalarial drug. This low bioavailability is attributed to its high rate of metabolism and its rapid elimination [6,7]. Different attempts have been made using different models such as encapsulations in nanoparticles [8,9] nanoemulsions [10,11], cyclodextrins [12], phospholipid complexes [13] or solid dispersions [14] all in a bid to improve the oral bioavailability and therapeutic efficacy of curcumin [15].

However, these strategies are characterized by several weaknesses, such as high costs, cytotoxicity, limited drug loading and entrapment efficiency, poor scale-up and the use of organic solvents. The bioavailability may be increased as a result of cooking or dissolution in oil [16]. Recently, self-emulsifying lipid-based systems were investigated as agents to solubilize the hydrophobic curcumin in the gastro-intestinal tract and to protect curcumin from enzymatic and/or chemical hydrolysis in order to improve the oral bioavailability of curcumin [17,18,19]. One of the factors that contribute to its low bioavailability is its rapid breakdown in the blood plasma via a retro-aldol reaction of the 1,3-dicarbonyl moiety of the molecule. A viable solution to the inherent problem of bioavailability of curcumin as an antimalarial agent which has not been explored yet is synthetic modification. Analogs of curcumin which will not breakdown readily in the blood plasma may be synthesized and screened for possible antimalarial activity. Monocarbonyl curcumins (synthetic analogs of curcumin) have been proposed to be studied in this work. The monocarbonyl curcumins are devoid of the CH₂CO group that is implicated in the retro-aldol breakdown of curcumin in the blood plasma (Figure 1).

It is thought that if the analogs of curcumin are more bioavailable and have considerable antimalarial activity then they will be better candidates for future development as antimalarial agents. Hemoglobin (Hb) digestion occurs within a highly specialized Plasmodium compartment referred to as the food vacuole (FV), which presents an acidic pH (5.0-5.4) and contains proteases responsible for Hb digestion [20]. These proteases include four aspartic proteases (plasmepsins) [21,22,23], three cysteine proteases (falcipains) [24,25,26,27], a metalloprotease (falcilysin) [28] and a dipeptidyl aminopeptidase 1 (DAPP1) [29]. The early events of hemoglobin degradation in the FV involve two closely related aspartic proteases from *P. falciparum*. Both proteases, termed plasmepsin (plm) I and II, hydrolyze the peptide bond between residues Phe33 and Leu34 in the α -chain of native hemoglobin, thus initiating hemoglobin degradation [30,31,32]. This initial cleavage most likely promotes protein unfolding and release of the heme moiety followed by further degradation by the plms, the cysteine protease falcipain, and the metalloprotease falcilysin [33,34]. It has also been established that [35], plm II is capable of degrading erythrocyte skeleton components spectrin, actin, and protein 4.1 at neutral pH and used specific antibodies to localize plm II to the periphery of the parasite during the merozoite stage, implying that plm II plays a role in mature merozoite escape from the erythrocyte host by cytoskeletal degradation [36]. Plasmepsin II (plm II) is the most extensively characterized of these enzymes since several crystal structures have been determined [37,38,39] and potent inhibitors developed [40,41,42,43,44]. Silva et al. [37] determined the first crystal structure of the plm family showing recombinant plm II complexed with pepstatin A, a known aspartic protease inhibitor whose inhibition constant for plm II is 0.006 nM. The structure of plm II has the typical bilobal shape and topology of eukaryotic aspartic proteases. Two topologically similar N- and C-terminal domains contact each other along the bottom of the binding cleft that contains the catalytic dyad

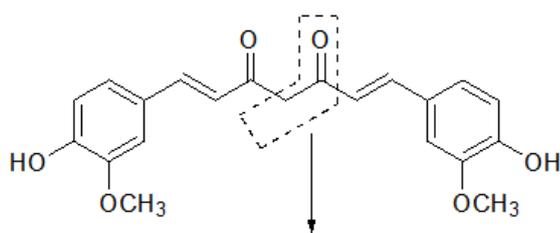
(Asp34 and Asp214). The presence of two plm II molecules in the crystallographic asymmetric unit suggested a high degree of interdomain flexibility of plm II, which was later confirmed by the resolution of different enzyme-inhibitor complexes. Many experimental peptidic and nonpeptidic plm inhibitors exist and have been described in detail, along with their K_i values and often the steps of their synthesis, in a number of papers [37], [45-66]. Some of these inhibitors were identified by high-throughput screening [61,67,68,69,70], by computational analysis of the plm's active site [56,62], or in natural products [57,71,72], though most are the result of rational drug design.

2. Materials and Methods

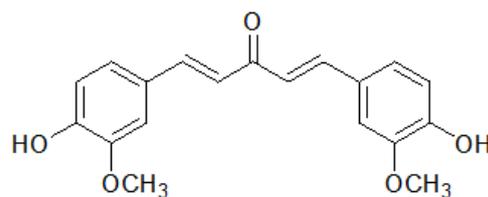
A set of 10 monocarbonyl curcumins along with their dinitrophenylhydrazone derivatives were docked with plm II in this study using autodock vina to estimate the binding energies of the compounds with plmII. The results were compared with that of curcumin docked with plm II under the same conditions. The monocarbonyl curcumins are bis benzylidene propanones thought to be easily synthesized from a Claisen-Schmidt condensation of 1 mole of acetone with two molar equivalents of an aromatic aldehyde (Figure 2). The dinitro phenylhydrazones are derivatives of the monocarbonyl curcumins analogs that are supposedly synthesized from the condensation of the monocarbonyl curcumins with 2,4-dinitrophenylhydrazine (Figure 3). The 3D structures of the compounds were prepared using chem3D and their energies were minimized with MM2 Force Field of Chem3D application interface and the ligands were saved as pdb files. MGL Tools 1.5.4 was used to prepare the ligands' pdbqt files, setting the number of rotatable bonds to maximum, since most of the mono carbonyl used in this study have fewer than eight rotatable bonds. 3D crystallographic structure of the plasmepsin II cocrystallized with pepstatin A – was obtained from the protein data bank (PDB ID: 1M43). PyMol was used to remove one of the dimeric chains and the pepstatin A molecule bound to it. Water molecules were also removed. The polypeptide chain was processed in MGL Tools 1.5.4 to obtain the grid.pdbqt files. The grid box of the macromolecule was obtained based on the binding site of plm II to the inhibitor, pepstatin A, in the downloaded PDB file. The grid box dimension in Armstrong units is (x, y, z) = (14, 20, 16) and centred at coordinates (x, y, z) = (55, 62, 26) The dockings were performed using Auto Dock Vina 1.1.2 which is a new generation of docking software from the Molecular Graphics Lab [73].

3. Results

In order to check the accuracy of the Vina docking program, we have docked the plmII cocrystallized ligand, pepstatinA (pepA) into the plmII binding site. *The docking results were satisfactory when we analysed the root mean square deviation values (RMSd), which showed lower values (i.e RMSd < 3Å), despite the different binding modes.*



Group present in curcumin that is absent in the monocarbonyl curcumin analogs



A monocarbonyl curcumin devoid of the CH_2CO group that is part of the curcumin's 1,3-dicarbonyl group implicated in the retro-aldol breakdown of curcumin in the blood plasma

Figure 1. Comparison of curcumin with its monocarbonyl curcumin analog

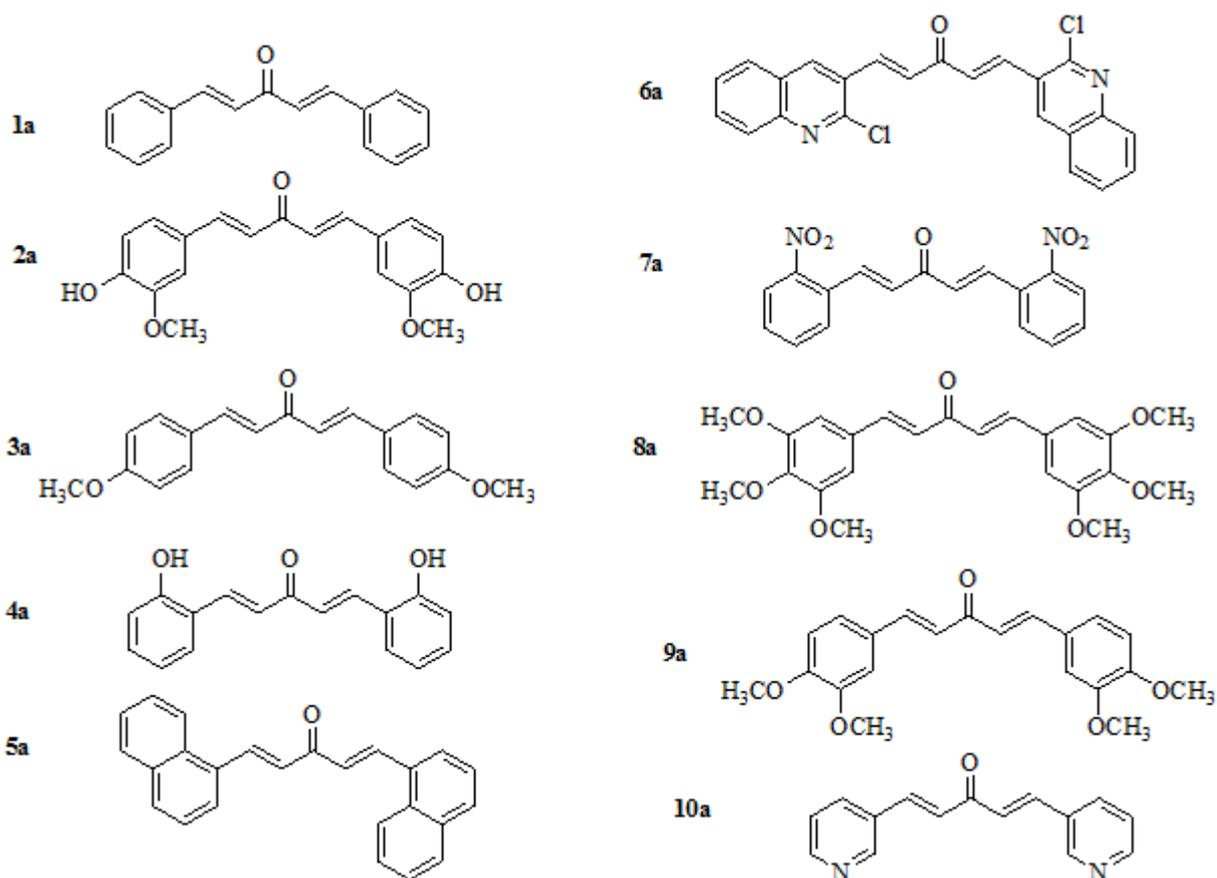


Figure 2. The structures of the monocarbonyl curcumin analogs of curcumin docked with plasmepsin II

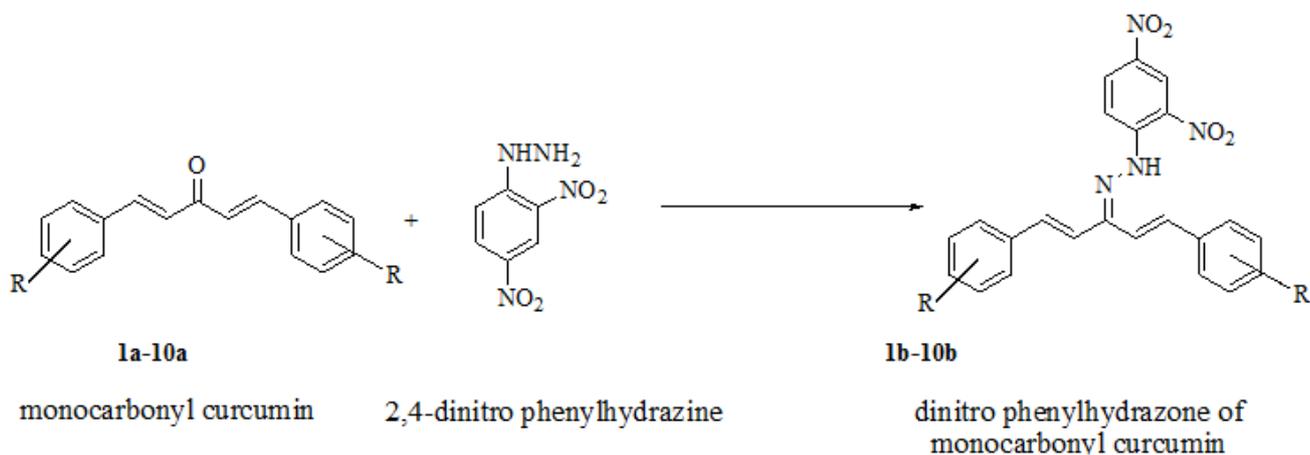


Figure 3. The condensation of the monocarbonyl curcumins with 2,4-dinitro phenylhydrazine to generate the corresponding 2,4-dinitro phenylhydrazones of the monocarbonyl curcumin analogs

Table 1. Autodock Vina Score of Ligands with PlmII, The Number of Hydrogen Bond Interactions and Amino Acid Residues Interacting in The Binding Site Via H-Bonding.

Compounds	Vina Score (kJmol^{-1})	Number of H-bonds	Residues of the PlmII binding site interacting with the ligands
Chloroquine	-28.42	2	Ser79, Asp214
Pepstatin A	-32.66	7	Ser79, Ser118, Tyr192, Gly216
Curcumin	-30.96	3	Ser218, Ala219
1a	-33.02	0	-
1b	-39.29	4	Ser79, Thr217, Ser218, Thr221
2a	-32.60	2	Ser79, Leu131
2b	-36.78	4	Ser79, Tyr192, Asp214, Thr221
3a	-32.19	0	-
3b	-37.62	4	Ser79, Ser218, Thr221
4a	-32.60	0	-
4b	-39.29	6	Tyr192, Asp214, Thr217, Ser218
5a	-39.71	0	-
5b	-44.73	3	Tyr192, Asp214, Thr217
6a	-36.78	0	-
6b	-42.64	4	Ser79, Ser118, Tyr192, Gly216
7a	-33.86	5	Ser79, Ser218, Ala219
7b	-41.80	7	Asn76, Ser79, Thr217, Ser218
8a	-30.10	2	Ser118, Thr217
8b	-32.60	5	Ser79, Tyr192, Asp214, Ser218, Thr221
9a	-30.51	2	Ser79, Tyr192
9b	-35.11	3	Ala38, Ser79, Tyr192
10a	-29.76	0	-
10b	-37.20	3	Ser79, Gly 216, Thr221

The results of docking studies using the AutoDock Vina scores observed for plmII and the 20 curcuminoids and their derivatives are summarized in Table 1. The output binding energy is in kcal/mol which has been converted to kJ/mol. Table 1 shows the number of hydrogen bond interactions between the ligand of the highest pose scores and the amino acids residues in the binding cavity, with residues Ser79, Tyr192, Asp214, and Ser218 appearing consistently in the conformational poses of protein-ligand complex. In all the docking runs, the DNP derivatives showed higher binding energies compared to the monocarbonyl curcumin counterparts (Figure 3).

The DNP derivatives are much larger than their parent monocarbonyl curcumin analogs, hence, it is expected that they possess a higher magnitude of van der Waal contribution to the binding energies. These derivatives also possess extra polar groups (-NO₂ groups) that augment the H-bond interactions in the binding site.

4. Discussion

Amongst the ligand with the highest binding affinities, **5b**, **6b** and **7b** appears to show the highest interaction energies. The set of compounds with the highest binding energies are **5b** (-44.73 kJ/mol), **6b** (-42.64 kJ/mol), **7b** (-41.80 kJ/mol), **5a** (-39.71 kJ/mol), **4b** & **1b** (-39.29 kJ/mol). Compounds **5b**, **6b**, **7b** and **10b** are the largest molecules in the compound set used in this study, hence, it is expected that since they fit into the binding pocket, they will elicit the largest hydrophobic interactions, which results to a substantial increasing in their binding energies. These compounds also form H-bond interactions with the residues Ser79, Tyr192, Asp214 and Ser218 in the binding pocket. Figure 4 shows the binding interaction of the compounds **5b** and **6b** in the plmII. It is notable that compound **6b** has H-bonds with exactly the same amino

acid residues as pepstatin A, Ser79, Ser118, Tyr192, Gly216.

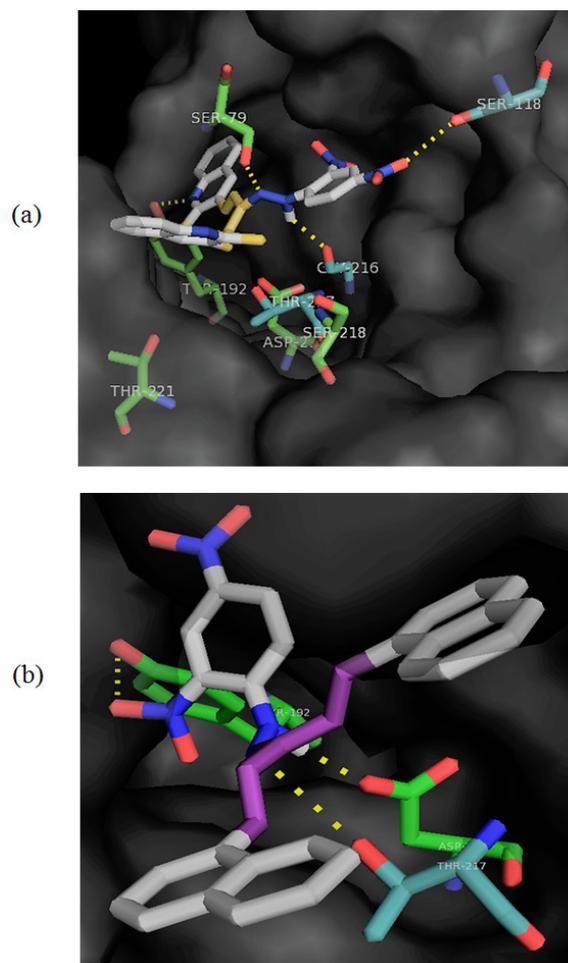


Figure 4. The structures of compounds **6b** (a) and **5b** (b) docked with plasmepsin II showing the amino acid residues interacting by H-bonding

5. Conclusion

Most of the compounds investigated in this study have a higher binding affinity compared to that of pepA for the plm II except compounds **2a**, **3a** and **4a** which are slightly less but comparable. The test compounds having a higher binding affinity gives insight into the extent to which the compounds might inhibit the enzyme plmII and therefore disrupt its metabolic function within the parasite and ultimately culminate in antimalarial activity. Most of the compounds docked with plmII have better binding profiles than curcumin and the high binding energies of the docked compounds reveal that these set of compounds include some that have high binding affinity for the plmII and interact by H-bonding with similar amino acid residues as pepA. These compounds may be good candidates for further exploration as antimalarial-plmII inhibitors. Of particular interest are the compounds **7b** (-41.8 kJ/mol, 7 H-bonds) that has as many H-bonding interactions as pepstatin A (-32 kJ/mol, 7 H-bonds) and much higher binding energy compared to that of pepA, compound **6b** (-42.64 kJ/mol) that interacts by H-bonding with the same residues as pepA but having a higher binding energy relative to that of pepA and compound **5b** that has the highest binding energy (-44.73 kJ/mol). If the monocarbonyl curcumin analogs and their derivatives have good oral absorption and are stable in the blood plasma, with the high binding affinity observed for some of these compounds for one of the key metabolic enzymes of the malaria parasite, then these class of compounds may have potential as lead compounds for the discovery of a new antimalarial drug.

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