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Integrated ligand and structure based studies of flavonoids as fatty acid biosynthesis inhibitors of *Plasmodium falciparum*

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ABSTRACT

A common feature pharmacophore with two hydrogen-bond acceptor and one aromatic hydrophobic feature has been generated using seven active flavonoids. Docking studies of these compounds well corroborates with the pharmacophore model. Therefore models could be useful for identification of potential novel FAS-II inhibitors.

Despite of the serious global effort to combat malaria, there were estimated 247 million malaria cases were reported in 2006, causing nearly a million deaths and among these, most of the cases were children under 5 years.[1] According to a published report, 109 countries were found endemic for malaria in 2008[1] and even in 2009, it is responsible for a child death in every 30 s.[2,3] The chemotherapeutic cure of malaria is becoming progressively more challenging with the fast development of resistance of the *Plasmodium falciparum* malaria parasites to many of the standard amine antimalarial drugs like chloroquine[4] and warrants the discovery of either new drugs or new analogues of existing drugs.

Novel metabolic pathways specific to *Plasmodium* and distinct from those of its human host can prove to be a good target for the development of novel antimalarials. In this context FAS-II inhibitors have been proved to be quite efficient due to the fact that an individual molecule can inhibit three enzymes in the same pathway thereby increasing the efficacy as well as decreasing the risk of resistance development.[5]

In view of above and considering the importance of flavonoid and its analogues as potent class of antimalarials, it appeared of interest to develop a pharmacophore model for finding out the essential structural requirements in this class of molecules for their antimalarial activity. In addition, interactions between *P. falciparum* enoyl-ACP-reductase (FabI) protein and the ligands used for pharmacophore model generation were also analyzed through molecular docking.

The common feature pharmacophore model was generated using seven most active molecules from the literature[6] (Fig. 1) using CATALYST common feature pharmacophore/HipHop algorithm.[7] Antimalarial activity of these compounds have been listed in Table 1.

The program CATALYST uses two different approaches for the generation of pharmacophore. The first method is usually applied to develop three-dimensional pharmacophore models, starting from a collection of molecules possessing a range of diversity both in structure and activity with the last spanning at least four orders of magnitude. The second method, called common feature pharmacophore or HipHop[8] is able to generate pharmacophore models only by identification of the common chemical features shared by the molecules and their relative alignment to the common feature set, without considering biological data. The structural analogy between the studied compounds, combined with their small activity range of only two orders of magnitude (3.2–141.1 μM), led to the conclusion that the common feature hypothesis generation was the most suitable approach that can be applied to this class of molecules. In fact, although the biological activity of this class of compounds represents a good result from their medicinal point of view, the activity range seems to be insufficient to develop a statistically significant quantitative model able to correlate the structural features of these compounds with their biological data. Therefore, the first approach for the pharmacophore generation was not considered suitable for this reason.

All compounds were built using ISIS Draw 2.5, imported to CATALYST window and optimized using CHARMm force field. A maximum of 255 conformations were generated within an energy threshold of 20.0 kcal/mol above the global energy minimum for

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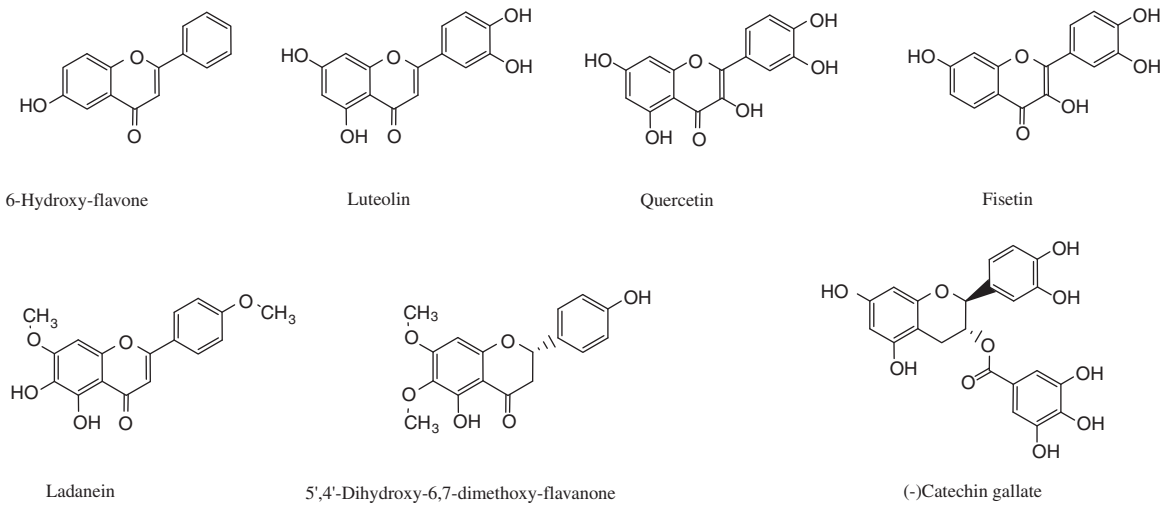

Figure 1. Structures of the molecules used in the HipHop generation.

Table 1
In vitro activity of flavonoids against FabI inhibition and growth inhibition of chloroquine-sensitive (NF-54) *P. falciparum* strains

Compound name	FabI IC_{50} (μM)	*P.f.* NF-54 IC_{50} (μM)
6-Hydroxy-flavone	na	5.3
Luteolin	2	10.7
Quercetin	1.5	10
Fisetin	1	8.2
Ladanein	na	9
5′,4′-Dihydroxy-6,7-dimethoxy-flavanone	na	8.8
(−)-Catechin gallate	0.3	3.2

na means that the compound was not active when measured at a concentration of 100 μM.

Table 2
The characteristics of generated hypotheses

Hypothesis	Features	Rank score	Direct hit	Partial hit	Max fit
Hypo-1	HYAr, HBA, HBA	56.011	1111111	000000	3
Hypo-2	HYAr, HBA, HBA	55.541	1111111	000000	3
Hypo-3	HYAr, HBA, HBA	54.289	1111111	000000	3
Hypo-4	HYAr, HBA, HBA	54.271	1111111	000000	3
Hypo-5	HYAr, HBA, HBA	53.808	1111111	000000	3
Hypo-6	HYAr, HBA, HBA	53.525	1111111	000000	3
Hypo-7	HYAr, HBA, HBA	53.187	1111111	000000	3
Hypo-8	HYAr, HBA, HBA	53.16	1111111	000000	3
Hypo-9	HYAr, HBA, HBA	51.628	1111111	000000	3
Hypo-10	HYAr, HBA, HBA	51.014	1111111	000000	3

HYAr = Hydrophobic Aromatic, HBA = H-bond acceptor, Direct hit = all the features are mapped. Direct hit = 1 means yes; Partial hit = partial mapping of the hypothesis. Partial hit = 0 means no.

all ligands within the Catalyst ConFirm using the 'Poling' algorithm.[9] Seven most active compounds of the dataset molecules associated with their conformations were submitted to the CATALYST hypothesis generation (HipHop). All conformers were searched for three-dimensional arrangements of chemical features (hydrophobic region, H-bond acceptor) common to all molecules of the training set.[10] Pharmacophore generation was carried out by setting the default parameters in the common feature pharmacophore generation procedure in CATALYST.

The common feature pharmacophore generation protocol produced 10 hypotheses. The characteristics of 10 hypotheses are listed in Table 2.

Since the top 10 HipHop models contained the same feature (i.e., one HYAr, two HBAL), first hypothesis (Hypo-1) seemed to be the most predictive model with highest rank score. Features of the HipHop model viz. one Hydrophobic Aromatic (HYAr) and two H-bond acceptor (HBA) feature were found to be important for effective FAS-II antagonism (Fig. 2A). From the mapping experiment it has been observed that all the active compounds considered for HipHop generation mapped well to the Hypo-1 indicating that the model provides reasonable pharmacophoric characteristics of the FAS II inhibitors (Fig. 2B).

The docking studies were performed using GOLD version 3.1.[11] Among all conformations of the ligands used in QSAR study the conformation of the pharmacophore based alignment was submitted to the docking studies. Since the inhibitory activity (IC_{50} value) of *P. falciparum* enoyl-ACP-reductase (FabI) showed the same trend as with the antimalarial activity of the chloroquine sensitive NF54 strain (Table 1), the crystal structure of *P. falciparum* FabI protein (PDB ID: 2OOS) was selected for the docking studies while the co-crystallized ligand was used for the selection of active site. Protein was prepared by protein preparation wizard available in Schrodinger[12] package. All ligands used in the pharmacophore generation were docked at the active site of the protein.

The docking studies at the FabI active site with the most active molecule (viz. catechin gallate) of the dataset clearly showed (Fig. 3A and B) that the hydroxy groups present at position 7 of the chroman moiety and at position 4′ of the trihydroxybenzoate group involved in H-bonding with the isoleucine (Ile-369), phenylalanine (Phe-368) and alanine (Ala-217) residue, respectively. The later was also involved in H-bonding with water molecule. At the same time the phenyl group at position 2 of the chroman moiety provided hydrophobic feature which occupy the hydrophobic pocket in the receptor. These findings well corroborates with the Hypo-1 where the importance of hydrophobic functionality at the active site has been described by HYAr feature while H-bond

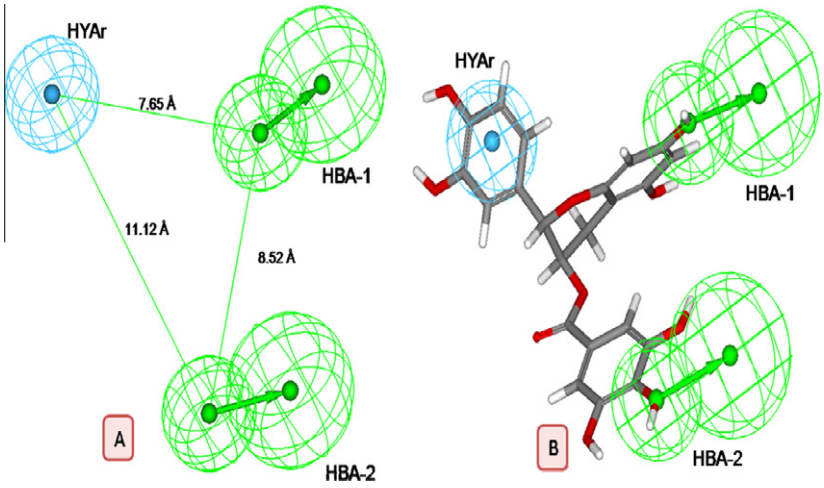

Figure 2. (A) Pharmacophore model along with their inter-feature distance. (B) Pharmacophore mapping of the most active molecule of the series (catechin gallate). HBAs and HYAr are coloured green and cyan, respectively.

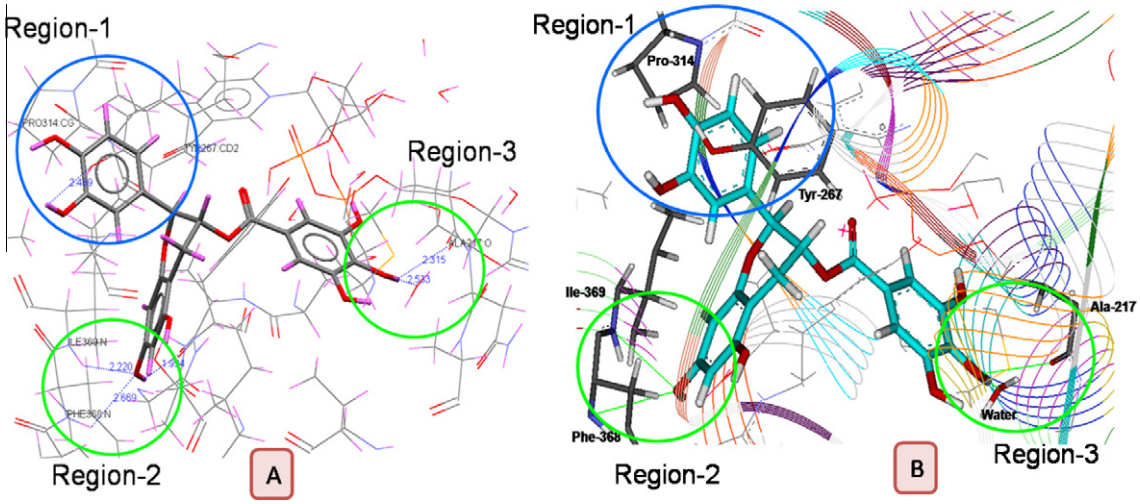

Figure 3. Docked pose of the (−)-catechin gallate molecule along with the interaction at the active site of the FabI protein as visualized in (A) GOLD and (B) CATALYST window. Region-1 corresponds to aromatic hydrophobic feature of the pharmacophore while Region-2 and Region-3 corresponds to the HBA-1 and HBA-2 feature of the pharmacophore.

interactions at the binding site has been well described by two H-bond acceptor feature of the pharmacophore.

In summary, a common feature pharmacophore has been generated using seven most active flavonoids consisted of two hydrogen-bond acceptor and one aromatic hydrophobic feature. Since the FabI inhibitory activity well explain the antimalarial activity of these compounds, these active flavonoids were further docked at the active site of the FabI protein where the docking study well corroborates with the pharmacophore model. Therefore this model may useful in finding new scaffolds that may aid in design and develop New Chemical Entities (NCEs) as potent FAS-II inhibitors for malarial chemotherapy.

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