ISSN 2249-622X

Asian Journal of Biomedical and Pharmaceutical Sciences 1 (6) 2012, 01-05

RESEARCH ARTICLE

Virtual Screening of Xanthones in Combating Malaria Targeting Plasmodium Falciparum Erythrocyte Membrane Protein 1(PfEMP1)

Saumya K. Patel[1*], Yogesh T. Jasrai[2], Linz-Buoy George[1*], Hyacinth N. Highland[1]

[1]*Department of Zoology, Univrsity Scool of Sciences, Gujarat University, Ahmedabad -380009, Gujarat, India.*

[2]*Department of Botany, Univrsity Scool of Sciences, Gujarat University, Ahmedabad -380009, Gujarat, India*

.

ABSTRACT

Malaria is the most important parasitic disease in humans, with transmission occurring in over 100 countries with a population of three billion people. It is caused by protozoan parasites of the genus Plasmodium. These parasites are transmitted from one person to another by the female anopheles mosquito. The Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) family plays a central role in antigenic variation and cytoadhesion of P. falciparum infected erythrocytes. In the present study, investigation of Xanthones as probable anti malarial molecules, was carried out targeted against Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) via molecular docking studies. The *in silico* effectiveness of Xanthones was studied based upon the interaction with the protein's active site residues with less binding energy. The interacting Xanthones were further filtered to predict the bioavailability and drug likeness properties. 3, 6-dihydroxyxanthone was shown to be a better interacting ligand with low binding energy (-66.16 kcal/mol) and passed all the physicochemical parameters for drug likeness. This work encourages the development of Xanthones with some chemical modifications to augment more efficacy and better activity as anti malarial drugs.

KEY WORDS: Anti malarial drugs, PfEMP1, Xanthones, Molecular docking, Bioavailability analysis

INTRODUCTION

Malaria is a disease of enormous importance by any standard of measure. Billions of people live in the regions where, according to recent figures from the World Health Organization, malaria causes 100 million clinical episodes and over 1 million deaths per year [1] the malaria parasite depends on both humans and mosquitoes to carry out its deadly cycle of life. These parasites are transmitted from one person to another by the female anopheles mosquito. Plasmodium develops in the gut of the mosquito and is passed on in the saliva of an infected insect each time it takes a new blood meal. When an infected mosquito bites a human, the parasite rapidly goes to the liver within 30 minutes. There the parasite starts reproducing rapidly in the liver. The parasites then enter into red blood cells and reproduce there after bursting, the parasites releases out and spread in the host's blood. It is injected by another mosquito and the life cycle continues. The increasing resistance of malaria parasites, in particular Plasmodium falciparum, to antimalarial drugs is a key factor in the persistence of this disease as a major worldwide public health threat. Various potential biochemical targets have been proposed and are being pursued for the de novo design of novel antimalarials [2] Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is a clonally variant adhesion protein that mediates binding of infected erythrocytes (IE) to blood microvasculature and other host cells [3] Adherence of IEs to microvascular endothelium is a major virulence factor and, in conjunction with the related phenomenon of rosetting with uninfected erythrocytes, prevents parasitized erythrocyte circulation to the spleen where parasites may be destroyed [4] The antimalarial potency of the xanthones correlated well with their ability to inhibit in vitro heme polymerization, suggesting that these compounds exert their antimalarial action by preventing hemozoin formation [5] In the present study, docking simulation was performed using Molegro software [6] with PfEMP1 as the target and computationally Xanthon compounds were docked into the receptor's binding site. Subsequently, the compounds were screened with ADME/T (absorption, distribution, metabolism, excretion and toxicity) filtering protocol to evaluate their drug likeness.

METHODOLOGY:

Docking studies and in silico bioavailability analysis were performed for 5 Xanthone molecules marine compounds with Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1).

[*]***Corresponding author:*** Saumya K. Patel ***/ Email:*** saumya50@yahoo.co.in

PREPARATION OF PROTEIN TARGET STRUCTURE AND LIGANDS:

The X-ray crystal structure of Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) was retrieved from the Protein Data Bank [8] The dataset comprised of 18 ligands, out of which 5 inhibitors having known inhibitory activity were taken from pubchem and chembase databases [9, 10] The ligand structures were subjected to energy minimization using Hyperchem 8.0.7(without reaction field) and the energy was minimized to Kcal/mol [11] Subsequently, the ligands were geometrically optimized using 3D cleaning utility.

DOCKING STUDIES:

The Docking simulations were performed by Molegro Software in which we considered Xanthones as ligand against PfEMP1 as target protein. Ligand dataset under study were docked separately into the binding site of the receptor using Molegro. The binding site was constructed which consist of all residues that have at least one atom within 3.5 Å from any atom in the co-crystallized inhibitor. This generally gives a good representation of the important residues in the binding pocket for a protein target.[7] To determine the optimal geometry of the ligand binding mode is done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the targets. The Highest Scoring solutions (best poses of low-energy) are returned for further analysis.

***IN SILICO* BIOAVAILABILITY ANALYSIS:**

Bioavailability analysis is based upon the prediction of various physicochemical properties proposed by Lipinski's Rule of Five (RO5) [12] and Ghose et al, 1999 [13] Lipophilicity, quantified as WlogP was analyzed using weighted approach with the help of logP plugins of Marvin Sketch. Topological Polar Surface Area (TPSA) was calculated using Polar Surface Area plugin. ADME/T test for the ligand dataset was performed using FAF-Drugs program available at Mobyle portal [14]

RESULTS AND DISCUSSION:

The ligand dataset was virtually screened with the protein targets using Molegro software and the binding energy values were analyzed for each docked conformation. Conformations having low energy and exhibited favorable hydrogen bonding with the amino acids side chain and its amide nitrogen was considered (Table 1). Binding energies of the protein-ligand interactions are important to describe how fit the ligand binds to the target macromolecule. Docking simulations of Xanthones against PfEMP1 protein target resulted in few best compounds that were evaluated based on the binding compatibility [docked energy (kcal/mol)] with the receptor (Fig.1). Ligands such as 3, 6-dihydroxyxanthone have higher binding affinity with the cavity present in PfEMP1 possessed the better energy (-66.16 kcal/mol) value than the others (Fig. 2). From this analysis, it is evident that this compound may exhibit better interaction than other inhibitors. Besides the better interaction with the receptor, the compound should possess acceptable physical properties and chemical functionalities in order to participate in lead optimization and selection of drug discovery process. Lipinski's RO5 and Ghose et al, 1999 profiling for drug likeness were carried out for the dataset. Compounds under study had a molecular weight of less than 500 which suggested better absorption and low level of allergic reactions. Hydrogen bond donors and acceptors were less than 5 and 10. WlogP values of dataset were found to be less than 5 which predicted low level of toxicity, non-specific binding and possible oral administration [15]

Topological polar surface area for the dataset were greater than 60 Å2 and lesser than 140 Å2 indicating a high possibility of complete absorption [16] 3,6-dihydroxyxanthone had passed all the physicochemical parameters with better values (Table 2) and have the greater possibility of participation in clinical trials and may exhibit better inhibitory activity.

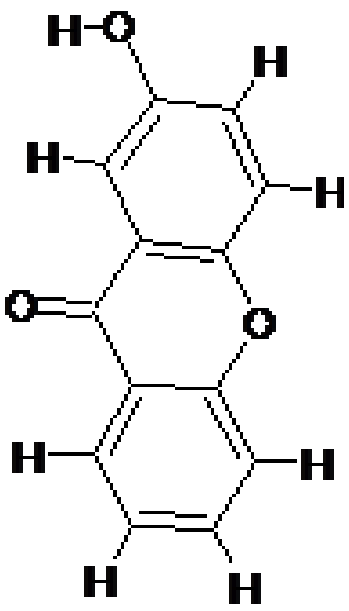
PROTEIN	LIGANDS	DOCKING ENERGY (KCAL/MOL)
Plasmodium falciparum erythrocyte membrane protein 1	2-hydroxyxanthone	-50.22
	3-hydroxyxanthone	-49.54
	3,6-dihydroxyxanthone	**-66.16**
	1,3-dihydroxyxanthone	-45.13
	2,3,4,5,6,7-hexahydroxyxanthone	-49.70

Table No.1: Docking Results of Xanthones as ligand against PfEMP1 as target.

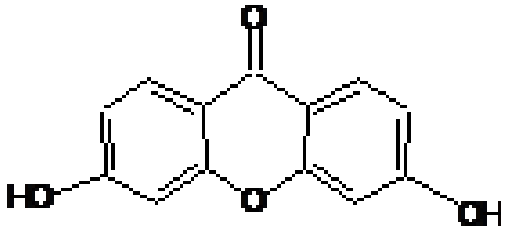
Sr. No.	LIGANDS	HD	HA	WlogP	MW	TPSA
1	2-hydroxy xanthone	1	3	2.91	212.1	46.53
2	3-hydroxy xanthone	1	3	2.91	212.1	46.53
3	3,6- dihydroxy xanthone	2	4	2.51	228.1	66.76
4	1,3-dihydroxyxanthone	2	4	2.51	228.1	66.76
5	2,3,4,5,6,7-hexahydroxyxanthone	6	8	2.17	292.1	147.6

Table No. 2: *in silico* bioavailability analysis of Xanthones

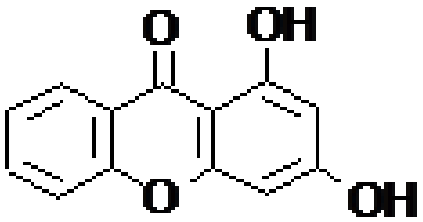
LEGENDS: HD-Hydrogen bond donor, HA- Hydrogen bond acceptor, WlogP-Weighted logP, MW-Molecular Weight and TPSA-Topological Polar Surface Area.



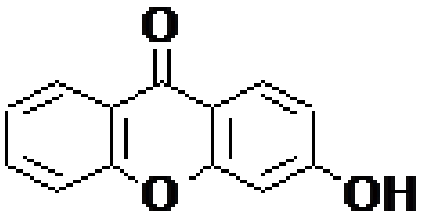
3-hydroxyxanthone



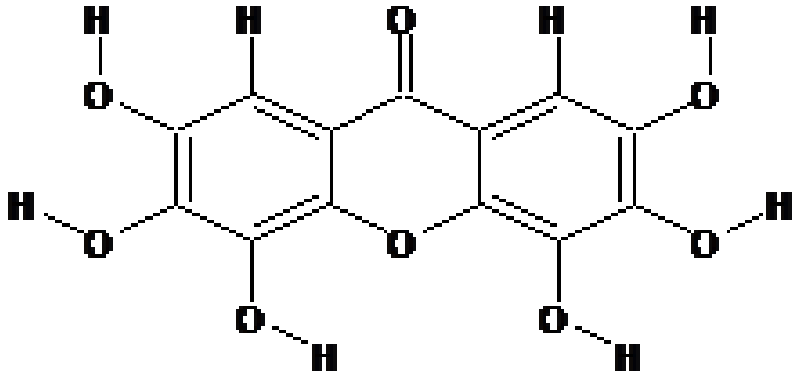
3,6dihydroxyxanthone 2-hydroxyxanthone



1,3dihydroxyxanthone



2-hydroxyxanthone



2,3,4,5,6,7-hexahydroxyxanthone

Figure No.1: Structures of Xanthones

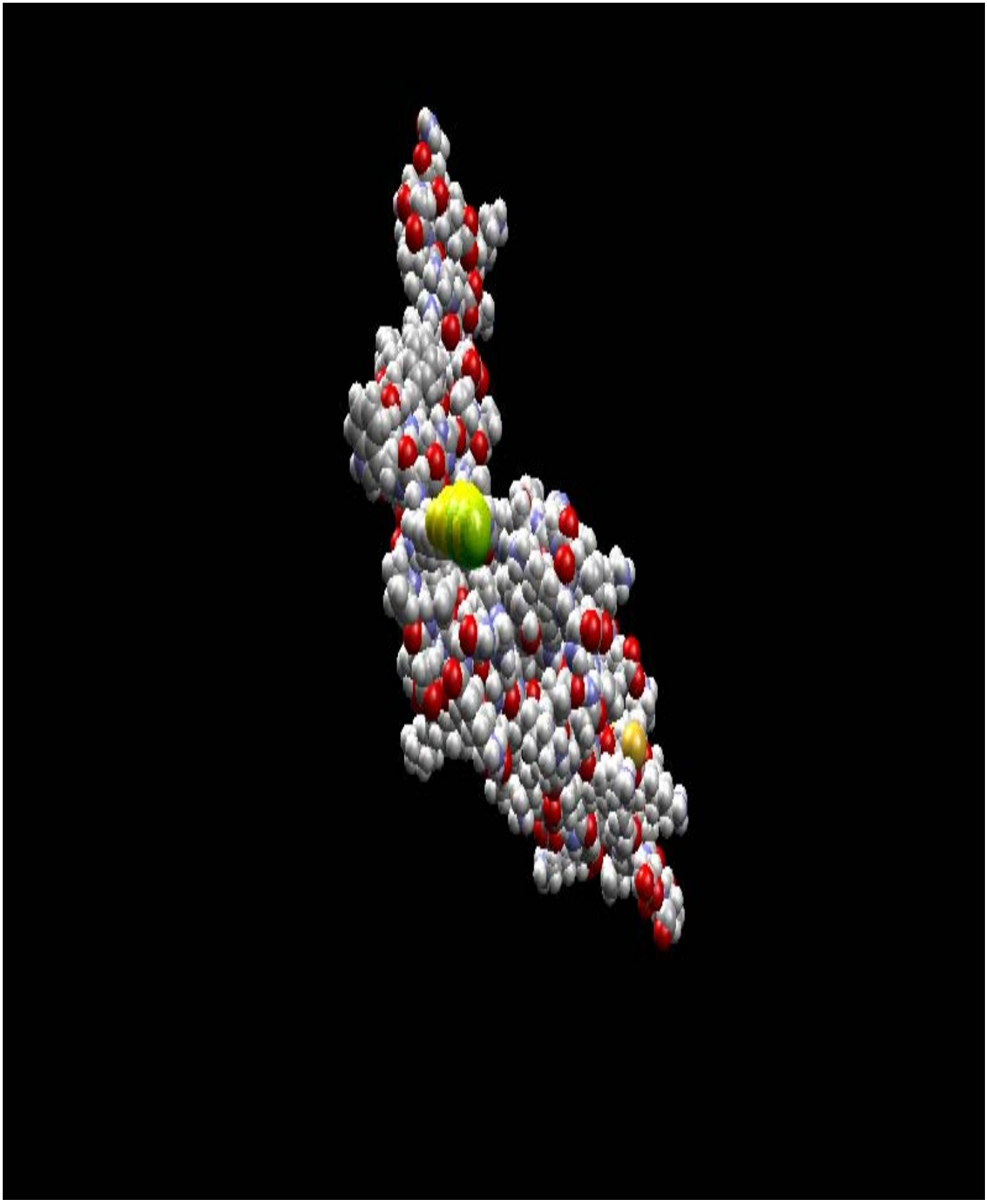


Figure No. 2: Docked conformations of ligand within the receptor active site-3,6-dihydroxyxanthone with PfEMP1

CONCLUSION:

The Protein-Ligand interactions play a significant role in structural based drug discovery/Designing. In the present work we have taken the receptor PfEMP1 and assessed Xanthone molecules for complication with it. Table1 shows the binding affinity values.In the present work, Xanthone compounds were docked with the receptors PfEMP1. The 3,6-dihydroxyxanthone established low binding energy and formed more number of H-bond interactions (-66.16 kcal/mol). Further, in silico bioavailability tests were carried out with physicochemical parameters and found that 3,6-dihydroxyxanthone had better values than known inhibitors. From these results, it is concluded that 3,6-dihydroxyxanthone could be a potential inhibitor and possessed the entire entire theoretical drug like properties. However, additional in vitro studies would help in characterizing the compounds in order to confirm the conclusions. This study also insists the importance of novel molecules showing selective interaction towards PfEMP1 will be useful strategies in malaria treatment .Keeping the above facts in consideration the experiments can be planned to understand & evaluate New Chemical Entity (NCE) from Xanthone compounds by targeting PfEMP1 by considering various pathways involving the role of PfEMP1 in malaria disease and also the assessment of the cytotoxiciy profile of Xanthones compounds which can be used as effective alternative anti malarial drug.

ACKNOWLEDGEMENTS:

The authors are thankful to Gujarat State Biotechnology Mission (GSBTM) for funding this project and special thanks to Dr. R.M. Rawal, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad for their assistance and guidance with this project.

REFERENCES:

1. World Health Organization Malaria Unit (1993) Bull. WHO 71,281-284.

2. Olliaro, P.L. and Yuthavong, Y. (1999). An overview of chemotherapeutic targets for antimalarial drug discovery. Pharm. Ther. 81: 91–110

3. Kraemer SM, Smith JD (2006) A family affair: var genes, PfEMP1 binding, and malaria disease. Curr Opin Micro 9: 374–380.

4. Miller LH, Baruch DI, Marsh K, Doumbo OK (2002) The pathogenic basis of malaria. Nature 415: 673–679.

5. Marina V. Ignatushchenkoa, R.W. Winterb,c, Hans Peter Bächinger, David J. Hinrichs b,c ,Michael K. Riscoea (1997) Xanthones as antimalarial agents ; studies of a possible mode of actionFEBS Letters 409 67-73

6. Molegro Virtual Docker, Licensed version at The Gujarat Cancer Research Institute,Asarwa, Ahmedabad

7. Patel S.K., Kumar S.P., Jasrai Y. T., Pandya H.A. and R.M. Rawal. (2011) Computational analysis of Naturally Occurring Marine Compounds (NOMC) targeting Gap Junctions and Cell Adhesive Molecules for the Identification of Anticancer Drug Targets. The Journal of Computational Intelligence in Bioinformatics, 4: 165-171

8. Protein Data Bank- RCSB, Repository of Biological Macromolecular Structures, http://www.rcsb.org

9. PubChem. Free database of chemical structures of small organic molecules and information on their biological activities. National Centre for Biotechnology Information (NCBI). www.pubchem. ncbi.nih.gov/. Last accessed on 18.11.2011

10. Chembase - Chemical Compounds Database www.chembase.com/

11. Hyperchem 8.0.7-Licensed version at The Gujarat Cancer Research Institute,Asarwa, Ahmedabad ,

12. Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J., (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug. Del. Rev. 41(1-3), pp. 3–26.

13. Ghose, A.K., Viswanadhan, V.N., and Wendoloski, J.J., (1999) A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemistry Libraries for Drug Discovery J. Combin. Chem., 1(1), pp. 55–68

14. Miteva, M.A., Violas, S., Montes, M., Gomez, D., Tuffery, P., Villoutreix, B.O., (2006) FAF-Drugs: free ADME/tox filtering of compound collections,Nucleic Acids Res.(Web Server issue), 34(1), pp. W738-44. http://bioserv.rpbs.jussieu.fr/FAFDrugs.

15. Mark Earll, (2005) A guide to Log P and pKa measurements and their use http://www.raell.demon.co.uk/chem/logp/logppka.html

16. Palm, K., Stenberg, P., Luthman, K., and Artursson, P., (1997) Polar Molecular Surface Properties Predict the Intestinal Absorption of Drug in Humans," Pharm. Res., 14(5), pp. 568-571.