INTRODUCTION

Malaria is an infectious disease. The most severe form of malaria is caused by blood borne malaria parasite *Plasmodium falciparum* as cerebral malaria. Quinine is the first natural antimalarial drug of choice for cerebral malaria action with a half period of 11-18 hrs. The development of parasite resistance to quinine has been slow. For several decades the gold standard for treatment of malaria was chloroquine (CQ), a structural derivative of quinine since 1940. However, the continuous use of CQ as monotherapy, it was reported that CQ resistance strains of *P. falciparum* have developed and rendering this drug increasing ineffective. Artemisinin is the most recent advance in the chemotherapy of malaria. However, despite being the fastest drug against all eryhrocytic stages of malaria parasite, artemisinin has a very short elimination half life (~1 hr), which precludes their use for malaria prophylaxis. So, with the emergence of multi-drug resistant (MDR) parasite the WHO has advocated a policy of Artemisinin based combination therapy (ACTs) for treating falciparum malaria. This is one of the reason that ACTs particularly combination of artemisinin with a long-lasting drug are recommended.

The rationale behind ACTs is that the chance of parasites simultaneously developing resistance as a result of genetic mutation of two drugs with different modes of action is much lower than the chance of parasites developing resistance to a single drug. When developing an ACTs, the partner drugs should ideally be structurally unrelated, most slowly eliminated *in vivo* and should target those parasite that are yet to develop resistance. A recent rational approach of antimalarial drug design is characterized as ‘‘covalent bitherapy’’. This involves linking two molecules with individual intrinsic activity into a single agent, thus packaging dual activity into a single hybrid molecule. In view of this background, dihydroartemisinin-quinine hybrid is a new class of anti-malaria drug. Experimental study revealed that the hybrid is highly effective against cultured, asynchronous, blood-stage *P. falciparum* strains 3D7 and FcB1. As both components of the hybrid have independent mechanisms of action, resistance to a drug of this type may be less likely. However, a little is known about the biological target and its antimalarial activity. So this research work is the first approach where computational methodology is applied to predict the binding modes and anti malarial activity of artemisinin-quinine hybrid and its derivatives two putative bio systems; haeme polymerisation and vacuolar plasmepsins.

OBJECTIVES

The detailed objectives of the research work compiled in the thesis are as follows:

1. To study and analyze the interaction mechanism of artemisinin-quinine hybrid and its congeners complex with Fe-Protoporphyrin-IX.
2. To estimation of binding energy (ΔG_{bind}) by the method of docking molecular mechanics based on generalized Born/surface area (MM-GBSA) solvation model.
3. To study the mode of interaction of hybrid molecule with HAP enzyme.
4. To predict the binding mode & estimate the relative binding affinity of Art-Qui-OH with respect to two known inhibitors of Histo-aspartic Protease (HAP).
5. To design an adaptive inhibitor of plasmepsins family of *P.falciparum* considering HAP as the primary structure.

RESULTS

Haemozoin is produced as an end product of haeme released during the digestion of host haemoglobin by the malaria parasite and is believed to be a detoxification pathway in the parasite. So to better understand the mechanism of interaction and antimalarial activity of Art-Qui-OH & its structural derivatives, computer-aided docking procedures were performed between the drugs and its putative receptor Fe (II)-PPIX. The XP score of the experimental structure; dihydroartemisinin-quinine hybrid compound is -7.485 kcal/mol. Out of 34 derivatives; seven ligands among the library; two from C3-Artemisinin-Quinine hybrid, three from C10-Artemisnin-Quinine hybrid and two from Miscellaneous Artemisinin-Quinine hybrid have more negative Glide score with values from -7.600 to -8.913 kcal/mol.

For each of the seven ligands, the pose with the lowest Glide score was rescored using Prime/MM-GBSA approach. The ΔG_{bind} energies among these ligands vary in between -49.00 to -32.35 kcal/mol. The calculated relative binding energy ($\Delta\Delta G_{bind\text{-}cald}$) of the ligands was also obtained by using Art-Qui-OH as reference. The drop in calculated relative binding energy of the ligand provides a favourable energetic evaluation of the binding affinity. Interaction of Art-Qui-OH and its derivatives with Fe (II) PPIX (Iron (II)) involves binding between the endoperoxide bridges (O1 and O2) bridge of the hybrid to the front of the iron bridge of protoporphyrin-IX. The docking pose revealed O2–Fe as the shortest haeme–artemisinin distance and O1–Fe as the second shortest. Presence of quinine entity in the

hybrid structure helps to increase the potency of drug and enhanced the cellular uptake and simultaneously increase half life period of drug.

The study conclusively indicates that magnitude of the binding affinity can be a key factor that decides the activeness of an individual inhibitor. We propose a model for the binding mode and binding affinity of Art-Qui-OH and its derivatives with a putative receptor. This model will be of immense help for the rational design of new artemisinin based hybrid anti-malarial that can target the haemozoin formation.

Plasmodium falciparum ingests and degrades up to 75% of the host cell haemoglobin during its intra-erythrocytic stage. This is a massive catabolic process that takes place inside an acidic food vacuole. This involves a pathway of proteolytic enzymes both *in vitro* and *in vivo*, called plasmepsins (PMs). The *P.falciparum* genome encodes 10 aspartyl proteases; four of these plasmepsins are located in the food (digestive) vacuole (DV: I, II, IV and the Histo-aspartic protease). Histo-aspartic protease (HAP) is the most-divergent vacuolar plasmepsins, with no counterpart in other characterized species of *Plasmodium*. HAP activity was completely inhibited by the aspartic protease inhibitor Pepstatin-A while KNI-10006 is a weekly active inhibitor. So, as a proof-of-concept we conceptualize to evaluate *in silico* the binding mode and relative binding affinity of Art-Qui-OH with the Histo-Aspartic Protease (HAP) of *P.falciparum*.

Their molecular docking score values range from -7.397 to -8.777 kcal/mol. The relative docking scores (ΔG_{score}) of Art-Qui-OH with reference to HAP-Pepstatin A complex is 1.29 kcal/mole where as it is estimated to be 1.38 kcal/mol for HAP-KNI-10006 complex These results demonstrate that binding mode of Art-Qui-OH resembles to Pepstatin-A in compare to KNI-10006 in HAP active site. The docked complexes were rescored with MM-GB/SA and the relative binding energy ($\Delta\Delta G_{bind\text{-}cald}$) of Art-Qui-OH was calculated using Pepstatin-A and KNI-10006 as reference. The drop in calculated relative binding energy ($\Delta\Delta G_{\ bind\text{-}cald}$) for Art-Qui-OH revealed strong binding (2.43 kcal/mol); with a similar conformation to Pepstatin-A binding in compare to KNI-10006 conformation having a $\Delta\Delta G_{\ bind\text{-}cald}$ value of 3.44 kcal/mol. From the binding energy, the Ki value of the hybrid compound is calculated to be 10.15nM.

The mode of binding of Art-Qui-OH is drastically different and the hybrid interacts in the active site of the enzyme with O11 atom of artemisinin moiety forming H-bond with His32. The O11 atom of artemisinin and His 32 $N^{\varepsilon}2$ H-bond distance is 2.085 Å. The H-bond distance is less than the H-bond diatance (3.036 Å) formed between statine hydroxyl group and His32 residue in the active site of HAP. This revealed a strong binding affinity between ligand and binding site residue.

Art-Qui-OH exhibited a nanomolar binding affinity against Histo-Aspartic Protease. For that reason, the inhibitory activity of the hybrid was measured against the entire plasmepsins. Experimental study revealed that *Plasmodium falciparum* plasmepsin 1 (PMI) and plasmepsin 4 (PMIV) could complement for each other, whereas PfPM2 and HAP could functionally complement as the other pair. The Ki value of Art-Qui-OH complex is determined with molecular modelling analysis which indicates that that the value is 0.001 nM against plasmepsin II in compare to $3.91*10^{-8}$ nM for plasmepsin I and $6.3*10^{-6}$ nM for plasmepsin IV. The Ki values of the hybrid against PMI and PMIV are relatively insignificant. However the significantly low Ki value of Art-Qui-OH against PMIV raises a new possibility of designing an adaptive inhibitor. The binding mode of Art-Qui-OH resemble to that of Pepstatin-A binding in plasmepsin II. Interesting the central OH of the hybrid molecule is involved in the interaction with one of the catalytic Asp 34 residue.

The plasmepsins are key enzymes in the life cycle of the *Plasmodium* parasites responsible for malaria. In this chapter we have used the structural information of plasmepsins as well as computer aided drug design to a study the binding affinity a potent antimalarial compound. From our computational analysis it revealed that Art-Qui-OH fortuitously cross-reacts with at least two plasmepsins (PMII and HAP) if not all four of these, without toxicity to the host playing crucial role in the pathogenesis of malaria.

CONCLUSION

There is no doubt that the hybrid molecules show potent and novel anti malarial activity. The next major steps, therefore, is to experimentally analyze the antimalarial activity of the hybrid compound against the drug target i.e. vacuolar plasmepsins and haemoglobin derived Fe-Protoporphyrin IX, before it is promoted for the first clinical trial against malaria.

Ancillary Research Work

Computational identification of Sweet Wormwood (*Artemisia annua)* miRNA and their m-RNA targets

INTRODUCTION

The relatively low yield (0.01%-0.8%) of artemisinin in *Artemisia annua* is a serious limitation to the commercialization of the drug. Therefore, a better understanding of the molecular mechanisms involved in the artemisinin biosynthesis and regulation is needed. The regulation of key enzymes leading to increased artemisinin biosynthesis with metabolic engineering approach appears to be more promising than the other approaches like synthesis of artemisinin by semi-synthetic route. Plant miRNAs target a large number of genes with functions in a range of development processes, including metabolite biosynthesis.

OBJECTIVE

The present investigation was undertaken with the following objective:

1. Computational prediction of miRNA & its targets in a publically available EST data set of *Artemisia annua* using comparative genomics approach.

RESULTS

Using the homology search with previously known miRNAs from *Arabidopsis* and rice against expressed sequence tag (EST) database of *A. annua*, a total of six potential miRNAs were predicted, which belong to the miR414 and miR1310 families. Furthermore, eight potential target genes were identified in this species. Among them, seven genes encode proteins that play important roles in artemisinin biosynthesis. In addition, a gene coding for putative AINTEGUMENTA, which is involved in signal transduction and development, was also predicted as one of the targets.

CONCLUSION

This is the first *in silico* study to indicate that miRNAs target genes encoding enzymes involved in artemisinin biosynthesis, which may help to understand the miRNA-mediated regulation of artemisinin biosynthesis in *A. annua.*