Introduction

The telomerase reverse transcriptase (TERT) protein of Plasmodium falciparum is one of the few well-defined targets in malarial chemotherapy. This plasmodial protein contains all of the canonical motifs of RTs as well as conserved amino acids known to be critical for the RT activity. TERT contains unique and variable N- and C-terminal extensions that flank a central RT like domain (Figueiredo, 2005; Autexier and Lue, 2006). PfTERT has received considerable attention as it is the target of berberine and its derivatives used treatment of P. falciparum infection. The rapid emergence of antifolate resistant P. falciparum has unfortunately compromised the clinical utilities of the drugs, and thus highlights the urgent need to search for new effective antifolate antimalarials (Rastelli et al., 2000). The PfTERT predicted molecular weight is 280 kDa, which is almost the three times larger in size of other TERTs. In the N-terminal half of PfTERT has three motifs specific to TERTs: GQ/N, QFP and T (Figueiredo, 2005; Autexier and Lue, 2006; Lingner et al., 1997; Malik et al., 2000; Friedman and Cech, 1999). Amino acids known to be specific for telomerases were in PfTERT: Arg in motif 1, an aromatic residue (Phe or Tyr) following the two critical Asp residues in motif C and a motif similar to the Trp-X-Gly-XSer/Leu in motif E. Depending upon the difference in size of the Plasmodial TERT results from increased distance between nearly all motifs. Human Genome Center, Tokyo, Japan has found that PfTERT contains several NLS (Nuclear Localization Signal)-like motifs (Figueiredo, 2005). The k-nearest neighbors classifier (k-NN) algorithm predicts that PfTERT pri...
ary sequence refers to a protein that has 78.3% probability to be nuclear (Figueiredo, 2005; Xia et al., 2000). The blood stage cycle takes 48 hours to be completed. It begins with the invasion of non-infected erythrocytes by merozoites present in the bloodstream. When th erythrocyte, the parasite will undergo three stages: ring (0-18 hours), trophozoite (18-38 hours) and schizont (38-48 hours) stages. S phase begins in the trophozoite stage, 28-31 hours after merozoite invasion. Nuclear division occurs throughout schizogony, which leads to the production of up to 32 individual merozoites. At the end of the 48 hours cycle, erythrocytes burst releasing merozoites and the cycle starts a new (Figueiredo, 2005). It has been shown that telomerase activity is detectable by TRAP only in trophozoite and schizont stages (Srivilajajereon et al., 2002). During schizogony, the number of PfTERT foci increased proportionally to the number of nuclei. After schizonts become fully mature (48 hours) and merozoites are released into the blood stream, PfTERT was no longer detectable. PfTERT was chosen as it is a gene in P. falciparum that has the characteristics of the protein component of telomerase. It contains structural features that are common with the telomersases known in other species: RT motifs, telomerase-specific motifs and pI>=10. PfTERT contains several stretches of 10-20 basic amino acids, such as asparagines, which are encoded by A-rich codons (Figueiredo, 2005). Among all P. falciparum polymerases, the TERT gene is the one that contains more A+T nucleotides, more repeats and a higher increase in size relative to the yeast orthologue, suggesting that PfTERT sequence is less functionally constrained than that of the other polymerases. These repeats are therefore more likely to reflect the propensity of the P. falciparum genome by polymerase slippage events due to the presence of stretches of A and/or T, instead of selection process that leads to increased gene length. As a result the extreme A+T richness of Plasmodia genomes (80% A+T for P. falciparum) may favour the increase in gene size and led to the accumulation of the repetitive regions in non-essential gene areas (Achaz et al., 2001; Levinson and Gutman, 1997). In P. falciparum cell extracts, telomerase can be efficiently inhibited by RT type of drugs, such as nucleoside analogues (i.e. berberine and its derivatives)(Figueiredo, 2005; Weinrich et al., 1997). These drugs are, now-a-days being tested on in vitro cultures and preliminary data show killing of P. falciparum parasites after 3-5 blood stage cycles at micromolar concentrations (Bottiu et al., 1998). However, they were unable to generate a knock-out of the PfTERT gene, supporting the idea that telomerase activity is needed for blood stage parasite proliferation (Cong et al., 2002). A prophylactic therapy based on plasmodial telomerase inhibitors might be possible (Arnot et al., 1998; Lin et al., 2008). Berberis aristata is commonly known as “Daru haldhi and Chitra” is spnous herb native to northern Himalaya region. The plant is commonly distributed from Himalayas to Sri Lanka, Bhutan and hilly areas of Nepal. Its hypoglycemc, antibacterial, antifungal, antipyretic, anti-inflammatory, anti-oxidant, hepatoprotective, antimalarial, anti-cancer was proven by pharmacological studies. The plant fruit is edible and it is rich in vitamin C. The phytochemical studies proves that the plant B. Aristata mainly contains yellow colored alkaloids berberine, oxyberberine, aramine and a protoberberine alkalioid palmatine, oxyecantine etc. which acts as inhibitory activity against PfTERT protein of P. falciparum. Berberine has been used for the treatment of malaria as an antimalarial drug (Srivilajajereon et al., 2002; Lin et al., 2008; Sheng et al., 1997; Vennerstrom and Klayman, 1988). Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzo|dioxolo|5,6 a|quinolizinium), a yellow benzyloquinoline alkaloid, is a constituent of Coptis chinensis (Cong et al., 2002). Berberine is chief alkaloid from roots and stem-bark of Berberis species. It is produced mostly from roots of B. aristata (5% in roots and 4.2% in stem-bark). B. ptilorius (0.43%), B. vulgaris, B. aquifolium, B. thunbergi and B. asiatica. The Chinese herbs, the primary sources are B. sargentiana, Phellodendron amurense and Coptis chinensis. Coptis chinensis rhizomes and its related species used as its substitutes have about 4-8% berberine, while Phellodendron amurense bark has about half as much, at 2.4% berberine (Sheng et al., 1997). The inhibitory effects of three protoberberine alkaloids on E. coli revealed that the sequence of their antimicrobial activity was greater in comparison to the others which is berberine > cepetine > palmatine. The pharmacological studies of berberine include anti-inflammation, antiadiarhetic, antimalarial, and even antimicrobial activities (Vennerstrom and Klayman, 1988; Janbaz and Gilani, 2000; Yan et al., 2007). In Vietnam, it is being collected from 14 medicinal plants and used in traditional treatment of the disease. Twenty-four extracts from these plants were found to have antiplasmodial effects inhibiting the growth of the chloroquine-resistant P. falciparum strain FCR-3 with EC50 values of less than 10 µg/mL. Telomerase activity is synchronized in P. falciparum during its erythrocytic cycle using the telomerase repeat amplification protocol (TRAP) (Srivilajajereon et al., 2002). It is reported that berberine extract inhibited telomerase activity in a dose-dependent manner over a range of 30-300 µM. It indicated that P. falciparum telomerase might be a potential target for future malaria chemotherapy (Srivilajajereon et al., 2002; Sharma et al., 2011; Dutta and Panse, 1962; Gardner et al., 2002; Singh et al., 2010). Hence to block the telomerase activity of the P. falciparum the docking study of the berberine derivatives were per-formed with newly modeled protein of TERT protein with the help of homology modeling.

**Materials and Methods**

**Sequence retrieval:** The pfTERT protein sequence was downloaded from NCBI (http://www.ncbi.nlm.
Template identification: The template identification for homology model building of the selected protein sequence was taken place in Exome Horizon. The protein “oxidoreductase–thione reductase” having PDB ID 1ONF was found to be the best template based on the e-value i.e. 1.00.

Homology modeling of P. falciparum TERT: The structure of the TERT protein of P. falciparum was obtained from homology modeling simulations. The model was constructed by alignment of P. falciparum TERT primary sequences of which the crystal structures are already known. The alignment of P. falciparum TERT sequence was carefully checked by using the available sequence alignment evaluation functions within MODELLER 9v8 and by superimposing the structurally conserved regions of the available TERT crystal structures.

Ligand preparation: The ligands were drawn using Moldraw tool of Exome™ Horizon in 2D and were converted into 3D before submission of docking. The Molecular formulae and the chemical properties of all the selected ligands were given in Table I. The ADMET (absorption, distribution, metabolism, excretion and toxicology) properties were studied and were given in Table II.

Protein-ligand docking studies: Protein-ligand docking is used to check the structure, position and orientation of a protein when it interacts with small molecules like ligands. Protein-ligand docking aims to predict and rank the structures arising from the association between a given ligand and a target protein of known 3D structure. Protein-ligand docking module is further divided into different parts for user convenience like receptor preparation, ligand preparation, binding site analysis, dock and analysis.

The inhibitors were docked into the active site of PfTERT, using the final structure obtained from the explicit solvent molecular dynamics calculations. The protein-ligand docking was performed using Lamarckian genetic algorithm using default parameter (Morris et al., 1998; Morris et al., 1996). This is the same as the standard genetic algorithm except that, before scoring, each conformation (gene) is subjected to energy minimization. The next population is then originated by members of energy-minimized population. The name “Lamarckian” refers to the failed genetic theory of Jean-Baptiste Lamarck, who held that an organism could pass on changed experienced in its lifetime to its offspring. This theory was eventually abandoned in favor of Mendel's now familiar laws of inheritance. The LGA (Lamarckian Genetic Algorithm) is faster than both simulated annealing and the standard genetic algorithm, and it allows the docking of ligands with more degrees of freedom. The number of automated docking runs, number of individuals in the population, maximum number of energy evaluations and number of generations were set to 10, 50, 2500 and 3000 respectively. All the molecules successfully docked to the binding site.

Results and Discussion

The analogues were successfully docked into the binding pocket. The binding energy was observed in the range of +186.20- -11.34 Kcal/mol. The key result in a docking log file (DLG) are the docked structure or conformation found at the end of each run, the energies of these docked structures and their similarities to each other. The DLG file provides docked conformations, orientations and the binding energies. The similarity of docked structures is measured by computing the root-mean-square deviation (RMSD) between the coordinates of selected molecular conformation with the molecular conformation having lowest interaction energy which is ranked on top. Clusters are created based on the comparison of conformations using RMSD values. The docking results consist of the PDBQT (The pdbqt format is 'pdb' plus 'q' for partial charge and 't' for atom type.) of the transformed 3D Figure 1: Binding energy calculation of the ligands Cartesian coordinates of the ligand atoms as docked to the receptor molecule (Ramachandran et al., 1963). The binding energy of the selected ligands were plotted in the graph and from the graph (Figure 1) the binding energy of all the active sites were observed among which the best ligand which shows better activity in all the active site was found to be cyano dihydroberine. The aminoacids and the drug interactions were given in the Figure 2a-i. In this study, it was targeted the putative Plasmoidal telomerase reverse-transcriptase gene, PfTERT. PfTERT contains the most conserved telomerase motifs, but interestingly is predicted to encode an unusually large TERT. PfTERT is not detectable in early ring forms (GI-like phase); however in parasites that have begun DNA synthesis, PfTERT forms a single discrete spot at the nuclear periphery. Attempts to disrupt PfTERT gene failed suggesting that telomerase is essential for the parasite viability. So the current research paper deals with the study of blocking the telomerase activity of the protein pfTERT which may result in blocking the DNA synthesis in the parasite. It was found an unknown gene of P. falciparum which is a protein component of telomerase, named here PfTERT. The protein sequence was taken as target sequence. The template identification process was taken place to find the desired template structure of the target sequence. The template was found to be crystal structure of P. falciparum glutathione reductase whose PDB ID is 1ONF. One
| SL No. | Ligand name          | Mol. formulae | Log P   | 2D Structure |
|-------|----------------------|---------------|---------|--------------|
| 1     | berberine betaine    | C_{20}H_{17}NO_{5} | -2.4922 | ![Structure 1] |
| 2     | Berberine            | C_{20}H_{24}NO_{4} | -2.6025 | ![Structure 2] |
| 3     | Coptisine            | C_{19}H_{14}NO_{4} | 0.60923 | ![Structure 3] |
| 4     | Cyanodihydroberberine| C_{21}H_{18}N_{2}O_{4} | 3.1896 | ![Structure 4] |
| 5     | Dihydroberberine     | C_{20}H_{19}NO_{4} | 3.883   | ![Structure 5] |
| Sl. No. | Ligand name | Mol. formulae | Log P  | 2D Structure |
|--------|-------------|---------------|--------|--------------|
| 6      | Methoxyhydroberberine | C_{26}H_{24}O_{10} | 1.11315 | ![2D structure](image1) |
| 7      | Oxycanthine  | C_{36}H_{46}N_{2}O_{5} | 7.28   | ![2D structure](image2) |
| 8      | Palmitine    | C_{21}H_{25}NO_{4} | 3.2    | ![2D structure](image3) |
| 9      | N-Methyltetrahydroberberinium iodide | C_{25}H_{30}INO_{4} | 3.27835 | ![2D structure](image4) |
| 10     | 9,10-dimethoxy-5,8-dihydro-6H-[1,3]dioxolo[4,5-g]isoquinoline[3,2-a]isoquinoline | C_{20}H_{17}NO_{5} | 3.27835 | ![2D structure](image5) |
homology model of the same was developed using Modeller 9v8 since there was no actual 3D structure in the PDB (Protein Data Bank). Berberine is an isoquino-line alkaloid, present in roots and stem-bark of Berberis species. Berberine based formulations, are widely used in traditional systems of medicine including, Ayurveda and traditional Chinese medicine. Berberine has treated wide range of pharmacological activities including; antihypertensive, anti-inflammatory, anti-oxidant, anti-depressant, anti-cancer, anti-diarrheal, cholangou-ge, hepatoprotective, antimicrobial and antimalarial (Singh et al., 2010). Recent studies, have thrown light on anti-diabetic and hypolipidemic activities of the alkaloid. Berberine has been proved clinically in the treatment of oriental sore, diarrhea, trachoma diabetes mellitus type 2, hypercholesterolemia and congestive cardiac failure. The present research work, discusses the antiplasmodial activity of barberine and its analogues which will be potential for drug-development.

Berberine has definite potential as drug, since it possesses diverse pharmacological properties. Previous studies established utility of berberine as antibacterial agent. As per recent studies, the striking effect of berberine is on DNA synthesis of P. falciparum.

| Ligand name                      | Lipinsky’s filter | Bioavailability | Lead likeliness | Ghose filter |
|----------------------------------|-------------------|-----------------|-----------------|--------------|
| Berberine betaine                | Green, 4          | Red, 7          | Red, 5          | Red, 3       |
| Berberine                        | Green, 4          | Red, 7          | Red, 5          | Red, 3       |
| Coptisine                        | Green, 4          | Red, 7          | Red, 5          | Red, 3       |
| Cyanodihydroberberine            | Green, 4          | Red, 7          | Red, 5          | Red, 4       |
| Dihydroberberine                 | Green, 4          | Red, 7          | Red, 5          | Red, 4       |
| Methoxyhydrnocarpin              | Green, 4          | Red, 7          | Red, 3          | Red, 3       |
| Oxyanthine                       | Yellow, 2         | Red, 5          | Red, 4          | -            |
| Palmatine                        | Green, 4          | Red, 7          | Red, 6          | Red, 4       |
| N-Methyltetrahydroberberinium iodide | Green, 4      | Red, 7          | Red, 4          | Red, 2       |
| 9,10-dimethoxy-5,8-dihydro-6H-[1,3]dioxolo[4,5-g] isoquin[3,2-a]isoquinoline | Green, 4 | Red, 7 | Red, 5 | Red, 4 |

Figure 1: The binding energy calculation of the drugs against the model
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Figure 2: The interactions of the berberine betaine (a), berberine (b), coptisine (c), cyanodihydroberine (d), dihydroberbeine (e), methoxyhydroberberine (f), oxycahine (g), palmitine (h), and 9,10-dimethoxy-5,8-dihydro-6H-[1,3]dioxolo[4,5-g] isoquinoline (i) against the proteins. The colors ( ) were represented as the interacting hydrogen bonds between the protein and the drugs.

References

Achaz G, Netter P, Coissac E. Study of intrachromosomal duplications among the eukaryote genomes. Mol Biol Evol. 2001; 18: 2280–88.

Arnot DE, Gull K. The Plasmodium cell-cycle: Facts and
questions. Ann Trop Med Parasitol. 1998; 92: 361–65.

Autexier C, Lue NF. The structure and function of telomerase reverse transcriptase. Ann Rev Biochem. 2006; 75: 493–517.

Bottius E, Bakhis N, Scherf A. Plasmodium falciparum telomerase: De novo telomere addition to telomeric and non-telomeric sequences and role in chromosome healing. Mol Cell Biol. 1998; 18: 919–25.

Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiol Mol Biol Rev. 2002; 66: 407–25.

Dutta NK, Panse MV. Usefulness of berberine (an alkaloid from Berberis aristata) in the treatment of cholera (experimental). Int J Med Res. 1962; 50: 732-36.

Figueiredo LM. The unusually large Plasmodium telomerase reverse transcriptase localizes in a discrete compartment associated with the nucleolus. Nucl Acids Res. 2005; 33: 1111–22.

Friedman KL, Cech TR. Essential functions of aminoterminal domains in the yeast telomerase catalytic subunit revealed by selection for viable mutants. Genes Develop. 1999; 13: 2863–74.

Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, Shal-lom SJ, Suh B, Peterson J, Angiuoli S, Perta M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, Mcfadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B. 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. Nature 2002; 419: 498-511.

Iwazaki RS, Endo EH, Ueda-Nakamura T, Nakamura CV, Garcia LB, Filho BP. In vitro antifungal activity of the berberine and its synergism with fluconazole. Antonie Van Leeuwenhoek. 2010; 97: 201-05.

Janbaz KH, Gilani AH. Studies on preventive and curative effects of berberine. Fitoterapia 2000; 71: 25-33.

Levinson G, Gutman GA. Slipped-strand mispairing: A major mechanism for DNA sequence evolution. Mol Biol Evol. 1997; 4: 203–21.

Lin JP, Yang JS, Wu CC, Lin SS, Hsieh WT, Lin ML, Yu FS, Yu CS, Chen GW, Chang YH, Chung JG. Berberine induced down-regulation of matrix metalloproteinase-1, -2 and -9 in human gastric cancer cells (SNU-5). In vivo. 2008; 22: 223-30.

Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. 1997. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science 1997; 276: 561–67.

Malik HS, Burke WD, Eickbush TH. Putative telomerase catalytic subunits from Giardia lamblia and Caenorhabditis elegans. Gene 2000; 251: 101-08.
Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian Genetic Algorithm and empirical binding free energy function. J Comput Chem. 1998; 19: 1639-62.

Morris GM, Goodsell DS, Huey R, Olson AJ. Distributed automated docking of flexible ligands to proteins: Parallel applications of AutoDock 2.4. J Comput Aided Mol Design 1996; 10: 293-304.

Qi M, Feng Y, Dai DZ, Li N, Cheng YS, Dai Y. CPU86017, a berberine derivative, attenuates cardiac failure through normalizing calcium leakage and down-regulated phospholamban and exerting anti-oxidant activity. Acta Pharmacologica Sinica. 2010; 31: 165-74.

Ramachandran GN, Ramakrishnan C, Sasisekharan V. Stereochemistry of polypeptide chain configurations. Int J Mol Biol. 1963; 7: 95-99.

Rastelli G, Sirawaraporn W, Sompongphisut P, Vilaivan T, Thebharanon Y, Yuthavong Y. Interaction of pyrimethamine, cycloguanil, WR99210 and their analogues with Plasmodium falciparum dihydrofolate reductase: Structural basis of antifolate resistance. Bioorg Med Chem. 2000; 8: 1117-28.

Sharma K, Ranjan B, Neelam C, Birendra S, Kumar SN. Berberis aristata: A review. Int J Res Ayurveda Pharm. 2011; 2: 383-88.

Sheng WD, Jiddawi MS, Hong XQ, Abdulla SM. Treatment of chloroquine-resistant malaria using pyrimethamine in combination with berberine, tetracycline or cotrimoxazole. East Afr Med J. 1997; 74: 283-84.

Singh A, Duggal S, Kaur N, Singh J. Berberine: Alkaloid with wide spectrum of pharmacological activities. J Nat Prod. 2010; 3: 64-75.

Sinha R, Kumar GS. Interaction of isoquinoline alkaloids with an RNA triplex: Structural and thermodynamic studies of berberine, palmatine, and coralyne binding to Poly(U). Poly(A) (*)Poly(U). J Physical Chem. 2009; 113: 13410-20.

Sriwilaijareon N, Petmim S, Mutirangura A, Pollongkittmongkol M, Wilairat P. Stage specificity of Plasmodium falciparum telomerase and its inhibition by berberine. Parasitol Int. 2002; 51: 99-103.

Vennerstrom JL, Klayman DL. Protoberberine alkaloids as antimalarials. J Med Chem. 1988. 31, 1084-87.

Wang YX, Wang YP, Zhang H, Kong WJ, Li YH, Liu F, Gao RM, Liu T, Jiang JD, Song DQ. Synthesis and biological evaluation of berberine analogues as novel up-regulators for both low-density-lipoprotein receptor and insulin receptor. Bioorg Med Chem Lett. 2009; 19: 6004-08.