In silico characterization of human-malarial parasite species based on their DHFR and GST targets leading to a change in binding conformations of anti-malarial drugs

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INTRODUCTION

Malaria worldwide is a substantial public health burden, and the countries where it is endemic exhibit economic crux (Ondeto et al., 2017; Tse et al., 2019). This results in the emergence of novel drug activity versus malaria pandemic (Escalante and Ayala, 1994; Cui et al., 2015). There are four Plasmodium species utterly responsible for invoking parasitic infection in humans viz P. falciparum, P. malariae, P. ovale, and P. vivax. Significantly, P. vivax being obligated for the extensive spread of the malarial infection worldwide. P. falciparum, however, is the most dangerous and causes the most fatalities. P. malariae and P. ovale are less widely distributed (Ramasamy, 2014). There are many contrasting characteristics among them yet these four human parasitic species are closely related (Carpenter et al., 1991; Oaks et al., 1991; Coulson et al., 2004).

Diverse reviews were published in the past few years stated the eventual future of anti-malarial drugs. In 2014, combinations of the anti-malarial drug were scrutinized with three drug candidates. In 2017, numerous reviews about currently used anti-malarial drug discovery and designing and all the other aspects of malaria in-depth primer were also published. A review emphasizing numerous novel anti-malarial drug candidates in drug discovery and designing was also published in early 2018 (Tse et al., 2019). In this study, we are mainly focusing on the action of quinines - Chloroquine, Mefloquine, and Proguanil on proteins...
Dihydrofolate reductase (DHFR) and Glutathione S-transferases (GST) of four human *Plasmodium species*. These quinines compounds are not used as front line treatment for malaria because of their side effects except for in severe cases of malarial treatment purpose where there is artemisinin inadequateness. However, quinines are still on WHO's Model List of Essential Medicines (MLEM). Chloroquine (Figure 1a) was directed to treat all forms of malaria and with few side effects amid 1940. In 1950, chloroquine resistance was firstly reported and over many years the other malarial strain/have also developed resistance. In the regions where resistance has developed against P. vivax, Chloroquine is administered as it’s on MLEM. Mefloquine (Figure 1b) was developed by the United States Army in the 1970s. Mefloquine is also on MLEM and is still being used as a drug today. It was originally innovated to treat chloroquine-resistant malaria and it was given as both a prophylactic and curative drug in malaria. In 1986, mefloquine resistance was firstly reported. It acts on the blood-stage of the parasite by disrupting hemoglobin digestion. Proguanil (Figure 1c) was reported as one of the first antifolate anti-malarial drugs in 1945. In 1991, Atovaquone was first reported for the treatment of malaria. Usually, a combination of these two drugs is used with the name of Malarone™. In 2000, these two drugs are showing an effective synergistic effect in the treatment of malaria was proven. Proguanil when administered as a single drug exploit as DHFR inhibitor by disrupting the synthesis of deoxymethylate (Tse et al., 2019).

DHFR is said to be a substantiated drug target for the treatment of parasitic disease- malaria (Anderson, 2005). Frequent drug resistance of *Plasmodium species* results in the evolution of novel drug candidates with a new mode of action. To maintain a high rate of replication, malarial parasites also require DHFR/folate and they are also capable of synthesizing or scavenging folate newly (Zhang and Rathod, 2013; Tse et al., 2019). In addition to drug resistance, GST is likewise involved in numerous diseases counting malaria (Westling et al., 1997; Sohail et al., 2010). In recent years, many researchers have distinguished the role of free radicals in malarial pathogenesis and other parasitic infections. *P. vivax* is known as an oxidative stress-sensitive strain of *Plasmodium*. Hence GST an oxidative enzyme fascinated benefits in the diagnosis and monitoring fields of malarial complications. There are several diseases including malaria linked with GST deficiency in humans, thus there is a booming perception about the role of GST target (Sohail et al., 2007).

**MATERIALS AND METHODS**

In this study, we have investigated four human *Plasmodium species* listed in Table 1 (Antinori et al., 2012; Ortiz-Ruiz et al., 2018). The complete sequence of target protein DHFR and GST from the above four *Plasmodium species* sequences were retrieved from the UniProt database. For protein structural analysis, only *P. falciparum* structure with two genes A and B was retrieved from PDB whereas the rest three species protein structures were obtained from homology modeling (Yang et al., 2012) using Phyre 2.0 since their structures are not available in PDB. These *Plasmodium* sequences were subjected to MSA using Clustal O (1.2.4) program so that the utmost number of residues are matched up on the basis of a particular scoring function.

Phylogenetic relationship was derived by the neighbor-joining (N-J) method, which is a distance-based method that allows calculation of the evolutionary distance between sequences. In the N-J method, the branch length represents the amount of inferred evolutionary changes taken place (Gascuel and Steel, 2006; Pavlopoulos et al., 2010).

These antimalarial drugs (Figure 1) were further docked using AutoDock vina and binding affinity was calculated in kcal/mol. The interactions of them were studies using Discovery studio visualizer.

**RESULTS**

MSA of DHFR for amino acid residue ranging from 60-637 (Figure 2) and GST for amino acid residue ranging from 60-211 (Figure 5) among *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax* was performed using Clustal Omega (1.2.4) that showed sequence similarity between them. The distance based method i.e. N-J method was also used to build a phylogenetic tree of tree data received from MSA. In MSA result amino acids resembling at various positions were denoted with symbols that give graphical interpretation at that respective residue.
Amino acid residues with the "*" sign show identical residues; "-" sign shows highly similar whereas "=" shows less similar residues at that particular site. Regions with no signs symbolize no similarity at that site. The N-J method was applied to create a unique parsimonious tree from the distance or guide tree data obtained from MSA. The DHFR protein from *P. malariae* and *P. vivax* was found to be the most closely related species among four of them. However, *P. vivax* was found to be the most distant species (Figure 3). The GST protein from *P. ovale* and *P. malariae* were found to be the most closely related species among four of them. However, *P. falciparum* was found to be the most distant species among them (Figure 6).

The 3D structure of protein DHFR and GST from four *Plasmodium species* were superimposed using PyMol. Superimposed structure of DHFR protein (Figure 4) shows (a) *P. falciparum* (green) with chain A and B, *P. malariae* (cyans) chain A, *P. ovale* (magentas) chain A and *P. vivax* (yellow) chain A. (b) *P. falciparum* (green) with chain A, *P. malariae* (cyans) chain A, *P. ovale* (magentas) chain A and *P. vivax* (yellow) chain A. (c) *P. falciparum* (green) with chain A, *P. malariae* (cyans) chain A, *P. ovale* (magentas) chain A and *P. vivax* (yellow) chain A. Mesh (Red) indicates the residues which are not aligned in that particular region causing deviation in protein 3D structure. PyMOL was used to identify similarities in protein folding pattern and the RMS of all superimposed protein structures were calculated those are shown in Table 2. On superimposing proteins there was not much difference in protein folding pattern was observed.

Molecular docking studies were executed using the AutoDock vina tool. Numerous drug interactions with proteins were observed using Discovery studio visualizer and binding affinity were also calculated in kcal/mol (Tables 3 and 4) which is as follows:-

Docking analysis showed interactions between Chloroquine and DHFR protein target in four *Plasmodium species* (Figure 8) (a) showing the interaction of chloroquine drug and *P. falciparum* target protein, forming two carbon-hydrogen bonds with Asn 340, Phe B: 499; two alkyl bonds at Phe A: 354 and Ile B: 544; Pi-alkyl bond with Lys B: 502; six Van der Waals interactions with Lys A: 353, Tyr B: 497, Ser B: 363, aspartic acid B: 361, Gln B: 542 and Ser B: 504; and only one Pi-sigma interaction with Ala B: 541. (b) showing interaction of chloroquine drug and *P. malariae* target protein, forming two Pi-alkyl bonds with His 611, Tyr 609; one conventional hydrogen bond interaction with glutamic acid 29 and thirteen Van der Waals interactions with Ile 606, Gln 607, Thr 35, Arg 37, Phe 31, Lys 18, 26, 386; Val 19, 196, 610; Asn 21, 608. (c) showing the interaction of chloroquine drug and *P. ovale* target protein, forming three Pi-sigma bonds with Ile 432, Phe 549, Tyr 433 and 10 Van der Waals interactions with Ile 408, Gln 50, Cys 519, Leu 516, Tyr 459, Phe 415, His 520, Gln 411 and Asn 436, 550. (d) showing the interaction of chloroquine drug and *P. vivax* target protein, forming two Pi-carbon bonds with His 614 and Lys 389; conventional carbon bond with Leu 390; Pi-alkyl bond with Phe 31 and ten Van der Waals interactions with glutamic acid 29, Thr 26, Asn 28, Tyr 612, Arg 393, Phe 391, Leu 384, 392; Ile 396, 609.

| Table 1: *Plasmodium species* their geographical distribution and host explicitness |
|---------------------------------|-----------------|-----------------|
| Species                        | Geographic Distribution | Host            |
| *P. falciparum*                | Tropics worldwide  | Human           |
| *P. malariae*                  | Worldwide         | Human           |
| *P. ovale*                     | Worldwide         | Human           |
| *P. vivax*                     | Tropics worldwide | Human           |
| *P. fragile*                   | Asian tropics     | Monkey          |
| *P. Knowlesi*                  | Asian tropics     | Human, Monkey   |
| *P. berghei*                   | African tropics   | Rodent          |
| *P. lophurae*                  | Old world         | Bird            |
| *P. maxicanum*                 | North America     | Lizard          |

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Docking analysis showed the interactions between Mefloquine and DHFR protein target in four *Plasmodium species* (Figure 9) (a) showing the interactions of mefloquine drug and *P. falciparum* target protein, forming Pi-donor hydrogen bond with Asn B: 340; Pi-Pi T-shaped bond with Phe A: 499; alkyl bond with Ala A: 541; five conventional hydrogen bonds with Lys B: 302 and 353; Phe B: 354; Ser A: 504; and Asn B: 338; three Van der Waals interaction with Gln B: 298; Lys A: 502 and Tyr A: 497; Gln A: 542 residue showed halogen (fluorine) interaction. (b) showing the interaction of mefloquine drug and *P. ovale* target protein, forming Pi-alkyl bond with Phe 31 and Pi-Pi T-shaped bond with Tyr 609; three conventional hydrogen bonds with Gln 29, Asn 28 and His 611; one carbon-hydrogen bond with Lys 386; eight Van der Waals interactions with Val 196, 610; Lys 18, Asn 608, Gln 607; Ile 606, Phe 388 and Leu 389; and two amino acid residues viz Lys 26, Leu 387 showing halogen (fluorine) interaction. (c) showing the interaction of mefloquine drug and *P. malariae* target protein, forming four conventional hydrogen bonds with Ser 28, His 627, Lys 609; Tyr 625, three alkyl bonds with Ile 622; six Van der Waals interactions with Gln 29, 623; Asn 624, Val 626, Ile 405, Tyr 31 and two halogen (fluorine) bonds with Lys 26, 403. (d) showing the interaction of mefloquine drug and *P. vivax* target protein, forming two alkyl bonds with Phe 31, Lys 18 and Pi-Pi T-shaped bond with Tyr 612, four conventional hydrogen bonds with Lys 389, His 614, Gln 29 and Asn 28; two halogen (fluorine) bonds with Leu 390, Thr 26 and six Van der Waals interactions with Val 613, Asn 611, Gln 610, Ile 610, Leu 392 and Phe 391.

Docking analysis showed the interactions between Proguanil and DHFR protein target in four *Plasmodium species* (Figure 10) (a) showing the interactions of proguanil drug and *P. falciparum* target protein, forming one conventional hydrogen bond with Lys A: 359; two Pi-anion bonds along with attractive charges were observed in aspartic acid A: 212 and 361 position as well as one unfavourable donor-donor bond was also conformed with aspartic acid A: 361; one Pi-alkyl bond with Tyr A: 214; seven Van der Waals interactions with Phe A: 360; Gln A: 364; Val A: 213; Tyr A: 365; proline A: 324; Gln A: 327 and Tyr A: 322. (b) showing the interaction of proguanil drug and *P. malariae* target protein, forming one unfavourable donor-donor bond with Asn 534; one Pi-sigma bond with Typ 417; Pi-Pi stacked bond with Phe 533; four alkyl bonds with Ile 416, Leu 500, Tyr 443 and Cys 503 as well as five Van der Waals interactions were seen at Ile 392, Gly 530, Asn 420, His 504, Gln 522. (c) showing the interaction of proguanil drug and *P. ovale* target protein, forming two alkyl bonds with Lys 21, Val 19; one Pi-anion bond and attractive charge with Glu 29; one unfavourable positive-positive bond with Lys 18; one conventional hydrogen bond with Thr 195, seven Van der Waals interactions with Arg 37, Thr 35, Lys 26, Val 626, Tyr 31, 625 and Asn 624. (d) showing the interaction of proguanil drug and *P. vivax* target protein, forming four alkyl bonds with Ile 419, Tyr 446, Leu 503 and cystine 506; Pi-Pi stacked bond with Phe 536; one Pi-sigma bond with Typ 420; one unfavourable donor-donor bond with Asn 537 was seen and five Van der Waals interaction with Ile 395, Gln 525, His 507, Asn 423 and Gly 533.

Docking analysis showed the interactions between Chloroquine and GST protein target in four *Plasmodium species* (Figure 11) (a) showing the interactions between Chloroquine drug and *P. falciparum* target protein, forming two alkyl bonds with Phe B: 110, Lys B: 175; one Pi-alkyl bond with Phe B: 110 and Pi-cation bond with Lys B: 175; two carbon-hydrogen bonds with Thr B: 113, Asn B: 111 and ten Van der Waals interactions were seen with Asn A: 111, 114 & B: 114, aspartic acid A: 171, Val A and B: 210, Thr A and B: 174, Tyr A and B: 211. (b) showing the interaction between Chloroquine drug and *P. malariae* target protein, forming one conventional hydrogen bond with Gln 104, four alkyl bonds with Leu 18, Phe 100, Val 103 and His 107; Pi-cation and carbon-hydrogen bond with Lys 15; Tyr 108 showing Pi-Pi-T-shaped bond whereas six Van der Waals interactions with Gln 73, Ser 72, Val 76, Ala 158, Asn 111 and 161. (c) showing the interaction between Chloroquine drug and *P. ovale* target protein, forming one conventional hydrogen bond with Gln 104; Pi-Pi-T-shaped bonds with Tyr 108; Pi-cation bond with Lys 15; six alkyl bonds with Val 76 and 103, Phe 100, Leu 18, Ala 158, His 107; four Van der Waals interactions at Asn 111, Gln 73, Ser 72 and Asn 161. (d) showing the interaction between Chloroquine drug and *P. vivax* target protein, forming two Pi-anion bond with Asn 32; two carbon-hydrogen bonds were seen with Phe 23 and Ala 24; eight Van der Waals interactions were seen with Tyr 196, Gly 27, Ile 28, Asn 192 and 195 and Arg 20, 34 and 200. There was also one unfavourable donor-donor interaction was seen with Tyr 30 and no conventional hydrogen bond was observed in this docking analysis.

Docking analysis showed the interactions between Mefloquine and GST protein target in four *Plasmodium species* (Figure 12) (a) showing the interactions between Mefloquine drug and *P. falciparum* target protein, forming conventional hydrogen bond with aspartic acid A: 11; one Pi-alkyl bond with proline B: 177; three alkyl bonds with Ala A: 41, Tyr A: 211.
Figure 2: MSA of DHFR target protein among all four *Plasmodium* species

Figure 3: Phylogenetic relationship of DHFR protein between four *Plasmodium* species

Table 2: RMS of DHFR and GST protein among three *Plasmodium species* viz *P. malariae*, *P. ovale* and *P. vivax* over *P. falciparum* was calculated using PyMol

<table>
<thead>
<tr>
<th><em>Plasmodium species</em></th>
<th>DHFR RMS</th>
<th>DHFR Residues</th>
<th>GST RMS</th>
<th>GST Residues</th>
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</thead>
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<tr>
<td><em>P. malariae</em></td>
<td>0.552</td>
<td>1089 vs 543</td>
<td>0.075</td>
<td>422 vs 205</td>
</tr>
<tr>
<td><em>P. ovale</em></td>
<td>0.555</td>
<td>1089 vs 543</td>
<td>0.078</td>
<td>422 vs 205</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>0.543</td>
<td>1089 vs 544</td>
<td>0.079</td>
<td>422 vs 205</td>
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</table>
Figure 4: Superimposed structure of DHFR protein

(a) Pf chain A & B (green), Pm, Po, Pv chain A (cyan, magenta, yellow)
(b) Pf, Pm, Po, Pv chain A (green, cyan, magenta, yellow)
(c) Pf, Pm, Po, Pv chain A (green, cyan, magenta, yellow) with highlighted dissimilar region

Figure 5: MSA of GST target protein among all four *Plasmodium* species

Figure 6: Phylogenetic relationship of GST protein between four *Plasmodium* species
Figure 7: Superimposed structure of GST protein

(a) Pf chain A & B (green), Pm, Po, Pv chain A (cyan, magentas, yellow)

(b) Pf, Pm, Po, Pv chain A (green, cyan, magentas, yellow) with highlighted dissimilar region

Figure 8: Interaction of Chloroquine with DHFR proteins present in (a) *P. falciparum* (b) *P. malariae* (c) *P. ovale* (d) *P. vivax* respectively
Table 3: Docking output showing binding affinity in kcal/mol between DHFR proteins of four *Plasmodium* species with three known anti-malarial drugs

<table>
<thead>
<tr>
<th>Species / Drugs</th>
<th><em>Plasmodium falciparum</em></th>
<th><em>Plasmodium malariae</em></th>
<th><em>Plasmodium ovale</em></th>
<th><em>Plasmodium vivax</em></th>
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</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>-5.7</td>
<td>-6.7</td>
<td>-6.3</td>
<td>-6.2</td>
</tr>
<tr>
<td>Proguanil</td>
<td>-6.2</td>
<td>-6.7</td>
<td>-7.3</td>
<td>-6.5</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>-7.8</td>
<td>-8.6</td>
<td>-7.8</td>
<td>-8.3</td>
</tr>
</tbody>
</table>

Table 4: Docking output showing binding affinity in kcal/mol between GST proteins of four *Plasmodium* species with three known anti-malarial drugs

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<tr>
<th>Species / Drugs</th>
<th><em>Plasmodium falciparum</em></th>
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<th><em>Plasmodium ovale</em></th>
<th><em>Plasmodium vivax</em></th>
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<tbody>
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<td>-6.8</td>
<td>-5.9</td>
</tr>
<tr>
<td>Proguanil</td>
<td>-7.6</td>
<td>-6.6</td>
<td>-6.6</td>
<td>-6.6</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>-8.2</td>
<td>-7.5</td>
<td>-7.4</td>
<td>-7.5</td>
</tr>
</tbody>
</table>

Figure 9: Interaction of Mefloquine with DHFR protein present in (a) *P. falciparum* (b) *P. malariae* (c) *P. ovale* (d) *P. vivax* respectively
and Leu B: 115; three halogen(fluorine) bonds with Gly A: 36, Phe A: 35, Gln B: 120; six Van der Waals interactions with Asn A: 40, Tyr B: 176, Lys B: 175, Ala 12, Phe A: 10 and 42. (b) showing the interaction between Mefloquine drug and *P. malariae* target protein, forming Pi-alkyl bond with Arg 200; three alkyl bonds with Tyr 196, Ala 24 and Tyr 30; Lys 29 is showing carbon hydrogen bond as well as alkyl bond formation; two halogen(fluorine) bond were seen with Arg 20 and Ile 28; four Van der Waals interactions were found with residues- Phe 23, Asn 199, Lys 201 and Arg 34. Aspartic acid 32 was found to build two bond interactions viz conventional hydrogen bond as well as one unfavourable acceptor-acceptor bond. (c) showing the interaction between Mefloquine drug and *P. ovale* target protein, forming two conventional hydrogen bonds one with Tyr 30 and Asn 199; halogen(fluorine) bond formation was seen with four residues like Tyr 30, Ile 28, Phe 23, aspartic acid 27; one Pi-Pi T-shaped bond with Tyr 196; two Pi-alkyl bonds with Tyr 30 and Ala 24 and five Van der Waals interaction with Lys 201, Asn 32, Arg 200, Asn 195, Ala 95 and Lys 29. (d) showing the interaction between Mefloquine drug and *P. vivax* target protein, forming two conventional hydrogen bonds with Asn 32; one carbon hydrogen interaction with Gln 29; one alkyl bond with Arg 200; three Pi-alkyl bonds with Tyr 196, Ala 24 and Tyr 30; two halogen (fluorine) bonds with Arg 20, Ile 28 and four Van der Waals interactions were found with Phe 23, Asn 199, Lys 201 and Arg 34.

Docking analysis showed the interactions between Proguanil and GST protein target in four *Plasmodium* species (Figure 13) (a) showing the interaction between Proguanil drug and *P. falciparum* target protein, forming three Pi-alkyl bonds each with Leu B:115, Phe A: 10 and Val A: 59 and one alkyl
bond with Tyr A: 9; Pi-Pi-T-shaped bond with Phe A: 45; two conventional hydrogen bond interactions were seen with Thr B: 121 and Gln B: 118; five Van der Waals interactions with Lys A: 49, Gln A: 58, Phe A: 56, Gln A: 57, Thr B: 122 and Asn B: 119. (b) showing the interaction between Proguanil drug and *P. malariae* target protein, forming one conventional hydrogen bond with Gln 104; one alkyl bond with Tyr 108; three Pi-alkyl bonds with Val 76, Leu 18 and Phe 100; His 107 showed Pi-Pi-T-shaped bond formation; six Van der Waals interactions with Asn 111, Val 103, Ile 19, Gln 73, Ser 72, Asn 161. His 107 and Lys 15 showed unfavourable positive-positive interactions along with formation of amide-Pi stacked bond and Pi-carbon bond respectively. (c) showing the interaction between Proguanil drug and *P. ovale* target protein, forming three Pi-alkyl bonds with Val 76, Leu 18, Phe 100; one alkyl bond with Tyr 108; seven Van der Waals interactions with Val 103, Ile 19, Ser 72, Asn 111 and 161, Gln 73 and 104. His 107 and Lys 15 showed unfavourable positive-positive interactions along with formation of amide-Pi stacked bond and Pi-carbon bond respectively. (d) showing the interaction between Proguanil drug and *P. vivax* target protein, forming one conventional hydrogen bond with Gln 104; three Pi-alkyl bonds with Val 76, Leu 18, and Phe 100; one alkyl bond with Tyr 108; six Van der Waals interactions with Asn 111, Val 103, Ile 19, Gln 73, Ser 72, Asn 161. His 107 and Lys 15 showed unfavourable positive-positive interactions along with formation of amide-Pi stacked bond and Pi-carbon bond respectively.

Figure 11: Interaction of Chloroquine with GST protein present in (a) *P. falciparum* (b) *P. malariae* (c) *P. ovale* (d) *P. vivax*
Multiple sequence alignment was done to study how closely related the malarial species are ancestral. The DHFR protein sequence of all the four Plasmodium species, when aligned with MSA, showed incorporation of gaps and less similarity between sequences whereas in the case of GST proteins highest sequence similarity with many * identical residues was seen. The phylogenetic tree which is drawn based on the of N-J method showed P. malariae - P. vivax and P. malariae - P. ovale is the most closely related species whereas P. ovale and P. falciparum is found to be most distantly related or divergent species in the case of DHFR and GST respectively. Not much structural divergence was seen in 3D structures on superimposing using PyMol.

On performing molecular docking studies, Proguanil showed unfavorable interactions with DHFR protein in P. malariae (Asn 534), P. ovale (Lys 18), P. vivax (Asn 537) whereas concerning GST protein in P. malariae, P. ovale, P. vivax with same residues viz His 107 and Lys 15 proguanil showed unfavorable interactions. Chloroquine also showed one unfavorable interaction with GST protein in P. vivax (Tyr 30). The highest binding affinity of chloroquine is observed with DHFR- P. malariae (-6.7 kcal/mol) and in GST- P. falciparum (-7.4 kcal/mol). Mefloquine showed maximum binding affinity towards DHFR- P. malariae (-8.6 kcal/mol) and GST- P. falciparum (-8.2 kcal/mol) compared to all other drugs.

**DISCUSSION**

Previous studies involving the analysis of the gene encoding P. falciparum DHFR from resistant parasites suggest that antifolate resistance arises from point mutations in the DHFR domain, mainly at posi-
Figure 13: Interaction of Proguanil with GST protein present in (a) *P. falciparum* (b) *P. malariae* (c) *P. ovale* (d) *P. Vivax* respectively

Virtual screening of 500,000 chemical compounds was performed by using FlexX against different plasmepsins (aspartic protease implicated in haemoglobin degradation). Experimental results have proved that some of the compounds selected from WISDOM-I function as sub-micromolar inhibitors against plasmepsin. The main goals of WISDOM project were to identify inhibitors to be tested in the experimental laboratories (Kasam et al., 2009). Drug targets from malaria were chosen initially, however, this could be expanded to determine ligand binding to any target protein. The crystal structure of DHFR enzyme from *P. vivax* was published by Kongsaeere et al. (2005), where they indicated that the principal difference between DHFR wild type and mutant, implicated in the antifolate resistance, is a structural change in
the chain of Asn-108, and this steric conflict is not present in \textit{P. falciparum}. Glutathione S-transferase of the malarial parasite \textit{P. falciparum} (PfGST) represents a novel class of GST isoenzymes. Since the architecture of the PfGST substrate binding site differs significantly from its human counterparts and there is only one isoenzyme present in the parasite, PfGST is considered a highly attractive target for antimalarial drug development (Hiller et al., 2006).

\textit{P. falciparum} parasites are increasingly drug-resistant, requiring the search for novel antimalarials with distinct modes of action. Enzymes in the glutathione pathway, including glutathione S-transferase (GST), show promise as novel antimalarial targets. By using reverse genetics, Colon-Lorenzo et al. provided evidence that GST is essential for survival of \textit{P. berghei} intra-erythrocytic stages and is a valid target for drug development (Colon-Lorenzo et al., 2020). Researchers have also studied Glutathione-S-transferases (GSTs) from chloroquine-resistant (CQR, K1) and -sensitive (CQS, T9/94) strains of \textit{P. falciparum} (Harwaldt et al., 2002; Al-Qattan et al., 2016). The enzymes from both strains exhibited the optimal pH for enzyme catalysis, at pH 7.5, and were stable at temperatures below 60 degrees C. They therefore proposed that GSTs from both malarial strains are identical in their functional domain but different in level of gene expression (Harwaldt et al., 2002; Al-Qattan et al., 2016). Yadav MK et al. analysed the possibility of using variable surface proteins as a common drug target in both the \textit{Plasmodium species} (Yadav and Swati, 2016). Sequence analysis of variable surface proteins showed a low-level conservation within as well as between the species. Amino acid composition analysis revealed higher frequency of hydrophilic amino acids as compared with that of hydrophobic residues. Structural alignment of variable surface proteins by superimposing them showed less conservation. The noted existence of structural differences showed that the variable surface proteins could not be used as a common drug target in both the malarial species (Yadav and Swati, 2016). The authors concluded that species-specific strategy may be followed for drug targeting against variable surface proteins of \textit{P. falciparum} and \textit{P. vivax}. Forlemu N et al. studies the effects of DHFR-TS mutations on interactions with antimalarial agents and sulfonamide ligands using AutoDock 4.2 molecular docking (Saitou and Nei, 1987; Mukinay and Forlemu, 2017). Three sulfonamides (SulfaC, SulfaH, and sulfaE) showed better or comparable interaction affinity (-7.0 to -9.5 kcal/mol) with PfDHFR-TS isoforms to antifolate drugs (artemisinin, primaquine, pyrimethamine). The active site was the preferred binding mode in all isoforms studied, with conserved polar residues (D54, S108, R122, Y170) stabilizing complexes formed. The affinities for all isoforms studied had similar magnitudes with each ligand. The findings indicated that mutations do not significantly impact binding.

Another study was conducted using molecular docking on 28 compounds belonging to 2,4-diaminoquinazoline and 2,4-diaminopteridine analogs (Bharatam and Adane, 2010). The authors used Glide, FlexX and GOLD programs and the X-ray crystallographic structures of the quadruple mutant (1J3K:pdb) and wild type (1J3L:pdb) \textit{P. falciparum} dihydrofolate reductase enzyme. The authors found that the bound ligand WR99210 was precisely reproduced by the docking procedures as demonstrated by low (<2.00 Å) root-mean-square deviations (Bharatam and Adane, 2010). The results indicated that most of the compounds dock into the active sites of both the wild type and quadruple mutant \textit{P. falciparum} dihydrofolate reductase enzymes.

**CONCLUSION**

There is a need to obtain new antimalarial drugs as malaria is one of the most important World health problems. This can be possible only by achieving a deep insight knowledge of \textit{Plasmodium species} mechanism. To gain comprehension of genomics and proteomics aspects of \textit{Plasmodium} is very crucial. Bioinformatics tools were used in our study to gain knowledge of evolutionary variations held on DHFR and GST proteins of different \textit{Plasmodium species}. Mefloquine exhibited the highest binding affinity (kcal/mol) with DHFR in all four \textit{Plasmodium species} whereas with respect to GST it exhibited the highest binding affinity in three \textit{Plasmodium species} except for \textit{P. malariae}. Docking analysis of receptor-ligand complex demonstrated that both mefloquine and chloroquine are capable of binding and inhibiting the DHFR and GST proteins of \textit{Plasmodium species}. Hence, further modification in Mefloquine and chloroquine may make them unique and potent inhibitor against DHFR/ GST among all four \textit{Plasmodium species} which will further help in controlling the worldwide spread of malaria.

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**Conflict of Interest**

The authors declare that there is no conflict of inter-

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