Molecular Docking: A Tool to Discover Potent Antimalarial Drug against Resistant *P. falciparum*

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Abstract

Malaria is one of the most prevalent parasitic diseases in tropical regions of the world which causes about 300-500 million clinical cases and 1.5-3 million deaths per year. Emergence of drug-resistant P. falciparum parasite is one of the major factors responsible for today's widespread occurrence of malaria which compromised clinical uses of the available antimalarial drugs such as chloroquine, cycloguanil and pyrimethamine. Dihydrofolatereductase enzyme is responsible for the replication of the parasite inside the human body. Antimalarial drugs work by inhibiting Dihydrofolatereductase enzyme. In certain regions the parasite was found to have developed resistance to certain anti-malarial drugs. Resistance found was due to the point mutations present on the pfdhfr gene sequence which is responsible for the production of Dihydrofolatereductase enzyme.

An In silico approach using docking of the receptor site of Dihydrofolatereductase enzyme with the available anti-malarial drugs was performed for prediction of potent drug. A molecular docking study was carried out on 28 compounds belonging to 2,4-diaminoquinazoline and 2,4-diaminopteridine analogs using Hex docking program and the X-ray crystallographic structures of the quadruple mutant (1J3K:pdb), Double mutant (1J3J:pdb) and wild type (1J3I:pdb) Plasmodium falciparum dihydrofolatereductase enzyme. The experimental conformation of the bound ligand WR99210 was precisely reproduced by the docking procedures as demonstrated by low (<2.00 Å) root-mean-square deviations. The results indicated that most of the compounds dock into the active sites of both the wild type and quadruple mutant P. falciparum dihydrofolatereductase enzyme.

Keywords: Malaria, Plasmodium falciparum, Drug resistance, Dihydrofolatereductase, Molecular Docking

1. Introduction

Malaria disease causes one to two million deaths each year-equal to150 to 300 deaths each hour (WHO, 2008; Breman, 2009). It is caused by protozoan parasites of the genus *Plasmodium*. The malarial 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. [1] In certain regions, however, the parasites have developed resistance to certain antimalarial drugs, particularly chloroquine. Patients in these areas require treatment with other more expensive drugs. The effectiveness of chloroquine against *P. falciparum* has declined as resistant strains of the parasite evolved[19-23].There are four major species of the malaria parasite of which *Plasmodium falciparum* causes the most virulent form of malaria and is responsible for more than 95% of malaria-related morbidity and mortality. Since the discovery of natural product quinine, structural modifications of its quinolone pharmacophore led to the development of most

effective antimalarial agents namely chloroquine (CQ),mefloquine and amodiaquine (AQ) and pyrimethamine-sulfadoxine (fansidar) was another best therapeutic option after CQ but rendered ineffective in most of malaria endemic regions due to spread of resistance. Currently, natural endoperoxideartemisinin and its semi-synthetic derivatives (atremether, arteether and artesunate) are the most potent and fast acting antimalarials effective against resistant strains of *P. falciparum*.

To keep pace with the continuously evolving resistant parasite, there is challenge and urgency to develop cost-effective and efficacious antimalarials with low potential of inducing resistance. [19-23]

The Dihydrofolatereductase (DHFR) is one of the well-defined and successfully exploited targets in malarial chemotherapy. Dihydrofolatereductase enzyme is responsible for the replication of the parasite inside the human body. These antimalarial drugs work by inhibiting Dihydrofolatereductase enzyme. Pyrimethamine and cycloguanil, the two important therapeutic drugs commonly employed for the prophylaxis and treatment of malaria target the DHFR. However, in the recent years, rapid spread of antifolate resistant *P. falciparum* seriously compromised the clinical utilities of these drugs and consequently necessitates the need to search for new potent antifolate antimalarials [37].

Molecular docking methods are widely used by pharmaceutical industries and academic institutes to study drug - target interactions in order to understand the basic electronic/steric features required for therapeutic action and to design new drug candidates with improved activities. The information generated acid residues in the binding pockets of targets, and also used to predict the corresponding binding affinities of ligands [35].

Toyoda *et al.* used molecular docking studies to identify potential antimalarial agents such as2-amino-1,4-dihydro-4,4,7,8-tetramethyl-s-triazino[1,2]benzimidazole and pyridoindole from commercially available compounds[36]. Rastelli *et al* .also used this approach to discover new classes of pfDHFR enzyme inhibitors which are structurally different from the classical antifolates [37]. Dasgupta*et al* .carried out ahigh-throughput *in silico*screening of database with consequent *in vitro* enzymatic assay and cellular culture studies [38]. They identified three novel biguanideanalogs which were found to be active against both the wild type and quadruple mutant *Pf*DHFR enzymes. Fogel *et al.* also employed the same approach to study the binding interactions of analogs of 3in the active sites of the wild type and multiple drug-resistant *Pf* DHFR enzymes [39]. Molecular docking also helps in target guided design and synthesis of lead compounds which could be developed as inhibitors of DHFR enzymes from different species including wild type and mutant strains of *Pf* DHFR enzymes [40].

Recently Ommeh *et al*. reported the antiplasmodial activities of the drug resistant (V1/S) strain of *P. falciparum* [41]. Based on their observations of the *in vitro* activity test of 7 in combination with dapsone, Ommeh *et al.* suggested that these compounds could competitively bind in the active site of the DHFR enzyme of *P. falciparum*, and act as DHFR inhibitors. But no molecular docking studies were carried out by them or other research groups to substantiate this suggestion. Thus, we were interested to study the binding modes of these compounds in the binding regions of quadruple mutant and wild type *Pf* DHFR enzyme (fig-2). Most of the compounds also have potentially flexible side chains. To the best of our knowledge, no similar study has been reported.

This prompted us to carry out the present study in order to examine their binding interactions and orientations in the active sites of the above mentioned enzymes. Thus, the binding modes (orientations), scores and their interactions with key amino acid residues will be used for the discussion.

1.1 Epidemiology

Co-infection with HIV and malaria does cause increased mortality; this is less of a problem than with HIV/tuberculosis co-infection, due to the two diseases usually attacking different age-ranges, with malaria being most common in the young and active tuberculosis most common in the old. Although HIV/malaria co-infection produces less severe symptoms than the interaction between HIV and TB, HIV and malaria do contribute to each other's spread. This effect comes from malaria increasing viral load and HIV infection increasing a person's susceptibility to malaria infection [5].

1.2 Drugs

There are a number of different types of malaria medicine that the healthcare provider can recommend. Most of the time, these medications can be taken by mouth. Patients with severe *Plasmodium falciparum* malaria, or who cannot take medications by mouth, can be given the treatment through an intravenous line (IV) [3]. In some countries, some antimalarial drugs are found in suppository form.

Specific medications used for treating malaria include [9-23]:

1. Chloroquine
2. Mefloquine (Lariam®)
3. Atovaquone-proguanil (Malarone®)
4. Sulfadoxine-pyrimethamine (Fansidar®)
5. Quinine
6. Doxycycline

In addition, the medication primaquine can be used to treat the forms of malaria parasites that may lay dormant in the liver; it can help prevent malaria relapses such parasites may cause. Pregnant women should not take primaquine. Also, people who are deficient in G6PD (glucose-6-phosphate dehydrogenase) should not take the drug. Patients should not take primaquine until a screening test has excluded G6PD deficiency [23]

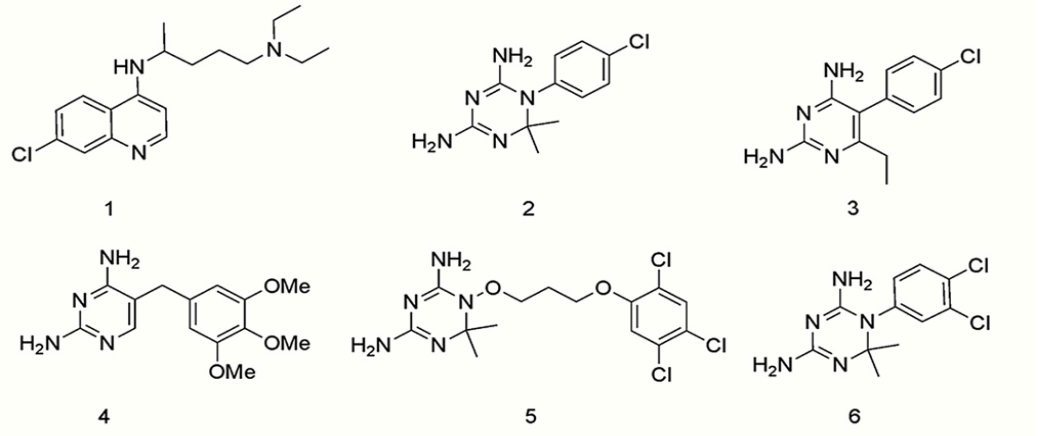

Figure 1: Structures of commonly used drugs for malaria

1.3 Drug Resistance

Though drugs are available for treatment of malarial infection from last few decades resistance has been observed to these drugs by the malarial species and to be more specific by *Plasmodium falciparum*. These resistances are found to be due to point mutations present on certain target genes like *pfcrt, pfdhfr* on which the drugs act. [23]

1.4 Mutations

Drug resistance is the ability of the parasite species to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limit of tolerance. Drug resistance is most commonly seen in *P. falciparum*. Resistance to chloroquine is most prevalent, while resistance to most other anti-malarials has also been reported. [9-23]

The point mutations observed on *pfdhfr* gene are as follows:

- At the site 51 asparagine is replaced by isoleucine i.e. N51I
- At the site 59 cysteine is replaced by arginine i.e. C59R
- At the site 108 serine is replaced by asparagine i.e. S108N
- At the site 164 isoleucine is replaced by asparagine i.e. I164N

Purpose of folate pathway is to produce reduced folate cofactors to act as donors of methyl groups necessary for synthesis of DNA base pairs and aminoacids. [33-35].
Folate antagonists. [27-32] reversibly binds to dihydrofolatereductase (DHFR), Inhibits the formation of reduced folates. Therefore results in the following consequences:

- Ineffective nucleic acid synthesis and DNA production
- Strand breakage and ineffective repair mechanism
- Ultimately cell death
- Specific for the S-phase of cell cycle

Longer exposure to methotrexate allows for more cells to be replicating and be exposed to cytotoxic effects of methotrexate. Intracellularly addition of glutamyl residues forms methotrexate polyglutamate. This has the following consequences:

- More likely to be formed with longer periods of drug exposure
- Greater binding affinity for DHFR
- Increases intracellular half-life of methotrexate
- Occurs more readily in malignant cells leading to sustained levels and prolonged duration of action. [33]

2. Materials and Methods

2.1 Materials:

2.1.1. Databases:

I. PDB

The crystal structure of the protein can be obtained from Protein Data Bank (PDB), which is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids and resource for studying biological macromolecules. [23]

2.1.2. Visualization Tools:

I. SPDBV

Swiss-PdbViewer (Version 4.o1) is an application that provides a user friendly interface allowing analyzing several proteins at the same time. It can open PDB/mmCIF/molSDF files. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Energy of each structure and its Ramachandran plot can also be computed.

II. CHIMERA

UCSF Chimera (Version 1.4) is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles. High-quality images and animations can also be generated. Chimera is segmented into a core that provides basic services and visualization, and extensions that provide higher level functionality

2.1.3. Structure Drawing:

I. ChemSketch

ChemSketch is a chemical structure drawing program developed by ACD/Labs. It has the ability to:

- Draw and view structures in 2D, or render in 3D to view from any angle
- Draw reactions and reaction schemes, and calculate reactant quantities
- Generate structures from InChI and SMILES strings
- Generate IUPAC systematic names for molecules of up to 50 atoms and 3 ring structures
- Predict logP for individual structures
- Search for structures in the built-in dictionary of over 165,000 systematic, trivial, and trade names

2.1.4. Docking Tools:

I. SCHRÖDINGER SOFTWARE SUITE[44]

Schrödinger software suite is drug design software using both ligand and structure-based methods. [48] Schrödinger provides accurate, reliable, and high performance computational technology to solve real-world problems in life science research.

II. HEX 6.1

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. *Hex* can also calculate protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes

2.1.5. Data set of compounds

A data set of 28 compounds consisting of 2,4-diaminoquinazoline, 2,4-diamino-5,6,7,8-teterahydroquinazoline and 2,4-diaminopteridine analogs were used in this study. The in vitro growth inhibitory activities of the compounds against multiple drug-resistant (V1/S strain) *Pf*DHFR enzyme have been reported in literature. [41]

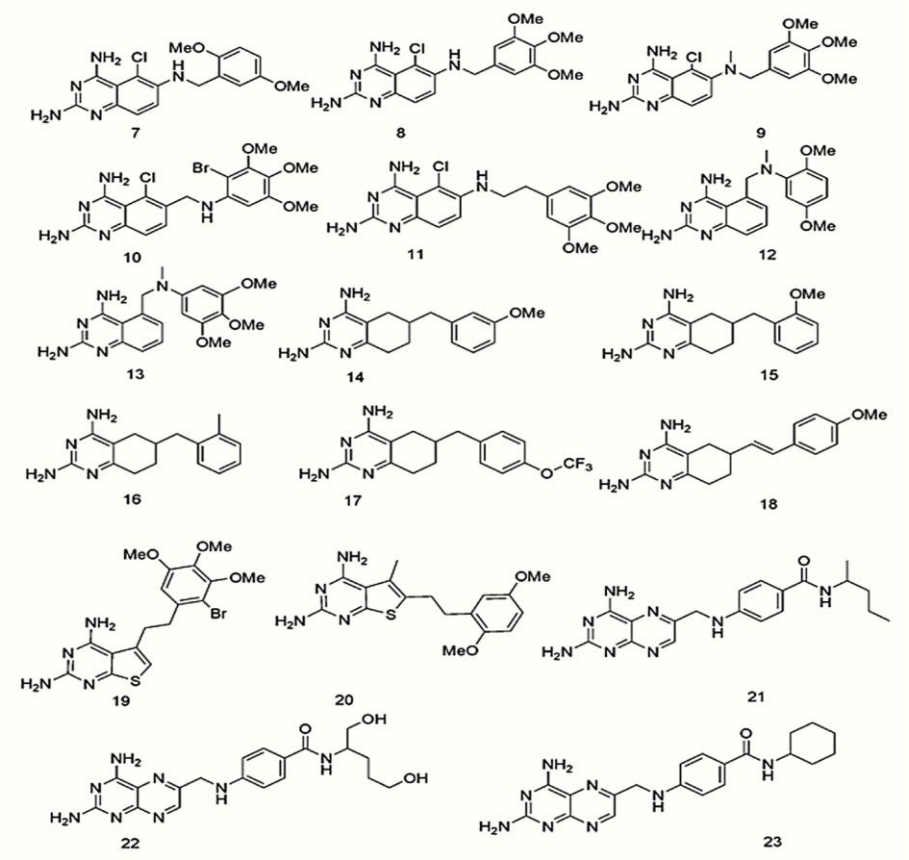

Figure 2.1: Chemical structures of the compounds used in study [41]

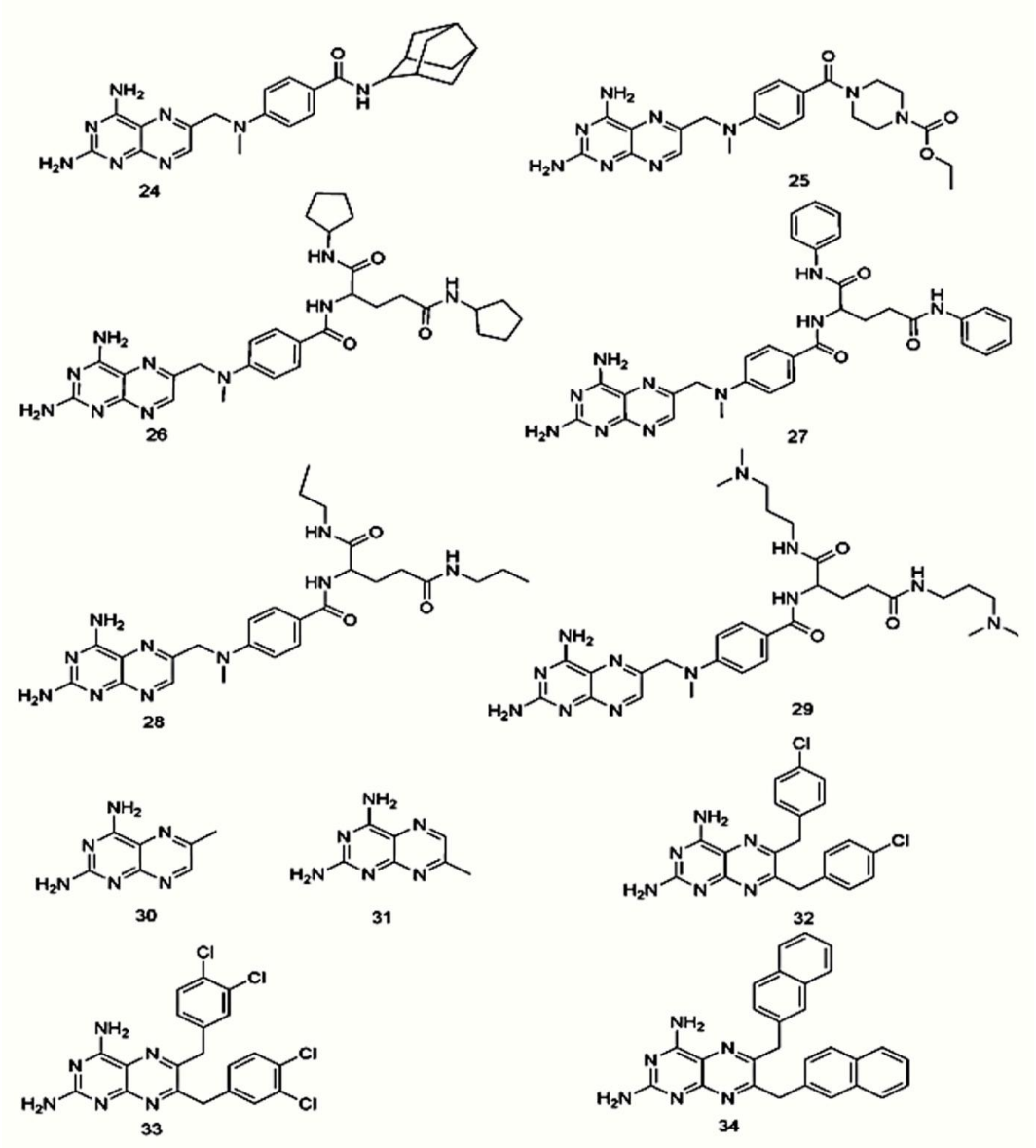

Figure 2.2: Chemical structures of the compounds used in study [41]

2.2 Methodology:

2.2.1 Protein Structure Preparation:

1. The Plasmodium falciparum Dihydrofolatereductase enzyme protein structures (wild type, double mutant, quadruple mutant) having PDB id as 1j3i; 1j3j and 1j3k were downloaded from RCSB PDB (protein data bank) in' .pdb' format [24].
2. The downloaded pdb structure was opened using Maestro Schrödinger, the structure was split into the components(i.e. bound ligands ,water, hydrogen and the protein structure free from all these) [43]
3. Minimization of structure was done by specifying formal charges by Gasteiger charge method [47].
4. Change Selenomethione to methionine(MSE to MET)
5. Mutate residue with incomplete side chains with ALA or GLY.
6. If alternate location keep only highest occupancy.

7. Add charge, delete solvent, and add hydrogen by steric method, His protonation.
8. Solvent was removed using SPDV

2.2.2 Ligand Structure Preparation

1. The compounds used in the study were built using ACD /ChemSketch
2. The structure of the compounds was saved in .mol2 format.
3. Minimization of the ligand structure was done using chimera
4. The compounds were saved in .pdb format.

2.2.3 Docking Protocol

Hex 6.1 docking software was used for the molecular docking studies of the antimalarial drugs with the three pfdhfr enzymes (wild, double mutant, quadruple mutant). Following protocol was followed:

1. The Receptor and the ligand were loaded in the Hex software.
2. Using the tools the ligand was placed closer to the receptor.
3. In the docking panel following options were selected:
   - Correlation Type… Shape+ electrostatics
   - FFT Mode… 3D Fast Lite
   - Post Processing… MM Minimization
   - Grid Dimensions … 0.6
   - Solutions… 500
   - Receptor Range… 45
   - Receptor Step size… 7.5
   - Ligand Range… 45
   - Ligand Step Size… 7.5
   - Twist Range… 360
   - Twist Step Size… 5.5
   - Distance Range… 40
   - Scan step… 0.75
   - Sub steps… 2
   - Steric Scan… 16
   - Final Search… 25
4. Generated results were saved as ‘both’.
5. The generated results were analysed for the binding energy.
6. By using Swisspdb viewer software hydrogen bonds were visualized.

3. Observations and Results

3.1 Quadruple Mutant (1J3K)

Table 1. Binding energies (E values) of the Drug analogues when docked with quadruple mutant pfDHFR enzyme using Hex 6.1 docking software.

Drug Analogue	E_{total}	E_{max}	E_{min}
7	-315.3	-126.23	-267.17
8	-313.2	-149.95	-277.54
9	-326.3	-158.31	-306.07
10	-380.4	-194.64	-353.88
11	-290.4	-136.38	-276.71
12	-218.6	-98.54	-188.17
13	-210.8	-102.62	-197.07
14	-236.2	-110.12	-195.68
15	-238.5	-109.55	-212.71
16	-241.9	-106.48	-213.07
17	-416.6	-191.42	-379.90
18	-241.3	-103.82	-210.83
19	-271.3	-139.06	-257.62
20	-237.5	-114.94	-210.35

Drug Analogue	E_{total}	E_{max}	E_{min}
21	-238.2	-102.47	-238.17
22	-303.1	-126.07	-256.10
23	-276.6	-125.77	-229.97
24	-331.4	-138.85	-245.42
25	-399.3	-151.41	-299.02
26	-332.2	-103.83	-261.10
27	-342.4	-115.65	-299.33
28	-301.6	-99.81	-263.71
29	-403.1	-122.58	-315.38
30	-172.2	-84.19	-146.08
31	-200.7	-70.45	-135.97
32	-382.7	-170.98	-345.71
33	-497.4	-235.86	-474.67
34	-305.7	-130.89	-289.12

Following compounds showed lowest E-values:

- 33: (-497.4)
- 17: (-416.6)
- 29: (-403.1)

3.2 Double Mutant (1J3J)

Table 2: Binding energies (E values) of the Drug analogues when docked with double mutant type pfDHFR enzyme using Hex 6.1 docking software.

Drug Analogue	E_{total}	E_{max}	E_{min}
7	-323.8	-265.58	-120.42
8	-296.0	-263.83	-141.55
9	-341.7	-312.48	-148.84
10	-351.9	-324.99	-183.92
11	-289.3	-266.92	-116.49
12	-240.3	-193.82	-92.47
13	-221.7	-191.67	-95.66
14	-215.9	-191.50	-105.70
15	-220.3	-188.00	-94.87
16	-218.6	-169.19	-92.59
17	-387.4	-333.66	-125.37
18	-266.4	-213.85	-88.70
19	-294.8	-226.77	-102.39
20	-225.4	-225.41	-106.75

Drug Analogue	E_{total}	E_{max}	E_{min}
21	-276.9	-211.15	-107.53
22	-268.8	-248.97	-121.63
23	-231.4	-231.43	-132.59
24	-307.5	-227.51	-132.79
25	-358.9	-278.40	-144.83
26	-355.0	-261.19	-115.68
27	-347.4	-306.22	-125.88
28	-306.4	-251.30	-111.91
29	-424.85	-306.91	-139.95
30	-185.5	-162.06	-80.23
31	-202.12	-168.10	-71.70
32	-333.2	-301.49	-151.99
33	-465.62	-449.98	-214.61
34	-304.61	-267.79	-122.37

Following compounds showed lowest E values:

33: (-465.62)

29: (-424.85)

17: (-387.40)

3.3 Wild Type (1J3I)

Table 3: Binding energies (E values) of the Drug analogues when docked with wild type pfDHFR enzyme using Hex 6.1 docking software.

Drug Analogue	E_{total}	E_{max}	E_{min}
7	-305.8	-241.49	-110.37
8	-278.5	-252.60	-129.73
9	-320.5	-287.03	-139.34
10	-303.9	-275.20	-149.37
11	-274.2	-246.69	-116.67
12	-267.0	-215.19	-93.67
13	-264.6	-210.29	-102.43
14	-272.2	-197.73	-99.97
15	-250.8	-194.19	-103.90
16	-237.9	-213.10	-114.46
17	-423.5	-328.01	-117.70
18	-259.57	-194.88	-96.87
19	-304.1	-237.95	-109.66
20	-293.0	-226.09	-109.82

Drug Analogue	E_{total}	E_{max}	E_{min}
21	-320.5	-247.21	-109.43
22	-246.0	-245.96	-123.68
23	-325.8	-245.52	-127.05
24	-271.3	-271.28	-135.87
25	-395.5	-296.86	-158.33
26	-363.89	-295.47	-126.22
27	-339.3	-286.63	-117.22
28	-311.2	-258.15	-105.83
29	-427.4	-304.68	-149.54
30	-185.0	-160.96	-79.50
31	-216.6	-158.99	-86.64
32	-341.1	-305.27	-136.36
33	-443.3	-428.91	-183.68
34	-288.7	-270.14	-121.75

Following compounds showed lowest E values:
33: (-443.3)
29: (-427.4)
17: (-423.5)

3.4 Interactions

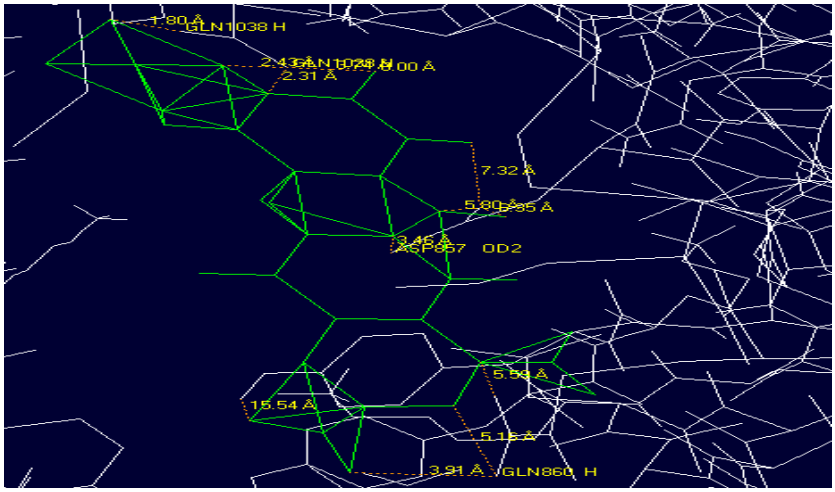

Figure 3: Binding interactions of the drug analog no. 17 with the wild type pfDHFR enzyme (PDB: 1j3i)

Table 4.1: Binding interactions of the drug analog no. 17 with the wild type pfDHFR enzyme (PDB: 1j3i)in terms of bond length (in A^o units).

GLN 860 H	3.91 A^o
GLN 1038 H	3.74 A^o
ASP 857 OD2	3.46 A^o
GLN 1038 H	1.80 A^o
GLN 1038 H	2.31 A^o
GLN 1038 H	2.43 A^o

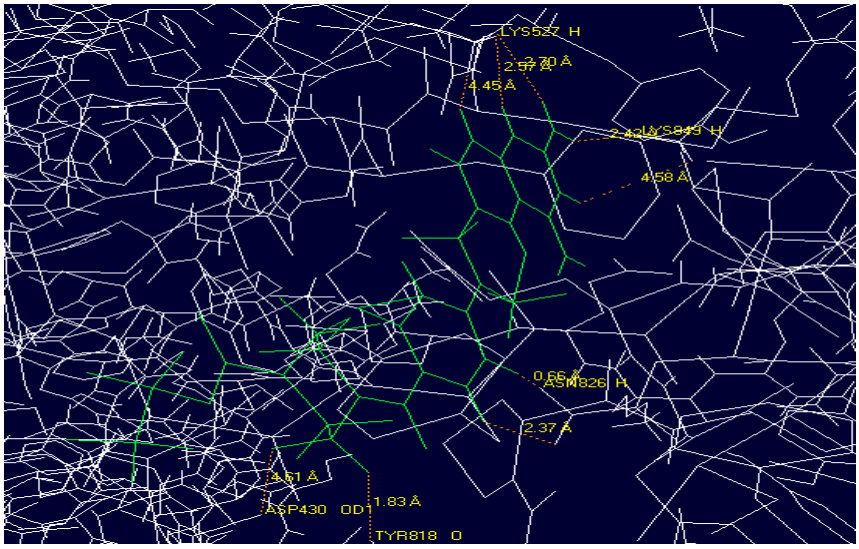

Figure 4: Binding interactions of the drug analog no. 33 with the wild type pfDHFR enzyme (PDB: 1j3i)

Table 4.2: Binding interactions of the drug analog no. 33 with the wild type pfDHFR enzyme (PDB:1j3i)in terms of bond length (in A° units).

ASN 826 H	0.66 A
LYS 849 H	2.42 A
TYR 818 O	1.83 A
ASN 826 H	2.37 A
LYS 527 H	2.57 A
LYS 527 H	2.70 A
LYS 527 H	4.45 A

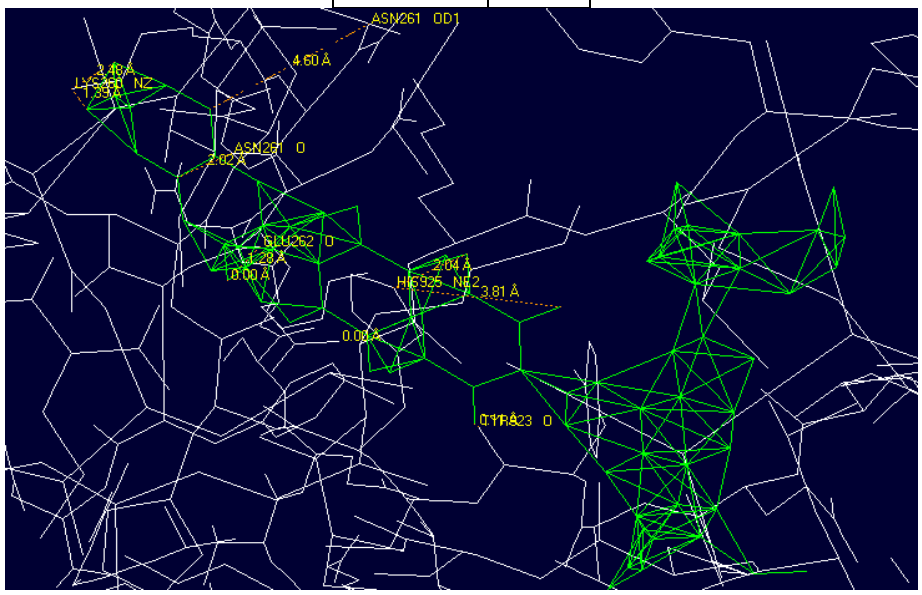

Figure 5: Binding interactions of the drug analog no. 29 with the wild type pfDHFR enzyme (PDB: 1j3i)

Table 4.3: Binding interactions of the drug analog no. 29 with the wild type pfDHFR enzyme (PDB: 1j3i) in terms of bond length (in A° units).

TYR923 O	0.11A
HIS925 O	5.03A
HIS925 NEZ	3.81A
HIS925 O	2.04A
LYS260 NZ	1.39A
LYS260 O	2.48A
ASN261 O	2.02A
ASN261 OD1	4.60A
GLU262 O	1.28A
GLU262 OE1	4.27A

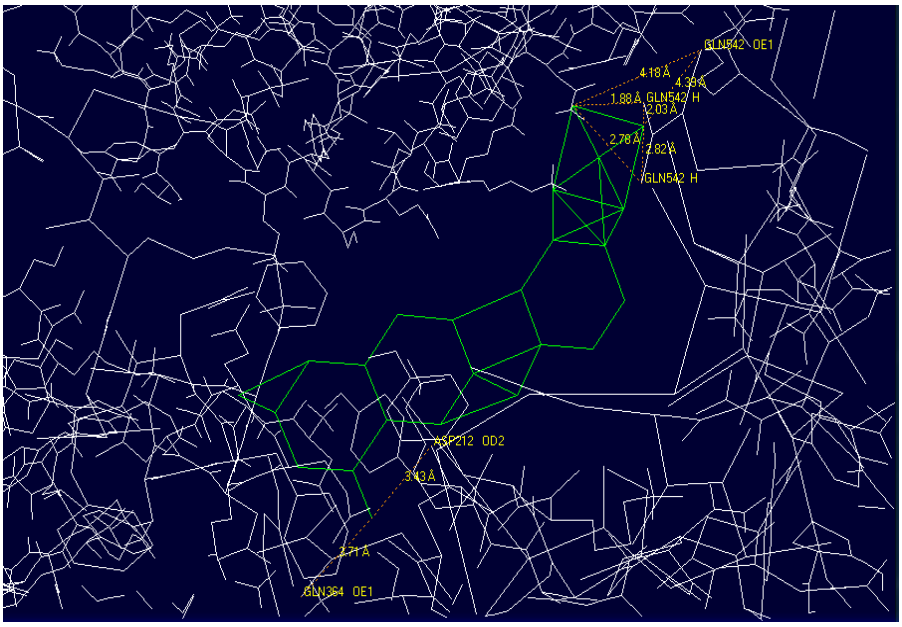

Figure 6: Binding interactions of the drug analog no. 17 with the mutant type (quadruple mutant) pfDHFR enzyme (PDB: 1j3k)

Table 4.4: Binding interactions of the drug analog no. 17 with the quadruple mutant type pfDHFR enzyme (PDB: 1j3k) in terms of bond length (in A° units).

GLN 542 H	1.88A
GLN 542 H	2.03A
GLN 542 H	2.78A
GLN 542 H	2.82A
ASP 212 OD2	3.43A
GLN 364 OE1	3.71A
GLN 542 OE1	4.18A
GLN 542 OE1	4.39A

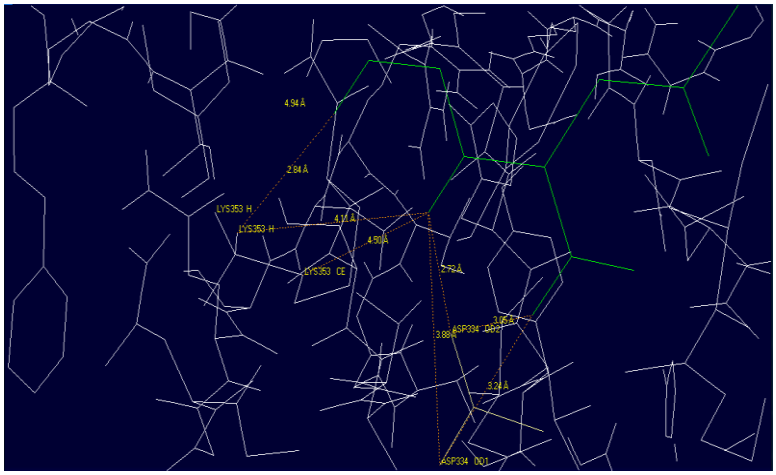

Figure 7: Binding interactions of the drug analog no. 33 with the mutant type (quadruple mutant) pfDHFR enzyme (PDB: 1j3k)

Table 4.5: Binding interactions of the drug analog no. 33 with the quadruple mutant type pfDHFR enzyme(PDB:1j3k)in terms of bond length (in A° units).

LYS 353 H	2.84A
LYS 353 H	4.11A
ASP 334 H	4.94A
LYS 353 CE	4.50A
ASP 334 OD1	3.88A
ASP 334 OD1	3.24A
ASP 334 OD2	3.05A
ASP 334 OD2	2.72A

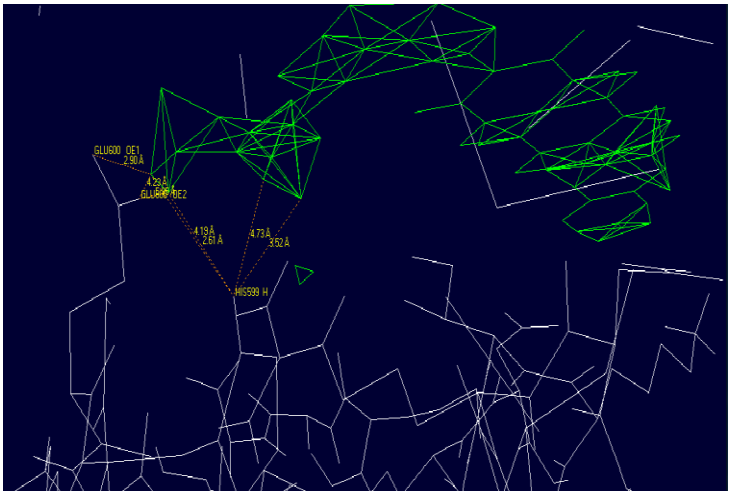

Figure 8: Binding interactions of the drug analog no. 29 with the mutant type (quadruple mutant) pfDHFR enzyme (PDB: 1j3k)

Table 4.6: Binding interactions of the drug analog no. 29 with the quadruple mutant type pfDHFR enzyme (PDB: 1j3k) in terms of bond length (in A° units).

ASN 34 H	2.47A
GLU 600H	4.76A
HIS 599 H	2.61A
ASN 565 OD1	4.24A
ILE 314 CG2	3.46A
ILE 314 CG2	1.24A
ASN 565 O	4.58A
ASN565 CA	2.78A

4. Conclusion

- On the basis of the binding energies (E values), those ligands (drugs) showing lowest E values were selected for the analysis.
- These compounds and their E values when docked with the wild type and mutant(Double mutant, quadruple mutant) are as follows:

Table 5: Binding energies (E values) of the selected drug analogues when docked with wild and mutant types of pfDHFR enzymes using Hex 6.1 docking software.

	29	**17**	**33**
Wild type	-427.4	-423.5	-443.3
Double mutant	-424.85	-387.5	-465.6
Quadruple mutant	-403.1	-416.6	-497.4

- All these Ligands showed increase in the E value when docked with the mutant types than when docked with the wild type pfDHFR enzyme.
- Also their interactions with the enzyme (binding interactions) were visualized using SPDBV.
- In this analysis, it was found that these ligands formed hydrogen bonds with the amino acid residues of the enzyme. Only the bonds with less than 5A were selected.
- It was observed that the ligands formed bonds with residues different than that observed in wild type (less than 5A)
- This might be due to the change in the conformation of structure caused due to the mutations.
- Thus on the basis of the decrease in the E value in the mutant types and binding interaction of the antimalarial drugs with mutant enzyme (protein); that was due to change in conformation of structure of the mutated protein, we can conclude that the antimalarial drug resistance was due to the mutations in the *pf*dhfr gene that is responsible for the production of the mutated *pf*dhfr enzymes which are resistant to the drugs.

On the basis of the lowest E value, it can also be concluded that the analogue number 33 is the most potent drug against the resistant malarial parasite. The same can be taken further for wet lab analysis for confirmation of results.

6. References

6.1. Journal Article

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