"DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS"

A PROJECT SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

BY

GAJJAR DHRUVIL D. (16BPH106)

Semester VIII

UNDER THE GUIDANCE OF

DR.VIVEK VYAS

INSTITUTE OF PHARMACY
NIRMA UNIVERSITY
SARKHEJ-GANDHINAGAR HIGHWAY
AHMEDABAD-382481
GUJARAT, INDIA

MAY 2020

"DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS"

CERTIFICATE

This is to certify that "DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS" is the bonafide work carried out by GAJJAR DHRUVIL (16BPH106), B.Pharm semester VIII under our guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2019-2020. This work is up to my satisfaction.

Guide:

Dr. VIVEK VYAS
M. Pharm., Ph.D.,
Designation,
Department of Pharmaceutical chemistry,
Institute of Pharmacy,
Nirma University

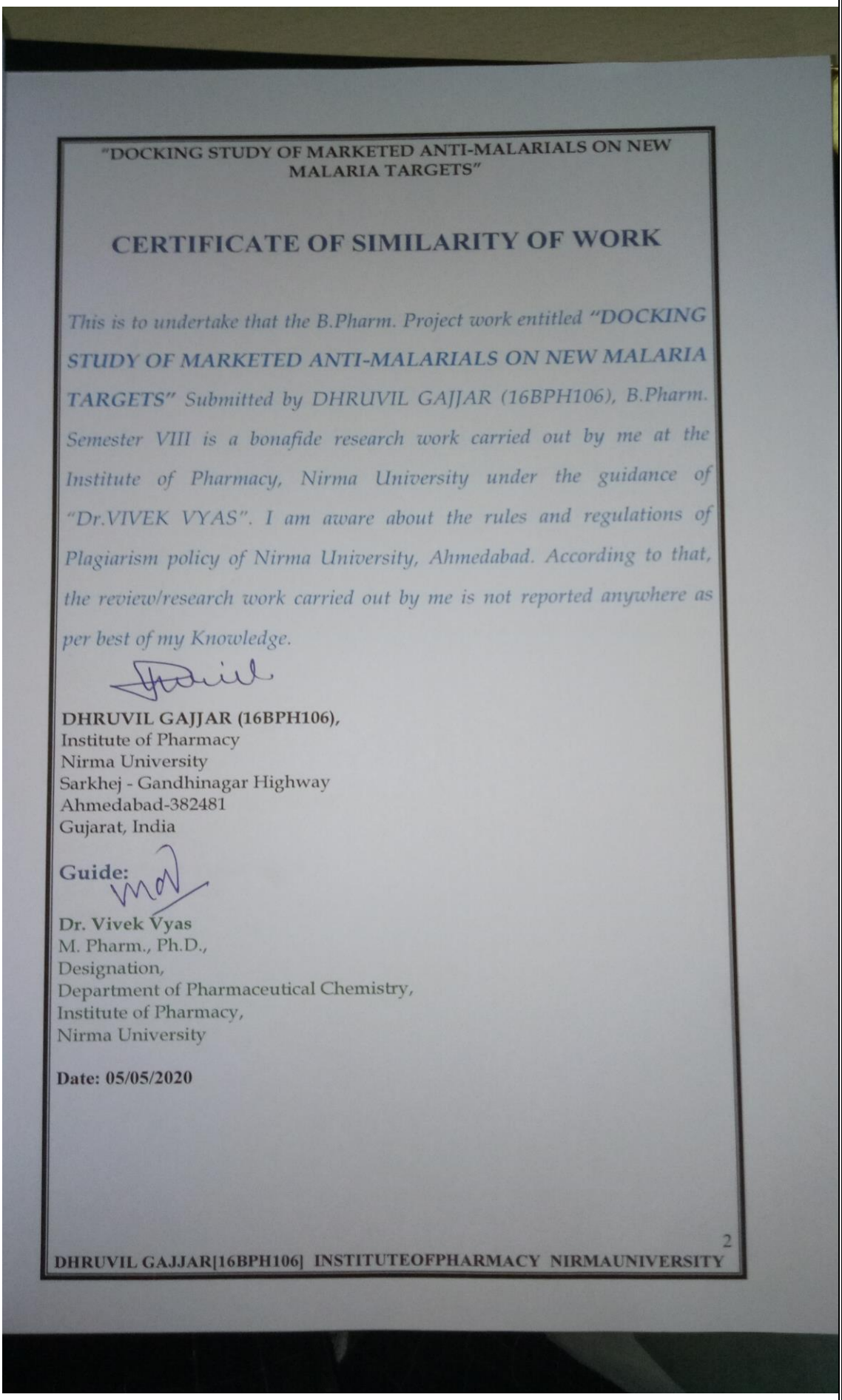
Dr. HARDIK BHATT
M. Pharm., Ph.D.,
Head, Department of Pharmaceutical chemistry,
Institute of Pharmacy
Nirma University.

17/7/2020

Prof.MANJUNATH GHATE
M. Pharm., Ph.D.,
Director,
Institute of Pharmacy
Nirma University.

Date: 05 /05/ 2020

1



"DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS"

CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work entitled "DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS" Submitted by DHRUVIL GAJJAR (16BPH106), B.Pharm. Semester VIII is a bonafide research work carried out by me at the Institute of Pharmacy, Nirma University under the guidance of "Dr.VIVEK VYAS". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the review/research work carried out by me is not reported anywhere as per best of my Knowledge.

DHRUVIL GAJJAR (16BPH106),
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Guide:

Dr. Vivek Vyas
M. Pharm., Ph.D.,
Designation,
Department of Pharmaceutical Chemistry,
Institute of Pharmacy,
Nirma University

Date: 05/05/2020

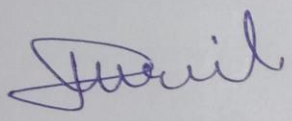
2

DHRUVIL GAJJAR[16BPH106] INSTITUTEOFPHARMACY NIRMAUNIVERSITY

"DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS"

DECLARATION

I,DHRUVIL GAJJAR (16BPH106), student of VIII[th] Semester of B.Pharm at Institute of Pharmacy, Nirma University, hereby declare that my project entitled "DOCKING STUDY OF MARKETED ANTI-MALARIAL ON NEW MALARIA TARGETS" is a result of culmination of my sincere efforts. I declare that the submitted project is done solely by me and to the best of my knowledge, no such work is done by any other person for the award of degree or diploma or for any other means. I also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.



DHRUVIL GAJJAR (16BPH106),
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Date: 05/ 05/ 2020

3
DHRUVIL GAJJAR[16BPH106] INSTITUTEOFPHARMACY NIRMAUNIVERSITY

"DOCKING STUDY OF MARKETED ANTI-MALARIALS ON NEW MALARIA TARGETS"

ACKNOWLEDGEMENT

To begin an acknowledgement without the mention of **Almighty** is like going into a voyage without a compass. I am very thankful to God for always behind me in my ups and downs. It is a delight to acknowledge those who have supported me over the last two year.

I owe to a debt of gratitude to **Dr. VIVEK VYAS** Assistant Professor, Institute of Pharmacy, Nirma University for the vision and foresight which inspired me to conceive this project. I am so thankful to have a caring, motivating, inspirational and friendly mentor like you. For me you are the **"BEST GUIDE FOREVER".** You are an excellent mentor and I appreciate all your hard work, it's meant so much to me. I am indebted to him for the encouragement and the freedom I enjoyed throughout.

How can I forget **TANVI SHUKLA**, best senior whom I ever met!! Without her support my docking wouldn't be completed and for that I am always thankful to her for helping me during my worst period of time.

I am deeply indebted and express my whole hearted thanks to **Prof. Manjunath Ghate,** Director of institute of pharmacy Nirma University and Also thankful to **Dr. Hardik bhatt** , head, department of pharmaceutical chemistry.

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

This thesis is only beginning of my journey.

Thank you...

DHRUVIL GAJJAR

16BPH106

4

DHRUVIL GAJJAR[16BPH106] INSTITUTEOFPHARMACY NIRMAUNIVERSITY

Table of Contents

ACKNOWLEDGEMENT ..... Error! Bookmark not defined.

CHAPTER 1 [ABSTRACT] **7**

CHAPTER 2 [INTRODUCTION] **9**

2.1- MORTALITY RATE IN A MONTH BY MALARIA 10
2.2- SOME ASPECTS OF MALARIA AS PER WHO & MMV 12
2.3- MECHANISM OF MALARIA 13

CHAPTER 3 [INTRODUCTION TO MOLECULAR DOCKING MATERIALS AND METHOD] **15**

3.1 - INTRODUCTION OF AUTODOCK SOFTWERE 16
3.2 - BASICS OF DOCKING STUDY 16
3.3 - EXAMPLE OF MULTI-STAGE SCREENING WORK FLOW 17
3.4 - DEALS WITH THE FLEXIBILITY OF PROTEIN 17
3.5 - PREPARATION OF RECEPTOR 17
3.6 - THE PREPARATION OF LIGAND 17
3.7 - DOCKING SOFTWERES 17
3.8 - SCHMATIC REPRESENTATION OF DOCKING 18
3.9 – DOCKING METHOD IN DOCKING SOFTWERE 18

CHAPTER 4 [SELECTED TARGETS FOR DOCKING] **19**

4.1 - DHFR 20
4.2 - CATALYSIS MECHANISM 21
4.3 - PROTONATION MECHANISM 22
4.4 – HOW DOCKING OCCUR IN DHFR ENZYME 23
4.5 - DHODH 25
4.6 – SITE OF ACTION 25
4.7 – A DRUG TARGET FOR ANTIMALARIALS{DHODH} 25

CHAPTER 5 [DOCKING RESULTS AND DISCUSSION] 26

5.1 - DOCKING OF 8-DHFR(DIHYDRO FOLATE REDUCTASE) ENZYME WITH NADPH [DIHYDRO-NICOTINAMIDE-ADENINE-DINUCLEOTIDE PHOSPHATE] LIGAND. 27

5.3 - DOCKING OF 8-DHFR (DIHYDRO FOLATE REDUCTASE) ENZYME WITH HYDROXY CHLOROQUINE LIGAND.....31

5.5 - DOCKING OF 8-DHFR (DIHYDRO FOLATE REDUCTASE) ENZYME WITH ARTEMISININ LIGAND. 34

5.7 - DOCKING OF 8-DHFR (DIHYDRO FOLATE REDUCTASE) ENZYME WITH PRIMAQUINE LIGAND. 37

5.9 - DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE ENZYME WITH HYDROXY CHLOROQUINE LIGAND.....39

5.11 - DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE ENZYME WITH MEFLOQUINE LIGAND. 41

5.13 - DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE) ENZYME WITH DAPSON LIGAND.....43

5.15 - DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE) ENZYME WITH DOXYCYCLINE LIGAND.....45

CHAPTER 6 [CONCLUSION] 47

CHAPTER 7 [SUMMERY]..... 49

CHAPTER 8 [BIBLIOGRAPHY]..... 51

CHAPTER 1

ABSTACT

1-ABSTRACT

Malaria is a very crusial disease as per the WHO (world health organisation). Malaria is one of the most pivotal parasitic diseases in humans and the malarial parasite transmission in above 100 countries of a population of five million people. Malaria is primarily happen by Genus Plasmodium protozoan parasites. But much of the transmission occurs by female anopheles mosquito. The other infecting species are a variety of hosts, including reptiles, birds, rodents and primates. There are several other medications commercially available but Proguanil is a malaria-fighting prophylactic agent. The malarial parasite such as Plasmodium falciparum and P.vivex ceases.
It replicates in the blood and inhibits a reductase of the enzyme dihydrofolate. Describe the common types of new antimalarial objectives in this article. There's several drugs available on the malaria market in this form of study but prefer some new drugs that have been tested on the targets. There's many different targets available but DHODH and DHFR showed strong potential and that's why we have preferred these targets for a docking study. Here we should take out a molecular docking study of the different malaria drugs and also know about the docking software. In the docking softwere we are going to make a whole docking study of the receptor and ligand. So in that docking I had used drugs which is from the different classes. These types of the drugs will informed us for the binding site of the drug. Thus I used to make a different coloumn and the content of the drugs for the binding. Anti malarial drugs will be docked and then described it binding site of the content. There are plenty of binding sites with the molecule which we will dock and from that which sites have a good potential to bind with the drugs and show a good interaction. In docking study we can know about the potency of the drug or their binding property with the drug. There are certain targets which will bind with the drugs and show some results.

Keywords : Malaria , Dihydrofolate reductase , DHFR , DHODH , molecular docking.

CHAPTER 2

INTRODUCTION

2.0 - INTRODUCTION

Malaria is caused by a genus protozoan plasmodium parasite and both humans and mosquito depend on malaria parasite. Malaria is a chronic condition andone that can be treated and prevented. Life can be preserved if very early and adequate diagnosis of such ailments. Which are the action is needed to stop the diseases and avoid or contain epidemics and other vital conditions is known Dihydrofolate reductase is a small enzyme that plays a significant role in developing DNA and other pathways, nevertheless.

The enzyme thymidylate synthase uses these carbon atoms to produce thymine bases as an important piece of DNA. It control the state of folate, a organic molecule that carbon atoms to enzymes that need them in their reactions of special importance. This will have to be reused after folate has released its carbon atoms. This is the work done by dihydrofolate reductase. Many anti-malarial treatments are used to treat malaria. Patients suffering from malaria were administered specific combinations of drugs. Proguanil is a prophylactic anti-malarial medication, which works by blocking development of a malaria parasite plasmodium falciparum vivex once it is in the RBC.

Malaria is a severe infectious disease that may have caused disease most frequently in hundreds of millions of people suffering every year. Malaria is caused by different Plasmodium species, including four well-known Plasmodium species triggering human malaria, namely P. falciparum, P.vivax, P.ovale and P.malariae. A fourth one is P.knowlesi has recently been recorded to cause human infection in many other Southeast Asian countries. P. falciparum affects the most severe illnesses and malaria deaths.

Parasites of erythrocytic malaria degrade hemoglobin as the main source of amino acids for its growth and survival. To provide amino acids for erythrocytic malaria parasite, the Plasmodium falciparum cysteine protease falcipain-2 hydrolyzes haemoglobin in the acid food vacuole. Furthermore, falcipain-2 has been used for further analysis. There are quite a number of antimalarial drugs.

The malarial study included the different types of the content which may includes the prevention protocol of the malaria and its importance of drugs.

The parasite has developed resistance to several antimalarial drugs, most notably chloroquine. There are so many drugs available for malaria (table-1). The main emphasis of this work is to identify the most appropriate drug molecule for the disease.

2.1 MORTALITY RATE IN A MONTH BY MALARIAL PARASITE

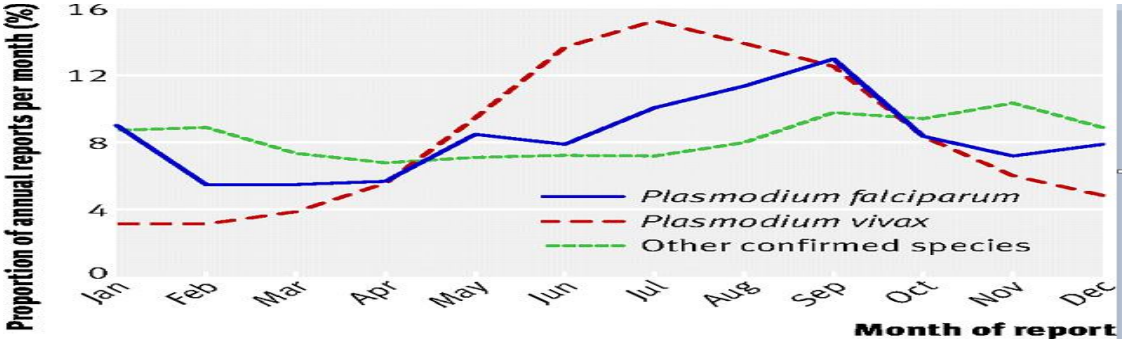
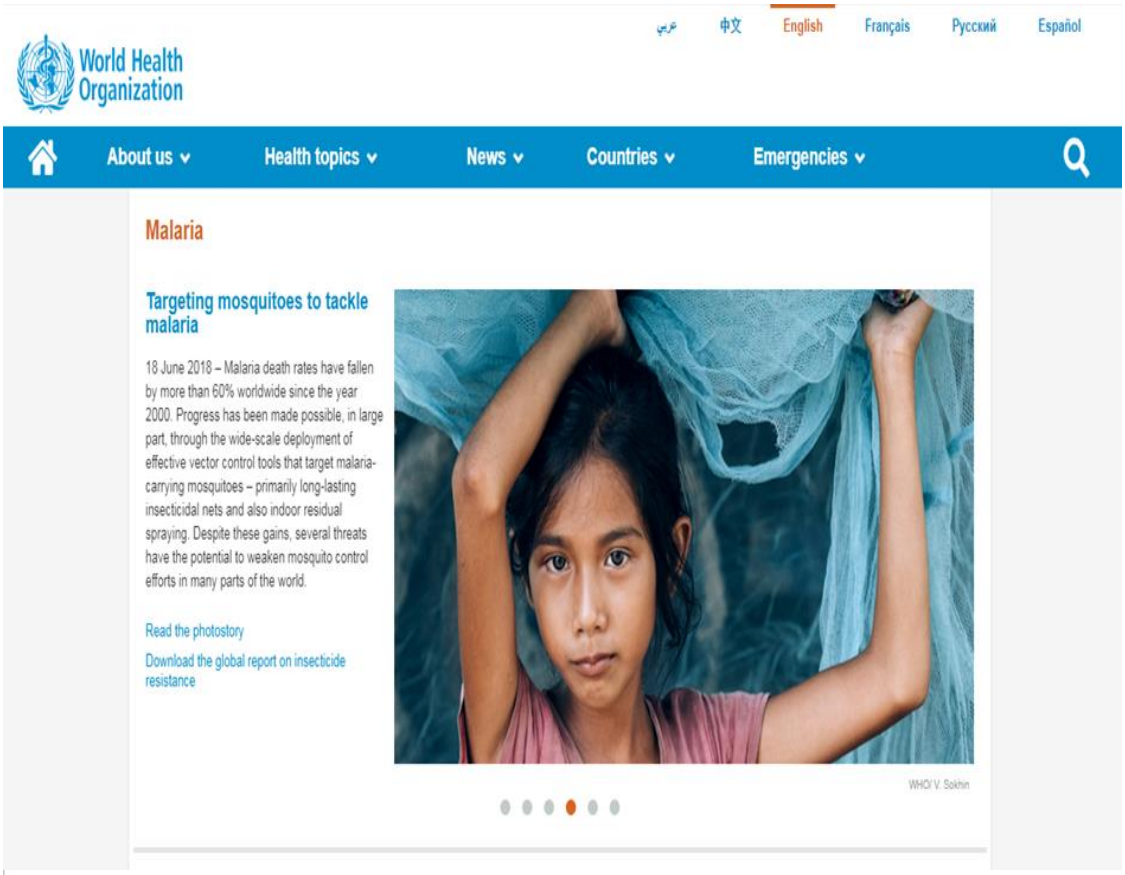


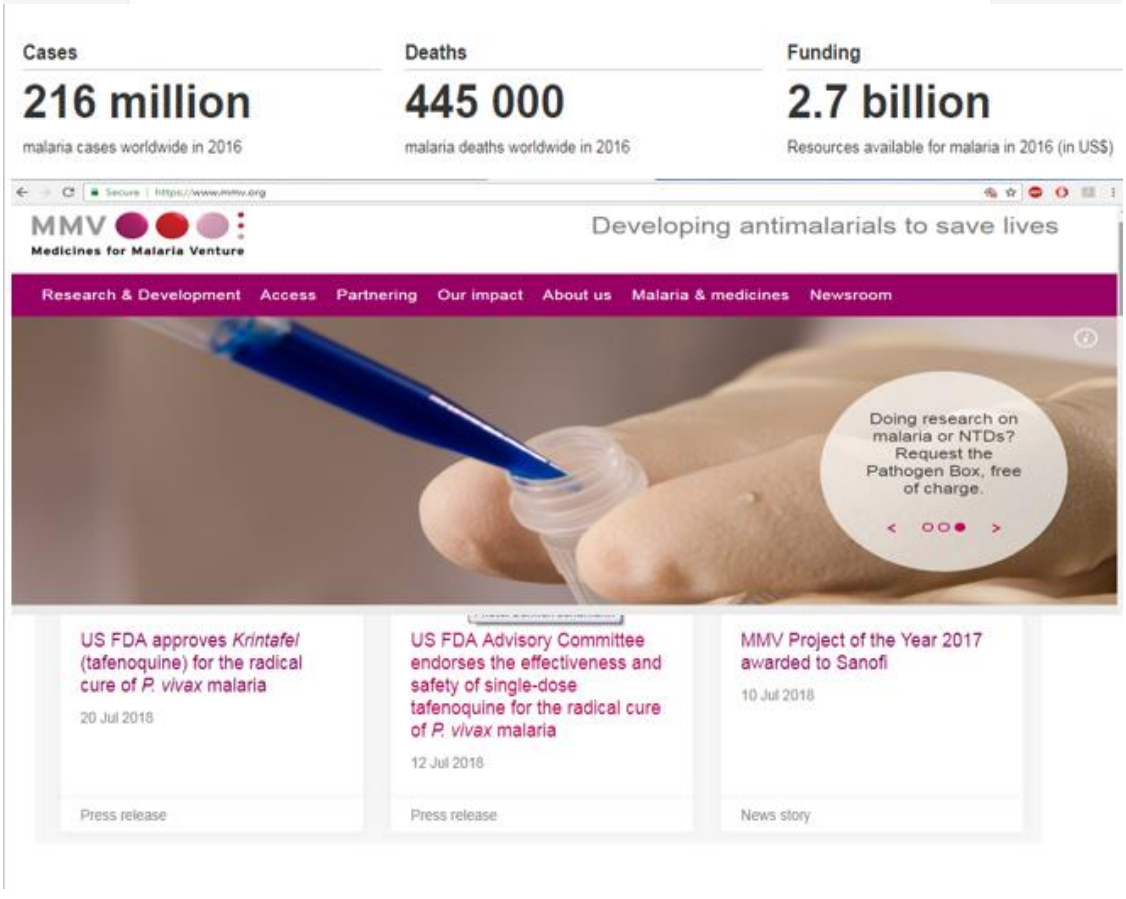
Table : 1 – Anti malarial drugs with binding energies.

Sr.No	DRUG NAME	BINDING ENERGY(Kcal/mol)	MW	Log P	H donor	H acceptor
1	Amodiaquine	-7.61	355.8611	2.6	2	4
2	Artemether	-6.32	298.3746	3.1	0	5
3	Artemisnin	-7.17	282.3322	2.8	0	5
4	Artesunate	-6.65	384.4208	2.5	1	8
5	Atovaquone	-7.79	366.8375	5.2	1	3
6	Chloroquine	-6.54	319.8721	4.6	1	3
7	Dapsone	-5.64	248.3009	1	2	4
8	Dihydroartemisnin	-6.85	284.3481	2.5	1	5
9	Mefloquine	-7.5	387.3122	3.6	2	9
10	Primaquine	-7.35	259.3467	2.2	2	4
11	Proguanil	-8.61	253.7312	1.5	3	5
12	Pyrimethamine	-6.82	248.7114	2.7	2	4

These are the some drugs with their binding energy. From these , we are taking some drugs and dock with the protein target and see the affinity towards the binding.

2.2 SOME ASPECTS OF MALARIA AS PER WHO AND MMV



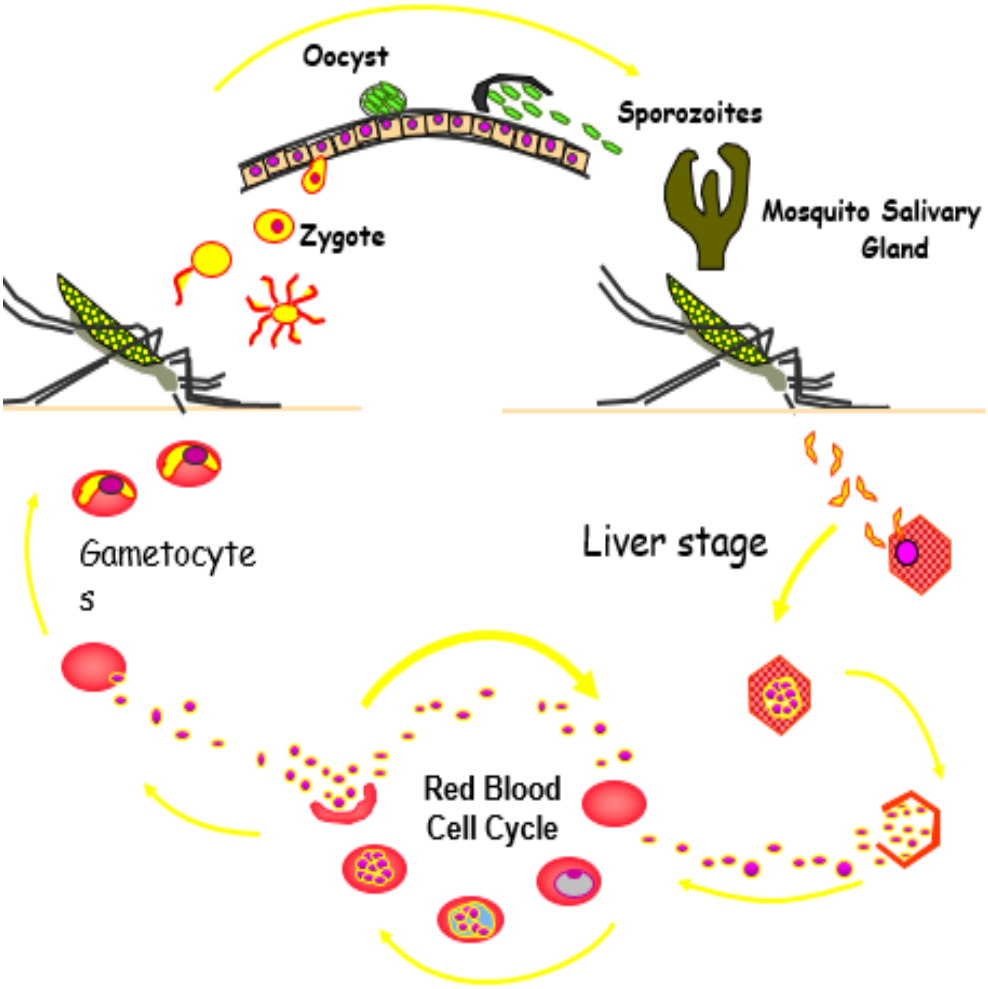


2.3 - INTRODUCTION: MECHANISM OF MALARIA

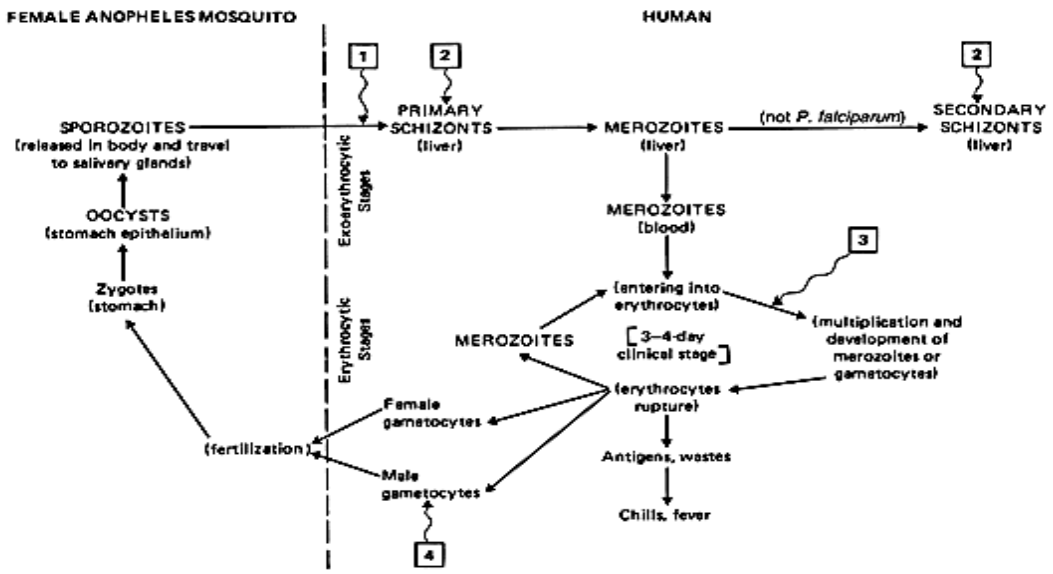
The first and the foremost reason is transmission of parasitic plasmodium to the patients by female anopheles mosquites.

LIFE CYCLE OF MALARIA PARASITES

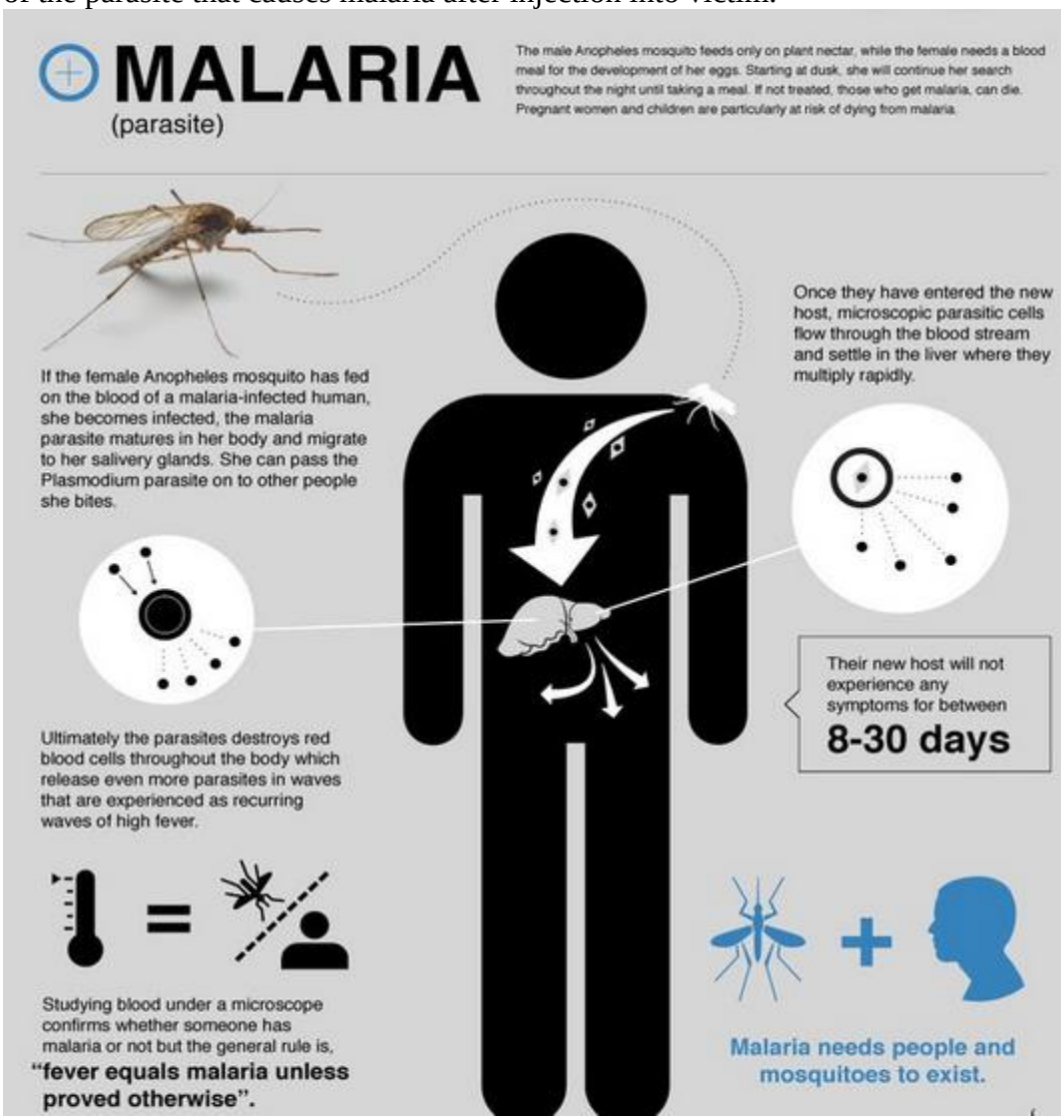
The most common diagram described about the transmission of malaria by the mosquitoes. First of all vector is anopheles mosquitoes and these mosquitoes goes inside the human. After that it makes a zygote and it is converted in to a oocyst. The oocyst is also introduced in to the sporozoites of the mosquito. Another reason is that it will be goes in to the salivary gland of the human and it is imparted in the liver stage. After these step is done, in the liver stage the RBCs will ruptured. These ruptured RBCs converted in the gametocytes and it will goes in the infected mosquitoes. This is the huge life cycle of the malaria.



This is a schematic diagram of the whole malarial life cycle.



This is the schematic representation of the life cycle of malarial parasite and the stages of the parasite that causes malaria after injection into victim.



CHAPTER 3

INTRODUCTION TO MOLECULAR DOCKING MATERIALS AND METHOD

3 – MOLECULAR DOCKING MATERIALS & METHOD

3.1 INTRODUCTION OF AUTO DOCK SOFTWERE

An autodock softwere is a free softwere for performing a docking study. In these softwere we can perform the whole docking such as preparing a receptor to prepare a ligand file. It gives a accurate result of the binding of ligand and receptor as well as give the good results of binding energies.

3.2 BASICS OF DOCKING STUDY

We are looking for the receptor (protein) and ligand rigid. In which , the binding of the protein and ligand rigid thus it can bind properly and show activity in the protein binding and drug.

The popular approach are the rigid receptor, flexible structure, these two are the proper ligand approaches and the receptor ligand approaches.

Newer approach , receptor and ligand flexible. These two are the advanced options of the docking study.

FAST, SIMPLE → SLOW, COMPLEX

Mainly, two types of the docking.

- **FIRST IS THE POSE GENERATION**

In that , place drug in binding site and basically we solved part included in this department.

- **SECOND IS THE SELECTION OF POSE**

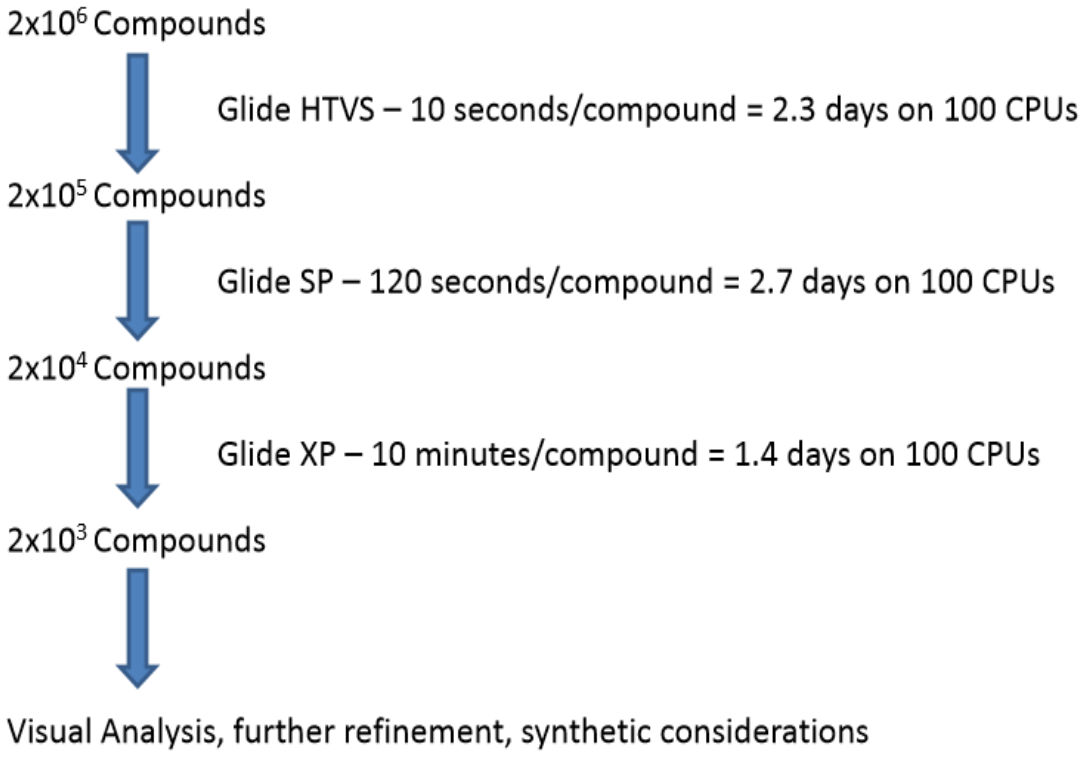
In this kind of method is little bit hard as compared to the pose generation and also done the determination of the proper pose.

The most famous site the focused on the research and famous function of the scoring will take longer and also done by the studies with multi-stages.

Virtual screening is one of the best part of the single compound will be too slower selected part.

These two are the main part of the molecular docking studies.

3.3 EXAMPLE OF MULTI-STAGE SCREENING WORK FLOW



3.4 DEALS WITH THE FLEXIBILITY OF PROTEIN.

- For the flexibility purpose we have to add the vdw ratio.
- Then we will use the flatter function.
- After that we can adding the mutation of the alanine amino acids.
- We can also add the structure of the receptor for the multiple input.
- Another part is the induced docking and for the glide and slower.

3.5 PREPARATION OF RECEPTOR

- Here we are going to explain the the preparation of the receptor.
- First of all doing the selection of the structure.
- Then we Are selecting a one chain and then remove an another chain.
- After that putting the different charges such as compute geister.
- Also remove the water molecule and metals.

3.6 THE PREPARATION OF LIGAND

- First of all open a ligand in the softwere.
- Then add the all hydrogens.
- Also calculate the charges and set charge field.
- Another thing is the combination of the pH range and adjust it.
- Then it saved as a ligand pdbqt file of the ligand.

3.7 DOCKING SOFTWERES

These are the some docking softwere, in which we can do a docking as our purpose.

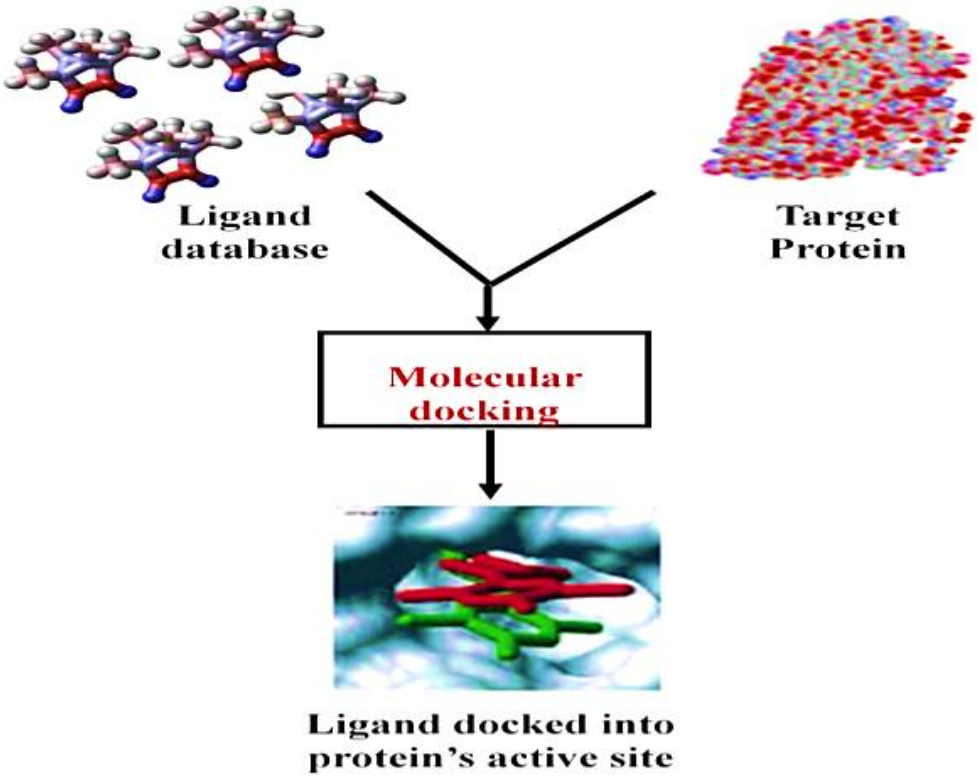
Free

- Auto dock is the free softwere which is use for the docking study.
- Another softwere is the swiss dock which is also used for the docking and the educational purpose.

PAID SOFTWERES

- First is the gold softwere.
- Second is the glide, which can be used in the different companies.

3.8 SCHEMATIC REPRESENTATION OF MOLECULAR DOCKING



3.9 DOCKING METHOD IN AUTO DOCK SOFTWERE

Here I m describe steps of docking in an autodock softwere.

1- Download a pdb file of receptor from the PDB and make a pdbqt file of the receptor.
2- Then make a ligand file as a same way of receptor preparation and named as a ligand pdbqt.
3- After that prepared a grid box and write a dimensions as a conf.txt file.
4- Once this all things are done then open a command prompt and give the commands.
5- After givan the successful command the result will be declared.
6- After it will be opened in the autodock vina result and show the binding energies.
7- After that we can see the different interactions of the ligand and receptor.

CHAPTER 4

SELECTED TARGETS FOR DOCKING

4. SELECTED TARGETS FOR DOCKING

There are plenty of targets available for malaria but here we are going to select some targets which have a strong potential like DHFR , DHODH etc.

1- DHFR
2- DHODH

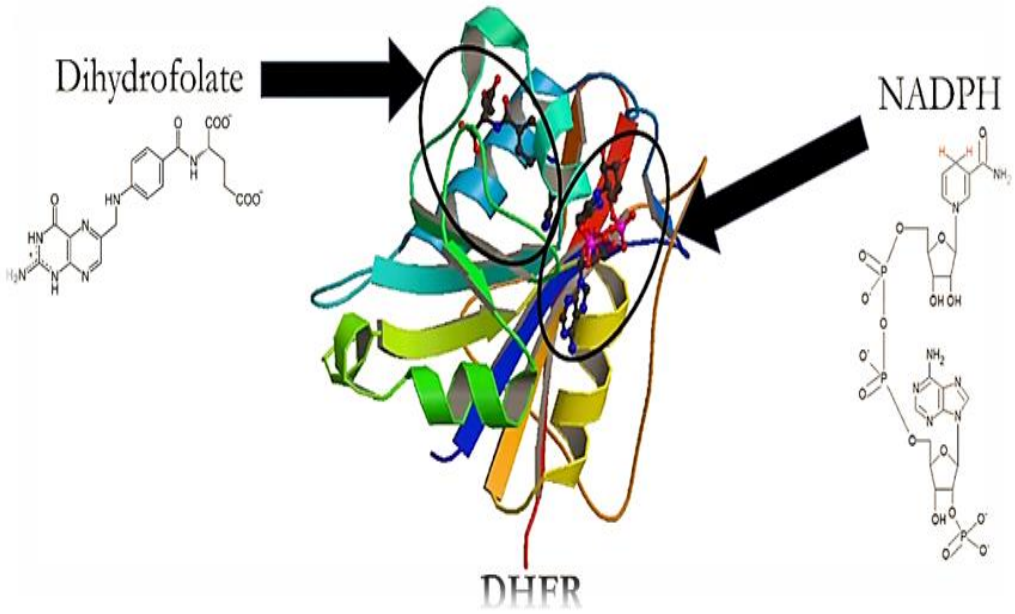
4.1 – [1] DHFR

Dihydrofolate reductase (dhfr) was first introduced by unintentionally such as the researchers searching for the folate dependent enzymes.

It is included the 1-C metabolism and its documented for the anti-pyretic and anti-biotic application.

Basically dhfr is the small protein with large binding site and also illustrates that DHF will bind to its co factor NADPH and its enzyme.

dihydrofolate reductase (DHF) binds to TWO biological such as substrate or NADPH.



Mainly this is focused on the structure of the di hydofolate reductase and its binding sites of the specific amino acids and its catalysis.

Another reason is that the compound will be inhibit DHFR and its application towards the anti-fungal and malarial agents.

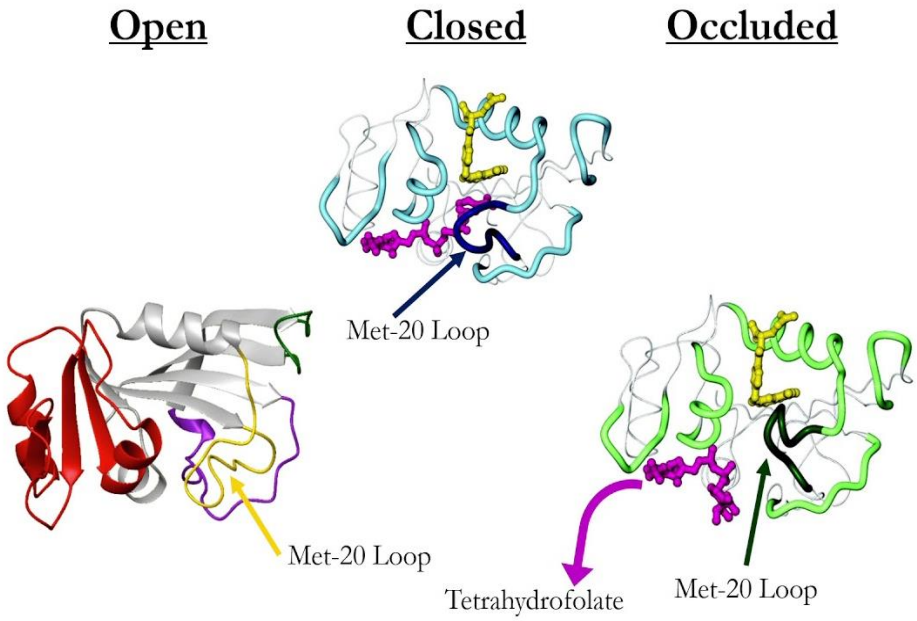
They also find the resistence of the reason and its way to overcome them the recent popular drugs under its classes and its clinical trials.

4.2 CATALYSIS (DHFR) MECHANISM :

- Here I had attached the three basic structures of the dhfr, open , closed and occluded respectively.

- DHFR catalysis involves the conversion of 7,8 dihydrofolate to 5,6,7,8 tetra hydeofolate reductase and this will done by using the NADPH as the co enzyme.

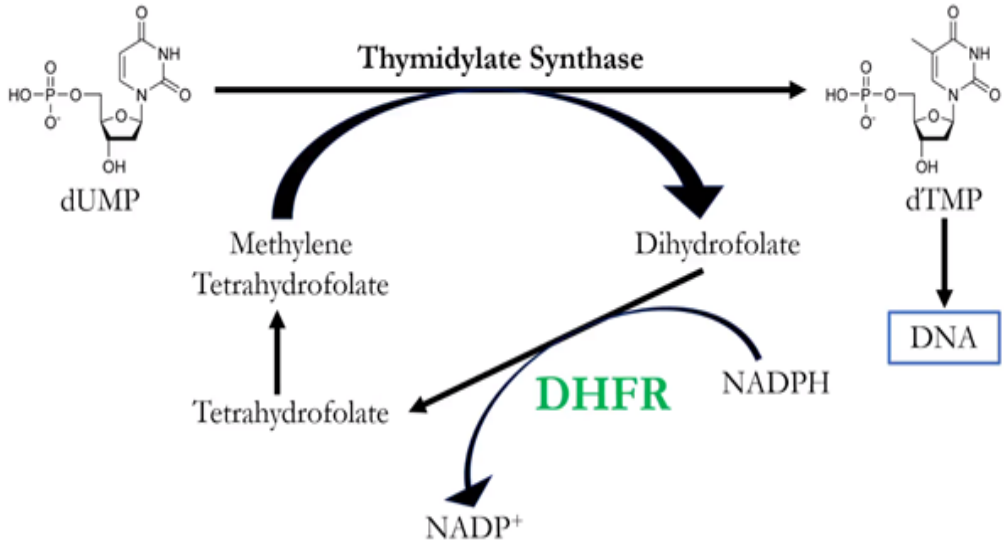
- The pivotal stage is the hydride molecule to substrate and it will utilizing the reaction mechanism with the specifying transition state.



You can think of DHFR like little biological recycle bin it takes a molecule that's produced during biological processes that the cells don't want and recycles it into a usable molecule specifically DHFR take dihydrofolate produced during DNA synthesis pathway and recycles it into tetrahydrofolate.

There are several biological pathway produced DHF as a product but one of the most important is a thymidine synthase pathway in which converts de oxy uranium monophosphate or dUMP into de oxy thymidine phosphate dTMP is an essential component of DNA synthesis.

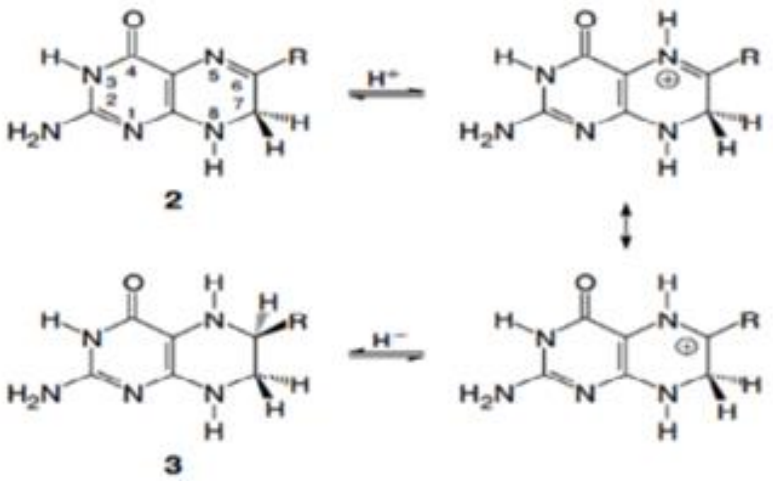
This pathway requires oxidation of methylene tetrahydrofolate into dihydrofolate and that methylene tetrahydrofolate comes from regular tetrahydrofolate .
If the reaction proceeded into infinity, you eventually developed dihydrofolate and THF depletion that blocked this DNA pathway from completely inhibiting DNA synthesis through inducing cell death, so how to replenish our tetrahydrofolate the **DHFR answer.**



This is the open and close structure of the DHFR and also shows the binding affinity of the substrate.

4.3 PROTONATION MECHANISM.

- In the study of the protonation there are two main stages occurred in the study.
- First is the substrate protonation and second is the transfer of hydride ions.
- In this type amino acids involves in the protonation and aspartic and glutamic acids will also took part in the humans. (glu 30 is the main site in the dhfr).

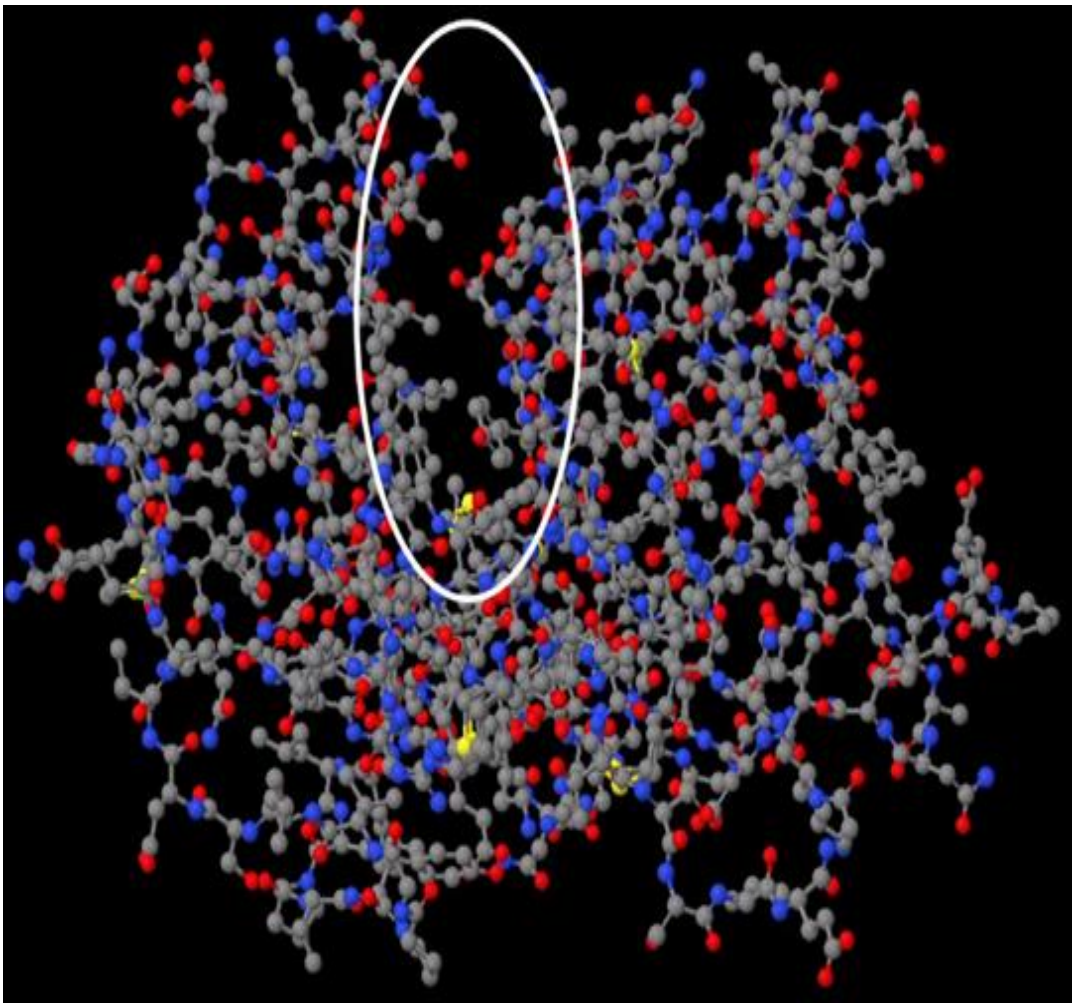


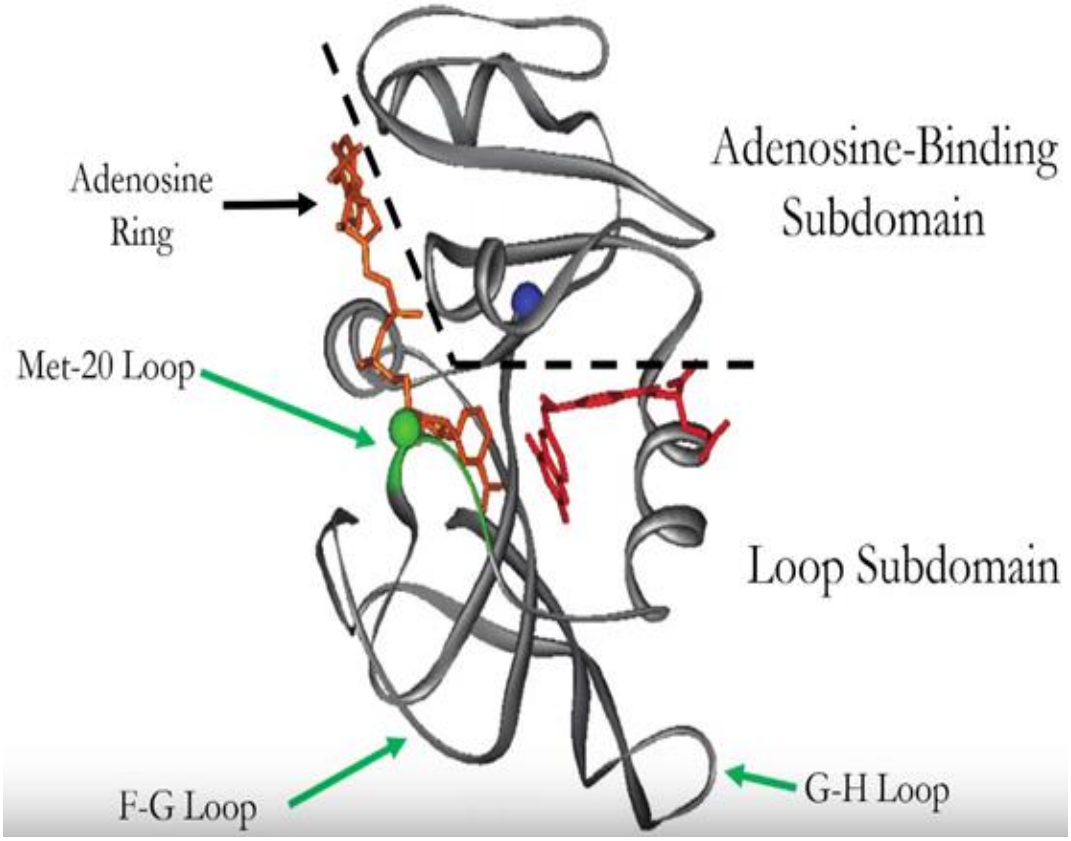
4.4 HOW DOCKING OCCUR IN DHFR ENZYME

- Aqueous environment surrounding DHFR , DHF and NADPH bind to DHFR,
- DHF and NADPH bind to active site which is shape like long grove that runs through the protein.
- The active site is located between DHFR's two subdomains the (1)adenosine binding subdomain .
- contain three short sequence of amino acids residues called loops.
- the (1) met 20 loop(Residue10-20)
- (2) FG loop (residue 117-131)and (3) GH loop(146-148)

met loop contain 9-24 ring structure in DHFR this loop of amino acids is pivotal for stabilizing nicotinamide structure ring and NADPH enzyme.

It illustrates in this page and next page, how docking occur in DHFR enzyme.





- In this picture we can see the different sites of the DHFR receptor also shows the binding sites of the receptor.

- These also contain the loop subdomain and amino site chain for the binding purpose.

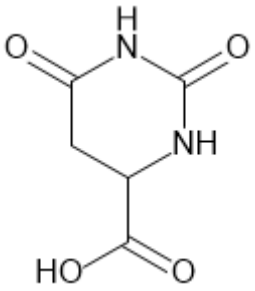
4.5 – [2] DHODH

An enzyme is dihydroorotate dehydrogenase (DHODH).That in humans chromosome 16 is encoded by the DHODH gene.
The protein encoded by this gene, in de novo pyrimidine biosynthesis.
Another protein is know as a inner mitochondrial protein (IMM) which is also used in this mechanism.

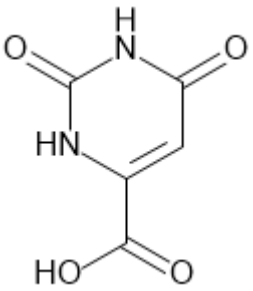
4.6 - SITE OF ACTION

- These all includes the conversion of the FMN to FMNH2.

dihydroorotate + O_2 **orotate + H_2O_2**



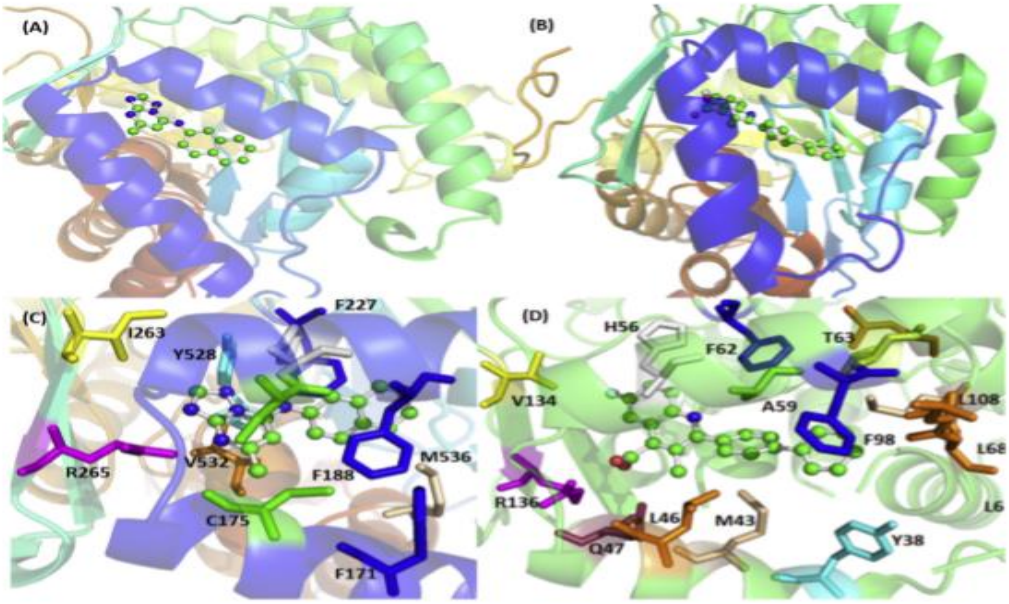
(**4,5-Dihydroorotic acid)**



(**Orotic acid)**

Basically it includes the two mechanism including dehydrogenation of the orotic acid by DHODH enzyme and these contains the two different classes.
1st is to break the orotic bond of the DHODH.
2nd is the breaking of bond including the iminium in to orotic acids.

4.7- A DRUG TARGET FOR ANTIMALARIALS [DHODH]



- Here the structure (A) illustrates the ribbon diagram of DSM1 which is bound to PfDHODH and this is the **ID (PDB ID 3I65.)**

CHAPTER 5

DOCKING RESULTS AND DISCUSSION

5.0 - DOCKING

Here I attach the docking study, procedure and results of the ligand and receptor binding. I took different types of the ligand and two receptors and then performed a docking. So here I will illustrates the all parameters related to docking and its results.

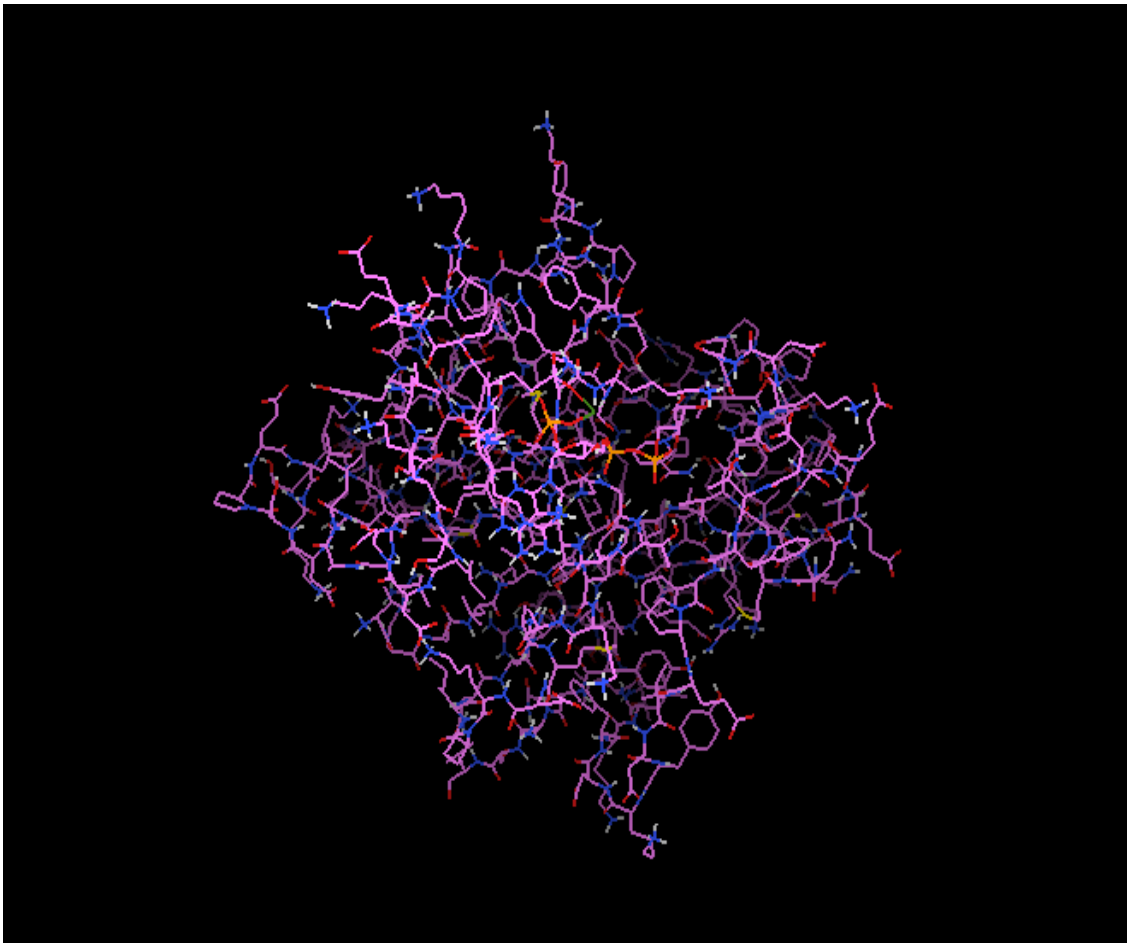
5.1- DOCKING OF 8-DHFR(DIHYDRO FOLATE REDUCTASE) ENZYME WITH NADPH [DIHYDRO-NICOTINAMIDE-ADENINE-DINUCLEOTIDE PHOSPHATE] LIGAND.

- Firstly I choose the receptor which is 8-DHFR and then it was downloaded from the RCSB PDB(PROTEIN DATA BANK).Basically it is the site where all kinds of receptors are available.

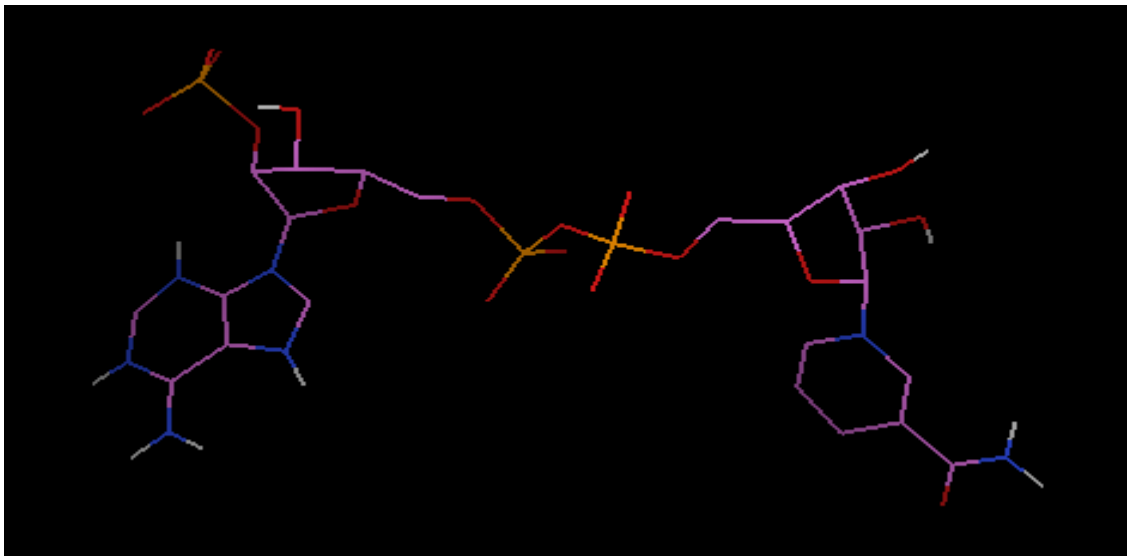
- Then the preparation of 8-DHFR was started and this file saved as a 8dhfr.pdbqt, after the preparation of this file showed in an autodock softwere.

- Same as above , the ligand was prepared and saved as 8-dhfrligand.pdbqt.

- Here I m attached the 2 file of ligand and receptor.



IMG : 1- RECEPTOR [8-dfr.pdbqt]



IMG : 2 - LIGAND [8dfrligand.pdbqt]

- After the preparation of these two files , prepared a grid file of the receptor ligand binding and saved as a **conf.txt** file.

8dfrconf.txt - Notepad

File Edit Format View Help

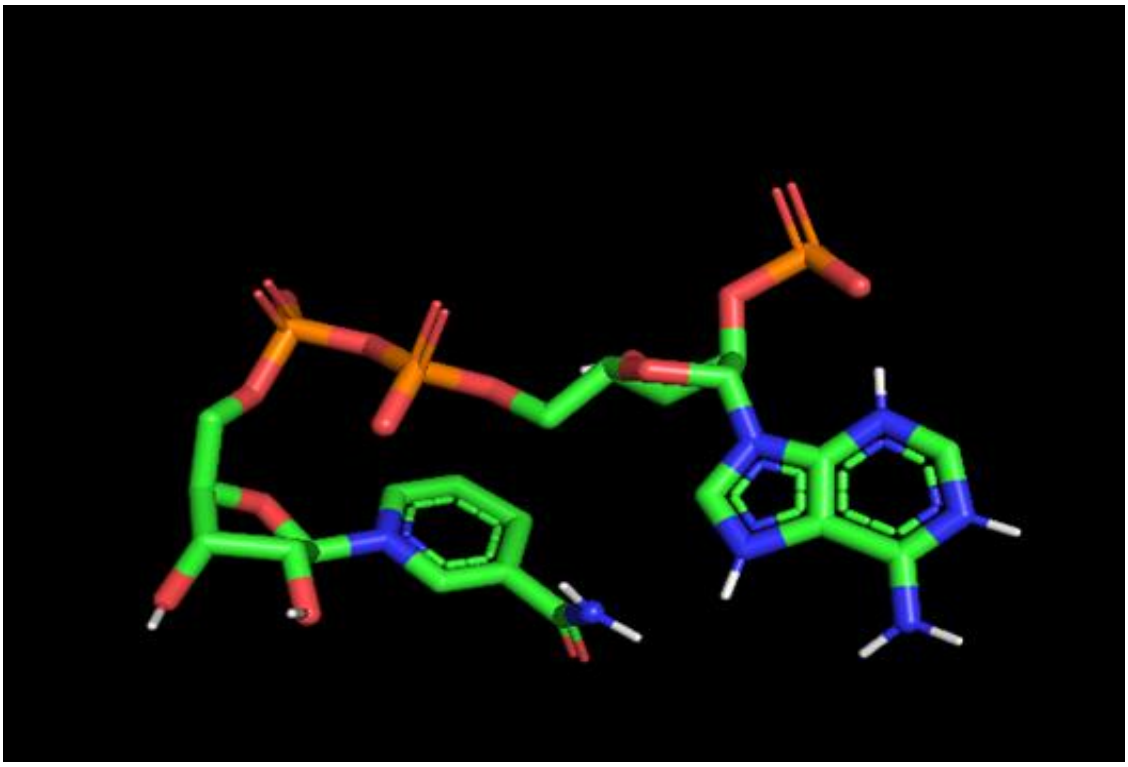
```
receptor = 8dfr.pdbqt
ligand = 8dfrligand.pdbqt

center_x = 23.088
center_y = 4.837
center_z = 12.307

size_x = 40
size_y = 40
size_z = 90

exhaustiveness= 8
```

IMG : 3 - 8dfrconf.txt

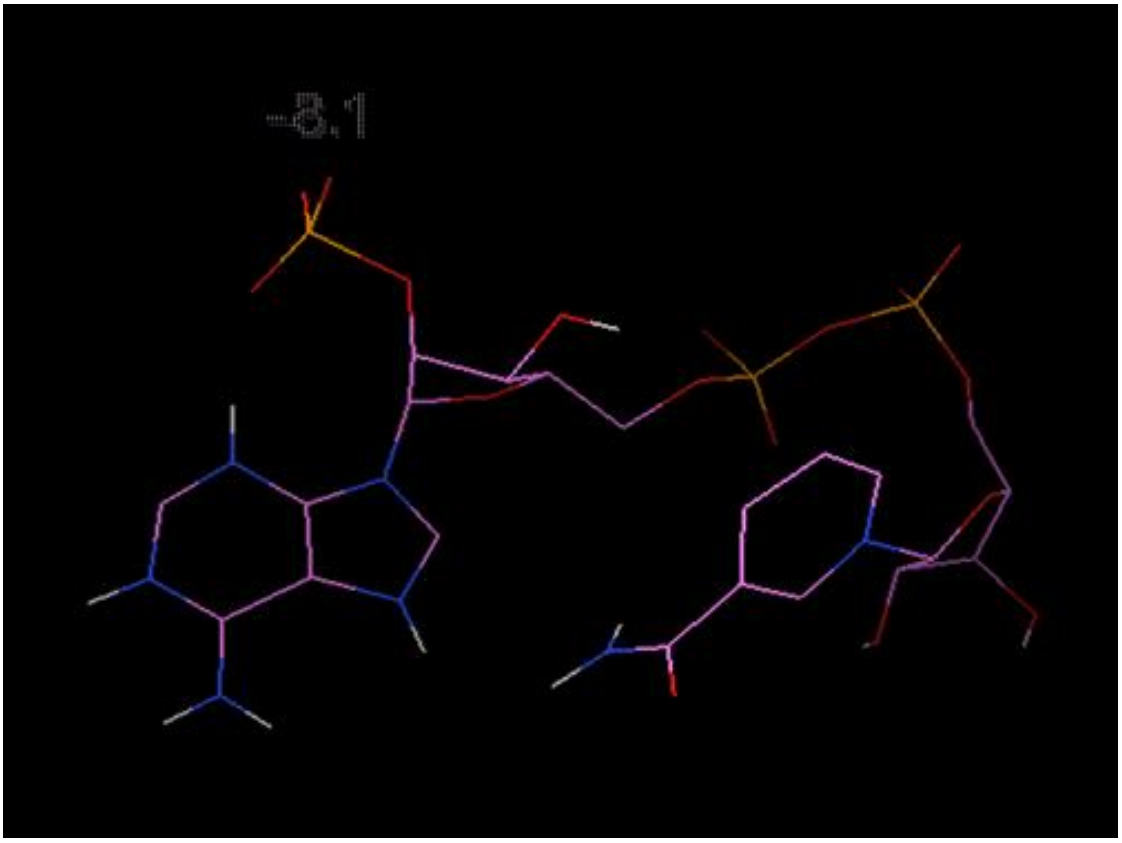
- After that , open the command prompt and give a command for docking of receptor and ligand file and then the output file is arrive.

- The output file is the result of the docking study and it give a idea about the binding energy and binding score.

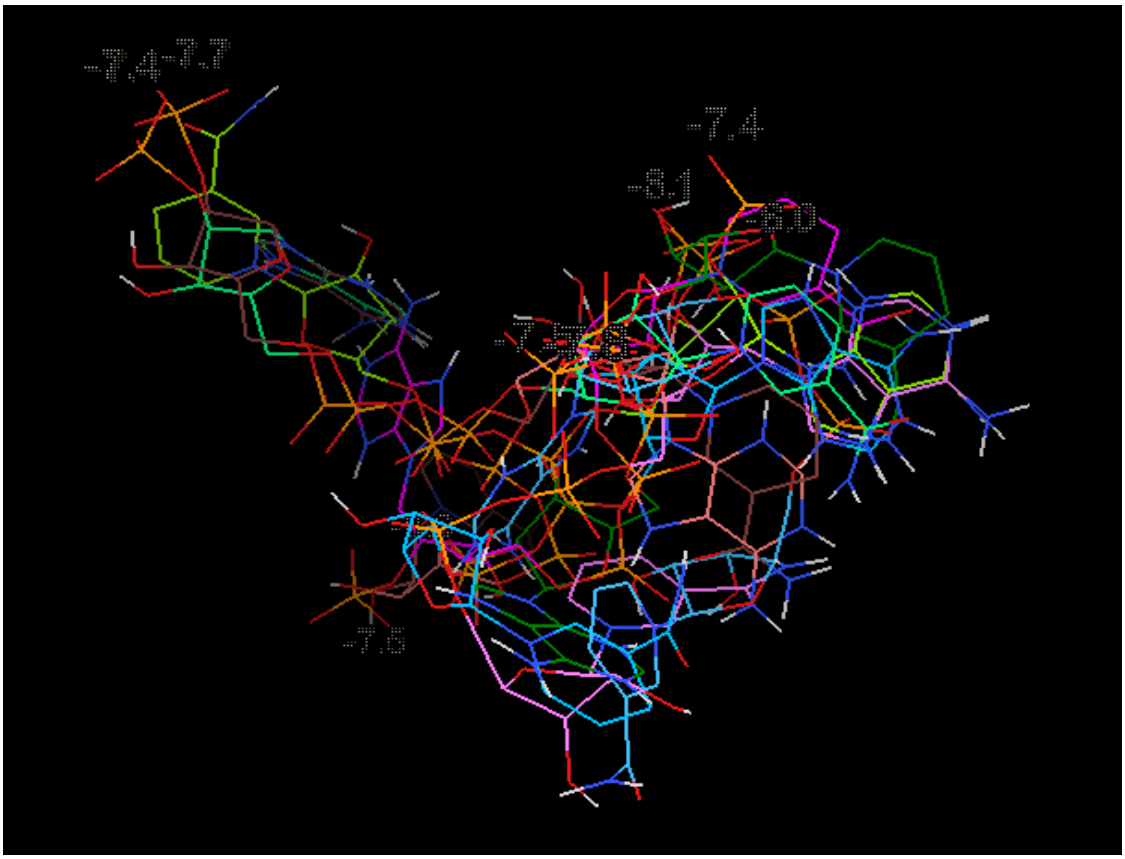
- Then it shows in the docking results and to show a binding sites as well as binding energy of the multiple molecule and the single molecule with multiple confirmation.



IMG : 4 - Single molecule with multiple conformation.



IMG : 5 - Binding energy with molecule

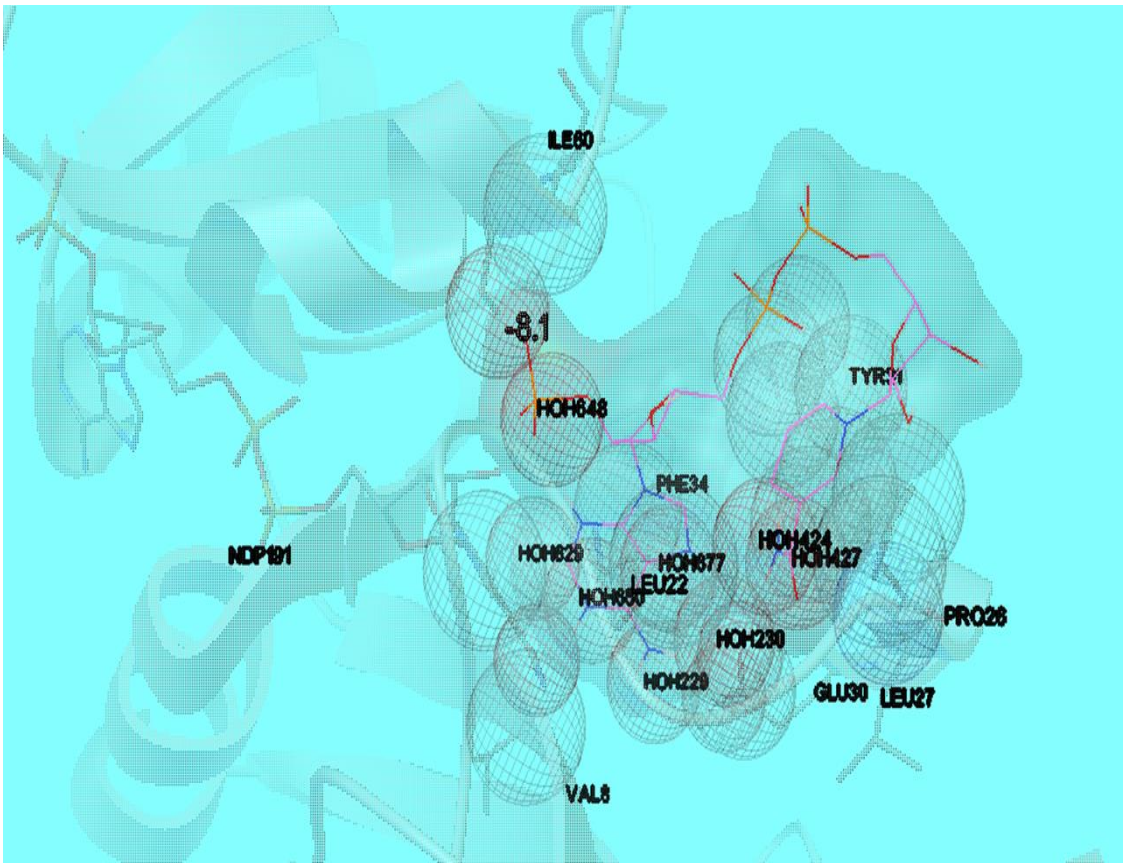


IMG : 6 - Docking of Multiple molecule

Command Prompt

```
C:\Users\ASUS\Desktop\vina> "\Users\ASUS\Desktop\vina\vina.exe" --config 8dfrconf.txt --log 8dfrlog.txt
#############################################################
# If you used AutoDock Vina in your work, please cite:      #
#                                                           #
# O. Trott, A. J. Olson,                                    #
# AutoDock Vina: improving the speed and accuracy of docking #
# with a new scoring function, efficient optimization and   #
# multithreading, Journal of Computational Chemistry 31 (2010) #
# 455-461                                                   #
#                                                           #
# DOI 10.1002/jcc.21334                                     #
#                                                           #
# Please see http://vina.scripps.edu for more information.  #
#############################################################

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Output will be 8dfrligand_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1343910944
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -8.1      0.000      0.000
   2        -8.0      2.831      8.913
   3        -8.0      5.319      6.908
   4        -7.8      3.638      7.308
   5        -7.7      7.964     10.349
   6        -7.5      4.911      9.632
   7        -7.5      2.653      4.200
   8        -7.4      7.846     10.411
   9        -7.4      6.828      8.908
Writing output ... done.
```

IMG : 7 - DOCKING RESULTS



IMG : 8 - Different binding sites with the Molecule

5.2- SUMMERY OF DOCKING.

- To be recapitulate, the NADPH ligand binds to the 8dhfr receptor is successful.
- We can conclude this from the good docking score[8.1] , the good docking score illustrates good binding sites of the receptor.
- In this result we can see that the ligand is bind with the different molecules of the amino acids.

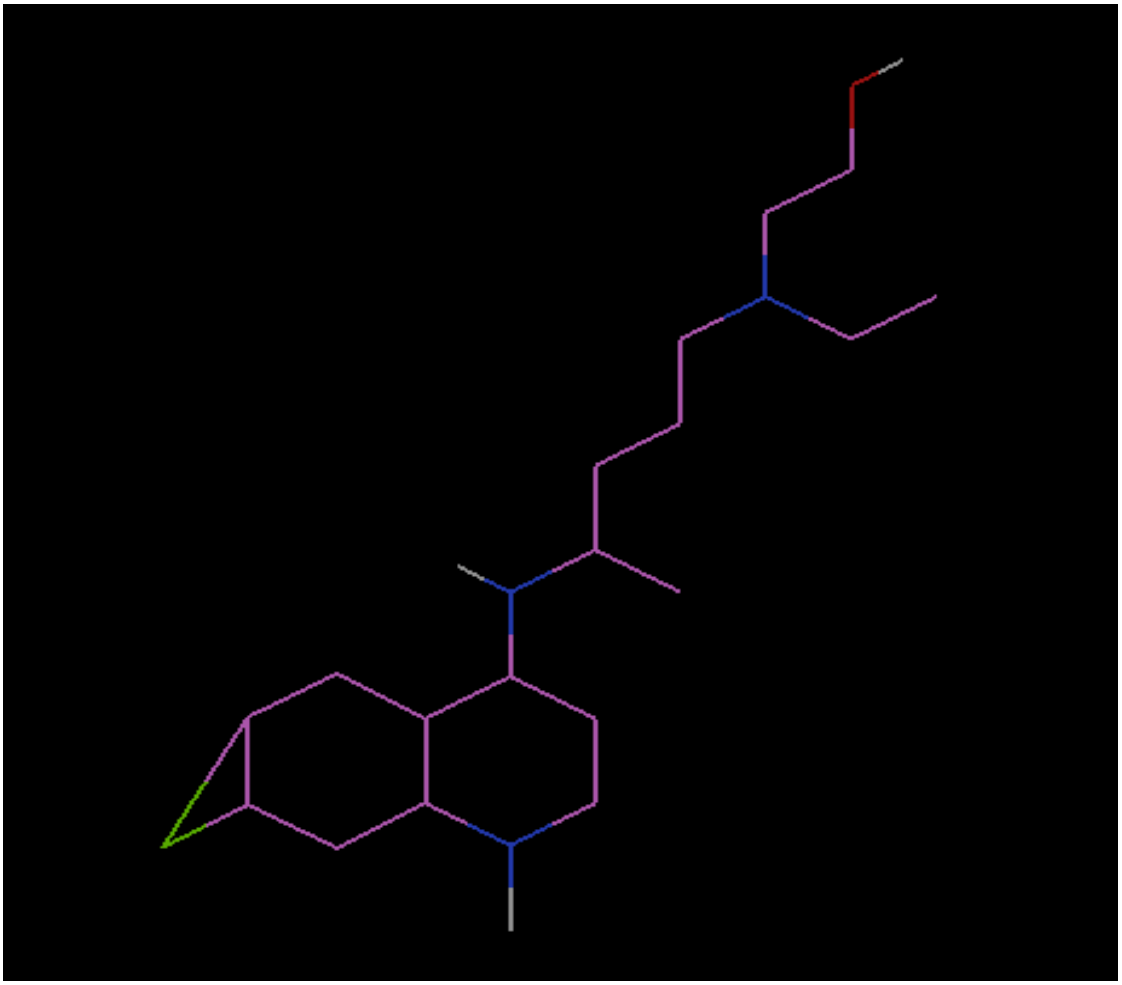
5.3 - DOCKING OF 8-DHFR (DIHYDRO FOLATE REDUCTASE) ENZYME WITH HYDROXY CHLOROQUINE LIGAND.

- The another docking study is the 8-DHFR enzyme with the Hydroxy chloroquine ligand.

- These docking is performed because too see the binding is occurred with this receptor or not.

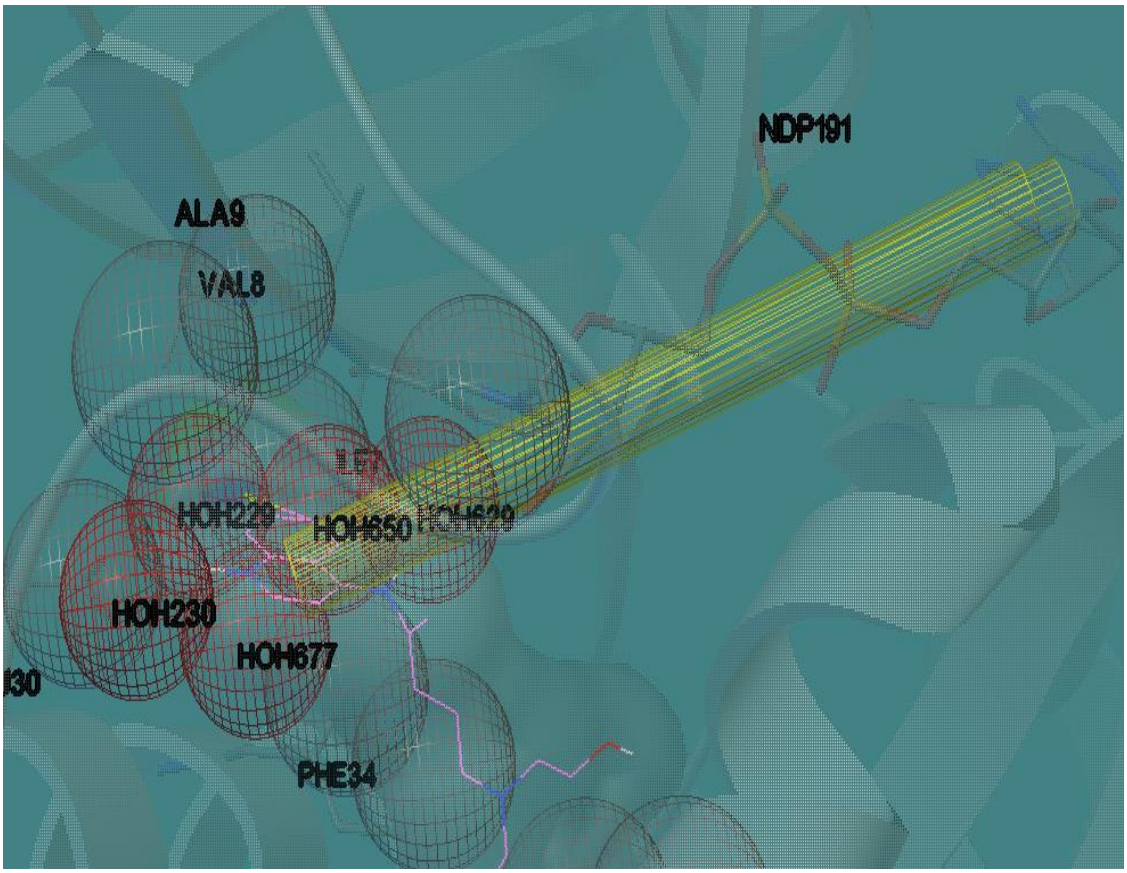
- This also performed in an autodock docking softwere.

- First of all I prepared a ligand file of this hydroxy chloroquine and then it docks with the 8-DHFR enzyme.

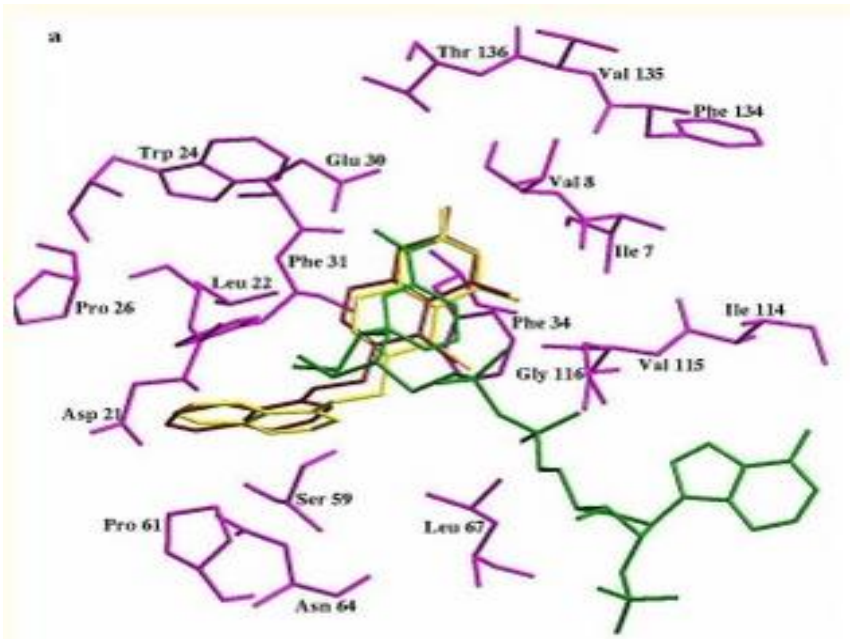


IMG : 9 - LIGAND STRUCTURE OF HYDROXY CHLOROQUINE

- This is the structure of ligand.pdbqt and it will bind with the different sites of the receptor (8-DHFR).
- I decided to dock hydroxyl chloroquine because it's the excellent drug for inhibiting the malaria.
- After that a docked it with the receptor and showed the multiple bonding of the ligand with the receptor.
- The successful completation of the docking study , two molecule structure is came out of it and its called the docking result.
- One is multiple molecule and another is a single molecule with multiple confirmation.
- These all are described in another page.

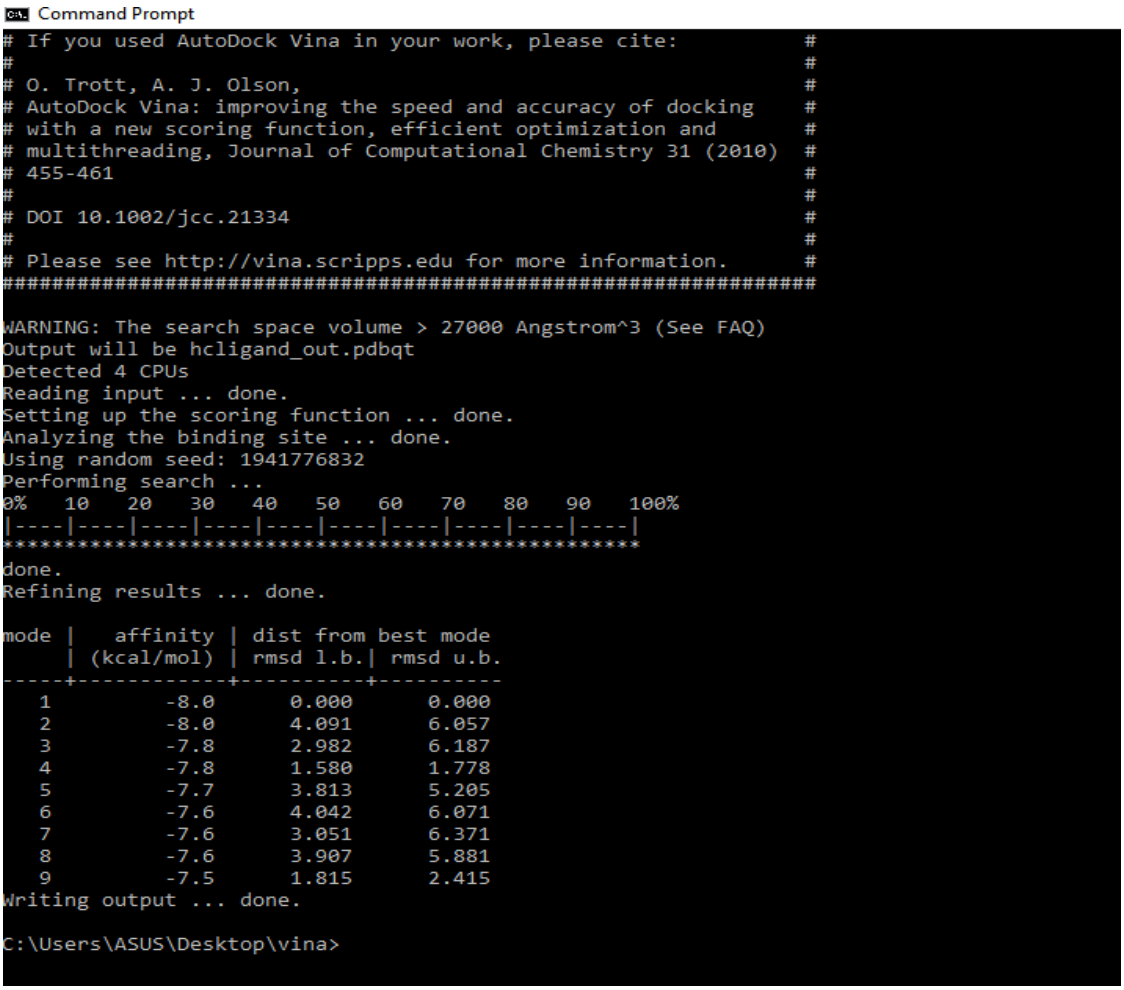


IMG : 10 - The different sites of binding



- In this picture described about the different sites where hydroxyl chloroquine is bind with multiple amino acid sites.

- The binding energy and the result of the hydroxyl chloroquine bind with the 8-dhfr receptor.



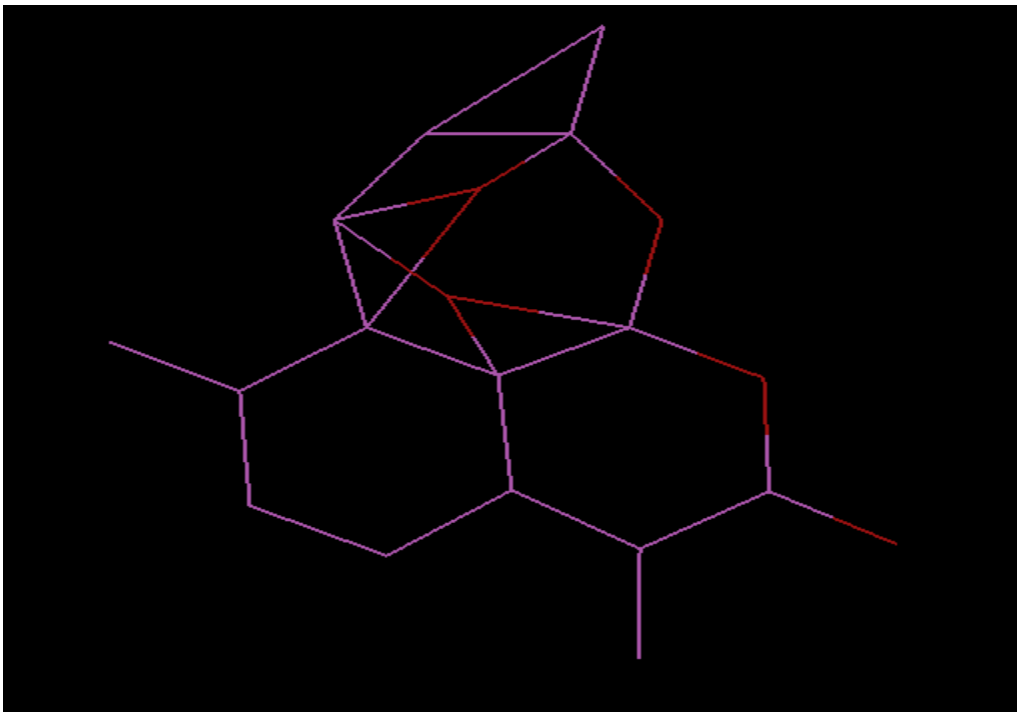
IMG : 11 - RESULTS OF HYDROXY CHLOROQUINE TO DHFR

5.4 SUMMERY OF THE DOCKING STUDY

- The docking score is 8 thus the drug binding to the receptor is excellent.
- The two of the molecule of the ligand shows the good score and they also binds very successfully.
- Binding with different aminoacid residues is aslo seen in the previous picture.

5.5 - DOCKING OF 8-DHFR (DIHYDRO FOLATE REDUCTASE) ENZYME WITH ARTEMISININ LIGAND.

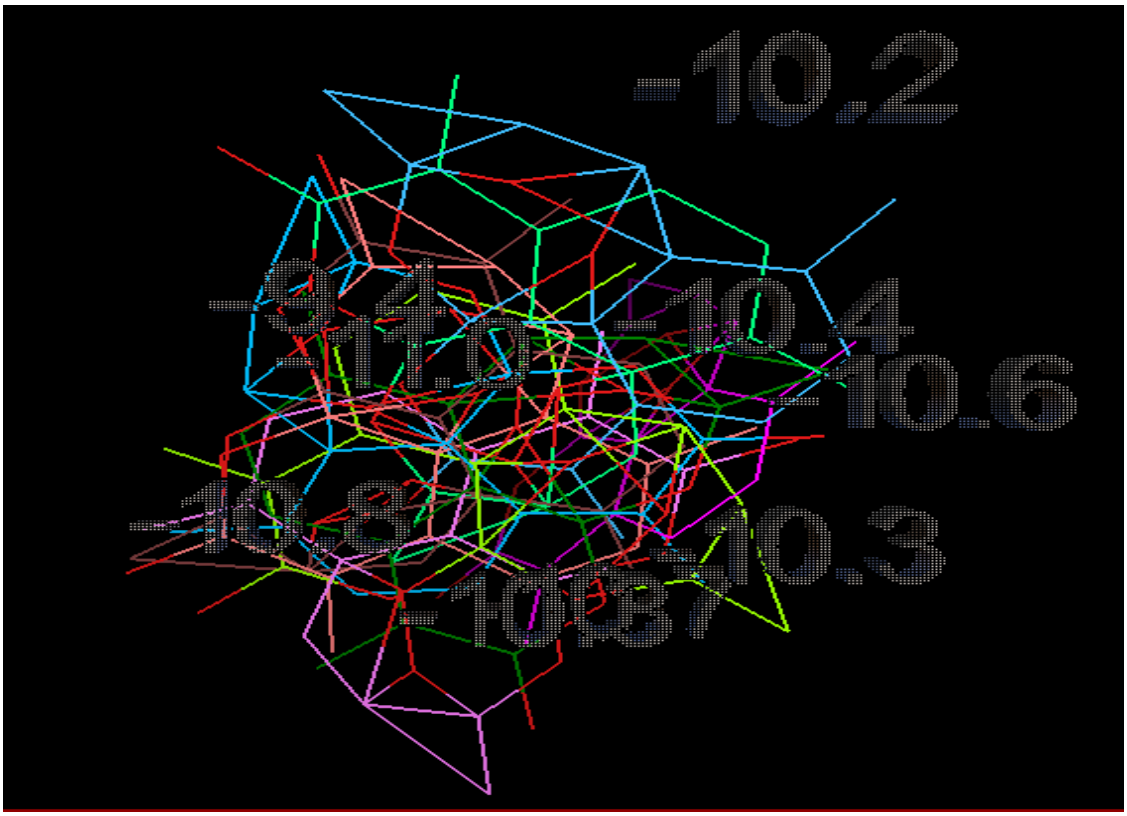
- Now the artemisinin drug is taken by me and it was docked with the same receptor and then showed an activity of these enzyme and drug.
- I decided to took these artemisinin derivative because its shows the most vulnerable effect against the malaria and it also used for adult as well as children.



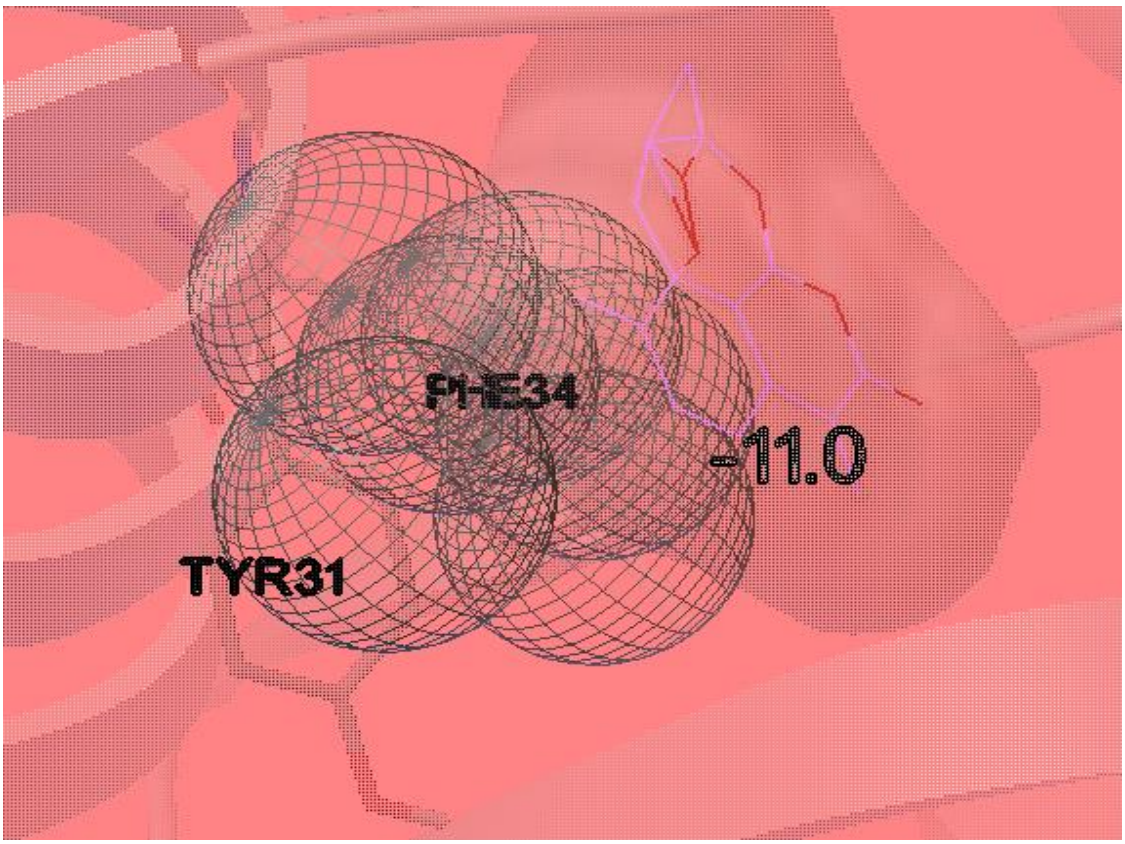
IMG : 12 - LIGAND PDBQT OF ARTEMISININ

- As we can see in this picture the ligand structure is completely ready for the docking study.
- This is the ligand in which we can see that the different ring structure of the artemisinin derivative.
- In that the main ring is the 7 membered ring of carbon and another attached to these ring.

IMG : 13 - Single structure with multiple confirmation



IMG : 14 - MULTIPLE MOLECULE DOCKED WITH THE RECEPTOR



IMG : 15 - Result and binding site of the drug

```
Command Prompt
##############################################################################
# If you used AutoDock Vina in your work, please cite:                       #
#                                                                            #
# O. Trott, A. J. Olson,                                                     #
# AutoDock Vina: improving the speed and accuracy of docking                 #
# with a new scoring function, efficient optimization and                    #
# multithreading, Journal of Computational Chemistry 31 (2010)               #
# 455-461                                                                    #
#                                                                            #
# DOI 10.1002/jcc.21334                                                      #
#                                                                            #
# Please see http://vina.scripps.edu for more information.                   #
##############################################################################

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Output will be arligand_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -288265548
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |    affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -11.0       0.000       0.000
   2         -10.8       0.945       3.202
   3         -10.7       1.581       4.203
   4         -10.6       2.169       4.853
   5         -10.4       1.692       2.992
   6         -10.3       2.157       4.418
   7         -10.3       1.527       3.177
   8         -10.2       2.096       3.743
   9          -9.4       1.131       3.120
Writing output ... done.

C:\Users\ASUS\Desktop\vina>_
```

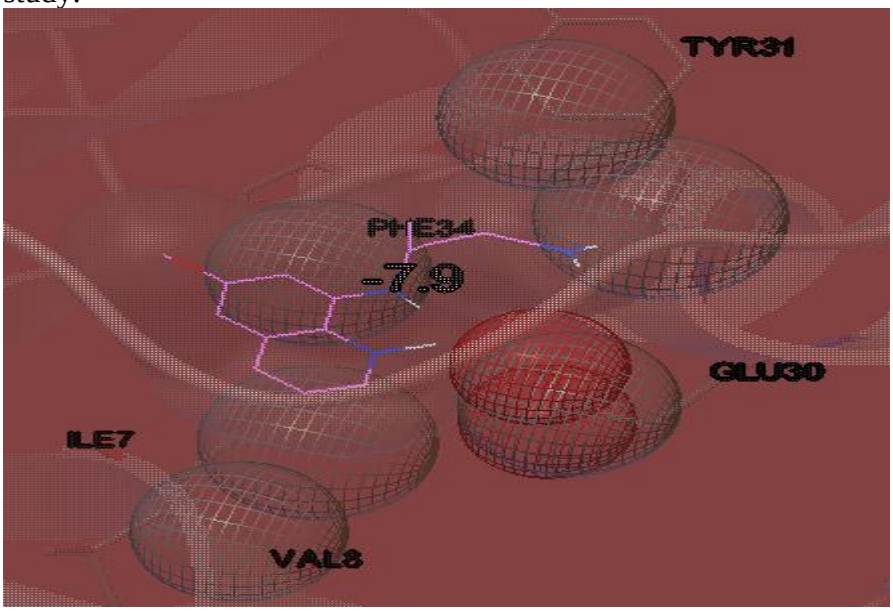
IMG : 16 - Result (binding energy)

5.6- SUMMERY OF THE DOCKING

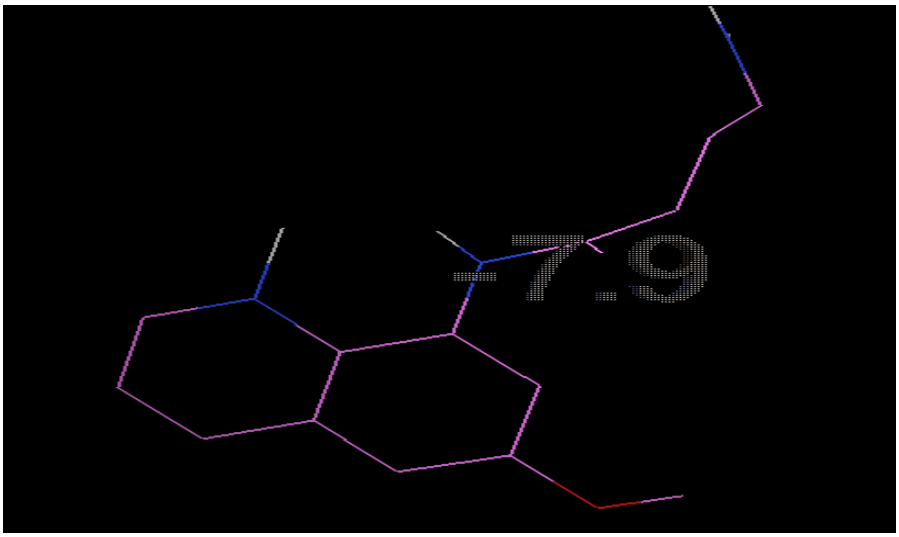
- The binding score of the ligand is 11 ad it also called as a docking score.
- good docking score illustrates the perfect binding of the ligand and receptor.

5.7 - DOCKING OF 8-DHFR (DIHYDRO FOLATE REDUCTASE) ENZYME WITH PRIMAQUINE LIGAND.

- The another molecule is the primaquine and it was selected for the docking study.



IMG : 17 - Result of autodock vina result.



IMG : 18 - SINGLE MOLECULE WITH ENERGY

Command Prompt

```
C:\Users\ASUS\Desktop\vina> "\Users\ASUS\Desktop\vina\vina.exe" --config
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Output will be prligand_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 277743352
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -7.9      0.000      0.000
   2         -7.9      2.159      3.785
   3         -7.8      2.032      4.396
   4         -7.7      1.360      4.279
   5         -7.6      2.539      4.800
   6         -7.5      2.318      4.448
   7         -7.5      2.281      4.271
   8         -7.4      3.112      4.569
   9         -7.3      1.614      2.762
Writing output ... done.
```

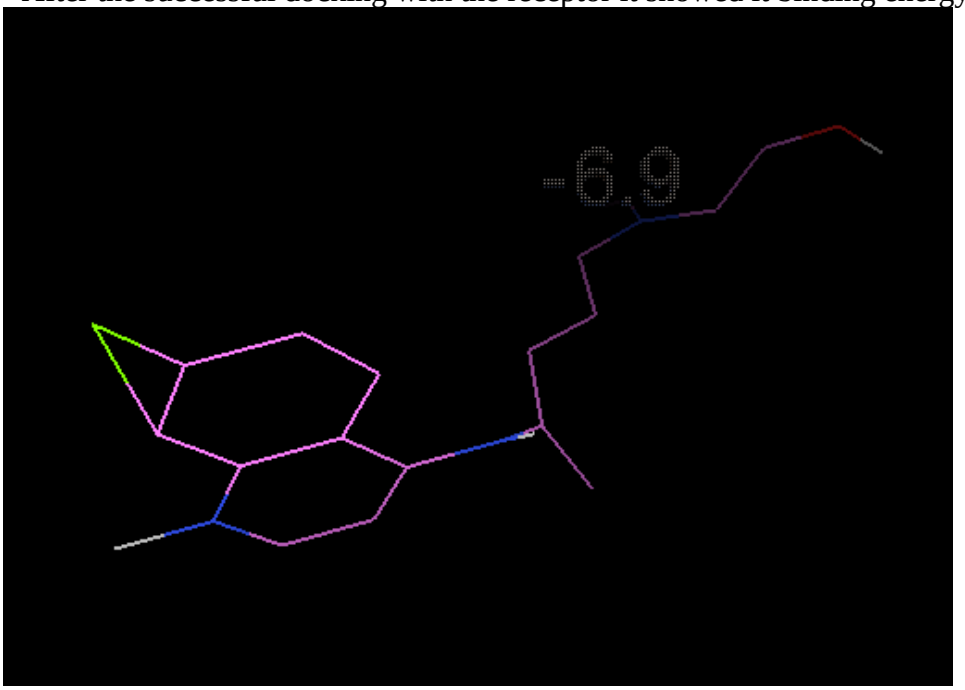
IMG : 19 - FINAL RESULT WITH ENERGY

5.8 DOCKING SUMMERY

- Docking score is 7.9 and the ligand is successfully binds with the receptor.
- primaquine shows the least docking score among the other drugs.

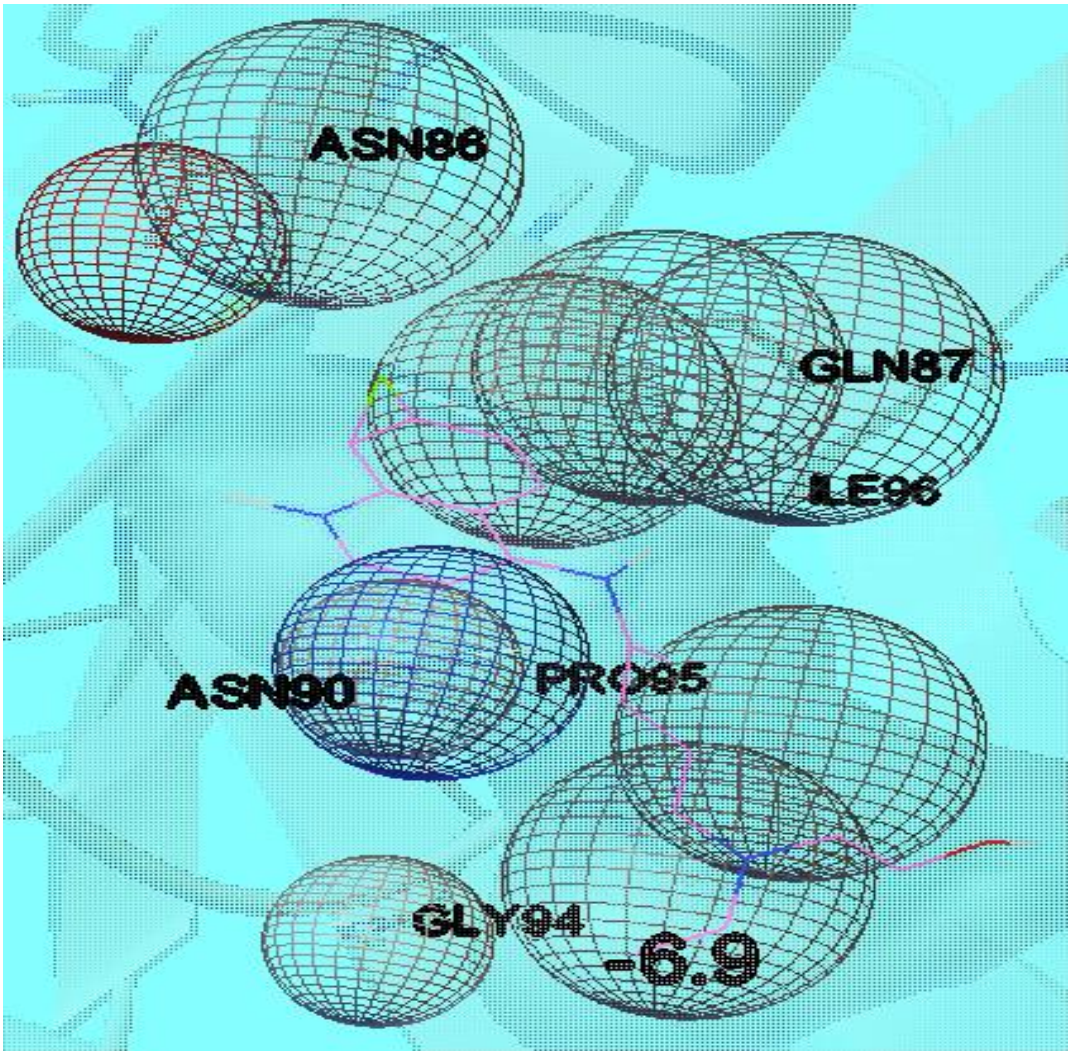
5.9 DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE ENZYME WITH HYDROXY CHLOROQUINE LIGAND.

- In this type of docking study Hydroxy chloroquine was taken and docked it with the new receptor like DHODH.
- After the successful docking with the receptor it showed it binding energy.



IMG : 20 - Result of an autodock vina and showed a single molecule with multiple confirmation.

IMG : 21 - Multiple molecule interaction



IMG : 22 – BINDING WITH AMINO ACIDS

- It shows the different binding sites of the ligand.
- Also shows the different sites of ligand binds with the multiple sites of the receptor and these all are the residues of amino acid.
- It also shows the different energies including the binding sites of receptor.

```
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Output will be 2hcligand_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 433329152
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1         -6.9      0.000      0.000
   2         -6.6     19.233     21.465
   3         -6.5      2.560      3.454
   4         -6.4      1.860      2.706
   5         -6.4      3.110      6.372
   6         -6.2      3.082      4.650
   7         -6.2     20.046     22.119
   8         -6.1      3.025      6.215
   9         -6.1     22.306     23.853
Writing output ... done.

C:\Users\ASUS\Desktop\vina>
```

IMG : 23 - DOCKING RESULTS

5.10 – SUMMERY OF THIS DOCKING.

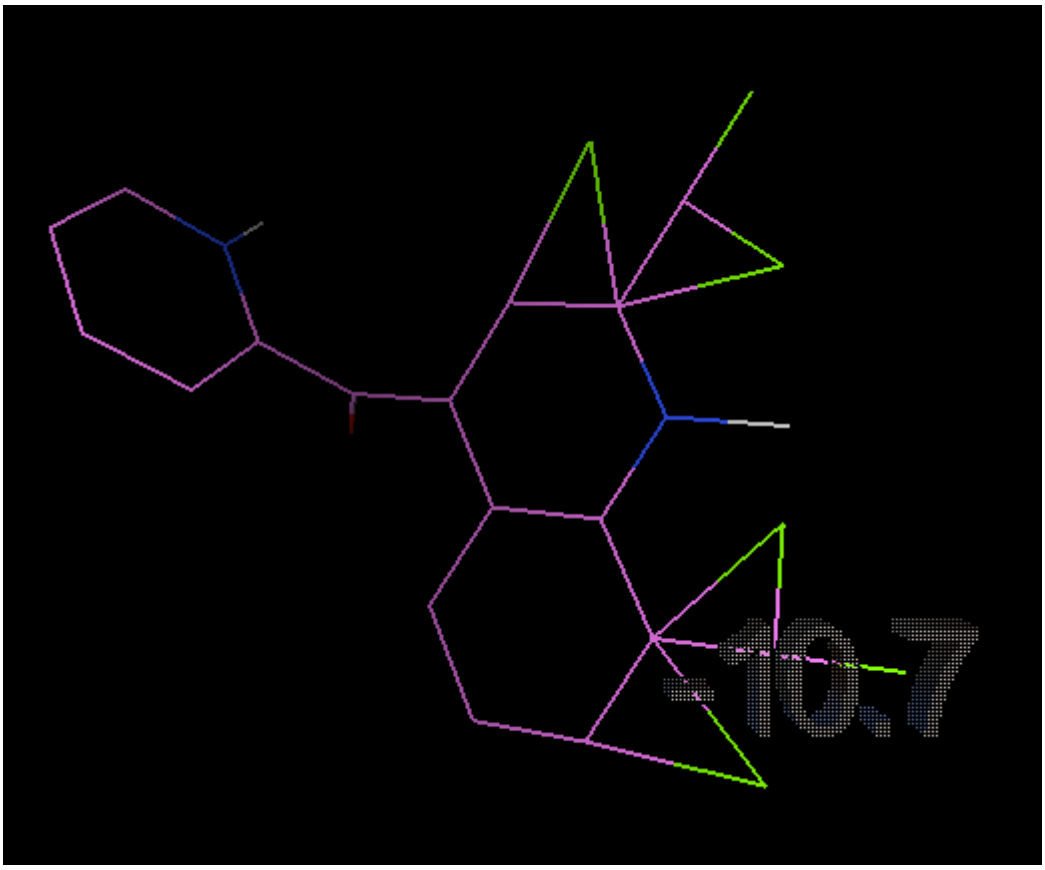
- This pictorial shows the different binding energies of the ligand receptor binding.
- It described the good docking score which is 6.9 thus the docking is successful.

5.11 - DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE ENZYME WITH MEFLOQUINE LIGAND.

- The another docking take place is the mefloquine with receptor DHODH.

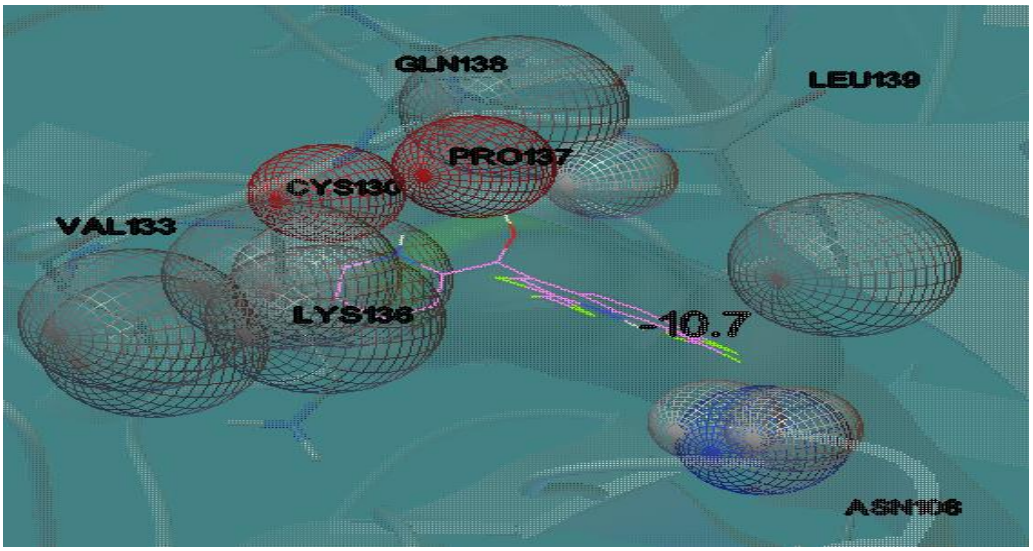
- In this study we will see the binding energy and specific amino acid binding site of the ligand.

- Basically mefloquine is from the quinolones derivative thus its binding should be great as compare the others drug from these class.



IMG : 24 - This pictorial shows the binding energy with one molecule bind to the receptor and another shows the docking results.

```
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -10.7      0.000      0.000
   2        -10.5      2.302      4.534
   3        -10.4      1.735      2.325
   4        -10.1     25.337     27.297
   5        -10.0     29.594     31.226
   6         -9.9     27.237     29.181
   7         -9.5     26.820     28.578
   8         -9.4      2.283      4.125
   9         -9.4      2.230      4.400
Writing output ... done.
```



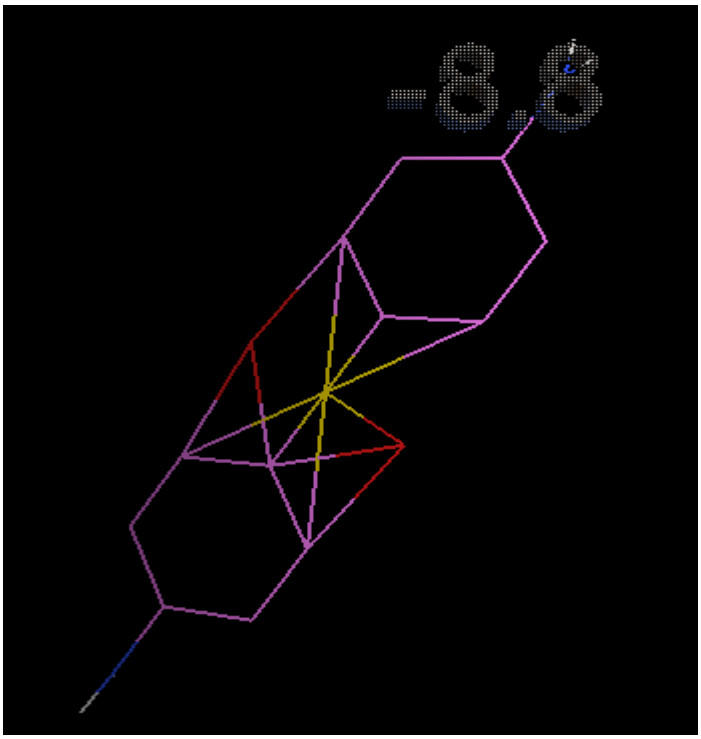
IMG : 25 - This shows the binding site of ligand with the receptor.

5.12 DOCKING SUMMERY

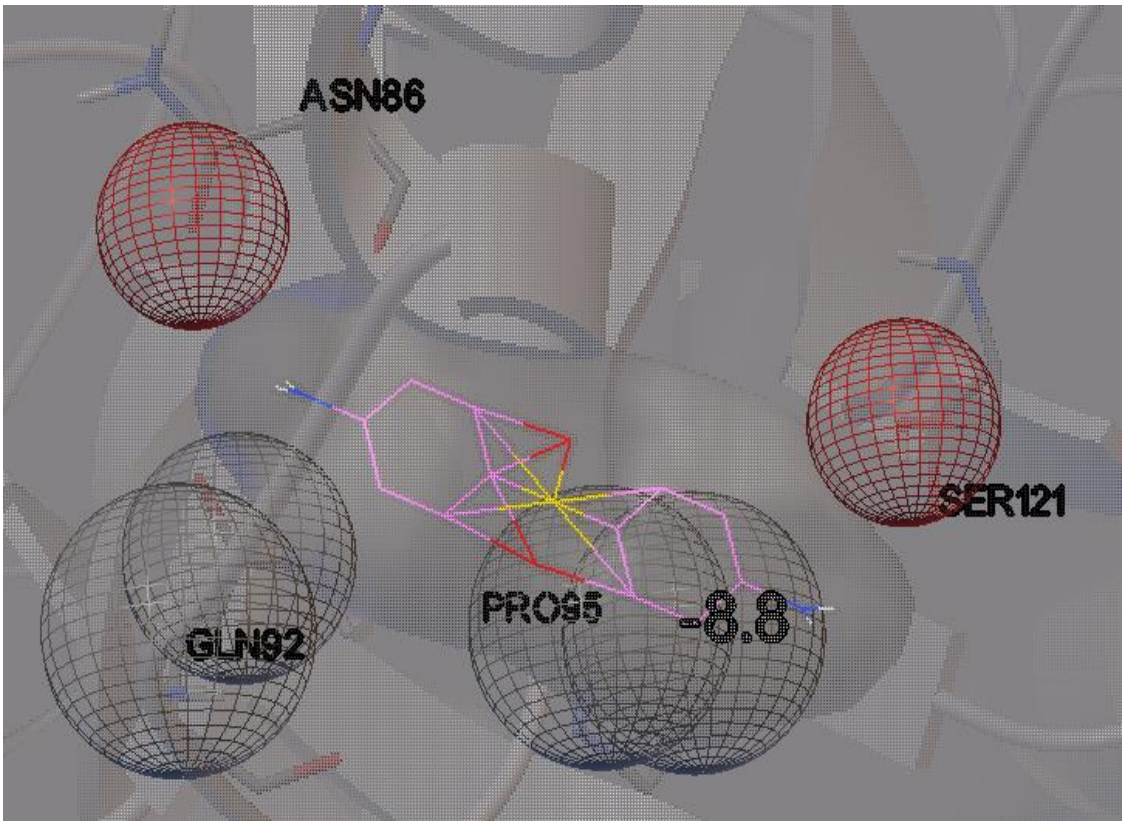
- In this docking shows the excellent binding score as per the prediction above.
- The docking score is 10.7 thus we gave it as a good binding site of ligand to the receptor.

5.13- DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE) ENZYME WITH DAPSON LIGAND.

- In this docking study includes another category of the malaria.
- To check the activity and binding energy of Dapsone with receptor DHODH.
- Here I will show the final output of the docking study and then give the summery of this docking.



IMG : 26 - This pictorial shows the binding energy of one molecule with different confirmation.



IMG : 27 - Binding site of the ligand with receptor

Command Prompt

```
WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Output will be dapligand_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1300171456
Performing search ...
0%    10    20    30    40    50    60    70    80    90    100%
|----|----|----|----|----|----|----|----|----|----|
**********************************************************
done.
Refining results ... done.

mode |    affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1          -8.8        0.000        0.000
   2          -8.8        0.000        4.703
   3          -7.8       21.957       23.598
   4          -7.7       21.989       23.480
   5          -7.6       27.177       28.400
   6          -7.6       27.181       28.198
   7          -7.0       21.279       22.206
   8          -7.0       21.291       22.156
   9          -7.0       22.698       24.433
Writing output ... done.

C:\Users\ASUS\Desktop\vina>
```

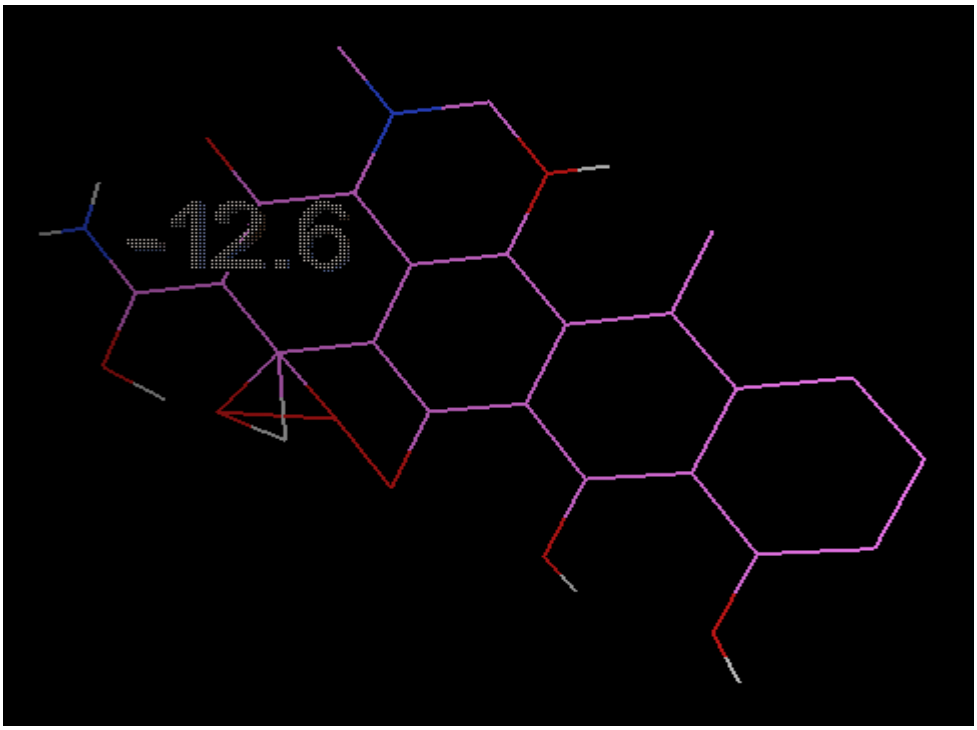
IMG : 28 - Docking result with the binding energy.

5.14 SUMMERY OF THE DOCKING

- In this docking study illustrates the good docking score (8.8) thus it shows the good activity with the receptor.

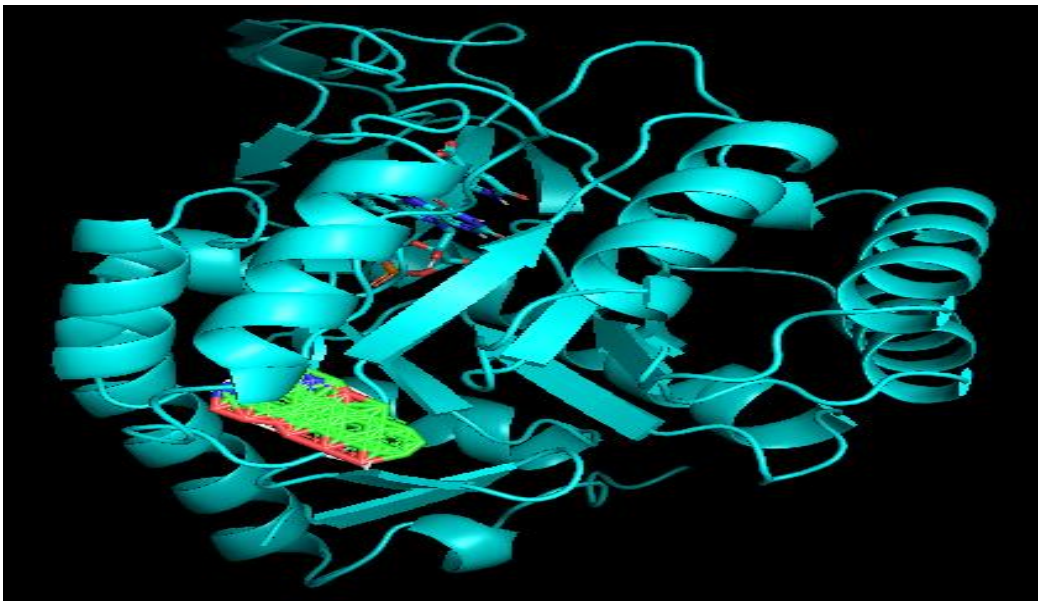
5.15 - DOCKING OF 2DOR (DIHYDROOROTATE DEHYDROGENASE) ENZYME WITH DOXYCYCLINE LIGAND.

- Doxycycline is a category from the miscellaneous category of the malaria.
- We show the docking study and the binding energy of the ligand with the receptor.

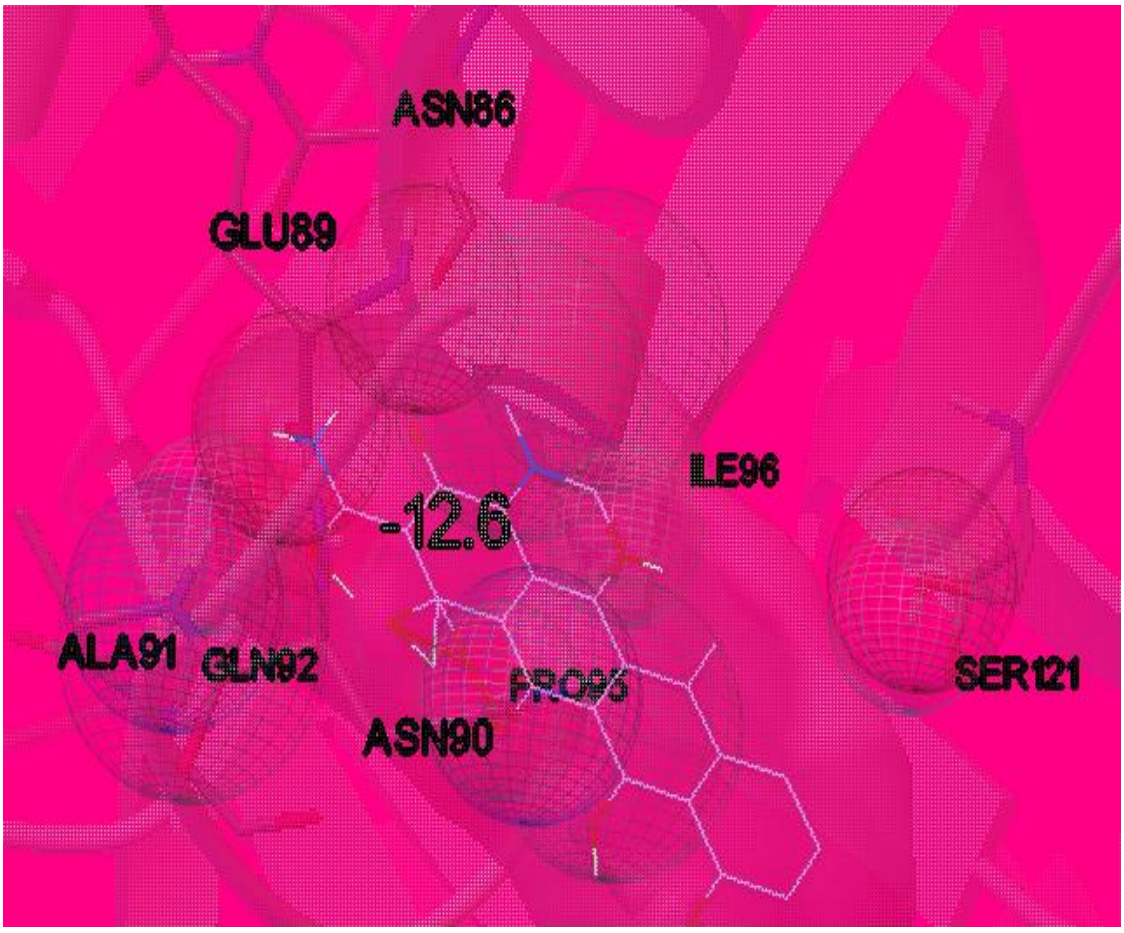


IMG : 29 - It shows the binding energy with one molecule and result.

```
mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -12.6      0.000      0.000
   2        -12.1      2.303      5.855
   3        -12.0     22.387     24.400
   4        -11.6      1.863      5.046
   5        -11.5     21.517     23.416
   6        -11.2      1.591      2.076
   7        -10.3      3.663      6.320
   8        -10.0     36.326     39.170
   9        -10.0      3.854      7.027
Writing output ... done.
```



IMG : 30 - Binding site of ligand with the receptor



5.16 - DOCKING SUMMERY OF THIS MOLECULAR DOCKING.

This molecular docking showed a successful with good docking score and docking score is **12.6** which is the highest score of anti-malarial drug category.

CHAPTER 6

CONCLUSION

6.0 CONCLUSION TABLE

(TABLE NO : 2)

Sr.No	LIGAND (class of drug)	RECEPTOR	BINDING ENERGY (Kcal/mol)	BINDING SITES
1	NADPH (adenine nucleotides)	8-dfr	-8.1	HOH648,PHE34,TYR, HOH424,LEU22,HOH680, HOH427.
2	HYDROXY CHLOROQUINE (aminoquinoline)	8-dfr	-8.0	NDP191,ALA9,VAL8, HOH677,HOH230.
3	ARTEMISININ (sesquiterpenes)	8-dfr	-11.0	PHE34,TYR31.
4	PRIMAQUINE (8aminoquinoline)	8-dfr	-7.9	GLU30,TYR31,PHE34, VAL8.
5	HYDROXY CHLOROQUINE (aminoquinoline)	DHODH	-6.9	ASN86,GLN87,ILE96. PR095,GLY94.
6	MEFLOQUINE (4-quinoline)	DHODH	-10.7	GLN138,VAL133,PRO137 CYS130,LEU139.
7	DAPSONE (sulfonamides)	DHODH	-8.8	ASN86,SER121,PRO95.
8	DOXYCYCLINE (miscellaneous)	DHODH	-12.6	ASN86,GLU89,ASN90, ILE96,SER121.

CHAPTER 7

SUMMERY

7.0 – SUMMERY

In this project I took two target of malarial disease. The one is DHODH and another one is DHFR. I understand from the literature that both targets having extremely good inhibition mechanisms. I tried old and as well as new antimalarial drug docking study with this potential targets of malaria. From this computational approach by AutoDock Vina software. I performed molecular docking study of DHODH and DHFR with different ligands and I identified different binding modes of structure with different amino acid interactions with different features like hydrophobic, hydrogen bonding, and vanderwaals interactions and from that I got the least binding energy interaction structure. From that I make a conclusion table in that described a binding site , energies.

CHAPTER 8

BIBLIOGRAPHY

1 - Aqvist, J.; Luzhkov, V.B.; Brandsdal, B.O. Ligand binding affinities from MD simulations. Acc. Chem. Res., 2002, 35, 358- 365.

2 - http://www.organic-chemistry.org/prog/peo (accessed January 2012)

3 - Davies, M.; Heikkila, T.; McConkey, G.A.; Fishwick, C.W.G.; Parsons, M.R.; Johnson, A.P. Structure-based design, synthesis, and characterization of inhibitors of human and Plasmodium falciparum dihydroorotate dehydrogenases. J. Med. Chem., 2009, 52(9), 2683-2693.

4 - Heikkila T.; Ramsey, C.; Davies, M.; Galtier, C.; Stead, A.M.W., Johnson, A. P.; Fishwick, C.W. G. Boa, A.N.; McConke G.A. Design and Synthesis of Potent Inhibitors of the Malaria Parasite Dihydroorotate Dehydrogenase J. Med. Chem., 2007, 50, 186-191.

5 - Boa, A. N.; Canavan, S.P.; Hirst, P.R.; Ramsey, C.; Stead, A. M. W.; McConkey, G.A. Synthesis of brequinar analogue inhibitors of malaria parasite dihydroorotate dehydrogenase. Bioorg. Med. Chem., 2005, 13, 1945–1967.

6 - Heikkila, T.; Thirumalairajan, S.; Davies, M.; Parsons, M.R.; McConkey A.G.; Fishwick, C.W. G.; Johnson, A.P. The first de novo designed inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase. Bioorg. Med. Chem. Lett., 2006, 16, 88–92.

7 - Cowen, D.; Bedingfield, P.; McConkey G.A.; Fishwick, C.W.G.; Johnson, A. P. A study of the effects of substituents on the selectivity of the binding of Narylaminomethylenemalonate inhibitors to DHODH.Bioorg. Med. Chem. Lett., 2010, 20, 1284–1287.

8 - Booker, M.L.; Bastos C.M.; Kramer, M.L.; Barker, R.H.; Skerlj, R.; Sidhu, A.B.; Deng, X.; Celatka C.; Cortese, J.F.; Bravo, J.E.G.; Llado, K. N.C.; Serrano, A..E.; Barturen, I.A.; Jimenez-Díaz, M.B.; Viera, S.; Garutn, H.; Wittlin, S.; Papastogiannidis, P.; Lin, J.W.; Janse, C.J.; Khan, S.M.; Duraisingh, M.; Coleman, B.; Goldsmith, E.J.; Phillips, M.A.; Munoz, B.; Wirth, D.F.; Klinger J.D.; Wiegand, R.; Sybertz, E. Novel inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase with anti-malarial activity in themouse model. J. Biol. Chem., 2010 285(43), 33054–33064.

9 - Phillips, M. A.; Gujjar R.; Malmquist, N.A.; White, J.; Mazouni, F.E.; Baldwin, J.; Rathod, P.K. Triazolopyrimidine-based dihydroorotate dehydrogenase inhibitors with potent and selective activity against the malaria parasite Plasmodium falciparum. J. Med. Chem., 2008, 51, 3649–3653.

10 - Liu, S.; Neidhardt, E.A.; Grossman, T.H.; Ocain, T.; Clardy, J.Structures of human dihydroorotate dehydrogenase in complex with antiproliferative agents. Struct. Fold Des.,2000, 8, 25-33.

11 - Fairbanks, L.D.; Bofill, M.; Ruckemann, K.; Simmonds, H.A.Importance of ribonucleotide availability to proliferating Tlymphocytes from healthy humans.J. Biol. Chem., 1995, 270,29682-29689.

12 - Marcinkeviciene, J.; Rogers, M. J.; Kopcho, L.; Jiang, W.; Wang, K.; Murphy, D. J.; Lippy, J.; Link, S.; Chung, T. D.; Hobbs, F.; Haque, T.; Trainor, G. L.; Slee, A.; Stern, A. M.; Copeland, R. A. Selective inhibition of bacterial dihydroorotate dehydrogenases by thiadiazolidinediones. Biochem.Pharmacol.,2000, 60, 339-342.

13 - Tamta, H.; Mukhopadhyay, A. K. Biochemical targets for malaria chemotherapy.Current Research & Information on PharmaceuticalScience (CRIPS), 2003, 4, 6–9.

14 - Morand, P.; Courtin, O.; Sautes, C.; Westwood, R.; Hercend, T.; Kuo, E. A.; Ruuth, E. Dihydroorotate dehydrogenase is a target for the biological effects of leflunomide. Transplant.Proc., 1996, 28, 3088– 3091.

15 - Barrington, J., Wereko-Brobby, O., Ward, P., Mwafongo, W., & Kungulwe, S. (2010). SMS for Life: A pilot project to improve anti-malarial drug supply management in rural Tanzania using standard technology. *Malaria Journal*. https://doi.org/10.1186/1475-2875-9-298

16 - Jorgensen, W.L. The many roles of computation in drug discovery.Science, 2004, 303, 1813-1818.

17 - Krungkrai J, Krungkrai SR, Supuran CT; Carbonic anhydrase inhibitors: inhibition of Plasmodiumfalciparum carbonic anhydrase with aromatic/heterocyclic sulfonamides-in vitro and in vivo studies.Bioorg. Med. Chem. Lett 2008;18(20):5466–5471. [PubMed: 18805693]

18 - Mascia, L.; Turchi, G.; Bemi, V.; Ipata, P. L. Uracil salvage pathway in PC12 cells.Biochem.Biophys.Acta, 2000, 1524, 45–50.

19 - Bjo¨ rnberg et al., 1997; Hansen et al., 2004; Jensen &Bjornberg, 1998).ActaCryst. (2006). D62, 312-323 [doi:10.1107/S0907444905042642]

20 - 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, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea 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,Genome sequence of the human malaria parasite Plasmodium falciparunm, Institute for Genomic Research, 9712 Medical Center Drive, Rockville, Maryland 20850, USA.

21 - Langley DB, Shojaei M, Chan C, Lok HC, Mackay JP, Traut TW, Guss JM, Christopherson RI.Structure and inhibition of orotidine 5'-monophosphate decarboxylase from Plasmodiumfalciparum. Biochemistry 2008;47(12):3842–3854. [PubMed: 18303855]

22 - Bello AM, Poduch E, Liu Y, Wei L, Crandall I, Wang X, Dyanand C, Kain KC, Pai EF, Kotra LP.Structure-activity relationships of C6-uridine derivatives targeting plasmodia orotidinemonophosphate decarboxylase. J. Med. Chem 2008;51(3):439–448. [PubMed: 18189347]

23 - Majbritt Hansen,1,2Jérôme Le Nours,1 Eva Johansson,1,3Torben Antal,1,4 Alexandra Ullrich,5 Monika Löffler,5 and Sine Larsen1,Inhibitor binding in a class 2 dihydroorotate dehydrogenase causes variations in the membrane-associated N-terminal domain.

24 - MALCOLM J., GARDNER et al., Sequence of Plasmodium falciparum chromosomes 2, 10, 11 and 14, Nature 419, 531–534 (2002); doi:10.1038/nature01094.

B. Pharm Dhruvil

ORIGINALITY REPORT

2%	1%	0%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to The New Art College Student Paper	1%
2	edoc.unibas.ch Internet Source	<1%
3	Submitted to University of South Florida Student Paper	<1%
4	zombiedoc.com Internet Source	<1%
5	50breakthroughs.org Internet Source	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		