|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
NLM (National Library of Medicine ID: 101738825)
Index Copernicus Value 2019: 79.34
Volume 4, Issue 11; November: 2020; Page No. 97-106

DRUG REPURPOSING FOR MALARIA AND DENGUE USING COMPUTATIONAL MODELLING.

Prajakta Velankar[1], Sara Rehman[2], Yukti Thakkar[3]
Ramnarain Ruia Autonomous College[1,3], Jamia Hamdard[2]

Article Info: Received 25 September 2020; Accepted 26 November 2020
DOI: https://doi.org/10.32553/ijmbs.v4i11.1534
Corresponding author: Prajakta Velankar, Sara Rehman, Yukti Thakkar
Conflict of interest: No conflict of interest.

Abstract
By and by the world is in a battle with the diseases like Malaria and Dengue with no prompt medicines accessible the scourge brought about by the Malaria and Dengue is expanding step by step. A ton of researchers are continuing for the potential medication up-and-comer that could help the medical care framework in this battle. We present a docking-based screening using a quantum mechanical scoring of a library built from approved drugs ie Remdesivir, Hydroxy-chloroquine, Curcumin, Moroxydine, Artesunate Sulphate, Mefloquine, Doxycycline, Atovaquone, Indinavir, and compounds that are with Malaria and Dengue Mpro Proteins could display antiviral activity against these diseases. Clearly, these compounds should be further evaluated in experimental assays and clinical trials to confirm their actual activity against the disease. We hope that these findings may contribute to the rational drug design against Malaria and Dengue
Keywords: Malaria, Dengue, Drug Repurposings, Computer Aid Drug Design, In silico drug development

Introduction

Dengue and Malaria has become a significant public issue over the globe. As of twelfth of November 2020, more than 3.79 billion cases have been accounted for in 210 nations and domains (World meter, 2020). Dengue and Malaria are viewed as a major danger to worldwide general wellbeing. There is a pressing need to create an intense enemy of these diseases' specialists for the avoidance of the flare-up and stop viral contaminations. Repurposing of realized little particles is by all accounts an exceptionally productive path so as to create strong medications to battle Dengue and Malaria in this brief timeframe period.

Targeted protein for Dengue:
DengueV-2 Capsid ST148 inhibitor Complex

PDB ID 6VG5

6vg5 is a 2 chain structure with sequence from Dengue virus 2. the high-resolution cocrystal structure (1.5 A) of the DENV-2 capsid protein in complex with an inhibitor that potently suppresses DENV-2 but not other DENV serotypes. The results have uncovered an antiviral mechanism through inhibitor-induced tetramerization of the viral capsid and provided essential structural and functional knowledge for rational design of pan serotype DENV capsid inhibitors.

Protein- envelope protein heterodimer from the dengue 2 virus

PDB ID 3C5X

3c5x is a 2 chain structure with sequence from Den26 and Dengue virus 2. Envelope protein E binding to the host cell surface receptor is followed by virus internalization through clathrin-mediated endocytosis. Envelope protein E is subsequently involved in membrane fusion between virion and host late endosomes. Synthesized as a homodimer with prM which acts as a chaperone for envelope protein E. After cleavage of prM, envelope protein E dissociates from small envelope protein M and homodimerizes.

Dengue virus NS2B-NS3 protease A125C variant at pH 8.5

PDB ID 4M9F

4m9f is a 1 chain structure with sequence from Dengue virus 2. Dengue virus protease (NS2B-NS3pro) is essential for dengue virus infection and is thus a target of therapeutic interest. attention has focused on developing active-site inhibitors of NS2B-NS3pro. The flat and charged nature of the NS2B-NS3pro active site may contribute to difficulties in developing inhibitors and suggests that a strategy of identifying allosteric sites may be useful.

Dengue Virus Nonstructural Protein 5

PDB ID 5ZQK

5zqk is a 2 chain structure with sequence from Dengue virus 2 NS5 is a nonstructural protein essential for flavivirus RNA replication with dual MTase and RdRp enzyme activities and thus constitutes a major drug target. Insights into NS5 structure, dynamics, and evolution should inform the development of antiviral inhibitors and vaccine design. We found that NS5 from DENV2 can adopt a conformation resembling that of NS5 from JEV and ZIKV. Replacement of the DENV2 NS5 linker with the JEV and ZIKV NS5 linkers abolished DENV2 replication in cells, without significantly

impacting in vitro DENV2 NS5 enzymatic activities. We propose that heterotypic flavivirus NS5 linkers impede DENV2 NS5 protein-protein interactions that are essential for virus replication.

DENGUE 3 NS5 METHYLTRANSFERASE

PDB ID: 4CTK

This protein functions as a signal peptide for NS4B. It is involved in fusion of virus membrane with host membrane, virion attachment to host cell, and viral RNA genome replication. Targeting this protein with an inhibitory molecule could result in damage to viral replication, virion assembly and could eventually reduce the infection.

Dengue virus RNA helicase

PDB ID: 2BHR

The NS3 protein from Dengue virus is a multifunctional protein. It has protease, helicase, and nucleoside 5'-triphosphatase (NTPase) activities. Owing to these activities it plays an important role in viral replication. Thus, making it a very interesting target for the development of specific antiviral inhibitor

Proteins for Malaria

Circumsporozoite Protein - surface antigen

PDB ID : 2MSA

The sporozoite's major surface protein, Circumsporozoite protein (CSP), is a multifunctional protein required for sporozoite development and likely mediates several steps of this journey. Contacts between CSP and heparan sulfate proteoglycans (HSPGs) lead to the attachment of sporozoites to hepatocytes and trigger signaling events in the parasite that promote invasion of hepatocytes.

Glutathione S-Transferase

PDB : 1OKT

In contrast to many other organisms, the malarial parasite Plasmodium falciparum possesses only one GST isoenzyme (PfGST). This GST is highly abundant in the parasite, its activity was found to be increased in chloroquine-resistant cells, and it has been shown to act as a ligandin for parasito toxic hemin.

Apical Membrane Antigen 1

PDB :3SRI

The Moving junction contains two key parasite components: the surface protein Apical Membrane Antigen 1 (AMA1) and its receptor, the Rhoptry Neck Protein (RON) complex, which is targeted to the host cell membrane during invasion.

Plasmodium falciparum lactate dehydrogenase enzyme (PfLDH)

PDB ID: 3ZH2

The Plasmodium sps. depend mostly on anaerobic glycolysis for energy production. Lactate dehydrogenase (LDH) constitutes a major checkpoint of anaerobic glycolysis, by catalyzing the reduction of pyruvate into lactate. Thus, the Plasmodium falciparum lactate dehydrogenase enzyme (PfLDH) is a key enzyme for energy generation of malarial parasites. Inhibition of PfLDH could lead to death of the parasite. So, PflDH could be a potential antimalarial chemotherapeutic target.

Plasmodium falciparum Erythrocyte Membrane Protein 1(PfEMP1)

PDB ID: 2LKL

The protein PfEMP1 is involved in cell adhesion molecule binding and host cell surface receptor binding. Erythrocyte adherence is directly associated with severe malaria and increased disease lethality, and it is mediated by the PfEMP1 family.This protein is critical for cytoadherence. Targeting this protein could disrupt the parasite cytoadherence system and reduce the lethality.

Procedure:

1. Ligand Screening

For the initial Ligand screening purposes, a web-based tool named SwissADME (https ://www.swiss adme.ch/) was used to eliminate a few compounds according to Lipinski's rule of five parameters. For a compound to qualify as ligand it should Have < 500 Da molecular weight, a high lipophilicity i.e. value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less than 5. Any compound with more than 2 violations was ruled out for further study (Lipinski2004).

2. Protein Preparation and Active site Determination.

Required protein in pdb format was downloaded from the website **rcsb.org**, commonly known as the **Protein Data Bank**. 3D conformers of the ligand were downloaded from PubChem.

Using **PyMOL (Version 2.4.1)** software water molecules as well as native ligands from the protein were removed, defined as cleaning/purification of the protein for further application. **Using a web server called DeepSite** Active Pockets of the proteins were calculated. The results calculated by web server was in the form of different ids, centers and scores.

Scoring In deep site was using neural networking based on following instructions using DCNN architecture. https://academic.oup.com/bioinformatics/article/33/19/3036/3859178 Center values for the grid were selected keeping score greater than 0.98.

UCSF Chimera (Version 1.14) was used to prepare the receptor using DockPrep function. **Dock Prep** prepared structures for Docking using these functions:

- deleting water molecules
- repairing truncated sidechains
- adding hydrogens
- assigning partial charges
- writing files in Mol2 format

3. In silico Docking Using Auto dock Vina

Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular **Docking Studies**. Center values and size of the grid of different scores were used from **DEEPSITE** calculations done above.

Following Parameters were set in auto dock vina.

Receptor options –

- **Add hydrogens in Chimera** (**true**/false) – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar (potentially H-bonding) hydrogens to identify atom types for scoring purposes.
- **Merge charges and remove non-polar hydrogens** (**true**/false) – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the processed receptor (and there may not have been any lone pairs to start with)
- **Ignore waters** (**true**/false)
- **Ignore chains of non-standard residues** (**true**/false) – ignore chains composed entirely of residues other than the 20 standard amino acids.
- **Ignore all non-standard residues** (true/**false**) – ignore all residues other than the 20 standard amino acids.

For Ligands

- **Merge charges and remove non-polar hydrogens** (**true**/false) – note Auto Dock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the ligand output files
- **Merge charges and remove lone pairs** (**true**/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with)

Docking parameters

1. **Number of binding modes** (1-10, **10**) – maximum number of binding modes to generate
2. **Exhaustiveness of search** (1-8, **8**) – thoroughness of search, roughly proportional to time
3. **Maximum energy difference (kcal/mol)** (1-3,**3**) – maximum score range; binding modes with scores not within this range of the best score will be discarded.

The docking results were calculated by Auto dock vina using it's Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative sign and least RMSD values were chosen for further studies.

4. Residue Analysis

PyMOL was used for visualization of interactions of the docked structure at the ligand sites. **Discovery Studio 2020** was used to study the ligand interactions and total number of residues. It was also used to plot the 2D structure of the interactions and residues.

5. Statistical Analysis: Descriptive, estimation and Hypothesis testing with confidence interval of 95% was applied to data using formula 1 given below.

$$CI = \bar{x} \pm z\frac{s}{\sqrt{n}}$$

CI = confidence interval
$\bar{x}$ = sample mean
z = confidence level value
s = sample standard deviation
n = sample size

Formula 1 used for calculation of confidence interval

Results and Discussion:

Docking Results: Based on the above statics the docking result is summarized in Table 1 and the interactions are shown below.**Bioavailability Radar:** Further analysis included selected ligands viz. Remdesivir, Hydroxy-chloroquine, Curcumin, Moroxydine, Artesunate Sulphate, Mefloquine, Zanamivir, Doxycycline, Atovaquone, Indinavir, A more illustrated and comprehensive study was done using bio- availability radar. Bioavailability radar is descriptive tool to investigate the drug-likeness of the ligands based on six physicochemical properties. The radar that fits the shaded area are Orally bioavailable **Table 2**.

LIGANDS	Dengue Proteins	Malarial Proteins
REMDESEVIR	Yes	NO
ATOVAQUONE	NO	YES
ARTESUNATE	NO	YES
CURCUMIN	Yes	NO
HCQ	NO	YES
INDINAVIR	YES	NO
MOROXYDINE	NO	NO
DOXYCYCLINE	NO	YES
MEFLOQUINE	NO	YES

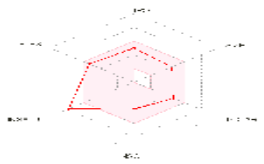
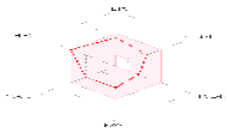
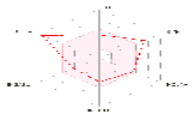
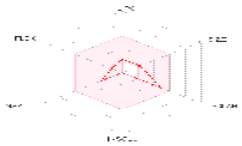
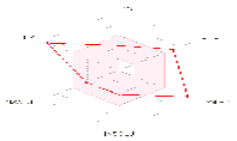
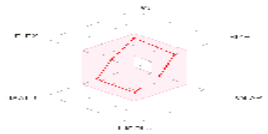
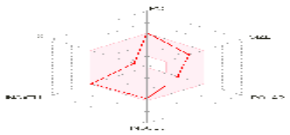
LIGAND	BIOAVAILABILITY SCORE	LIPINSKI RULE	RADAR
CURCUMIN	0.55	YES/0 VIOLATION	
HCQ	0.55	YES/0 VIOLATION	
INDINAVIR	0.55	YES/1 VIOLATION: MW>500	
MOROXYDINE	0.55	YES/0 VIOLATION	
REMDESIVIR	0.17	YES/2 VIOLATIONS: MW >500, NorO>10	
MEFLOQUINE	0.55	YES/0 VIOLATION	
ATOVAQUONE	0.85	YES/ 0 VIOLATION	
ARTESUNATE	0.56	YES/ 0 VIOLATION	
DOXYCYCLINE	0.11	YES/ 1 VIOLATION: NHorOH>5	

Table 2: *shows the bioavailability radar charts of selected ligands*

Molecular Docking:

The docking result was obtained from Autodock vina in the form of Dock score for all the three proteins docked with above mentioned ligands, average docking Score of each ligand aggregated to average dock score of three proteins were taken Fig 1. Standard deviation and Confidence interval were calculated, based on the confidence interval minimum value of dock score for each ligand was calculated Table 3. All the dock scores above the minimum score were considered for further evaluations.

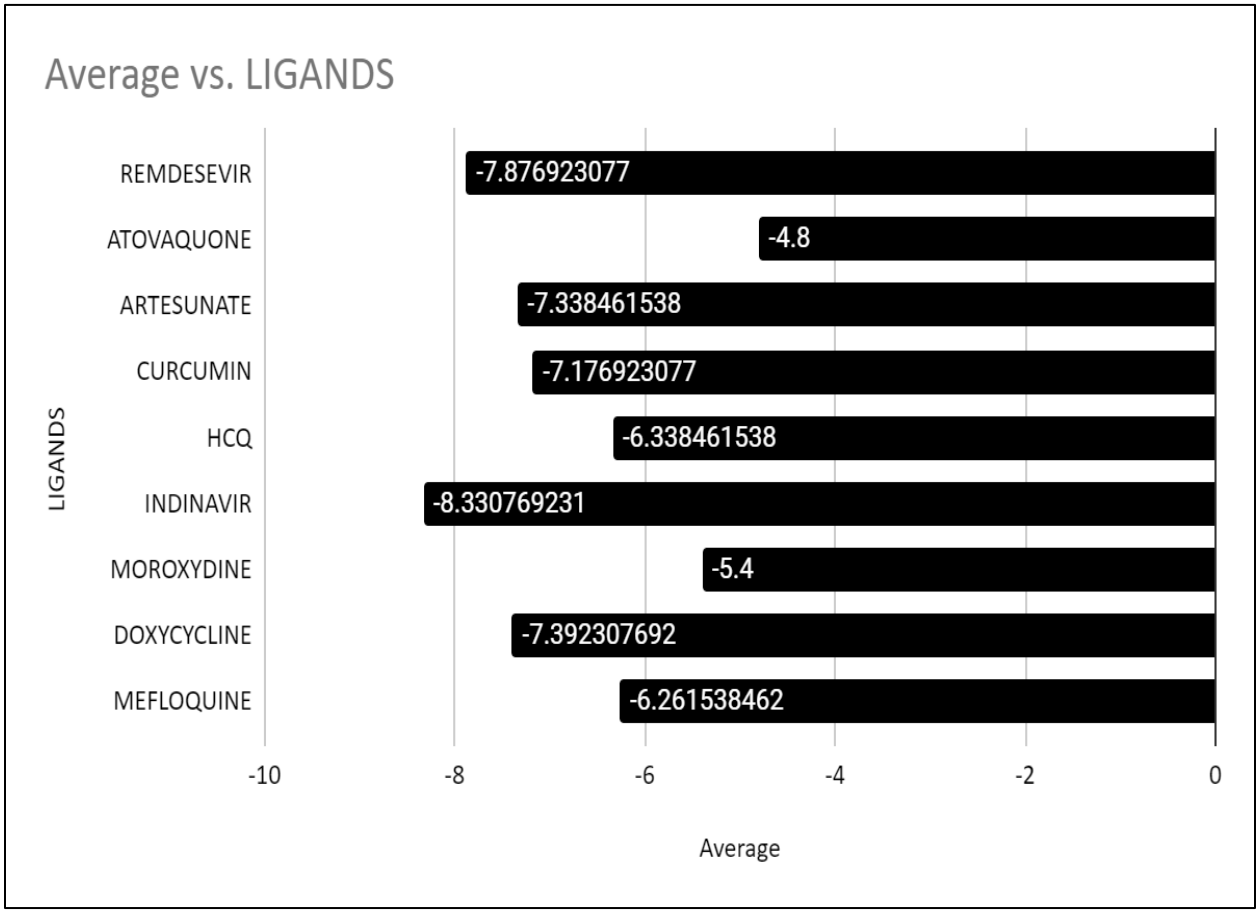


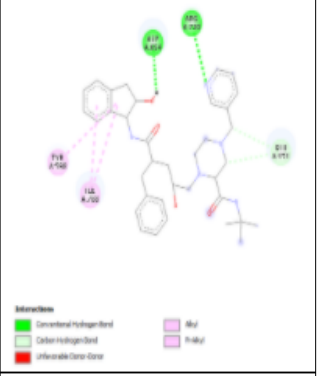
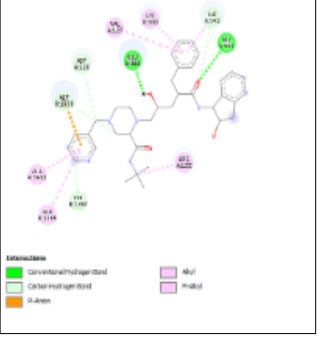
Figure 1: shows the average aggregate dock score for 3 proteins with respective ligands.

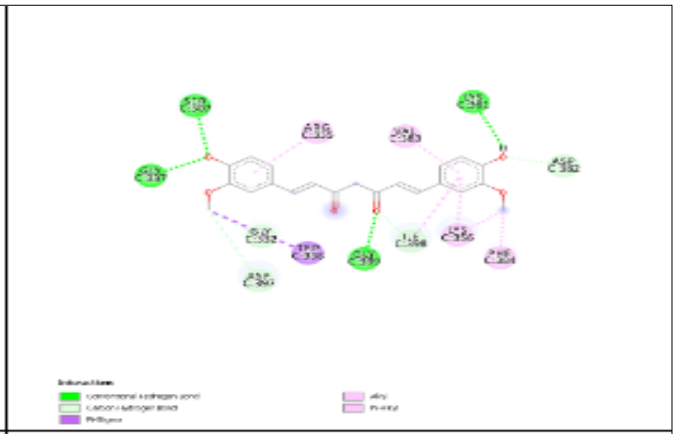
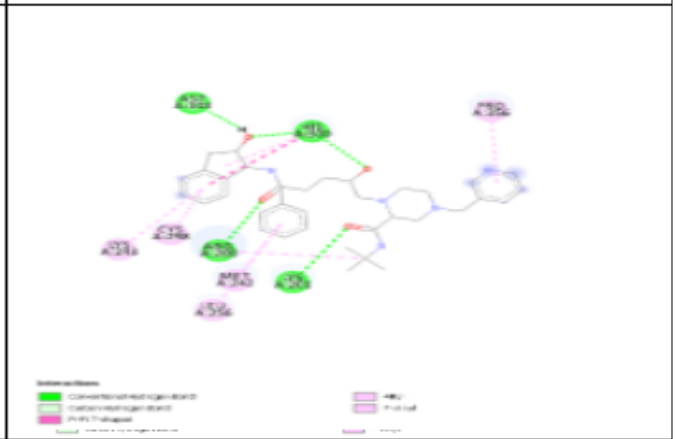
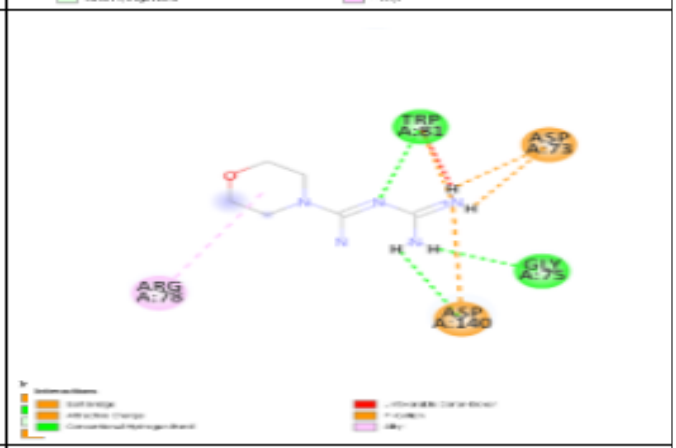
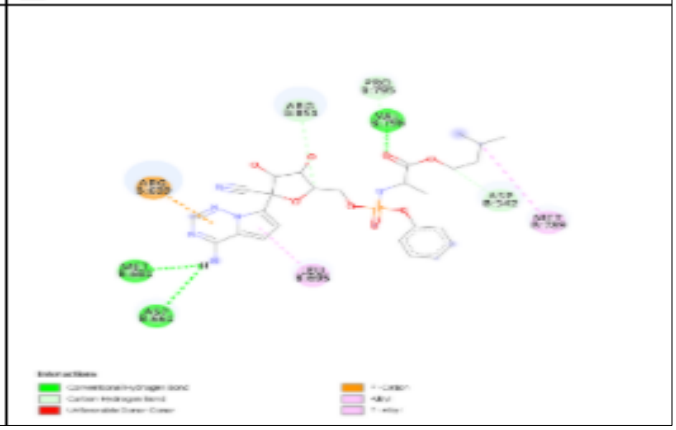
Table 2

LIGANDS	Average	Standard Deviation Sample	AVG Sample Size	Confidence Interval 95%	Min Score in 95% Confidence
REMDESEVIR	-7.876923077	0.5717718749	13	0.345518286	-7.531404791
ATOVAQUONE	-4.8	0.4672615256	13	0.282363314	-4.517636686
ARTESUNATE	-7.338461538	0.6292038111	13	0.3802240577	-6.958237481
CURCUMIN	-7.176923077	0.8115354235	13	0.4904059483	-6.68651713
HCQ	-6.338461538	0.3927222546	13	0.2373196833	-6.101141855
INDINAVIR	-8.330769231	0.7028330947	13	0.4247177884	-7.906051443
MOROXYDINE	-5.4	0.3674234614	13	0.2220317756	-5.177968224
DOXYCYCLINE	-7.392307692	0.6701511502	13	0.404968287	-6.987339405
MEFLOQUINE	-6.261538462	0.4839739345	13	0.2924625216	-5.96907594

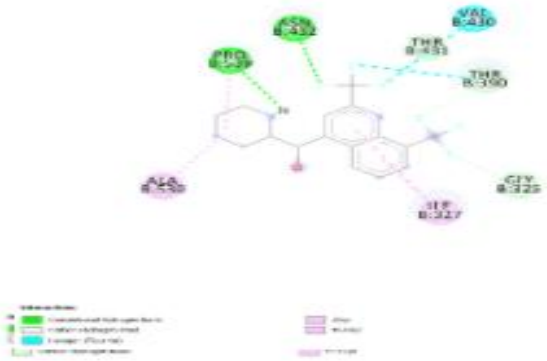
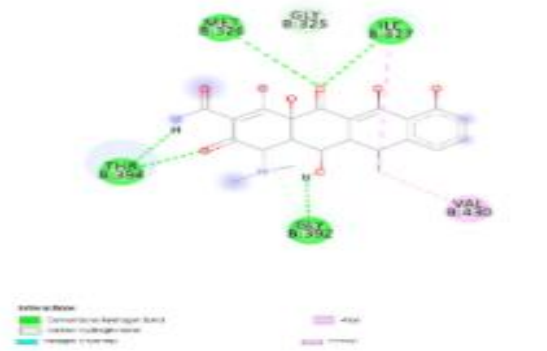
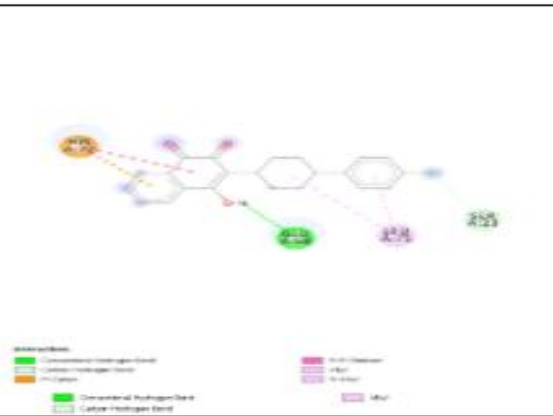
Table 3 *shows the standard deviation, confidence interval at 95% with minimum dock score.*

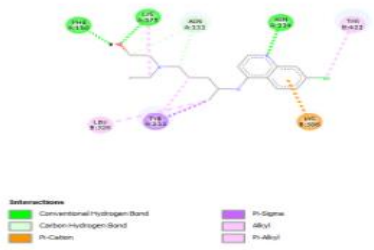
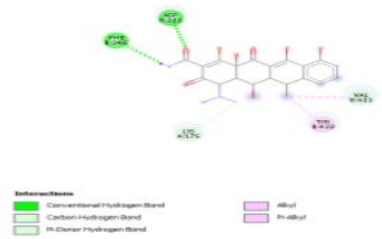
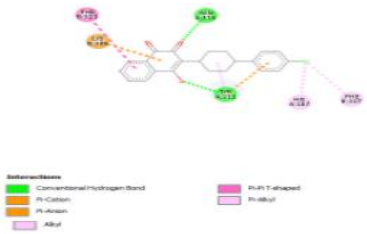
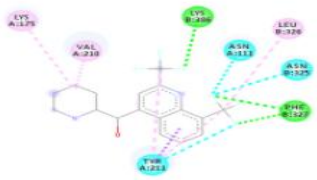
Dengue Docking Results

Dock Score	PDB-Id	ligands	Interaction
-8.1	5zqk [A] (0)	Curcumin	
-8.4	5zqk [A] (1)	Curcumin	
-8.3	5zqk [A] (0)	Indinavir	
-9	5zqk [A] (1)	Indinavir	

-8.3	4CTK_1	Curcumin	
-9.1	4CTK_1	Indinavir	
-8.5	2BHR_0	Remdesivir	
-8.9	4CTK_1	Remdesivir	

Malaria Docking Results

Dock Score	PDB ID	Ligand	Interactions
-7.3	3ZH2	Mefloquine	
-7.4	3ZH2	Doxycycline	
-7.8	3ZH2	Atovaquone	
-7.2	3ZH2	Artesunate	

-7.8	1OKT	HCQ	
-9.2	1OKT	Artesunate	
-9.3	1OKT	Atovaquone	
-8.9	1OKT	Mefloquine	

Conclusion:

All nine ligands were studied using bioavailability radar. Our results proposed Remdesivir, Curcumin, and Indinavir showed best docking result for Dengue Mpro Proteins with PDB id's mentioned above whereas Hydroxy-chloroquine, Artesunate Sulphate, Mefloquine, Doxycycline, Atovaquone, showed best docking result for Malarial Mpro Proteins with PDB id's mentioned above. To find the effectiveness and to propose the exact mechanism in-vitro studies can be encouraged on these findings.

Acknowledgement:

We would like to thank our supervisor, Professor Bharat Kwatra,from Invenzion Labs. whose expertise was invaluable in formulating the research questions, methodology and drawing Conclusions. His insightful feedback and Guidance pushed me to sharpen our thinking and brought our work to a higher level.

References

1. Cheung, Y. W. *et al.* Structural basis for discriminatory recognition of Plasmodium lactate dehydrogenase by a DNA aptamer. *Proc. Natl. Acad. Sci. U. S. A.* **110**, (2013).
2. Mayer, C., Slater, L., Erat, M. C., Konrat, R. & Vakonakis, I. Structural analysis of the Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) intracellular domain reveals a conserved interaction epitope. *J. Biol. Chem.* **287**, (2012).
3. Xu, T. *et al.* Structure of the Dengue Virus Helicase/Nucleoside Triphosphatase Catalytic Domain at a Resolution of 2.4 Å. *J. Virol.* **79**, (2005).
4. Coutard, B. *et al.* Assessment of Dengue virus

helicase and methyltransferase as targets for fragment-based drug discovery. *Antiviral Res.* **106**, (2014).
5. Vulliez-Le Normand, B. *et al.* Structural and functional insights into the malaria parasite moving junction complex. *PLoS Pathog.* **8**, (2012).
6. Fritz-Wolf, K. *et al.* X-ray structure of glutathione S-transferase from the malarial parasite Plasmodium falciparum. *Proc. Natl. Acad. Sci. U. S. A.* **100**, (2003).
7. Lai, Y. H. The climatic factors affecting dengue fever outbreaks in southern Taiwan: An application of symbolic data analysis. *Biomed. Eng. Online* **17**, (2018).
8. Kularatnam, G. A. M. *et al.* Evaluation of biochemical and haematological changes in dengue fever and dengue hemorrhagic fever in Sri Lankan children: A prospective follow up study. *BMC Pediatr.* **19**, (2019).
9. Li, L. *et al.* The flavivirus precursor membrane-envelope protein complex: Structure and maturation. *Science (80-.).* **319**, (2008).
10. Xia, H. *et al.* A cocrystal structure of dengue capsid protein in complex of inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **117**, (2020).
11. El Sahili, A. *et al.* NS5 from Dengue Virus Serotype 2 Can Adopt a Conformation Analogous to That of Its Zika Virus and Japanese Encephalitis Virus Homologues. *J. Virol.* **94**, (2019).
12. Studio, D. Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 4.5. *Accelrys Softw. Inc.* (2015).
13. Schrödinger, L. The PyMol Molecular Graphics System, Versión 1.8. *Thomas Holder* (2015).
14. Morris, G. M. *et al.* Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **30**, (2009).
15. Jiménez, J., Doerr, S., Martínez-Rosell, G., Rose, A. S. & De Fabritiis, G. DeepSite: Protein-binding site predictor using 3D-convolutional neural networks. *Bioinformatics* **33**, (2017).
16. Pettersen, E. F. *et al.* UCSF Chimera - A visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, (2004).
17. Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Research* vol. 28 (2000).
18. Daina, A., Michielin, O. & Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules.