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Virtual Screening of compounds to 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) from *Plasmodium falciparum*

Kamal Kumar Chaudhary & C.V.S. Siva Prasad*

Division of Applied Sciences & IRCB, Systems Biology lab, Indian Institute of Information Technology Allahabad, Deoghat, Jhalwa, Allahabad 211012, India; C.V.S. Siva Prasad – E mail: shiva@iiita.ac.in; Phone: +91-(0) 532 2922000; Fax: +91-(0) 532 2430006; *Corresponding author

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Abstract:
The 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) protein (Gen Bank ID AAN37254.1) from *Plasmodium falciparum* is a potential drug target. Therefore, it is of interest to screen DXR against a virtual library of compounds (at the ZINC database) for potential binders as possible inhibitors. This exercise helped to choose 10 top ranking molecules with ZINC00200163 [N-(2,2di methoxy ethyl)-6-methyl-2, 3, 4, 9-tetrahydro-1H-carbazol-1-amine] a having good fit (-6.43 KJ/mol binding energy) with the target protein. Thus, ZINC00200163 is identified as a potential molecule for further comprehensive characterization and in-depth analysis.

Keywords: AAN37254.1, Virtual screening, Molecular Docking, *Plasmodium falciparum*, Druggability.

Background:
Plasmodium falciparum is a protozoan parasite, and one of the species of *Plasmodium* that causes malaria disease in humans **[1].** It is a life-threatening disease and carried through female *Anopheles* mosquito **[1].** It causes high mortality and morbidity rate in human malarial infections (98% in Africa) and more dominant in Sub-Saharan Africa. It strikes around 100 tropical countries of the world, almost 1 million people die due to malaria annually **[2].** Maximum death due to malaria is caused by *P. falciparum* species. Resistance developed in this species against antimalarial drugs has significantly hampered malaria control process in the last 50 years **[1, 2].** Extensive analysis of the new target and new inhibitors to control malarial infection is needed. Simultaneously target should be non-human orthologous along with small or no effect on human **[3].** Target should be essential protein for pathogen, and should be no-homologous to the human host **[4, 5].** Objective of this study is to identify potential drug target as well as novel inhibitors also for treatment of malaria. Recent protein-protein interaction (PPI) studies have suggested that conservation of molecular networks provides the information that proteins with high degrees of connection are more probably to be essential for survival than proteins with lesser degrees. In this work MEP pathway is taken into consideration which is absent in humans but plays essential roles into mosquitoes. 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) is a fundamental enzyme of MEP pathway and described as target of fosmidomycin drug of malaria **[6].** Ligand-binding site was predicted, which is a key step in order to look into the function and molecular mechanism of a protein. Ligand-binding sites give key information about drug designing process through computational and Structural analysis **[7].** All of this information about the potential drug

target and new inhibitor would assist the drug discovery process.

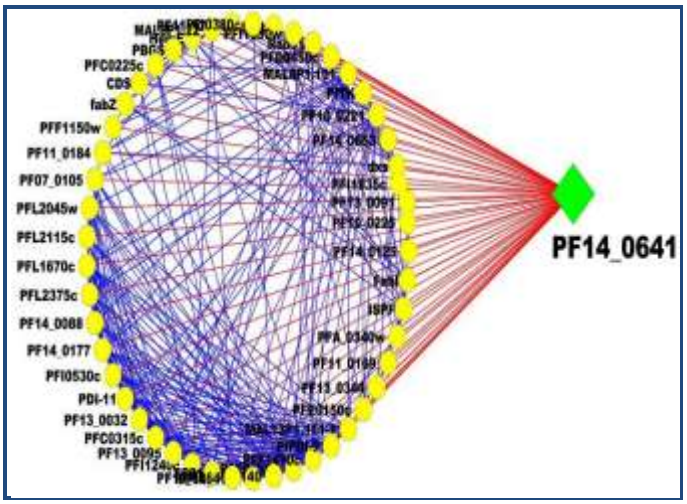

Figure 1: Graphical representation of protein interacting with 1-deoxy-D-xylulose 5-phosphate reductoisomerase (PF14_0641) protein highlighted in green. All yellow circles represent its interaction partners release of STRING database. This representation was performed using Cytoscape software

Methodology:

Protein-protein interaction network

Protein-Protein Interaction (PPI) Networks, where proteins are nodes and their interactions are edges. PPI networks are the most intensely studied networks in biology. In this respect lot of PPI detection methods are available to identify such interactions. Protein interaction and network analysis of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (PF14_0641) protein was performed and represented by using Cytoscape software (http://www.cytoscape.org/).

3D Structure of potential drug target

The structure for 1-deoxy-D-xylulose 5-phosphate reductoisomerase (Gen Bank ID: AAN37254.1) from *Plasmodium falciparum* was acquired in the UniProtKB/Swiss-Prot protein sequence database Accession no. Q8IKG4 (DXR_PLAF7). BLASTp **[8]** was performed against Protein Data Bank (PDB) **[9, 10].** BLASTp was used to optimize specific similarity measures. A 100% similar structure of *Plasmodium falciparum* protein was found in the Protein Data Bank (PDB ID: 3AU8_A). The sequence was downloaded from protein data bank, which is an archive for crystal structures of biological macro molecules. After downloading structure 3D coordinates from PDB database validation of the structure was done by using Ramachandran plot.

Active site prediction

Active site is the region of the target protein responsible for its activity and made up of different kinds of amino acid residues. Active site prediction was done by web server operated software Pocket Finder (http://www. modelling.leeds. ac.uk/pocketfinder/), DoGSite Scorer (http://dogsite. zbh.uni-hamburg.de/) **[11]** and CASTp (http://sts-fw.bioengr.uic.edu /castp/calculation.php) **[12].** Pocket Finder is used to detects and compare pocket with ligand binding site. DoGSite Scorer detects binding site and pockets in the protein structure; simultaneously they analyze its druggability by taking into consideration of its geometric and physiochemical properties **[13].** CASTp is used for detection and location of protein surface topography and measure pockets and voids on the 3D structure of the protein **[14].**

Protein preparation

The Open Eye module "Make Receptor" prepares the protein structure as receptor site for virtual screening of ligands. Active site within protein was detected and put into box of dimensions 15.89 Å x 22.87 Å x 32.28 Å and a total volume of 11732 Å3.

Ligand preparation

11826 lead and drug like molecules were downloaded between a range of mol. Wt. 35 to 350 and xlogP -4 to 3.5 from Zinc database **[15]** in mol2 format. These molecules used for virtual screening and docking further **[16].**

Virtual screening

FRED module of the Open Eye software uses exhaustive search algorithm for virtual screening of the ligands to bind the protein receptor **[17].** The Top-10 molecules were accepted as the best docked molecules against the receptor and once again rescored with high optimization along with true short poses. Chemgauss4 scoring function observes the shape and hydrogen bonding interactions on the protein, although the chemgauss4 is an amended version, more previous observes hydrogen bond geometry with hydrogen bond networks **[17, 18].** It is based on FRED 3.0 chemgauss4 scoring and lowest score of chemgauss4 results as the best docked molecules with hypothesis used as drugs in the future into account.

Molecular docking

The Molecular docking study employed to identify the binding affinity and interaction energy of the molecules with the target protein. Molecular docking was carried out by using AutoDock 4.0 software. Based on predicted binding residues **[19],** grid box was constructed and molecular docking was performed using tools like Autogrid and AutoDock. Applying AutoDock 4.0, top five molecules were docked to the 3AU8_A. By removing all the water molecules, co-factors and ligands from the protein structure and checked the macromolecule for the polar hydrogens and assigns atomic Kollman charges and atomic solvation parameters. Torsion bonds of the ligands were selected and defined. To evaluate the binding energy on the macromolecule coordinate a three dimensional grid box of 74 x 88 x 84 Å3 with spacing of 0.3 Å was created using Auto Grid which calculates the grid map representing the bound ligand in the actual target site **[20].**

Ligplot

It is a computer based program that automatically gives schematic 2-D representations of protein-ligand complexes interactions for a given PDB file. Only those interactions are shown, which was mediated by hydrophobic contacts and by hydrogen bonds. Hydrogen bond interactions are showed by dashed lines, while hydrophobic interactions are symbolized by an arc with spokes radiating towards the ligand atoms. The contacted atoms are shown with spokes radiating back.

Toxicity Prediction

The toxicological investigation of top five virtually screened molecules were carried out through OSIRIS Property explorer (*http://www.organic-chemistry.org/prog/peo/*), it provides drug relevant property such as cLogp, Molecular weight, Solubility, Drug Likeness, Drug Score, Mutagenicity, Irritancy, and Reproductive effect etc**. [21].** Top five screened molecules were subjected to the toxicity prediction and results were analyzed by keeping in view of drug like parameters.

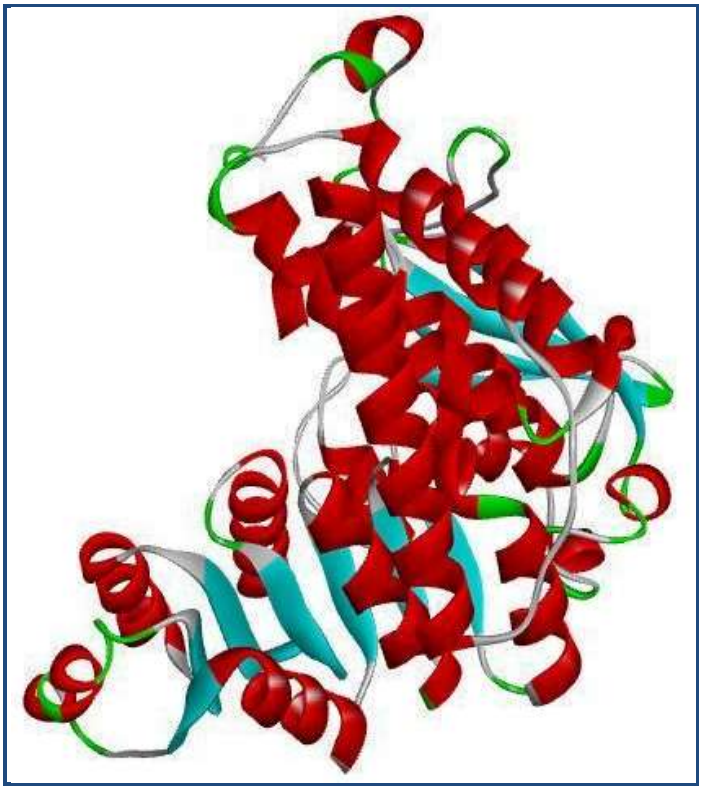

Figure 2: 3D structure of 1-deoxy-D-xylulose 5-phosphate reductoisomerase

Results:

Protein-protein interaction network

In PPI network maps, nodes represent protein and edges represent a physical interaction between two proteins **(Figure 1).**

Structure validation of Target protein

Blast search was performed for Gen Bank ID: AN37254.1 against Protein Data bank (PDB), and 100% similar sequence was received as PDB ID: 3AU8_A **(Figure 2).** It belongs to 1-deoxy-D-xylulose 5-phosphate reductoisomerase from *Plasmodium falciparum* species. Therefore there was no need to model the structure but the structure was validated by the Ramachandran plot which shows the details of the stability of the protein structure. According to the Ramachandran plot analysis 92.7% residues are falling in the most favorable regions.

Active Site Prediction

Comparative studies for active site residues were done by Pocket Finder, CASTp and DoGSite Scorer. The residues were observed common in the ealier repoted literature **[22, 23]** also **Table 1 (see supplementary material).**

Virtual Screening

The protein structure was prepared as a receptor site by preparing the active sites in the protein and defining its inner and outer contours. A library of lead and drug like molecules was screened with the help of FRED module of Open Eye software against receptor molecule 3AU8_A. The results are depicted in **Table 2 (see supplementary material)** according to their chemgauss4 scores to evaluate the complementarity of ligand poses within the binding site. After virtual screening against DXR active site, top ten molecules from 11826 molecules were selected based on chemgauss4 score **(Table 2).** ZINC00200163 [N-(2,2-dimethoxyethyl)-6-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-amine] placed in top position because of its least chemgauss4 score -13.075439, hydrogen bond energy -6.14 with 98% better scores. From selected top ten molecules, top five molecules have been selected for, further detail docking study with target protein by Autodock 4.0 software.

Molecular Docking and validation

Molecular docking was performed between DXR and top five selected screened molecules. Reference molecule like Fosmidomycin, Artemether, Mefloquine, and Quinidine was taken for Comparative analysis **Table 3 (see supplementary material).** It was found that ZINC00200163 [N-(2,2-dimethoxyethyl)-6-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-amine] molecule showed the best interaction with least binding energy (-6.43 KJ/Mol), which was better than other molecules. The H-bond interaction was studied through PyMol software. The numbers of H-bonds were calculated with bond length between atoms of protein-ligand docked complex. The molecular docking of the hits showed the binding and interaction energy. H-bond pattern and confirmed inhibition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase was analyzed which is directly linked to the antimalarial activity.

LigPlot

The LigPlot analysis was used to understand the exhaustive interaction pattern between the docked ligands and the active site residues. LigPlot is a requisite tool to understand hydrophobic interactions as well as hydrogen bonding pattern **[24]. Figure 3** shows LigPlot analyses for the top five virtually screened molecules which were discussed previously in docking simulations. The same H-bond interactions as seen in docking results were obtained for the five molecules. Thus, Ligplot analyses were particularly useful in knowing the hydrophobic interaction pattern.

Toxicity Prediction analysis

The toxicological studies of top five virtually screened drug candidates **Table 4 (see supplementary material)** suggest encouraging results. While analyzing the toxicological outputs by OSIRIS Property explorer (http://www.organic-chemistry.org/prog/peo/), among all five proposed drug candidates, ZINC00200163 is found to be best because it does not show any toxic property. In addition, this drug candidate was found to be preeminent in terms of cLogp, Molecular

weight, Solubility, Drug Likeness, Drug Score, Mutagenicity, Irritancy, and Reproductive effect etc.

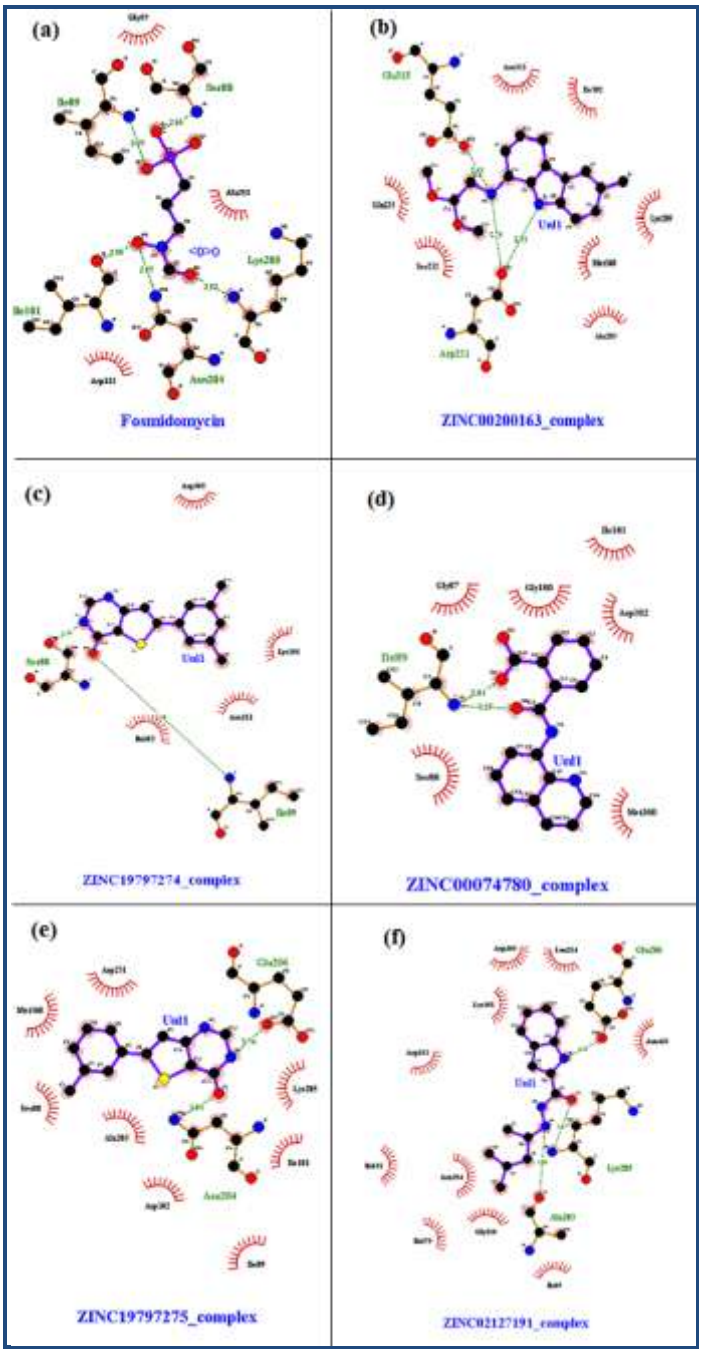

Figure 3: LigPlot analyses results: Schematic 2D representation of ligand–protein interactions were analyzed among receptor and reference top five molecules **(a)** Fosmidomycin **(b)** ZINC00200163 **(c)** ZINC19797274 **(d)** ZINC00074780 **(e)** ZINC19797275 **(f)** ZINC02127191. Hydrogen bond forming residues were shown in lines with hydrogen bonds shown as dotted lines and residues interacting by hydrophobic interactions were represented as lines in red.

Discussion:

Novel drug targets are needed in order to design new effective drugs against Malaria. Potential drug target should be a molecule essential for pathogens and non-essential for human, therefore inhibition of essential proteins would kill the pathogen but have no effect on human **[3].** Isoprenoids is crucial for respiration, membrane structure, allelochemical interactions, and growth regulation **[25, 26].** Isopentenyl diphosphate is a precursor of isoprenoids and is produced by the 2-C-methyl-derythritol 4-phosphate (MEP) pathway in plastids of plant and protozoa **[23, 24].** DXR is a fundamental enzyme of MEP pathway which is absent in human and reported as the drug target for fosmidomycin drug molecule of malaria **[6].** The 3D structure of DXR from *Plasmodium falciparum* was downloaded from PDB database (PDB ID: 3AU_8) and validated by computational tools viz. PROCHEK, ERRAT, VARIFY 3D and WHATCHECK. Overall validation results confirmed that the 3D structure was good. Active site of the DXR was predicted with the help of Pocket Finder, CASTp and Dog-Site Scorer and it was compared with the already reported literature **[25]** showing ALA 203, ASN 204, GLU 206, ASP 231, SER 232, GLU 233, LYS 301, ILE 302, ASP 305 amino acids residues in the active site in the DXR protein. Our prediction with various active site prediction software are corroborating. Virtual Screening was performed for DXR protein against the database collected from ZINC database. Screened molecules were subjected to the docking with the DXR protein. Some of the commercially available inhibitors like Fosmidomycin, Artemether, Mefloquine, and Quinidine were taken as reference molecules for comparative analysis with the screened molecules. Molecular docking result of the DXR with the screened molecules and reference molecules were analyzed. Top five screened inhibitors showed comparatively lesser binding energy as compared to the commercially available molecules. Out of five screened molecules, ZINC00200163 [N-(2,2-dimethoxyethyl)-6-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-amine] has proved as best ligand because of its least binding energy (-6.43 KJ/Mol) followed by ZINC19797274 [6-(3,5-dimethylphenyl)-3H,4H-thieno[3,2-d]pyrimidin-4-one], ZINC00074780 [2-(quinolin-8-ylcarbamoyl)benzoic acid], ZINC19797275 [6-(3-methylphenyl)thieno[3,2-d]pyrimidin-4(3H)-one], and ZINC02127191 [N'-(1,3-dimethylbutyl)-1H-indole-2-carbohydrazide]. The in-silico toxicological studies were carried out for above said virtually screened proposed drug candidates (Table 4) and different parameters were analyzed and found ZINC00200163 is not showing any toxicity to humans.

Conclusion:

This study documents the identification ZINC00200163 [N-(2,2-dimethoxyethyl)-6-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-amine] as potential candidate for the inhibition of 1-deoxy-D-xylulose5-phosphate reductoisomerase (DXR) using structure aided docking based virtual screening tools and databases. ZINC00200163 is characterized with low binding energy and better binding for further consideration.

Acknowledgement:

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Conflict of interest statement:

We declare that we have no conflict of interest.

References:
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[2] Parija SC & Praharaj I, *Indian J Med Microbiol.* 2011 **29**: 243 [PMID: 21860103]
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[6] Odom AR & Van Voorhis WC, *Mol Biochem Parasitol.* 2010 **170**: 108 [PMID: 20018214]
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[9] http://www.rcsb.org.
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Supplementary material:

Table 1: Comparing active site residues obtained in Pocket Finder, CASTp and DogSiteScorer. The residues highlighted are common in all three and earlier reported literature.

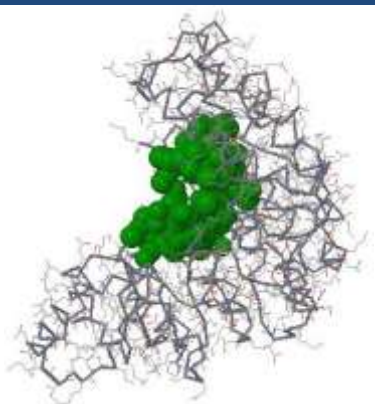
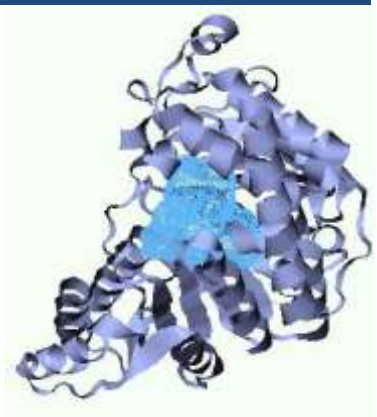
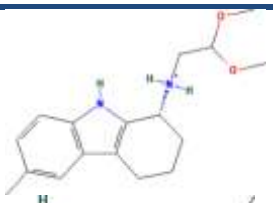
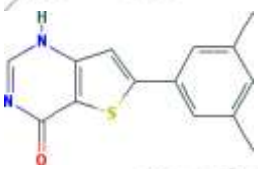
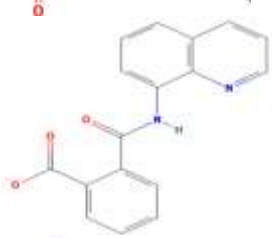
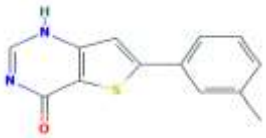
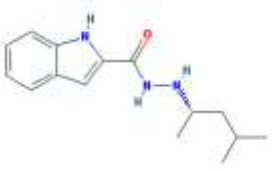
Pocket Finder	CASTp	DogSiteScorer
		
GLY 87, SER 88, ILE 89, GLY 90, ILE 179, GLY 180, ILE 181, ASP 182, ALA 203, ASN 204, LYS 205, GLU 206, ASP 231, SER 232, GLU 233, LYS 301, ILE 302, ASP 305	**GLY 87, SER 88, ILE 89,GLY 90, GLY 180, ILE 181, ASP 182, SER 183, PHE 184, ALA 203, ASN 204, LYS 205, GLU 206, VAL 209, ASP 231, SER 232, GLU 233, LYS 301, ILE 302, ASP 305**	**ALA, ARG, ASN, ASP, CYS , GLN, GLU, GLY, HIS, ILE, LEU, LYS, MET, PHE, PRO, SER, THR, TRP, TYR, VAL.**

Table 2: Results of Top 10 Molecules after Virtual Screening with ZINC ID, IUPAC name, 2D structure and chemgauss4 score.

Sr.No.	ZINC ID	Common name	Structure	Chemgauss4 score
1.	ZINC00200163	N-(2,2-dimethoxyethyl)-6-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-amine		-13.075439
2.	ZINC19797274	6-(3,5-dimethylphenyl)-3H,4H-thieno[3,2-d]pyrimidin-4-one		-12.663762
3.	ZINC00074780	2-(quinolin-8-ylcarbamoyl)benzoic acid		-12.591833
4.	ZINC19797275	6-(3-methylphenyl)thieno[3,2-d]pyrimidin-4(3H)-one		-12.186634
5.	ZINC02127191	N'-(1,3-dimethylbutyl)-1H-indole-2-carbohydrazide		-11.654129

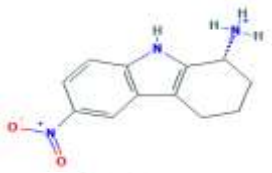
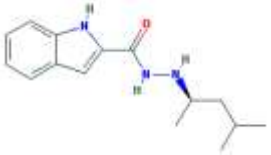
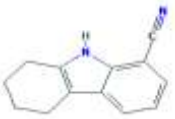
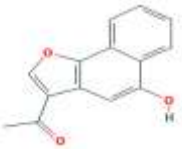
6.	ZINC00208551	6-nitro-2,3,4,9-tetrahydro-1H-carbazol-1-amine		-11.472092
7.	ZINC04569008	N-(2,4-dihydroxy-6-methyl-5-pyrimidinyl)-1-naphthamide		-11.441998
8.	ZINC02127190	N'-(1,3-dimethylbutyl)-1H-indole-2-carbohydrazide		-11.434769
9.	ZINC00208675	2,3,4,9-tetrahydro-1H-carbazole-8-carbonitrile		-11.168977
10.	ZINC02472134	1-(5-hydroxynaphtho[1,2-b]furan-3-yl)ethanone		-11.135767

Table 3: Docking results of Reference and top five screened molecules with auto dock

Sr. No.		Molecules	Binding Energy (KJ/mol)	Ligand Efficiency	Electrostatic Energy	Hydrogen Bonds
1.		Fosmidomycin	-2.72	-0.25	-0.9	2
2.		Artemether	-4.29	-0.2	-0.05	1
3.	Reference	Mefloquine	-4.6	-0.18	-2.45	4
4.	Molecules	Quinidine	-3.02	-0.13	-1.17	1
5.		ZINC00200163	-6.43	-0.31	-4.44	2
6.	Screened Molecules	ZINC19797274	-4.06	-0.23	-0.13	0
7.		ZINC00074780	-3.56	-0.16	-0.15	2
8.		ZINC19797275	-4.15	-0.24	-0.18	1
9.		ZINC02127191	-3.65	-0.19	-0.2	2

Table 4: Various critical properties of drug candidates derived from OSIRIS Property explorer

Sr. No.	ZINC ID	cLogp	Solubility	Molecular Weight	Drug likeness	Drug Score	Mutagenic	Tumorigenic	Irritant	Reproductive Effect
1.	ZINC00200163	1.63	-2.48	306	2.7	0.88	No	No	No	No
2.	ZINC19797274	3.54	-5.35	256	-3.13	0.26	Yes	No	No	No
3.	ZINC00074780	2.9	-3.85	292	-3.08	0.21	Yes	No	Yes	Yes
4.	ZINC19797275	3.23	-5.01	242	0.59	0.56	No	No	No	No
5.	ZINC02127191	3.07	-3.89	273	0.12	0.22	Yes	No	Yes	No