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Homology modeling and docking analysis of Prodigiosin from *Serratia marcescens*

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ABSTRACT

Prodigiosin is a powerful red pyrrole pigment produced by several bacteria, especially in *Serratia marcescens*. Prodigiosin has a wide biological activity profile with antifungal, immunosuppressive and anti-proliferative activity. Investigations were made in the present study to identify the activity of Prodigiosin against virus and bacteria related affected diseases. 3D modeling of HBV, HIV, HCV, *Pl.vivax*, and H1N1proteins were performed by comparative modeling approach using PDB ID's:1QGT, 1ESX, 1CU1, 1V0B, 2WR3 as template in MODELLER program. The best models were chosen based on Procheck analysis, energy minimized and applied for active site description in QsiteFinder. The Ligand-Protein interactions were calculated by autodock tool to monitor the probable drug targets and their applications.

Introduction

Natural plant pigments like curcumin, lycopene, anthocyanins, and β-carotene possess anti-tumor activities. Most of the plants have a lengthy life cycle and are able to produce pigments as seceondary metabolites from microorganisms as an additional option. Prodigiosin is a miraculous red pyrrole pigment produced by several bacteria, most notably in *Serratia marcescens*. (Bennett and Bentley, 2000**)**. The Prodigiosin red pigment was first initiated in the species of *Serratia marcescens* by Kraft in 1902 (Harned, 1954). The Prodigiosin is a distinctive member of a group of compounds with a common pyrrolyldipyrrolylmethene (PPM) skeletal core among different alkyl substituent's such as a linear carbon atoms (Furstner, 2003), 3 nitrogen atom of with 2 are secondary (4a and 14a) and 1 is tertiary (9a), a family of naturally secreting pyrrole red pigments that are secondary metabolites from microorganisms (Figure 1). Prodigiosins has a wide-range of biological profile and recently received better consideration for their reported antibacterial, antifungal, antiprotozoal, antimalarial, immunosuppressive and anticancer properties (Williamson et al., 2006, Williamson et al., 2007). Prodigiosin is a prodiginine family member which has emerged as promising anticancer drugs and is currently in clinical trials (Espona-Fiedler et al., 2012). These properties of the compound at low toxicity levels have again flamed up a new prospect for research. In the way of study we try to investigate the activity against the Hepatitis B virus genotype B2 (HBV) (P0C6G7) (Okamoto et al., 1988), Human immunodeficiency virus (HIV) (P05928) (Wain-Hobson et al., 1985), Hepatitis C virus (HCV) (B0B3F1) (Piodi et al., 2007), Plasmodium vivax (*Pl.vivax)* (Q9XZD6) (Speranca et al., 2001) and Influenza A virus (H1N1) (E1AWL9) (Zhou et al., 2010) with molecular modeling and docking studies.

Materials and methods

Ligand preparation

Chemical structure of ligand was taken from Pubchem compound database http://www.ncbi.nlm.nih.gov/search. Three dimensional structures for Prodigiosin were generated using ACD/ChemSketch, (freeware version 10.00). ChemSketch, chemically smart drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to generate the structure of the ligands. Using draw mode of Chemsketch, the ligands were generated and three dimensional optimizations were done and then saved in .mol file. Geometry optimizations and energy minimization of the ligands were performed by PRODRG2 server (Schüttelkopf etal., 2004).

Template Alignment and Homology Modeling

The three dimensional structures are not available thus the targeted protein sequences were retrieved from NCBI databases (http://www.ncbi.nlm.nih.gov/protein/). The target sequences were imported in BLAST search tool and the template structures were identified (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1990). The tertiary structure of the target models were predicted using Homology modeling by Modeller 9V5 (. Eswar et al., 2006). It was done using four input files-target ali, template PDB, model default – python script (default), align 2d.py-python script (default) (Sali et al., 1993). The tertiary structures generated by modeler were validated using Ramachandran validation methods. The ramachandran plot was generated by PROCHECK program in SAVS Server (Laskoswki et al., 1993).

Active binding site prediction

The probable binding sites of preferred target receptors were searched using Q-site Finder to predict the ligand binding site (http://www.bioinformatics.leeds.ac.uk/qsitefinder). It works

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by binding hydrophobic (CH3) probes to the protein, and finding clusters of probes with the most favorable binding energy. They consist of active sites on protein surfaces and voids covered in the interior of proteins. The individual probe sites relate most closely to the favored high-affinity binding sites on the protein surface. The favorable binding sites relate to locations where a putative ligand could bind and optimize its van der Waals interaction energy (Laurie et al., 2005).

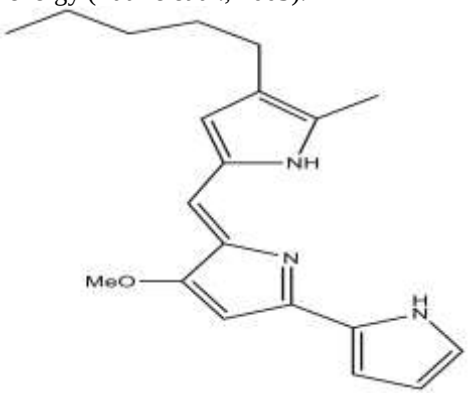

Figure 1. A) 2D structure of prodigiosin

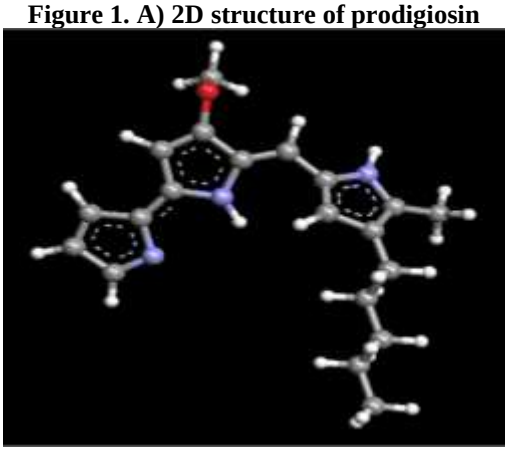

Figure 1. B) 3D structure of prodigiosin

Docking Analysis

Assessment of the antimalarial activity against *P. vivax* and antiviral activity against HBV, HCV, H1N1, and HIV were done using Auto dock tools (Morris et al., 2009) (ADT) v1.5.4 and Autodock v4.2 programs; (Autodock, Autogrid, Autotors, Copyright-1991e2000) from the Scripps Research Institute http://www.scripps .edu/mb/olson/doc/autodock. The searching grid extended above the preferred target proteins; polar hydrogen's were added to the ligand moieties. Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the nonpolar hydrogen's were merged with the carbons and the internal degrees of freedom and torsions were set. Prodigiosin was docked to target protein models (HBeAG, VPR, HCV, CRK2, HA –H1N1) with the molecule considered as a rigid body and the ligands being flexible.

The search was extended over the active sites of the receptor protein. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 Å. The search was carried out with the Lamarckian Genetic Algorithm (Morris et al., 2009); populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values, with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most trustable solution.

Results and Discussion

Homology Modeling and Validation

Homology modeling is generally the process of selection when a clear relationship of homology between the sequence of target protein and at least one identified structure is found. This approach would provide reasonable results based on the hypothesis that the three dimensional structures of two proteins will be similar if their sequences are correlated (Kroemer et al., 1996). The sequences of HBeAg, VPR_HV1BR, NS3/4A, CDC2H_PLAVI, Hemagglutinin was searched in Protein Knowledgebase (UniProtKB) site giving results as Hepatitis B virus genotype B2 (HBV) (P0C6G7), Human immunodeficiency virus (HIV) (P05928), Hepatitis C virus (HCV) (B0B3F1), Plasmodium vivax (*Pl.vivax)* (Q9XZD6), Influenza A virus (H1N1) (E1AWL9) respectively. The whole sequences of all the probable targets were retrieved in FASTA format and the protein BLAST for each complete protein sequences were executed. Among the target sequences with known three-dimensional structures, the PDB:1QGT, 1ESX, 1CU1, 1V0B, 2WR3 showed the highest sequence identity with HBV (95%), HIV (91%), HCV (83%), *Pl.vivax* (93%), H1N1 (63%). At this point of sequence identity, it is fine adequate to use crystallographic structure of the identified template, in order to attain high quality alignment for structure prediction by homology modeling.

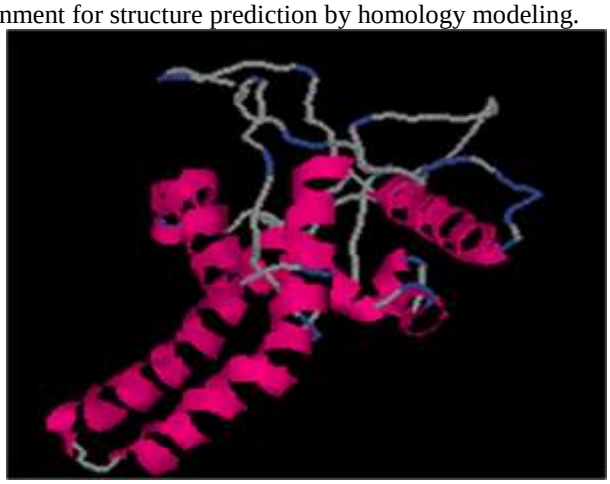

Figure 2. A) Homology model of Hepatitis B virus genotype B2 (HBV)

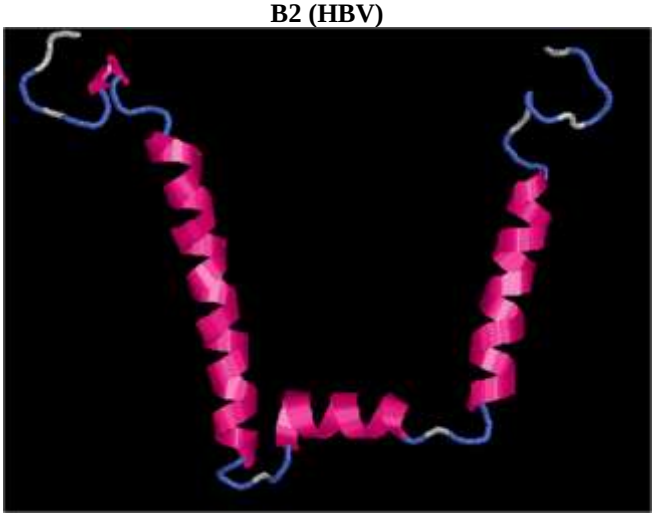

Figure 2. B) Homology model of Human immunodeficiency virus (HIV)

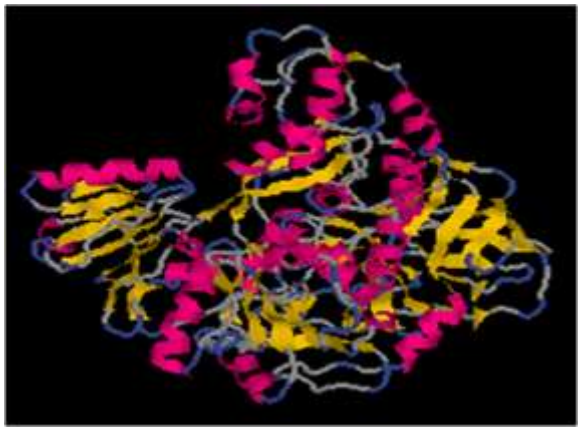

Figure 2. C) Homology model of Hepatitis C virus (HCV)

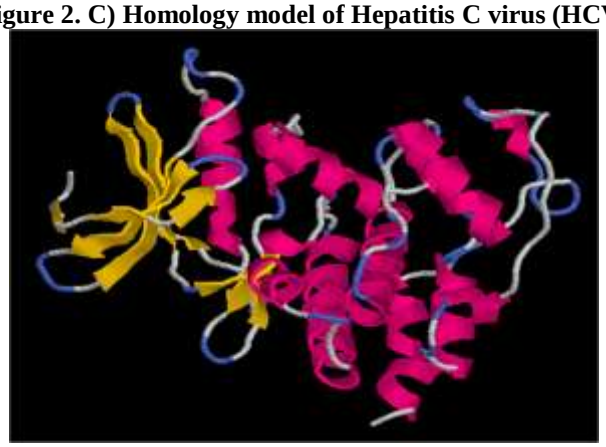

Figure 2. D) Homology model of Plasmodium vivax (*Pl.vivax)*

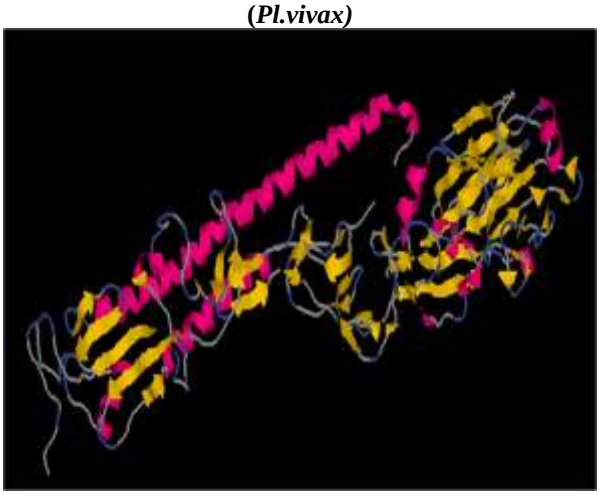

Figure 2. E) Homology model of Influenza A virus (H1N1)

The three-dimensional structure provides important imminent into molecular function and also enables the analyses of its interactions with suitable substrates or inhibitors. Five models were constructed by protein prediction tool MODELLER (Eswar et al., 2006)and the models are showed (Fig. 2. (a, b, c, d, e)). The developed predicted models were checked for psi and phi torsion angles using the Ramachandran plots. The ramachandran plot was generated by PROCHECK program in SAVS Server (Laskoswki et al., 1993). The Ramachandran plot showed 91.3% residues in most favorable region, 7.1% in allowed region, 1.6% in additional and disallowed region for HBV, 82.9% residues in most favorable region, 12.2% in allowed region, 4.9% in additional and disallowed region for HIV, 93.9% residues in most favorable region, 5.8% in allowed region, 0.3% in additional and disallowed region for HCV, 93.3% residues in most favorable region, 5.9% in allowed region, 0.9% in additional and disallowed region for *Pl. vivax*, 85.4% residues in most favorable region, 12.1% in allowed region, 2.2% in additional and disallowed region, 0.2% in disabled region for H1N1 respectively. The results revealed that majority of the amino acids are in a phi–psi distribution consistent with right handed α-helix and reliable to be good quality model (Figure 3 (a, b, c, d, e)).

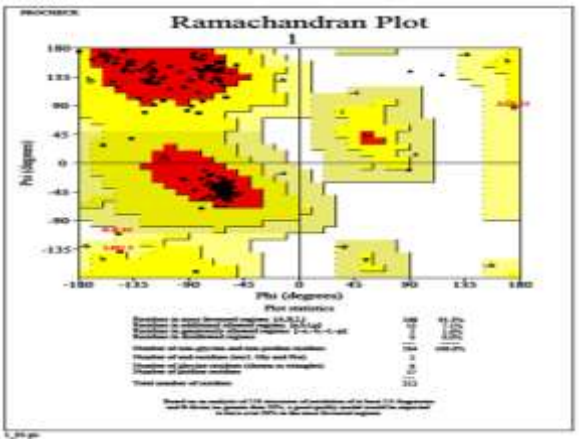

Figure 3. A) Ramachandran plot analysis of homology model Hepatitis B virus genotype B2 (HBV)

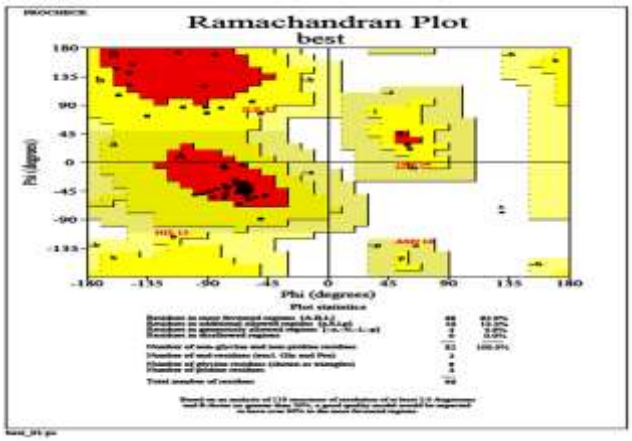

Figure 3. B) Ramachandran plot analysis of homology model Human immunodeficiency virus (HIV)

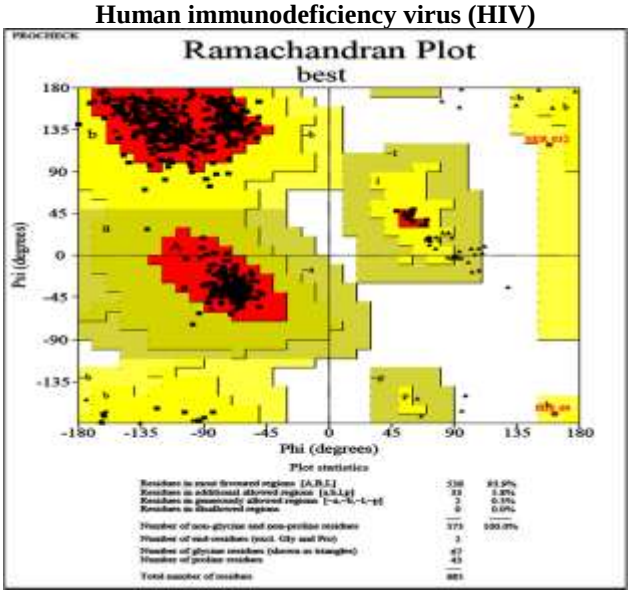

Figure 3. C) Ramachandran plot analysis of homology model Hepatitis C virus (HCV)

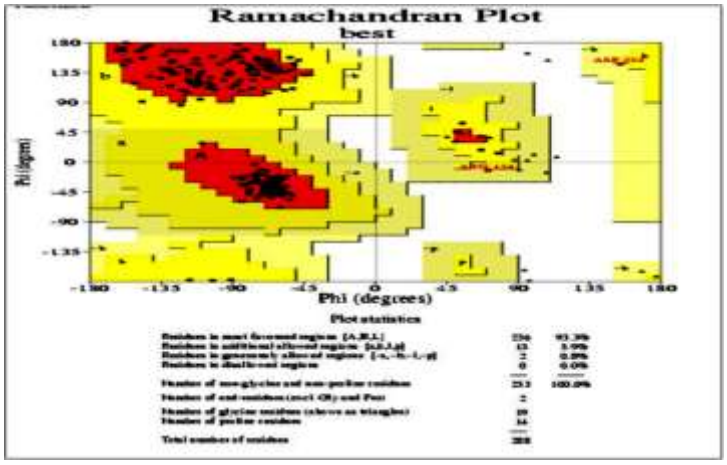

Figure 3. D) Ramachandran plot analysis of homology model Plasmodium vivax (*Pl.vivax)*

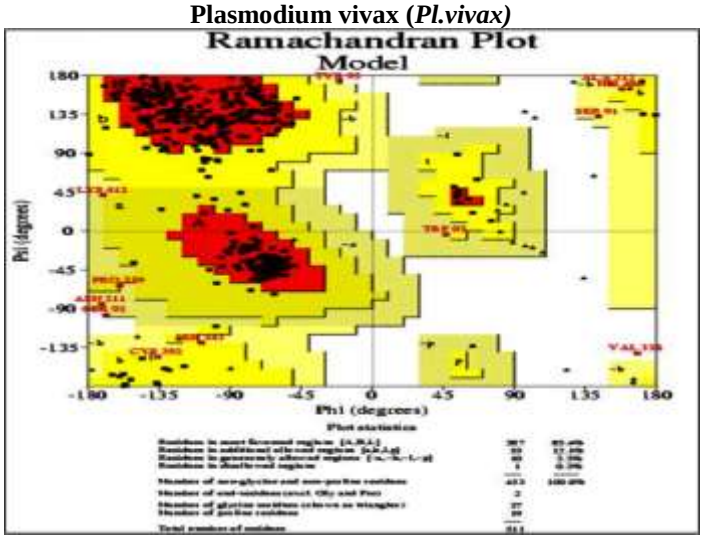

Figure 3. E) Ramachandran plot analysis of homology model Influenza A virus (H1N1)

Active site prediction

In the field of molecular modeling, docking is a process which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengaur et al., 1996) Knowledge of the preferred orientation in turn may possibly used to predict the potency of connection or binding affinity between two molecules by means of scoring functions. The protein–ligand complexes make available greater insights in structure based drug design; for that reason a protein–ligand composite was developed. It provides a more comprehensive and accurate image of the interactions and structural complementarities between the ligand and the active amino acids site. The protein–ligand complex in generated homology models were developed by active sites selection procedures. The active site for the modeled target proteins were predicted using Q SITE FINDER (Lanrie and Jackson, 2005). The active site residues are as follows

HBV: LEU8, ILE9, ILE10, SER11, PHE52, PRO54, TRP131, SER135, THR138, PHE139, TYR147, PRO167, ILE168, LEU169

HIV: MET1, GLU2, ALA4, PRO5, GLU6, ASP7, GLN11, ARG12, GLU13, PRO14, ASN16

HCV: GLN9, THR10, ARG11, GLY12, LEU13, THR16, SER20, ASP25, VAL35, LEU36, SER37, THR38, THR42, PHE43, THR54, GLY58, ALI59, ARG62, THR63, LEU64, ALA65, TRP85, ARG109

Pl. vivax: ILE10, GLY11, GLU12, GLY13, VAL18, LYS20, ALA30, HIS81, LEU82, ASP83, GLN84, ASP85, LYS87, LYS88, GLN129, LEU132

H1N1: LYS60, GLY63, VAL64, ALA65, PRO66, LEU67, HIS68, TRP77, SER88, THR89, ALA90, SER91, SER92, TRP93, SER94, ILE96, LEU122, SER123, SER124, VAL125, SER126, SER127, PHE128, GLU129, ARG130, MET274.

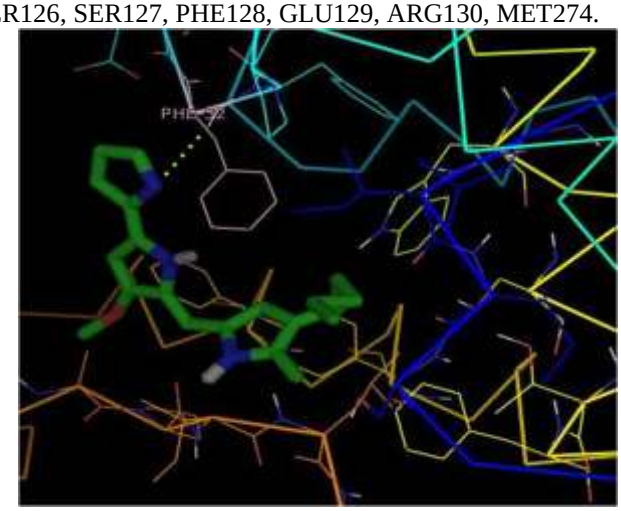

Figure 4. A) Binding analysis of prodigiosin-homology model Hepatitis B virus genotype B2 (HBV)

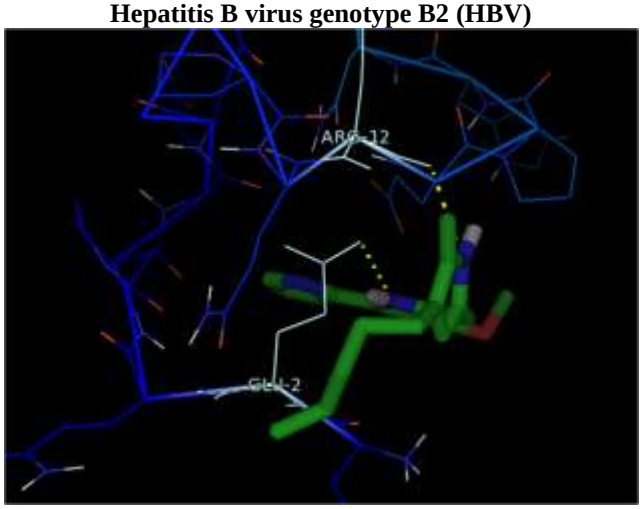

Figure 4. B) Binding analysis of prodigiosin- homology model Human immunodeficiency virus (HIV)

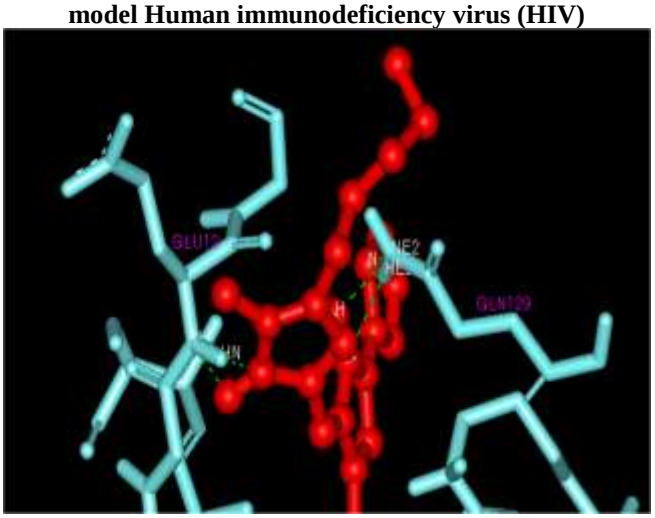

Figure 4. C) Binding analysis of prodigiosin- homology model Plasmodium vivax (*Pl.vivax)*

Table 1 Docking interactions

S. No	Target protein	Residue	Atom	Prodigiosin	Distance A°
1.	HBeAg (HBV)	PHE52	O	N	2.05
2.	VPR (HIV)	GLU2 ARG12	OE1 O	N H	2.30 2.97
3.	NS3/4A (HCV)	No Interaction			
4.	CRK-2 *(Pl.vivax)*	LN129 GLU12G GLN129	HN HE21 HE21	N N N	1.88 2.08 2.24
5.	HA (H1N1)	SER92 SER92 TRP 93	O OG N	H N N	2.03 3.10 3.08

Table 2 Docking score for the drug targets

S. No	Drug target	DOCKING SCORE (Kcal/Mol)	No. of Hydrogen bonds formed
1.	HBeAg (HBV)	-8.87	1
2.	VPR (HIV)	-8.14	2
3.	NS3/4A (HCV)	No interaction	
4.	CRK-2*(Pl.vivax)*	-10.50	3
5.	HA (H1N1)	-15.71	2

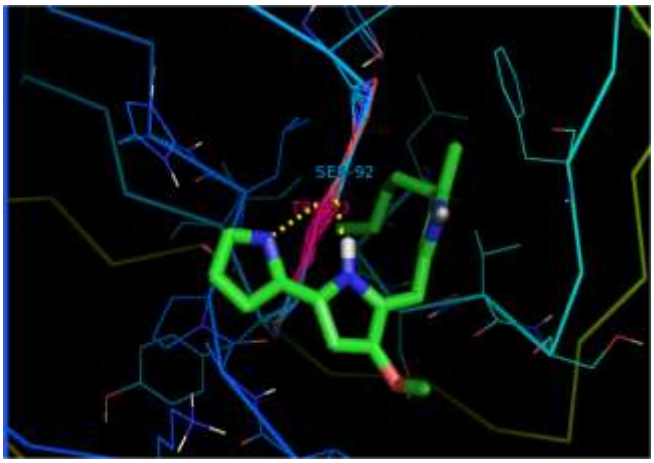

Figure 4. D) Binding analysis of prodigiosin- homology model Influenza A virus (H1N1)

Binding Interactions

After analyzing the active sites, molecular docking of the target proteins model was performed in order to make clear its structural and functional importance in terms of protein-ligand binding. Prodigiosin, the renowned Antibiotic, was successively docked on to the active sites of our target homology models.

Table 1 shows docking of ligand Prodigiosin on to the active sites of protein models, NH at 4a position interact where the PHE52 with the distance 2.05A° for HBV, tertiary nitrogen at 9a position interact where the GLU2, ARG12 with the distance 2.30A°, 2.97A° for HIV, GLU12 and GLN129interact with nitrogen at 14a and tertiary nitrogen at 4a positions with the distance 1.88A°, 2.08 A° and 2.24 A° for Pl. vivax, secondary nitrogen at 4a and tertiary nitrogen at 9a positions interact with SER92, TRP 93 with the distance 2.03 A°, 3.10 A°, 3.08 A° for H1N1. Typically in the molecule three nitrogen atoms were showed the binding interactions with the modeled proteins. The analysed active sites were directly involved in biding with the ligand and there was no binding intraction with the active sites of HCV. Table 2 shows the results of the docking experiments in terms of calculated free energy of binding. The antibiotic prodigiosin as ligand gives the binding score of -8.87, -8.14, and -10.50, -15.71kcal/mol alongside with the target protein models (Figure 4 (a, b, c, d)).

Conclusion

In the current research the three dimensional models of HVB, HIV, HCV, PL.vivax and H1N1were developed in order to bring about its molecular homology modeling and docking interaction studies. The homology models were validated and supplementary used for docking analysis with the antibiotic prodigiosin. The finalized docking results were analyzed for binding model and revise conformational analysis for the ligands. The result suggested a promising activity against all of our target models.

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