International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 2 Issue 6‖ June2013 ‖ PP.35-41

Homology Modeling and Docking Studies of Neuraminidase Protein of Influenza a Virus (H1N1) virus With Select Ligand – A Computer Aided Structure Based Drug Design

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ABSTRACT: *An outbreaks of influenza A virus subtype H1N1 also known as swine flu. Inhibition of influenza A virus to avoid morbidity and mortality is of main concern during epidemics and of major concern during pandemics. To design drugs for treating these epidemics is urgency. In this study, we employed the new sequence to build the neuraminidase sequence structure (PDB-ID: 3CL2 A chain) by homology modeling , which has been checked for high reliability by verify score and Ramachandran plot . Its accuracy has been verified by the Saves server. The model structure was employed for docking. Known inhibitor taken from the literature docked, and docked at the binding site. Obtain molecule Epicatechin gallate , Xylopine , 4-Amino-neu5AC2en, Sialic acid, Marchantin have been given score of -174.3 kcal/mol , -148.5 kcal/mol , -142.3 kcal/mol , -138.7 kcal/mol , -131.4 kcal/mol. This is greater than known inhibitor. Zanamivir and Oseltamivir have been given score of -155.9 kcal/mol, and -132.7 kcal/mol molecules were employed for similarity search from zinc database. To the candidate molecules with drug likeness property can be considered for the test invitro. And finally it can act as lead compound for the future development and optimization*.

KEYWORDS: *Neuraminidase protein, Homology modeling, Docking, Inhibitor.*

I. INTRODUCTION

Swine influenza (also called pig influenza, swine flu, hog flu and pig flu) is an infection by any one of several types of swine influenza virus. SIV is respiratory disease of pig caused by type A influenza virus that regularly cause outbreak of influenza in pigs. (Dawood, et al., 2009) Swine influenza virus is any strain of the influenza family of virus that is endemic in pigs. As a 2009, the known SIV strain include influenza c and the subtype of influenza A known as H1N1, H1N2, H3N1 and H2N3. The classical swine flu virus was first isolated from a pig in 1930 human infections with swine flu virus are of concern as influenza virus that infect pigs are different from human influenza . Thus influenza vaccine made against human strain of influenza virus is generally not expected to protect people from influenza strain circulatory in pigs. Two types of surface glycoprotein form the main antigenic determinants of influenza A virus namely Hemagglutin (HA) and Neuraminidase (NA). HA is responsible for viral attachment to the infected cell through surface bound sialic acid (SA) moieties, while NA is responsible for hydrolyzing the glycosidic bond that connect SA with the cell membrane resulting in viral detachment. Neuraminidase enzymes are glycosidic hydrolase enzymes which cleaves the glycosidic linkage of neuraminic acid. The viral neuraminidases are frequently used as an antigenic determinants found on the surface of the influenza virus. Some variants of the influenza neuraminidase confer more virulence to the virus than other. The committee made several recommendations for improving the methods, for validating the results, and for making cross comparisons to understand where the two approaches differed. The initial work was based on very limited data from a handful of industrialized countries. More countries, including some from middle- and low-income countries, are expected to provide data to the project in early 2012 enabling the investigators to produce credible estimates by late in this same year. It is hoped that the results of these efforts will allow public health policy makers to better understand the impact of the 2009 influenza pandemic and better prepare for future events (WHO Technical Consultation: H1N1pdm Mortality Estimates, 25-26 October 2011). NA exists as a mushroom shape projection on the surface of the influenza virus. It has a head consisting of four co-planer and roughly spherical subunits and a hydrophobic region that is embedded within the interior of the virus membrane. Neuraminidase protein id 3CL2 A (http://www.pdb.org/) chains is modeled by Swiss model. Sequence identity of the 3CL2 A chains 91.948 and the E value of the 3CL2 A chains are 0. Cavity of the 3cl2 A chain Neuraminidase protein is vol = 189.952, vol =10.752. NA cleaves sialic acid residue from viral protein, preventing aggregation of virus. NA has been targeted in ligand based enzyme inhibitor design programmes that have resulted in the production of two drugs Zanamivir (relenza) and

Oseltamivir (Tamiflu).Administration of neuraminidase inhibitors is a treatment that limits the severity and spread of viral infections.

II. LITERATURE REVIEW

Influenza virus: The flu virus is an RNA virus. The genomes codes for five viral protein and made of eight fragments. The virus has a lipid envelope with two glycoprotein present. Influenza spread around the world in seasonal epidemics resulting in the death of between 250,000 and 500,000 people every year, up to million in some pandemics years. Three influenza pandemics occurred in the 20th centuary and killed tens of million of people, with each of these pandemics being caused by the appearance of a new strain of the virus in human. Often , these new strain appear when an existing flu virus spread to human from other animal species or when an existing human strain picks up new genes from a virus that usually infects birds or pigs. In April 2009 a novel flu strain evolved that combined genes from human, pig and bird flu, initially dubbed "swine flu" and also known as influenza a/h1n1. Emerged in Maxico, The United States, and several other nations. The world health organization officially declared the outbreak to be pandemics on 11 June 2009. (http://www.who.int/)

III. METHODOLOGY

Sequence alignment:

The protein sequences of Neuraminidase protein were obtained from NCBI (http://www.ncbi.nlm.nih.gov/). The homologous structure of neuraminidase was identified, which was used as template for the homology modelling. The sequence alignment was done using the online version of Clustal W (http://www.ebi.ac.uk/Tools/msa/clustalw2/)

Homology modeling:

BLASTP was used to identify the most suitable template for homology modeling of Neuraminidase protein. The available structure of Neuraminidase protein from swine flu in the Protein Database (PDB entry 3CL2). The homology modeling was carried out using CPH SERVER3.2 Server (http://www.cbs.dtu.dk/services/CPHmodels/, a comparative protein modeling program, was used for homology modeling to generate the 3-D structures of Neuraminidase protein for H1N1. The energy computations were done with the GROMOS 96 by implementation of Swiss-PdbViewer (http://www.expasy.org/spdv).

Validation of the generated models:

Different structure verification programs such as PROCHECK, VERIFY3D and SAVES (http://nihserver.mbi.ucla.edu/SAVES/) were used to evaluate the 3D-model of Neuraminidase protein. The above mentioned validation programs validate the predicted structure by checking various parameters. While PROCHECK, a structure verification program relies on Ramachandran plot **[fig : 1]**, determines the quality of the predicted structure by assessing various parameters such as lengths, angles and planarity of the peptide bonds, geometry of the hydrogen bonds, and side chain conformations of protein structures as a function of atomic resolution. The Verify3D determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.) and comparing the results to valid structures.

Inhibition site identification:

Prediction of binding sie by Q site finder in it the involved residue are ser – (246), arg –(152) , ser – (294), arg –(292) , ile – (222), arg -(118), glu -(119), gln-(136) ,thr-(242) , val-(149), lys-(150), asp-(151), asn-(325), pro-(326), tyr-(347), gly-(348), ser-(246), trp-(408), ile-(427) ,gly-(429), lys- (432) Predicted site 1.

Generating novel ligand

The structure of the fragment, i.e. the "seed molecule" was revealed on the basis of previous studies of available inhibitors for Neuraminidase protein. The fragment "Epicatechin gallate " was identified on the basis of "Lipinski's Rule of Five" and may therefore represent suitable starting point for evolution of good quality lead compounds. The docking analysis of "Epicatechin gallate " compound with Neuraminidase protein was carried out by Molegro virtual docker docking software. The conformation of the pre-placed "seed" ensuring the binding affinity decides the manner that ligands would be grown with Chemsketch software**.**

IV. RESULT

Virtual screening

Out of 50 novel ligands generated, 9 ligands were selected on the basis of maximum binding affinity measured in kcal/mol. The selected 9 ligands were then analyzed for drug- relevant properties based on "Lipinski's rule of five" **[Table:2]** . Molsoft: DrugLikeness and molecular property explorer

(http://www.molsoft.com/mprop/), and moloinspiration tool (http://www.molinspiration.com/cgibin/properties) **[Table:3].** On the basis of binding affinity and drug like properties, one ligand was finally screened.

Lead optimization:

The goal of lead optimization is to improve the effectiveness of initial 'hits' from primary screening. There are many approaches to lead optimization, all of which have the same theme: reduce off-target effects, create an improved ADME profile and improve a compound's efficacy.

Protein-ligand docking:

The docking of ligands to the Neuraminidase protein for swine flu H1N1 was performed using Molegro virtual docker. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using the software, hydrogen atoms were added to the Neuraminidase protein and it's all bonds of ligands were set to be rotatable. The grid box was used around the catalytic triad to cover the entire enzyme binding site and accommodate ligands to move freely The NA and Zanamivir interaction has observed with minimal dock score -155.968 kcal/mol. **[fig: 2]** and Oseltamivir has been docked with -132.72 kcal/mol **[fig: 3].**After docking we select one inhibitor molecule in the basis of binding energy. The binding energy of Epicatechin gallate is -174.3 kcal/mol. **[fig: 4]**.The best conformation was chosen with the lowest docked energy **[Table: 1],** after the docking search was completed. The interactions of complex Neuraminidase protein-ligand conformations, including hydrogen bonds and the bond lengths were analysed using Swiss-PdbViewer4.0 and pymole software. The continuing mutation which is occurring in various strain of many eventually make the current drug ineffective for the influenza flu. The H1N1 swine flu is an impetus to develop new powerful drug against various influenza virus. Neuraminidase protein participates in the swine flu disease. Present drugs are not much more effective to cure this disease and have many side effects also. I am concluding my result the target protein having influenza neuraminidase. It consists of six, four stranded beta sheets, 3 helix and loops .When the different ligand docked with protein. It checks all possible orientations and conformation for all set of ligands. The docking results are best interaction with neuraminidase protein. After docking we find the binding energy of main drug zanamivir is -155.9 kcal/mol and the oseltamivir is -132.7 kcal/mol. We have search many inhibitor molecule of H1N1. After docking we select one inhibitor molecule in the basis of binding energy. The binding energy of Epicatechin gallate is (-174.3 kcal/mol).

V. DISCUSSION

Neuraminidase protein (NA) is the surface glycoprotein forms the main antigenic determinants of influenza A virus. The work has been started with the Neuraminidase Protein. NA sequence has been retrived from NCBI and the structure is modeled by CPH server. CPH server generated models of NA protein, model has been considered best because it has maximum core region, and minimum disallowed region and minimum energy. Then we using Saves server to verify the structure by verify score plot, procheck and Ramachandran plot. After this active site have been predicted using the Q – site finder which resulted in to 10 active site each having specific volume, area and amino acid residues. the site 1 which having maximum volume has following amino acid arg (118), glu (119), gln (136) , thr (148) , val (149) , lys (150) , asp (151) , asn (325) ,pro (326). Towards finding suitable inhibitors for neuraminidase protein the binding energy of NA prescribe drug for swine flu i.e zanamivir (ZMR) and oseltamivir (OTV) has been done by Molegro Virtual Docker is an integrated platform for predicting protein – ligand interaction. The NA and zanamivir interaction has observed with minimal dock score -155.968 kcal/mol. And oseltamivir has been docked with -132.72 kcal/mol. on the other hand we search the other inhibitor molecules like Marchantin , 4 –Amino-neu5AC2en , Xylopine , Epicatechin gallate , Sialic acid , Benzoic acid , Neuraminic acid by the research paper. The docked energy of NA into each of the inhibitor molecules such as Epicatechin gallate, Xylopine, 4-Amino-5neuAC2en, Sialic acid, Marchantin. Neuraminic acid have been -174.3 kcal/mol , -148.5 kcal/mol , -142.3 kcal/mol , -138.7 kcal/mol , -131.4 kcal/mol , -129.8 kcal/mol. Among these inhibitor molecules, Epicatechin gallate, 4-Amino-5neuAC2en, Xylopine has obeyed Lipinski's rule of 5. Druglikeness determines wheather particular molecule is similar to the known drugs. Out of all the inhibitors molecules Epicatechin gallate has been docked with minimum -174.3 kcal/mol at effector region forming hydrogen bonding with amino acid residue to the candidate molecules with drug likeness property can be consider for the test in vitro and finally it can act as lead compound for the future development and optimization.

VI. ACKNOWLEDGEMENTS

I want to acknowledge Dr. Durg Vijay Singh former lecturer Department of Bioinformatics, UIET, and CSJM University for his Kind support and guidance.

Docking result

S.no	inhibitor	Moldock score (-kcal/m)	H- bond(kj/mol)
1	zanamivir	-155.968	-24.12
2	oseltamivir	-132.72	-7.095
3	marchantin	-131.401	-7.1347
4	Epicatechin gallate	-174.3	-27.758
5	Benzoic acid	-81.3968	0
6	Sialic acid	-138.702	-14.559
7	Neuraminic acid	-129.818	-24.437
8	xylopine	-148.519	-17.156
9	4-amino-neu5AC2en	-142.332	-12.925

Table 1: Docking result of the inhibitor molecule.The NA and zanamivir interaction has observed with minimal dock score -155.968 kcal/mol. And oseltamivir has been docked with -132.72 kcal/mol. on the other hand we search the other inhibitor molecules like Marchantin , 4 –Amino-neu5AC2en , Xylopine , Epicatechin gallate , Sialic acid , Benzoic acid , Neuraminic acid by the research paper. The docked energy of NA into each of the inhibitor molecules such as Epicatechin gallate, Xylopine, 4-Amino-5neuAC2en, Sialic acid, Marchantin. Neuraminic acid have been -174.3 kcal/mol , -148.5 kcal/mol , -142.3 kcal/mol , -138.7 kcal/mol , -131.4 kcal/mol , -129.8 kcal/mol.

Lipinski's Rule of Five

Ligand	Mw(g/mol)	Log p	H bond donor	H bond acceptor
Marchantin	440.487	6.293	3	5
Epicatechin gallate	442.37	2.53	7	10
Benzoic acid	122.12	1.848	1	2
Neuraminic acid	267.23	4.344	7	9
Xylopine	295.33	3.287	1	4
Sialic acid	305.26	4.131	7	10
4-Amino-neu5AC2en	290.26	-3.498	6	8
Oseltamivir	312.40	6.25	2	5
Zanamivir	332.30	3.642	7	8

Table 2: Lipinski's rule of five helps in distinguishing between drug like and nondrug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying .

Bioactivity score of ligand

ligand	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzymes inhibitor
zanamivir	**0.47**	**0.03**	**-0.24**	**-0.18**	**0.55**	**0.99**
oseltamivir	**0.12**	**0.10**	**-0.33**	**-0.14**	**0.29**	**0.47**
marchantin	**0.14**	**0.09**	**0.08**	**0.19**	**0.09**	**0.16**
Epicatechin gallate	**0.17**	**0.02**	**0.65**	**0.34**	**0.13**	**0.25**
Benzoic acid	**-2.20**	**-1.57**	**-2.49**	**-2.05**	**-2.32**	**-1.60**
Neuraminic acid	**0.03**	**0.46**	**-0.13**	**0.10**	**0.30**	**0.73**
xylopine	**0.43**	**0.13**	**-0.14**	**-0.22**	**-0.07**	**0.21**
Sialic acid	**0.03**	**0.17**	**-0.26**	**0.10**	**0.25**	**0.62**
4-amino–neu5AC2en	**0.31**	**0.06**	**-0.20**	**-0.12**	**0.35**	**1.11**

Table 3. Calculation of druglikeness score using mol inspiration online tool .structure of inactive molecules and to identify substructure features typical for active molecules. The larger the values of the score is the higher is also probability that the p-articular molecule will be active the bioactivity score of all ligand are given.

Validate the model structure by saves server

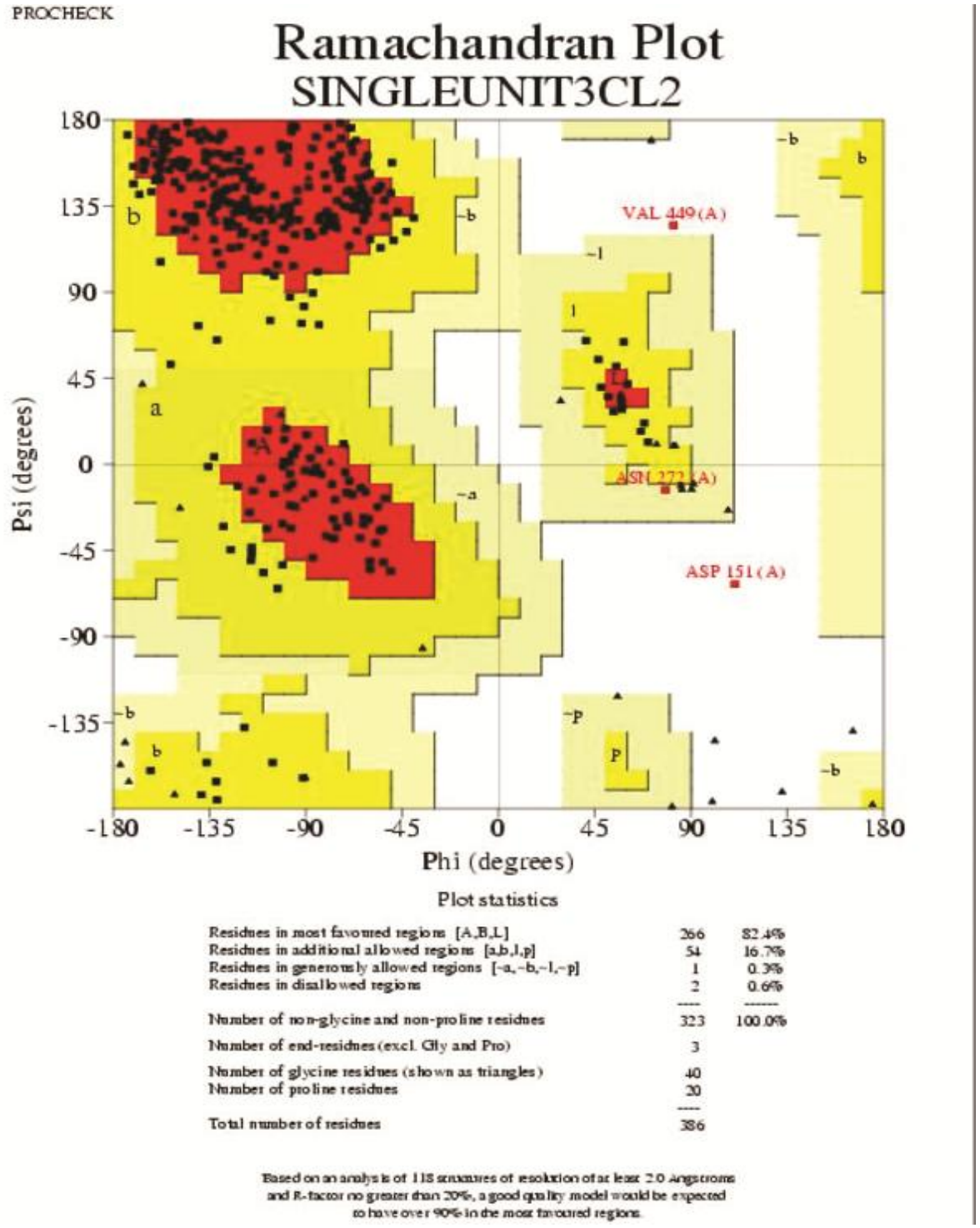

Figure 1: Verify-3D 100.00% of the residue an averaged 3D -1D SCORE >0.2

Docking study of Zanamivir Interaction:

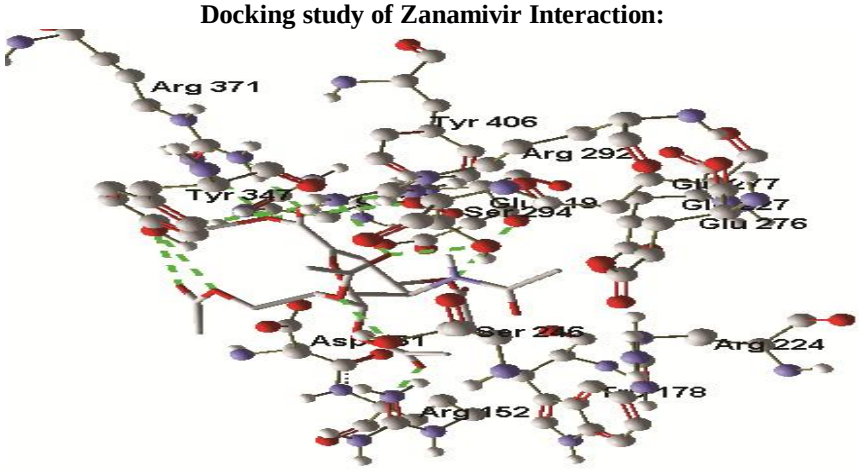

Figure 2: Predicted binding site on protein: ser – (246), gly – (429), Tyr – (406), arg – (292).
No of hydrogen bond: Tyr – (347), ser – (294), asp – (151).
Moldock score: -155.9 kcal/mol

Docking study of oseltamivir Interaction:

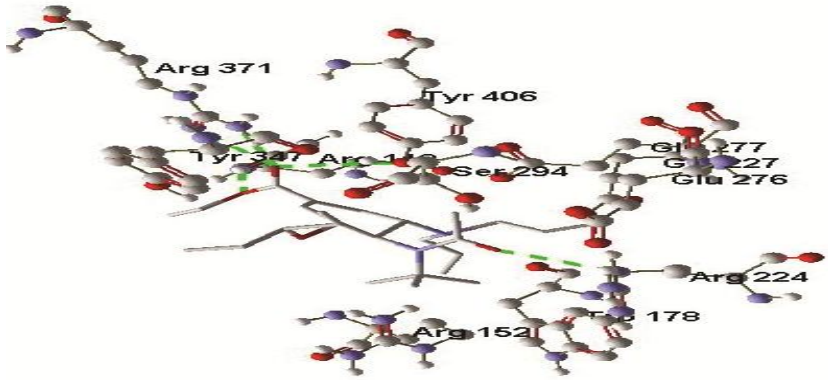

Figure 3: Predicted binding site on protein: Tyr – (406), ser – (294), arg – (224)
No of hydrogen bond: arg – (152), Tyr – (347).
Moldock score: -132.7 kcal/mol

Docking study of Epicatechin gallate Interaction –

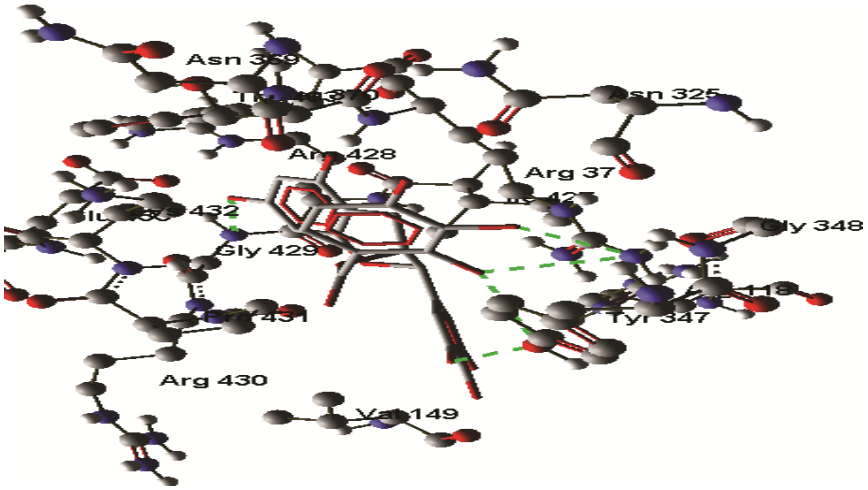

Figure 4:Predicted binding site on protein – gly – (429), pro – (431), cys – (432), arg – (428).
No of hydrogen bond – Tyr – (347), gly – (429).
Moldock score – -174.3 kcal/mol

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