Chapter-4

Molecular Docking Studies on Non-Structural (NS) Proteins

Modern computational-aided drug design established a novel platform by which researchers perform in-depth in silico simulation prior to labor-extensive wet-lab validation (Zhang, 2009). It comprises of two major parts corresponding to the information of molecular source it utilizes: structure-based (or receptor-based) drug design and ligand based drug design. Structure-based drug design, which relies on the knowledge of biological target structures, aims to discover small molecules/peptides leads with desired chemistry properties, and orchestrate the following experimental validation and lead optimization. Structure-based approach provides mechanism-based basis, where potential ligands are excavated using receptor-dependent parameters (Jorgensen, 2004).The spirit of structure-based drug identification and optimization is to identify biologically active compounds from "compound forests" with higher efficiency and hit enrichment by utilizing the information from protein structures.

Generally speaking, molecular docking is a computational approach that predicts the orientation of a small molecule (ligand) in stable complex with a macromolecular target (receptor) using specific scoring functions. It has become the basis of structure-based virtual screening and has shown to be influential in current pharmaceutical design which is more economic than experimental screening. Docking is overly a "standard" procedure when protein-ligand complex structure is available. The advantages of structure-based virtual screening are obvious: quick and minimal manual interventions. Docking-based virtual screening has played pronounced roles in the lead identification and optimization in the history.

The family *Flaviviridae* contains three genera, the *flaviviruses*, the *pestiviruses*, and the hepatitis C viruses. Many members within the *Flavivirus* genus are arthropod-borne pathogens that cause significant human morbidity and mortality. The global emerging *flaviviruses* include the four serotypes of Dengue (DENV), Yellow Fever (YFV), West Nile (WNV), Japanese Encephalitis (JEV), and tick-borne encephalitis viruses (TEV) (Burk,e et al., 2001).

Triaryl pyrazoline is antiviral drug, molecular formula $C_{20}H_{15}C_1N_{20}S$, molecular weight 366.8639 g/mol, XLogP3 is 3, hydrogen bond donor count is 0, hydrogen bond acceptor count 3 and rotatable bond count is 3.Triaryl pyrazoline Inhibits flavivirus infection

in cell culture. The inhibitor was identified through high-throughput screening of acompound library using a luciferase-expressing West Nile Virus (WNV) infection assay. It specifically suppressed viral RNA synthesis in WNV. Analyses of the compound in the Dengue Virus replicon systems showed that it weaklysuppressed viral translation but significantly inhibited viral RNA synthesis (Francesc, et al., 2006).

Ribavirin also known as Virazole, Rebetol, Tribavirin, Vilona, Ribasphere. Molecular formula $C_8H_{12}N_4O_5$ with molecular weight 244.20468 g/mol. Ribavirin is a nucleoside antimetabolite antiviral agent that blocks nucleic acid synthesis and is used against both RNA and DNA viruses. Ribavirin is a nucleoside analog antiviral compound. The chemical classification of ribavirin is nucleoside analog. Ribavirin is a synthetic nucleoside analog of ribo furanose with activity against hepatitis c virus and other RNA viruses. Ribavirin is incorporated into viral RNA, thereby inhibiting viral RNA synthesis, including virual genome mutations, and inhibition normal viral replication. XLogP3 is -1.8 hydrogen bond donor count is 4, hydrogen bond acceptor count is 7 and rotatable bond count is 3.

Castanospermine also known as Castanospermine, 6-Epicastanospermine, 6,7-Diepicastanospermine. Molecular formula $C_8H_{15}NO_4$ with molecular weight 189.209 g/mol. XLogP3 is -2.2, hydrogen bond donor count is 4, hydrogen bond acceptor count is5 and rotatable bond count is 0.Castanospermine inhibit Dengue virus infection by disrupting the folding of the structural proteins prM and E, a stepcrucial to viral secretion. *Flaviviruses* including all four serotypes of Dengue Virus, Yellow Fever Virus, and West Nile Virus. Using in vitro assays demonstrated that infections by all serotypes of Dengue Virus were inhibited by Castanospermine. In contrast, Yellow Fever Virus and West Nile Virus were partially and almost completely resistant to the effects of the drug. Importantly, Castanospermine prevented mortality (Kevin, et al., 2005).

6- Azauridine also known as Azauridine, 6-Azuridine, 6-Azauriacil Riboside, Riboazauracil and Riboazauratsil. It has Molecular formula $C_8H_{11}N_3O_6$ with Molecular weight 245. 18944 g/mol. 6-Azauridine is a triazine nucleoside used as an antineoplastic antimetabolite. It interferes with pyrimidine biosynthesis thereby preventing formation of cellular nucleic acids. As the triacetate, it is also effective as an anti-psoriatic. It is a

synthetic triazine analogue of uridine with antimetabolite activity. 6-azauridine inhibits de novo pyrimidine synthesis and DNA synthesis and is converted intracellularly into mono, di, and triphosphate derivatives, which incorporate into RNA and inhibit protein synthesis. XLogP3 is -2.1 hydrogen bond donor counts are 4, hydrogen bond acceptor counts are 7 and rotatable bond counts are 2.

Mycophenolic acid also known as Mycophenolate, Myfortic, Melbex and Mycophenolseacure. It has molecular formula $C_{17}H_{20}O_6$with molecular weight of 320.3371 g/mol. It belongs to antibiotic substance derived from pencilliumstoloniferum and related species. It blocks de novo biosynthesis of purine nucleotides by inhibition of the enzyme inosine monophosphate dehydrogenase. Mycophenolic acid is important because of its selective effects on the immune system. It prevents the proliferation of t-cells, lymphocytes and the formation of antibodies from B-cells. It also may inhibit recruitment of leukocytes to inflammatory sites. Mycophenolic acid is an antimetabolite immunosuppressant. Mycophenolic acid is an antineoplastic antibiotic derived from various penicillium fungal species. Mycophenolic acid is an active metabolite of the product mycophenolate mofetil. Mycophenolic acid inhibits inosine monophosphate dehydrogenase, preventing the formation of guanosine monophosphate and synthesis of lymphocyte DNA that results in inhibition of lymphocyte proliferation, antibody production, cellular adhesion, and migration of T and B lymphocytes. Mycophenolic acid also has ant bacterial, antifungal, and ant viral activities. XLogP3 is 3.2 hydrogen bond donor counts are 2, hydrogen bond acceptor counts are 6 and rotatable bond counts are 6.

PyRx is Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process - from data preparation to job submission and analysis of the results. While it is true that there is no magic button in the drug discovery process, PyRx includes docking wizard with easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design. PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for Rational Drug Design (Suvannang, et al., 2011; Syahdi, et al., 2012; Muhammad and Fatima 2015; Lin, et al., 2015; Kumar, et al., 2015)

In this chapter we performed the molecular docking studies using auto dock vina to non-structural proteins of viruses (DENV, WNV, YFV) with selected drugsusing auto dock vina in PyRx molecular docking off line software and the highest binding affinity drug with the three viruses non-structural proteins were characterise through molecular visualisation in Pymol visualizer to know their bonding interactions. The selected drugs for this docking study was Mycophenolic acid, Castanospermine, Triaryl pyrazoline, Ribavirin, 6-azauridine because all these drugs act on flaviviruses.

The molecular docking studies of selected therapeutic drugs with the Dengue, West Nile, and Yellow Fever Virus's non-structural proteins revealed that only Triaryl pyrazoline drugs showed the highest binding affinity with the least binding energy values than other drugs. Selected all drugs were docked with the all proteins first, then, only the highest binding affinity drug showed interactions and molecular visualisation are notified.

Triaryl pyrazoline (TPZ) drug showed-6.7kcal/mol binding affinity with the Dengue Virus NS1 protein with no hydrogen bond interactions. The drug binding site or surrounding amino acids of drug was Ser94, Ile $_{96}$, Ala_{98}, Glu_{99}, $Asn1_{39}$, Thr_{222}, Ile_{246}, Tyr_{247}, Thr_{264}, Ala_{265}, Thr_{268}, His_{269}, Ser_{348}, Val_{350}, and Ser_{351}. TPZ interact with the active site of NS2A protein of Dengue with -7.5kcal/mol binding affinity with one hydrogen bond interaction. The drug forms hydrogen bond with Alanine amino acid atom O_{23} (oxygen) through nitrogen. TPZ showed -7.8kcal/mol binding affinity with NS2B protein of Dengue. It showed one hydrogen bond interaction with Thr_{102} amino acid, O_{23} atom through O (oxygen).In NS3 active site the drug TPZ showed -8.3kcal/mol binding affinity with one hydrogen bond interaction. The O_{23} of leucine26, hydrogen bond interacted by N (nitrogen) of drug. TPZ showed -6.7kcal/mol binding affinity with NS4A of Dengue virus without any hydrogen bond interactions, the surrounding amino acids to TPZ was Leu $_{6}$, Ile_{7}, Ile_{10}, Gly_{11}, Leu_{31}, Met $_{56}$, Leu_{57}, Leu_{58}, Gly_{75}, Glu_{101} and His_{103}. NS4B protein was interacted by TPZ with -6.7kcal/mol binding affinity without any hydrogen bonds. The surrounding amino acids to drug was Ala_{18}, Ile_{64}, Thr_{67}, Ala_{68}, Ala_{70}, Asn_{71}, Ser_{86}, Val_{112}, Leu_{113}, Leu_{115}, Val_{116}, Leu_{126}, Ala_{128}, Ala_{134} and Lys_{136}. The NS5 protein of Dengue Virus showed -6.6kcal/mol binding affinity with single hydrogen bond interaction. The aspergine 729 amino acid forms hydrogen bond with oxygen of drug. The hydrogen bond interactions with amino acids with drugs and its bond length and

bond angels were represented in table 8. The molecular interactions of the drug with active site of the non-structural proteins were represented in figure 25.

The same drugs Triaryl pyrazoline showed the highest binding affinity with the West Nile Virus non-structural proteins.TPZ showed the -8.6kcal/mol binding affinity with the West Nile Virus non-structural protein NS1 without any hydrogen bond interaction. The drug binding site surrounding amino acids in NS1 includes as Pro_{8}, Phe_{9}, Leu_{11}, Gly_{12}, Leu_{13}, Met_{14}, Val_{15}, Ala_{78}, Met_{116},Tyr_{118}, Tyr_{119}, Asp_{120}, Glu_{128}, Val_{129}.NS2A protein of West Nile Virus has -8.0kcal/mol binding affinity with TPZ with two hydrogen bond interactions. Carbon atom of aspergine 67 formed the hydrogen bond interaction with nitrogen atom of drug TPZ. Second hydrogen bond interaction in between drug TPZ and NS2A was hydrogen atom 23 of glutamic acidnumber 64 amino acid of NS2A protein forms the bond with carbon atom of carbon atom of TPZ. The drug showed -8.0kcal/mol binding affinity with NS2B protein of West Nile Virus without any hydrogen bond interactions. The drug binding siteamino acids of the NS2A proteins includes Ala_{10}, Phe_{16}, ile_{17}, Val_{18}, Val_{44}, Ser_{46}, Gly_{47} Met_{52}, Trp_{53}, Ile_{54}, Glu_{55}, and Arg_{56}. The NS3 protein of the West Nile virus showed the one hydrogen bond interaction with -7.6 kcal/mol binding affinity. Nitrogen atom of ser_{129} aminoacid showed hydrogen bond interaction with (O) oxygen atom of drug. NS4A protein, drug TPZ interaction has -7.6 kcal/mol binding affinity without any hydrogen bond interactions. The drug binding site of the NS4A protein contained the amino acids includes Ile_{3}, Gly_{4}, Leu_{5}, Glu_{22}, Ala_{23}, Thr_{26}, Tyr_{28}, Phe_{68}, Phe_{69}, Lys_{79}, leu_{82}, Gly_{84}, Gly_{88}, leu_{87}, Thr_{91}. Binding affinity -7.7kcal/mol was showed in between NS4B of West Nile and drug TPZ with one hydrogen bond interaction. Tyr_{125} aminoacid atom oxygen 23 forms hydrogen bond interaction with oxygen atom of the drug TPZ. NS5 protein has -7.7kcal/mol binding affinity by drug TPZ with two hydrogen bond interactions. The amino acid $Threonine_{123}$, atom nitrogen 26 forms the hydrogen bond interaction with oxygen of drug. Lysine $amino\ acid_{127}$, nitrogen atom of drugs forms another hydrogen bond. All hydrogen bonded angels and lengths were represented in table 9. The molecular visualization of all proteins of West Nile with drug molecules was represented in figure 26.

The non-structural proteins of Yellow Fever Viruses docking results showed that no one protein in Yellow Fever not shown hydrogen bond interactions. The NS1 protein

showed -7.2kcal/mol binding affinity with drug Triaryl pyrazoline without any hydrogen bond interactions. The drug surrounded amino acids are Asp_1, Gly_3, Ala_5,Asn_7, gly_{16}, Asp_{17}, Ile_{19}, Phe_{20}, Ile_{21}, Arg_{23}, Ala_{187}, val_{188}, Asn_{189} and Lys_{192}. The NS2A protein has -7.9kcal/mol binding affinity with drug without any hydrogen bond interactions. The drug binding site amino acids in NS2A protein noted as Arg_{101}, Leu_{102}, ARG_{99}, LEU_{95}, THR_{94}, LEU_{115}, GLY_{117},VAL_{118}, MET_{119}, VAL_{43}, LEU_{42} and GLU_{64}. The NS2B protein showed has -8.0kcal/mol binding affinity with drug TPZ without any hydrogen bond interactions. The amino acids in NS2B proteins drug binding site are Phe_{28}, Val_{44}, Ala_{45}, Gly_{46}, Arg_{47}, Val_{48}, Asp_{49}, Leu_{51}, Glu_{52}, Lys_{54} and Leu_{56}. The NS3 protein also not shown any hydrogen bond interactions with drug but it has -8.9kcal/mol binding affinity. The drug binding site amino acids in NS3 protein was Gln $_{460}$, his_{292}, $seer_{456}$, ser_{457}, leu_{294}, cys_{491}, Lys_{435}, pro_{436}, asp_{414}, Ile $_{415}$, ala_{416}, Thr $_{229}$, pro $_{228}$. The NS4A protein showed -8.5kcal/mol binding affinity by drug without any hydrogen bond interactions. The amino acids of NS4A are Leu_{12}, Asp_{14}, phe_{15}, Asn_{42}, Ala_{43}, leu_{44}, Met_{55}, Val_{54}, Phe_{57}, Ile_{58}, Leu_{59} and Ala_{60} surrounded to drug. The TPZ showed -8.1kcal/mol with NS4B protein, not shown any hydrogen bond interactions. The amino acids are surrounded to the drug was Leu_{32}, Ile_{34}, Gln_{36}, Ile_{84}, Pro_{85}, Ile_{86}, Met_{87}, Lys_{88}, Arg_{138}, Glu_{158}, Glu $_{159}$, Ala_{160}, Pro_{161}, Pro_{164}. Ns5 protein has -7.6kcal/mol binding affinity with drug, not shown any hydrogen bond interactions. The amino acids present in drug binding site are Gly_{457}, Ile $_{475}$, Tyr $_{477}$, Gly_{604}, Arg_{483}, Ala_{409}, Arg $_{405}$, Arg $_{796}$, Trp_{799}, Lys_{458}, Arg_{459} (Table 10). The molecular visualization of all proteins of Yellow Fever Virus with drug molecules was represented in figure 27.

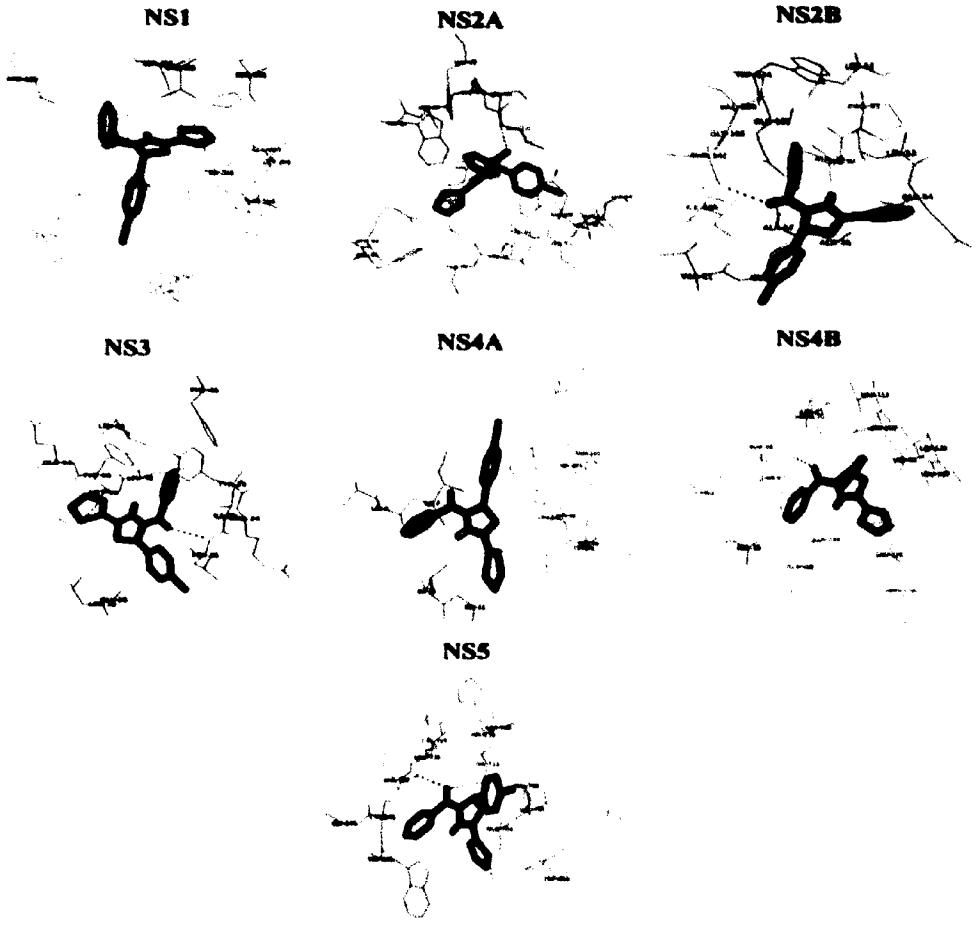

Figure 25: The molecular docking of Triaryl pyrazoline with non-structural proteins (NS1, NS2A, NS3, NS4A, NS4B and NS5) of Dengue Virus using auto dock vina in PyRx. The molecular visualizations was done in Pymol molecular visualizer (the middle green colour indicative for Triaryl pyrazoline and surrounding moieties are amino acids of protein. The red colour dots indicative for hydrogen bonds)

Table 8: The molecular interactions of the Triaryl pyrazoline with non-structural proteins of Dengue Virus.

S.No	Protein Name	Drug compound Triaryl pyrazoline			
		Binding affinity	**Hydrogen bond Interactions/ surrounding amino acids**	**Bond length**	**Bond angle**
1	NS1	-6.7	Ser94, Ile 96, Ala98, Glu99, Asn139, Thr222, Ile246, Tyr247, thr264, Ala265, Thr268, His269, Ser348, Val350, Ser351	-	-
2	NS2A	-7.5	Ala23 C-O23-------- $NG_2$225-CZ	2.9	107.5
3	NS2B	-7.8	Thr102 C-O23-------- $OG_2$755-CB	3.1	47.5
4	NS3	-8.3	Leu 26 C-O23-------- N 197-C	3.4	99.5
5	NS4A	-6.7	Leu 6, Ile7, Ile10, Gly11, leu31, Met 56, Leu57, Leu58, Gly75, Glu101, His103	-	-
6	NS4B	-8.0	Ala18, Ile64, Thr67, Ala68, Ala70, Asn71, Ser86, Val112, Leu113, Leu115, Val116, leu126, Ala128, Ala134, Lys136.	-	-
7	NS5	-6.6	Asn729 C-O 23---------- N 5831- C	3.3	104

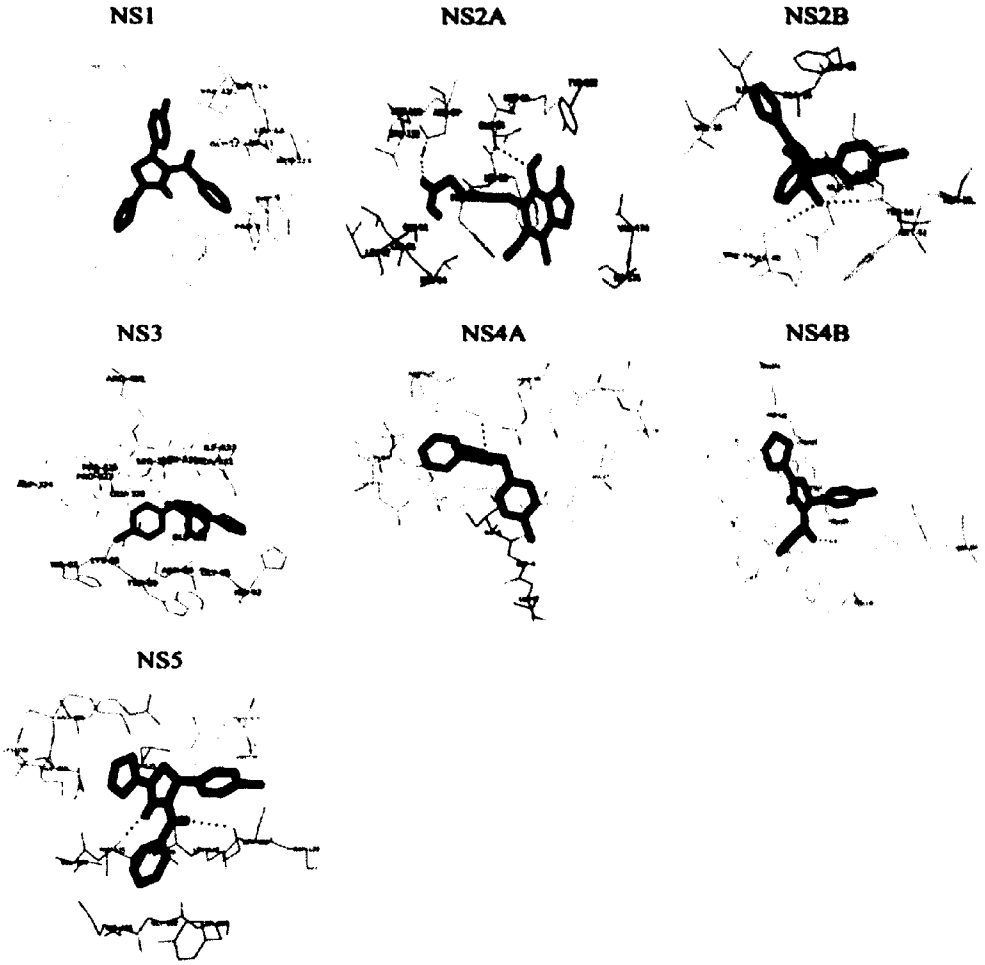

Figure 26: The molecular docking of Triaryl pyrazoline with non-structural proteins (NS1, NS2A, NS3, NS4A, NS4B and NS5) of the West Nile Virus using auto dock vina in PyRx. The molecular visualizations was done in Pymol molecular visualizer (the middle green colour indicative for Triaryl pyrazoline and surrounding moieties are amino acids of protein. The red colour dots indicative for hydrogen bonds)

Table 9: The molecular interactions of the Triaryl Pyrazoline with non-structural proteins of West Nile Virus

S.No	Protein Name	Drug compound Triaryl pyrazoline			
		Binding affinity	**Hydrogen bond Interactions/ surrounding amino acids**	**Bond length**	**Bond angle**
1	NS1	-8.6	Pro8, Phe9, Leu11, Gly12, Leu13, Met 14, Val15, Ala78, Met 116,Tyr118, Tyr119, Asp120, Glu128, Val129	-	-
2	NS2A	-8.0	Asn67 C-O 20----------ND_2 501- CG Glu64 O-H 23---------- CD 470-CG	3.1 3.5	95.6 110.7
3	NS2B	-8.0	Ala10, Phe16, ile17, Val18,Val 44, Ser46, Gly47 Met52, Trp53, Ile54, Glu55, Arg56	-	-
4	NS3	-7.6	Ser 329 C-N----O1-C7	3.1	119.2
5	NS4A	-7.6	Ile3, Gly4, Leu5, Glu22, Ala23, Thr26, Tyr28, Phe68, Phe69, Lys79, leu82, Gly84, Gly88, leu87, Thr91	-	-
6	NS4B	-7.7	Tyr 125 c-o23--------oh 952-cz	2.7	98.6
7	NS5	-7.7	Thr 123 H-HN26-------OG996-CB Lys 127 C-O23----------NZ1013-CE	2.3 3.1	167.9 112.8

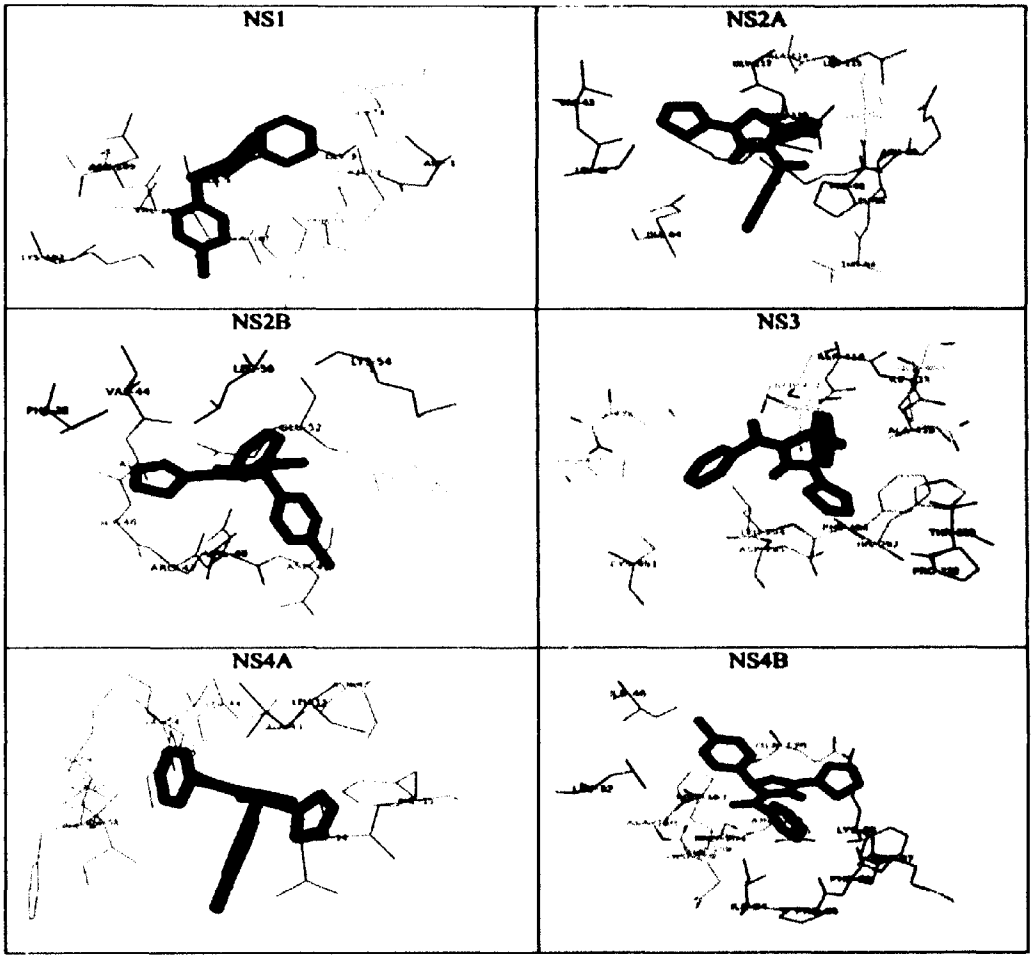

Figure 27: The molecular docking of Triaryl pyrazoline with non-structural proteins (NS1, NS2A, NS3, NS4A, NS4B and NS5) of yellow fever virus using auto dock vina in PyRx. The molecular visualizations was done in Pymol molecular visualizer (the middle green colour indicative for Triaryl pyrazoline and surrounding moieties are amino acids of protein. The red colour dots indicative for hydrogen bonds)

Table 10: The molecular interactions of the Triaryl pyrazoline with non-structural proteins of Yellow Fever Virus

S.No	Protein Name	Hydrogen Bonging Interactions		Bond Length(A)	Bond Angle	Binding Energy (kcal/mol)
		Protein	Ligand			
1	NS 1	Asp1, Gly3, Ala5,Asn7, gly16, Asp17, Ile19, Phe20, Ile21, Arg23, Ala187, val188, Asn189, lys192		-	-	-7.2
2	NS 2A	Arg 101, Leu 102, Arg99, Leu 95, Thr 94, Leu115, Gly117, Val118, Met119, Val43, Leu42, Glu64.		-	-	-7.9
3	NS 2B	Phe28, Val44, Ala45, Gly46, Arg47, Val48, Asp49, Leu51, Glu52, Lys54, Leu56		-	-	--8.0
4	NS 3	Gln 460, His292, Seer 456, Ser457, Leu294, Cys 491, Lys435, Pro436, Asp 414, Ile 415, Ala416, Thr 229, Pro 228		-	-	-8.9
5	NS 4A	Leu12, Asp14, phe15, Asn42,Ala43, leu44, Met55, Val54, Phe57, Ile58, Leu59, Ala60		-	-	-8.5
6	NS 4B	Leu32, Ile34, Gln36, Ile84, pro85, ile86, Met87, Lys88, Arg138, Glu158, Glu 159, Ala160, Pro161, Pro164		-	-	-8.1
7	NS 5	Gly 457, Ile 475, Tyr 477, Gly604, Arg483, Ala409, Arg 405, Arg 796, Trp799, Lys458, Arg459		-	-	-7.6