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Effect of Mutation of RNA-dependent RNA Polymerase (RdRp) of Hepatitis C Virus on Affinities of Dasabuvir: Computational Study

(Pengaruh Mutasi RNA-dependent RNA polymerase (RdRp) pada Virus Hepatitis C terhadap Afinitas Dasabuvir: Kajian Komputasi)

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ABSTRACT

Hepatitis C Virus (HCV) is one of the infectious diseases that has posed a serious threat to global public health for the past few decades. HCV is an RNA virus that infects the human liver and can lead to chronic liver damage, cirrhosis, and even liver cancer. Treatment for HCV infection has made rapid advancements in recent years, particularly with the development of more effective antiviral drugs. One of the drugs used in HCV therapy is dasabuvir. Dasabuvir is an RNA-dependent RNA polymerase (RdRp) inhibitor that functions to inhibit the replication of the HCV virus. The RdRp enzyme in HCV is represented by NS5B, and dasabuvir specifically targets this enzyme. Several reports have revealed mutations in HCV NS5B due to the use of dasabuvir. This study conducted a computational mutation analysis on NS5B of HCV resulting from dasabuvir usage. The research findings indicate that mutations in the HCV polymerase induced by dasabuvir usage lead to changes in dasabuvir's conformation and binding energy. Some mutations decrease binding energy, such as mutations C316N, C451S, and N411S. However, on the other hand, there are mutations that increase binding energy, such as M414V, A553V, and C445F. The decrease in binding energy is supported by increased hydrogen bonding interactions with Asp318, Gln446, and Tyr448, as well as the formation of new hydrogen bonds, such as hydrogen bonding with Ser288 in C451S and Arg200 in C451S. Meanwhile, the increase in binding energy is supported by decreased binding interactions with Asp318 and pi-pi interactions with Phe193. Hydrogen bonding with Asn291 also decreases, as seen in A553V, and is even lost in C445F. Future work will be devoted for designing new dasabuvir derivatives which having better affinited to NS5B of HCV.

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INTRODUCTION

Treatment for HCV infection has made significant progress in recent years, especially with the development of more effective antiviral drugs. One of the drugs used in HCV therapy is dasabuvir. Dasabuvir is an RNA-dependent RNA polymerase (RdRp) inhibitor that functions to inhibit the replication of HCV. The RdRp enzyme in HCV is represented by NS5B, and dasabuvir specifically targets this enzyme (Bukh, 2016).

Several reports have revealed mutations in NS5B of the HCV due to the use of dasabuvir. Mutations in NS5B of the HCV have become a major concern in the treatment of HCV infections related to dasabuvir use. This is due to several important reasons, including drug resistance issues, as HCV has a high mutation rate, allowing it to quickly develop resistance to antiviral drugs, including dasabuvir (Chen, Li, Ren, & Hu, 2016). Mutations in NS5B can result in a form of the virus that is no longer responsive to this drug, making treatment less effective.

Another issue is related to treatment optimization, where understanding mutations in HCV NS5B related to dasabuvir can help researchers and healthcare practitioners optimize individualized treatment. Identifying specific mutations can assist in selecting the most suitable therapy for particular patients, which can improve the chances of recovery.

The next issue is related to the development of new drugs, where a deep understanding of mutations in NS5B can aid in the development of stronger and more effective drugs to combat HCV infections. By comprehending how mutations affect NS5B activity, researchers can design drugs that are more resistant to viral resistance.

Another aspect is related to virus spread prevention, where studies on NS5B mutations can play a critical role in efforts to prevent HCV infection spread. By understanding how the HCV virus can undergo genetic changes, infection prevention and control efforts can be adjusted more effectively.

Therefore, a better understanding of mutations in the RNA-dependent RNA polymerase enzyme NS5B of the hepatitis C virus as a result of dasabuvir drug use has significant implications for HCV treatment, prevention, and control efforts. Further research on this topic can help enhance the effectiveness of therapy, reduce drug resistance, and ultimately help address the global threat posed by HCV. This study conducted a computational mutation analysis of NS5B in the HCV resulting from the use of dasabuvir (Kohli, Shaffer, Sherman, & Kottilil, 2014).

The use of dasabuvir was well known to have induced several mutations on NS5B of HCV. The mutations include C316N, N411S, M414V, C445F, C451S, and A553V. Those mutations have impacted the use of

dasabuvir, thus the molecular aspect of how the mutation affect the interactions of the drug with NS5B in the HCV urgently need to be understood. This study will address the issue by emplying computational tool.

METHODS

The virtual screening procedures used in this study included extra precision (XP) docking, molecular dynamics simulations, and MM-GBSA energy calculations. Docking was performed to understand how dasabuvir binds to the wild type (WT) protein and mutant proteins.

The crystal structure of the HCV NS5B virus bound to dasabuvir was obtained from the RCSB Protein Data Bank with PDB ID 4MKB at a resolution of 1.9 Å (Schoenfeld et al., 2013). The protein structure was prepared using the Protein Preparation module of Maestro 10.3 (Sastry, Adzhigirey, Day, Annabhimoju, & Sherman, 2013) which involved removing water and non-interacting ions, adding missing atoms to residues, and determining the optimal protonation states for histidine residues.

The mutant protein structures, namely C316N, N411S, M414V, C445F, C451S, A553V, were prepared by introducing specific point mutations into the WT protein system.

The dasabuvir structure was prepared by adjusting its ionization state at pH 7, determined through pKa calculations using the Epik module (Sastry et al., 2013). The lowest tautomeric state of dasabuvir was selected and optimized to minimize potential energy and address undesired contacts. This optimization process used default parameters, including the use of the OPLS3 force field with a maximum of 2500 iterations (Arba et al., 2023) .

A receptor grid file was built using the protein-ligand complex for the WT, with a van der Waals scaling factor of 1. The binding site of dasabuvir was defined using the natural ligand position as a reference. Subsequently, the optimal dasabuvir molecular structure was substituted into the receptor protein grid for the WT. The docking conformations of each WT and mutant protein system were used to build molecular dynamics (MD) simulation systems conducted using the Desmond simulation package. System neutrality was achieved by adding Na+ ions with a 0.15 M NaCl concentration. The OPLS_2005 force field was used for protein-ligand interaction modeling (Banks et al., 2005) .

The Gaussian split Ewald k-space method [41] was used to handle long-range electrostatic interactions by applying periodic boundary conditions. A 10 Å cutoff was set for non-bonded interactions. To speed up calculations, non-bonded forces were computed using the r-RESPA integrator (Stuart, Zhou, & Berne, 1996). Frames were saved at 50.0 ps intervals for further analysis.

Molecular dynamics simulations were carried out for 100 ns using the NPT ensemble with default settings in Desmond (Jorgensen, Maxwell, & TiradoRives, 1996). Temperature control was achieved using the Nosé–Hoover coupling scheme (Ikeguchi, 2004) with a coupling constant of 1.0 ps, while pressure was controlled using the Martyna-Tuckerman-Klein chain coupling scheme with a coupling constant of 2.0 ps. Bond constraints were maintained using M-SHAKE (Bailey & Lowe, 2009).

The Gaussian split Ewald k-space method was used to handle long-range electrostatic interactions by applying periodic boundary conditions. A 10 Å cutoff was set for non-bonded interactions. To expedite calculations, non-bonded forces were computed using the r-RESPA integrator (Stuart, Zhou, & Berne, 1996). Frames were saved at 50.0 ps intervals for further analysis. Molecular dynamics simulation analysis included the calculation of root-mean-square deviation (RMSD) values for protein Cα and ligand positions over the 100 ns simulation. The RMSD plot confirmed simulation convergence.

For MM-GBSA binding energy calculations, the Molecular Mechanics-General Born Surface Area (MM-GBSA) approach was used. This method employs the Generalized Born model with an implicit solvent model (Li et al., 2011). The OPLS3 force field was used, with charge models based on CM1A-BCC and extensive parameterization of bond charge correction (BCC) terms (Harder et al., 2016). The binding free energy was calculated as described in previous paper (Patel et al., 2022)

RESULTS AND DISCUSSION

Dasabuvir is one of the drugs used in the treatment of hepatitis C virus infection. This drug is used in combination with other medications to treat hepatitis C virus infection, particularly the genotype 1 hepatitis C. Dasabuvir functions as an inhibitor of the hepatitis C virus polymerase (NS5B), an enzyme required for the replication of the hepatitis C virus in the human body.

In its development, the use of dasabuvir induces several mutations in the polymerase, such as C316N, N411S, M414V, C445F, C451S, and A553V (Akaberi et al., 2018; Cento, Chevaliez, & Perno, 2015; Eltahla, Luciani, White, Lloyd, & Bull, 2015; Kati et al., 2015; Schoenfeld et al., 2013; Sorbo et al., 2018)

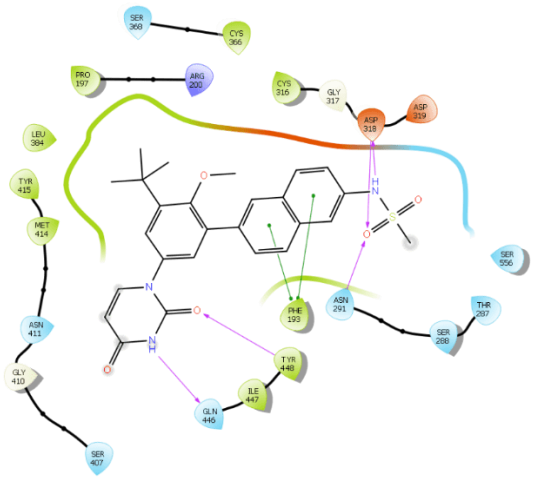
Molecular docking results of dasabuvir with wild-type (WT) and mutant proteins show that the binding energy of dasabuvir to WT is -9.730 kcal/mol. The binding energy of dasabuvir to the C451S mutant protein decreases to -10.060 kcal/mol, while the binding energy of dasabuvir to the C316N, M414V, C445F, A553V, and N411S mutants slightly increases to -9.604 kcal/mol, -8.923 kcal/mol, -8.912 kcal/mol, -9.620 kcal/mol, and -9.146 kcal/mol, respectively. Table 1 displays the binding energies resulting from dasabuvir docking to WT and mutant proteins.

Table 1. Binding energies of dasabuvir docking to WT and mutant proteins.

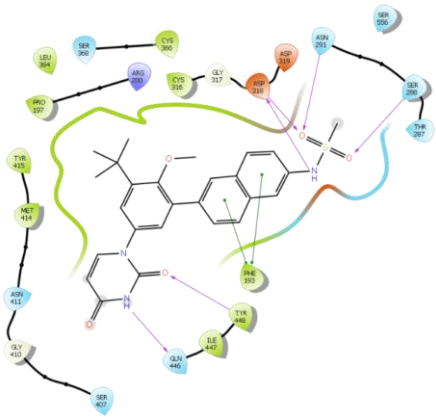
Mutation	Extra precision docking (kcal/mol)	ΔXP (kcal/mol)
WT	-9.730	0
C316N	-9.604	0.126
M414V	-8.923	0.807
C445F	-8.912	0.818
A553V	-9.620	0.11
N411S	-9.146	0.584
C451S	-10.060	0.33

Dasabuvir interactions with WT include hydrogen bonding interactions with Asp318, Gln446, Tyr448, and Asn291, as well as pi-pi interactions with Phe193. Hydrogen bonding and pi-pi interactions with the same amino acids are also observed in mutant proteins A553V, C451S, C316N, N411S, C445F, and M414V, with the addition of a new hydrogen bond with Ser288 observed in dasabuvir's interaction with these mutant protein systems. Figure 1 illustrates dasabuvir interactions with WT and mutant proteins.

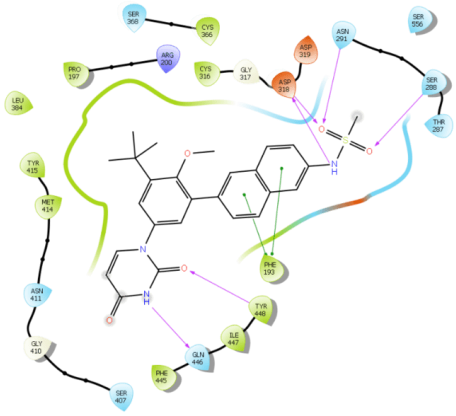
WT



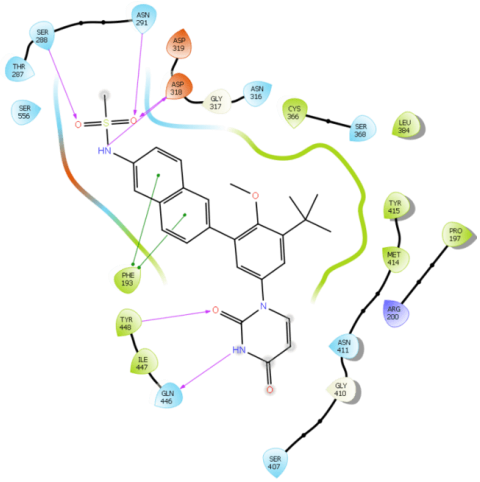
A553V



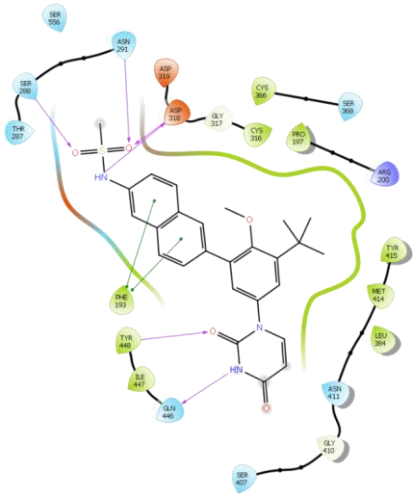
C445F



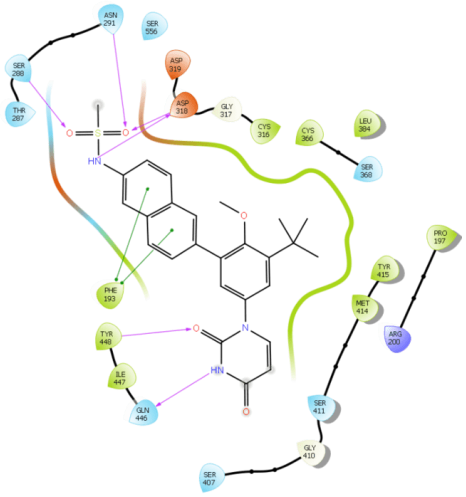
C316N



C451S



N411S



M414V

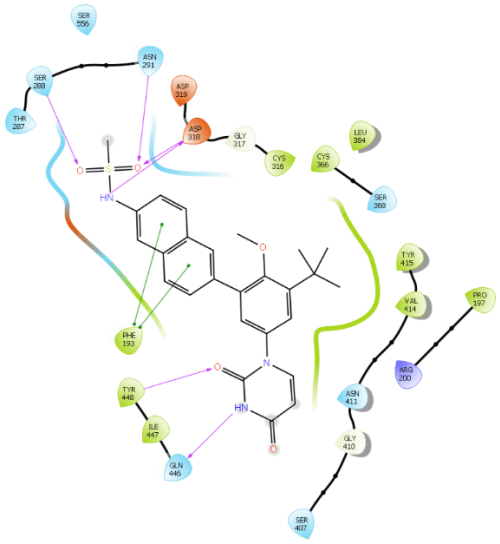
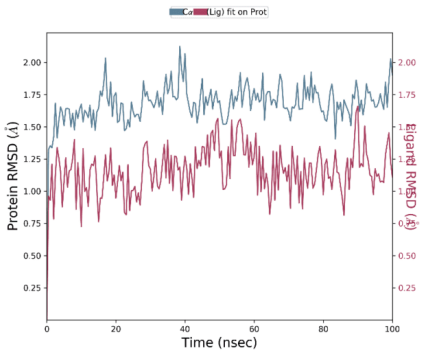


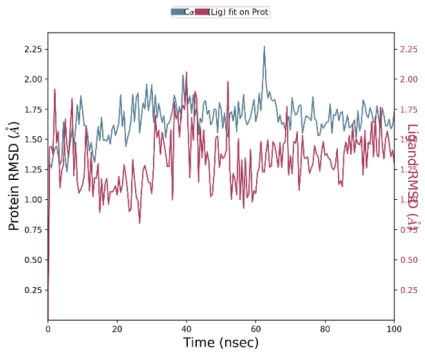
Figure 1. Interaction of dasabuvir with WT and mutant proteins.

Furthermore, using the conformation of each system, a 100 ns molecular dynamics simulation was conducted. The root mean square deviation (RMSD) values are displayed in Figure 2.

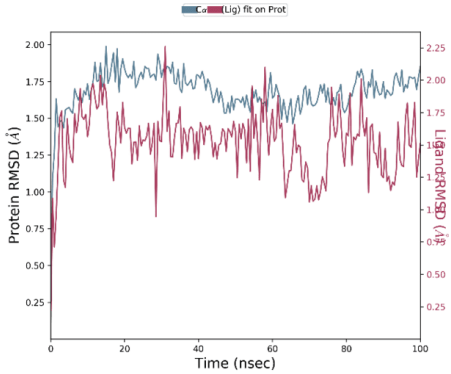
WT



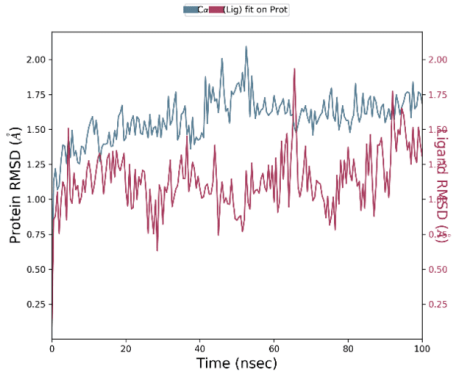
A553V



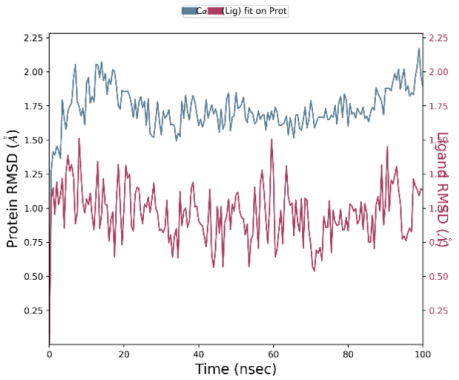
C445F



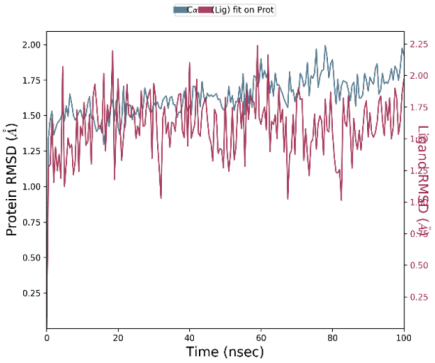
C316N



C451S



N411S



M414V

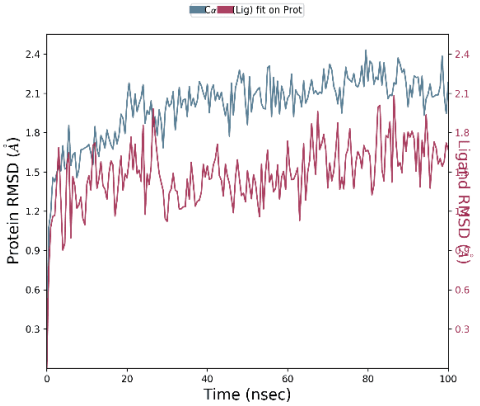


Figure 2. The RMSD of each system.

Furthermore, based on molecular dynamics simulation trajectories, the binding energy of each system was calculated and the results are shown in Table 2.

Table 2. The binding energy of each system.

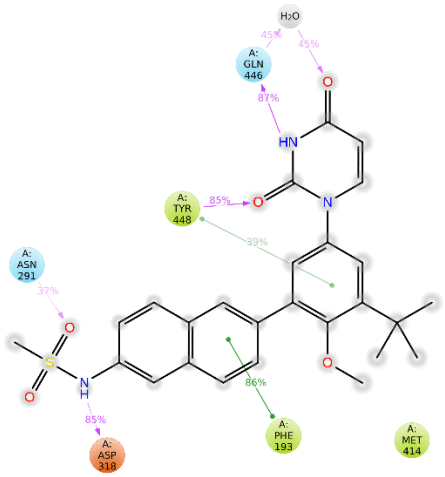
System	ΔE_{vdw}	ΔE_{ele}	ΔE_{lipo}	ΔG_{bind}
WT	-60.57	5.83	-21.91	-76.65
A553V	-63.28	10.36	-21.41	-74.33
C316N	-63.01	7.75	-21.95	-77.21
C445F	-60.61	7.16	-21.27	-74.73
C451S	-60.95	3.56	-21.59	-78.99
M414V	-59.34	5.89	-22.12	-75.57
N411S	-58.29	3.69	-22.53	-77.13

The increase in binding energy in C316N to 77.21±5.84 kcal/mol from -76.65±6.95 in WT is supported by an increased percentage of hydrogen bonding with Asp318 (91% compared to 85%), Gln446 (96% compared to 87%), and Tyr448 (97% compared to 85%) as shown in Figure 3. In C451S, the binding energy increases to -78.99±4.78 kcal/mol compared to WT, with an increased hydrogen bonding

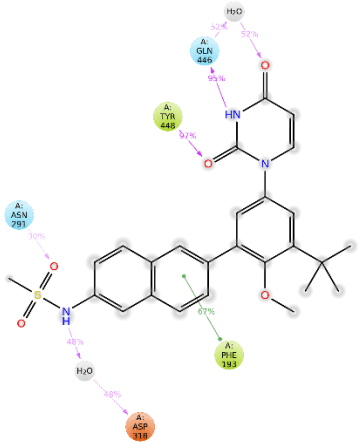
interaction with Asp318 (96%), Gln446 (98%), Tyr448 (99%), and the formation of a new hydrogen bond with Ser288 (39%). In N411S, the binding energy increases to -77.13±4.38 kcal/mol compared to WT, with increased hydrogen bonding interactions with Asp318 (99%), Gln446 (98%), Tyr448 (98%), Asn291 (90%), and the formation of a new hydrogen bond with Arg200 (45%). Additionally, hydrogen bonding mediated by water with Thr287 (42%) is observed. Furthermore, pi-pi interaction with Tyr448 also increases to 75% compared to 39% in WT.

In M414V, the binding energy decreases to -75.57±4.30 kcal/mol compared to WT, supported by a decrease in hydrogen bonding interactions with Asp318 (58%) and Gln446 (81%). Decreases also occur in pi-pi interactions with Phe193 (76%) and the loss of pi-pi interaction with Tyr448. Decreases in interactions also occur in hydrogen bonding with Asp318 (48%), Asn291 (30%), pi-pi interaction with Phe193 (67%), and the loss of pi-pi interaction with Tyr448, resulting in a decreased binding energy of -74.33±5.34 kcal/mol in the A553V mutant. A similar scenario occurs in the C445F mutant, where the binding energy decreases to -74.73±4.71. Hydrogen bonding interactions decrease to 77% with Asp318, and hydrogen bonding with Asn291 is lost. In the C445F mutant, pi-pi interaction with Phe193 is lost, although there is an increase in pi-pi interaction with Tyr448 at 69%.

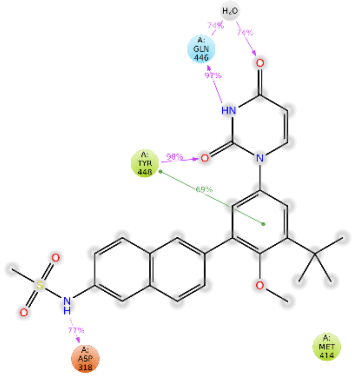
WT



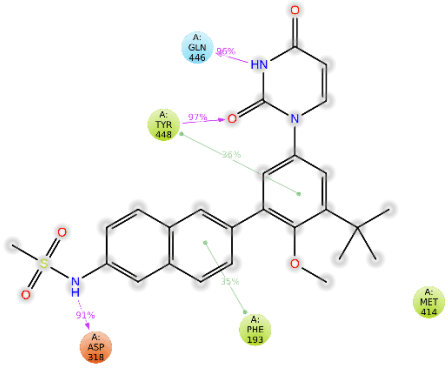
A553V



C445F



C316N



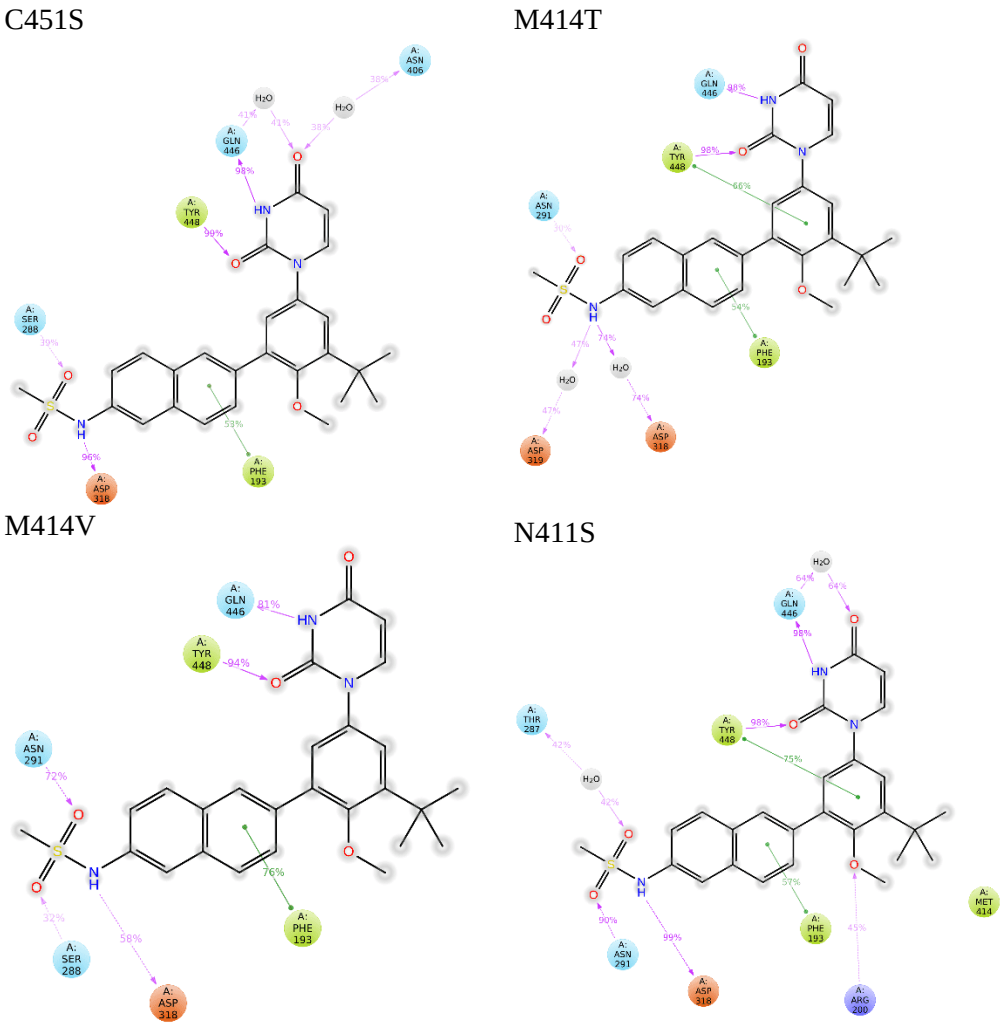


Figure 3. Protein-ligand interactions during a 100 ns molecular dynamics simulation.

Similar works have recorded a change in drug affinities toward protein with noted mutation. Arba et al. (2023) reported single-point mutation modulates the Pimodivir binding in Polymerase basic 2 protein (PB2) of Influenza A virus (IAV) (Arba et al., 2023). The binding of **GS-461203 was also varied due to** multiple mutations within the RNA-dependent RNA Polymerase (RdRp) of Hepatitis C Virus (Arba et al., 2022).

CONCLUSION

Mutations in the HCV polymerase resulting from dasabuvir usage induce changes in dasabuvir conformation and its binding energy. Some mutations decrease binding energy, such as the C316N, C451S, and N411S mutations. However, on the other hand, there are mutations that increase binding energy, like M414V, A553V, and C445F. The decrease in binding energy is supported by an increase in hydrogen bonding interactions with Asp318, Gln446, and Tyr448, as well as the formation of new hydrogen bonds, such as the hydrogen bond with Ser288 in C451S and Arg200 in C451S. Meanwhile, the increase in binding energy is supported by a decrease in binding interactions with Asp318 and pi-pi

interactions with Phe193. Hydrogen bonding with Asn291 also decreases, as seen in A553V, and is even lost in C445F. As far as we know, our present works is the first study which reveals the details interactions of dasabuvir with the WT and mutant proteins of NS5B of HCV, which might be useful in the design of new derivatives of dasabuvir with better affinities.

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CONFLICT OF INTEREST

The author declare that there is no conflict of interest

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