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Molecular docking analysis of fluoroquinolones and other natural and synthetic compounds with the HCV NS3 helicase

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Abstract:
It is of an interest to document the molecular docking analysis of fluoroquinolones and other natural and synthetic compounds with the HCV NS3 helicase. Data shows that three fluoroquinolones interacted with the NS3 helicase in the catalytic region, targeting some of the

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amino acids known to play a crucial role in NS3 helicase activity. Similarly, binding energy showsthat the fluoroquinolones were comparable to the thiazolpiperazinyl derivatives, while superior to several of the synthetic and natural derivatives. The results show three fluoroquinolones to be potent helicase inhibitors that can be repurposed as supplemental therapy against HCV especially in cases non-responsive to DAAAs.

Keywords: HCV; NS3 helicase; antiviral activity; fluoroquinolones.

Background:

Hepatitis C virus (HCV) is still a major public health issue in the 20th century, responsible for about 71 million infections worldwide **[1]**. Out of the 10 proteins encoded in the HCV genome **[2]**, HCV non-structural protein 3 (NS3), which is a helicase belonging to RNA helicase super-family 2 enzymes, is one of the most crucial proteins **[3]**. The N-terminal domain of protein possesses serine protease activity in the presence of the NS4A cofactor protein, while the C-terminal exhibits helicase activity **[4]**. The N-terminal protease and C-terminal helicase domains of HCV NS3 are interdependent, and both enzymatic activities are essential for HCV replication, assembly, and pathogenesis **[5]**. Although current treatment therapy, comprising of directly acting antiviral agents (DAAA), is highly effective **[6]**, still virus in 4-5% of the individuals do not respond to the therapy **[7]**. Furthermore, numerous reports have identified the emergence of drug resistance mutations, which can affect the activity of DAAAs **[8]**. These observations warrant the search for alternate or supplemental antiviral agents that can exhibit superior antiviral activity **[9]**. Several studies have reported the inhibitory activity of synthetic and natural compounds against HCV helicase **[10]**. Similarly, fluoroquinolones, which are broad-spectrum DNA gyrase and topoisomerase IV (helicase) enzyme inhibitors, have also been reported by us and others to exhibit inhibitory activity against the HCV helicases **[11-13]**. Therefore, it is of interest to document the Molecular docking analysis of fluoroquinolones and other natural and synthetic compounds with the HCVNS3 helicase.

Materials and Methods:

HCV NS3 helicase structures:

The HCVNS3 helicase crystal structures (genotype 1a: PDB ID:1A1V and genotype 1b: PDB ID: 1CU1) wereretrieved from PDB database **[14]** in *.pdb* format (**Figure 1**).The structures wereedited to remove water molecules and any bound ligands in Discovery Studio Visualizer version 4.0 (DSV4.0; Dassault Systems BIOVIA, Discovery Studio Visualizer, version 4.0, San Diego: Dassault Systems, 2020) and thereafter saved in PDB format. The polar hydrogen and Kollman charges were added to the structures using Autodock tools **[15]**, and structures were saved in *.pdbqt* format.

HCV NS3 helicase inhibitors:

We compared the efficacy of 20 previously published synthetic and natural NS3 helicase inhibitors, namely, Ring-expanded (fat) nucleoside analogs **[16]**, DRBT **[17]**, manoalide **[18]**, AICAR analog (compound 4) **[19]**, thiazolpiperazinylderivatives, TBBT **[17]**, cholesterol sulfate-1 **[20]**, NS3 peptide (p14) **[21]**, QU663 **[22]**, compound 17, and tropolone derivatives **[23]** against selected fluoroquinolones, namely moxifloxacin, ofloxacin, and sparfloxacin that were previously reported to exhibit superior inhibitory activity against HCV NS3 helicase as compared to other fluoroquinolones tested **[24]**.Structures of these drugs were retrieved from the PubChem database in SDF format **[25]** and converted into PDB format using OpenBabel software **[26] (Figure 2)**.

Table 1: Docking energies and interacting amino acids involved in the drug-protein complexes: The table shows, each inhibitor, its binding energy, and NS3 helicase amino acid(s) it targets. Genotype 1a (1A1V) and 1b (1CU1) NS3 helicase amino acids commonly targeted by different inhibitors have been underlined.

	Names of drugs	Highest docking energy KJ/Moles	Docking energy (Range; Kcal/mol)	Interacting amino acids
		Natural and synthetic inhibitors		
1A1V	Fatsol_503436	-8.5	-8.5 to -7.8	Threonine 433
	DRBT	-8	-8 to -6.8	Aspartate 296, Proline 230, Threonine 295
	Manoalide	-8.8	-8.8 to -8.0	Valine 432
	AICAR analogue (compound 4)	-8	-8.0 to -7.8	Glutamine 434
	Fatsol_44354609	-7.8	-7.8 to -7.1	Aspartate 296, Proline 230, Threonine 295, Glutamine 493
	Compound 17	-10.3	-10.3 to -9	Threonine 295
	Tropolone derivatives	-7.9	-7.9 to -7	Glutamine 460
	Acridone derivative	-11.4	-11.4 to -9.9	Threonine 295, Threonine 433, Glutamic acid 493, Glutamine 493
	Thiazolpiperazinyl Derivative 1	-9.7	-9.7 to -8.8	Proline 348, Tyrosine 350, Lysine 373
	Thiazolpiperazinyl Derivative 2	-11.1	-11.1 to -10.1	Glutamic acid 376, Tyrosine 350, Proline 348
	Thiazolpiperazinyl Derivative 3	-10.6	-10.6 to -9.4	Aspartate 296
	Thiazolpiperazinyl Derivative 4	-9.6	-9.6 to -8.9	Aspartate 296, Threonine 295
	Thiazolpiperazinyl Derivative 1	-9.8	-9.8 to -8.7	None
	TBBT	-6.0	-6.0 to -5.4	Glutamine 460
	Cholesterol sulfate1	-8.7	-8.7 to -7.8	Threonine 295, Aspartic acid 412, Glutamic acid 493, Histidine 293
	NS3 peptide (p14)	-4.6	-4.6 to -3.6	Glutamic acid 493, Glutamine 434
	QU663	-9.7	-9.7 to -9.0	Threonine 295, Threonine 433, Histidine 293, Glutamine 460 and Glutamine 434, Glutamic acid 493, Aspartate 296, Alanine 295.
		Fluoroquinolones		
	Moxifloxacin	-9.1	-9.1 to -7.7	Aspartate 296, Histidine 293, Threonine 295, Proline 230, Glutamine 460, Glutamic acid 493
	Ofloxacin	-8.6	-8.6 to -7.3	Aspartate 296, Glutamine 434
	Sparofloxicin	-9.2	-9.2 to -7.1	Threonine 433 and Threonine 295, Proline 230
		Natural and synthetic inhibitors		
1CU1	Fatsol_503436	-7.5	-7.5 to -7.2	Threonine 295, Proline 482, Aspartate 484, Valine 456
	DRBT	-7.7	-7.7 to -6.9	Tyrosine 502, Threonine 298, Alanine 497, Tryptophan 501, Asparagine

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Manoalide	-8.7	-8.7 to -8.2	Alanine 1111, Lysine 373, Histidine 1110
AICAR analogue (compound 4)	-7.5	-7.5 to -7.0	Cystine 341, Aspartate 454, Arginine 481, Proline 482, Glutamine 434
Fatsol_44354609	-7.8	-7.8 to -7.3	Lysine 352, Serine 1007, Valine 1035
Compound 17	-10.4	-10.4 to -9.7	Glutamine 1434, Valine 1490, Threonine 1295, Aspartate 1296, Proline 1230, Methionine 1415, Alanine 1234, Phenylalanine 1238
Tropolone derivatives	-8.4	-8.4 to -8	Valine 629, 630 and 524, Cysteine 525, Arginine 155, Glutamine 526, Aspartate 437, Phenylalanine 438, Methionine 485
Acridone derivative	-11.9	-11.9 to -9.7	Glutamate 1376
Thiazolpiperazinyl Derivative 1	-10.9	-10.9 to -9.6	None
Thiazolpiperazinyl Derivative 2	-10.9	-10.9 to -9.6	Lysine 1373 and Lysine 1380, Glutamate 1376, Proline 1348, Leucine 1377
Thiazolpiperazinyl Derivative 3	-10.2	-10.2 to -9.3	None
Thiazolpiperazinyl Derivative 4	-9.6	-9.6 to -8.5	Glutamate 376, Tyrosine 350.
Thiazolpiperazinyl Derivative 1	-11.8	-11.8 to -10.7	Tyrosine 350, Lysine 373, Proline 348, Glutamate 376
TBBT	-6.0	-6.0 to -5.3	Methionine 485, Glycine 484, Proline 482, Arginine 481, Valine 456, Aspartate 454
Cholesterol sulfate1	-7.7	-7.7 to -7.3	Valine 1432, Aspartate 1412, 1454 and 1487, Methionine 1485, Arginine 1481, Proline 1452, Glycine 1453, Glutamine 1434, and Glutamate 1453
NS3 peptide (p14)	-4.3	-4.3 to -3.7	Serine 1208 and Sreine 1211, Glycine 1207 and 1209, Threonine 1212, Lysine 1210, Aspartate 1290, Glutamate 1291
QU663	-8.2	-8.2 to -7.7	Threonine 295, Glycine 484, Methionine 485, Arginine 481 and Arginine 461, Aspartate 296 and Aspartate 412
		Fluoroquinolones	
Moxifloxacin	-9.1	-9.1 to -7.9	Aspartate 296 and Aspartate 454, Valine 456, Glycine 484, Methionine 485, Histidine 293, Serine 294, Cysteine 431, Threonine 295
Ofloxacin	-9.2	-9.2 to -7.5	Histidine 293, Serine 294, Valine 456, Aspartate 454, Glycine 484, Methionine 485
Sparofloxicin	-9.0	-9.0 to -8.1	Alanine 1111 and 1005, Lysine 373 and 380, Leucine 377, Aspartate 1112, Proline 348, Phenylalanine 349, Tyrosine 1006, and Tyrosine 350, Glutamate 376.

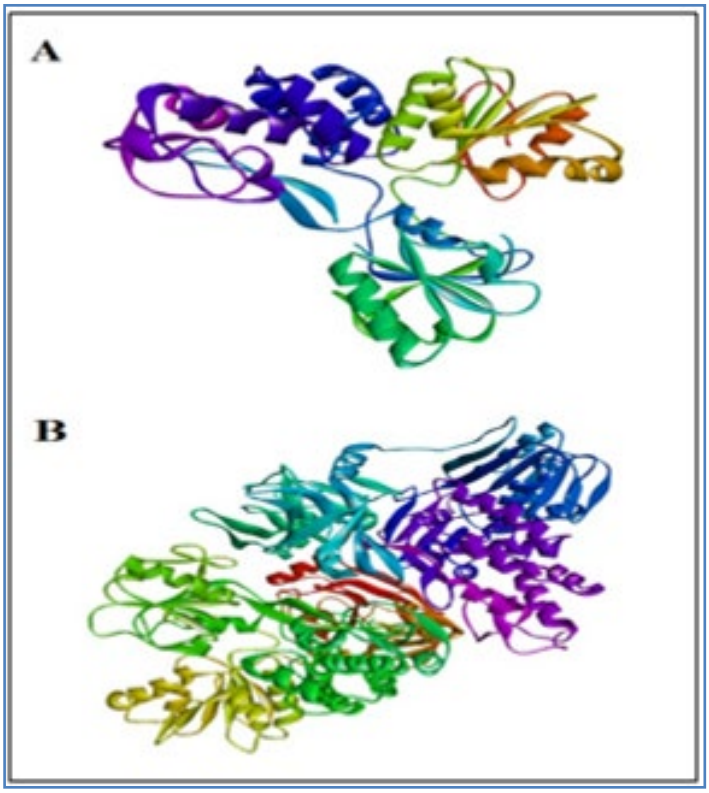

Figure 1: HCV NS3 helicase structures: The structure of NS3 helicase from **(A)** genotype 1A (PDB ID: 1A1V) and **(B)** genotype 1B (PDB ID: 1CU1) are shown.

Molecular docking to analyze binding mode of inhibitors with HCV NS3:

For the analysis of drug-protein interactions, molecular docking simulations were performed. Molecular docking studies and conformational analysis were performed using a web-based version of Autodock Vina, Webina 1.0.3 **[27]**, and interactions were analyzed in DSV 4.0 software. For docking studies, the X, Y, and Z grid box centers were set to 19 Å, 71Å, and 111 Å, respectively, while the X, Y, and Z box size-was set to 116 Å, 81 Å, and93 Åto cover the entire length of the protein. We adopted a blind docking approach to allow drugs to bind anywhere on the protein **[28]**. Molecular docking was performed using standard precision protocols with default parameters of Webina 1.0.3. Out of the stimulated interactions, the top 10 poses were selected based on docking energies, for these selected poses, further analysis of the interaction(s) between NS3-drug binding was carried out. Visualization of docking poses and analysis of drug-protein interactions were performed using DSV4.0.

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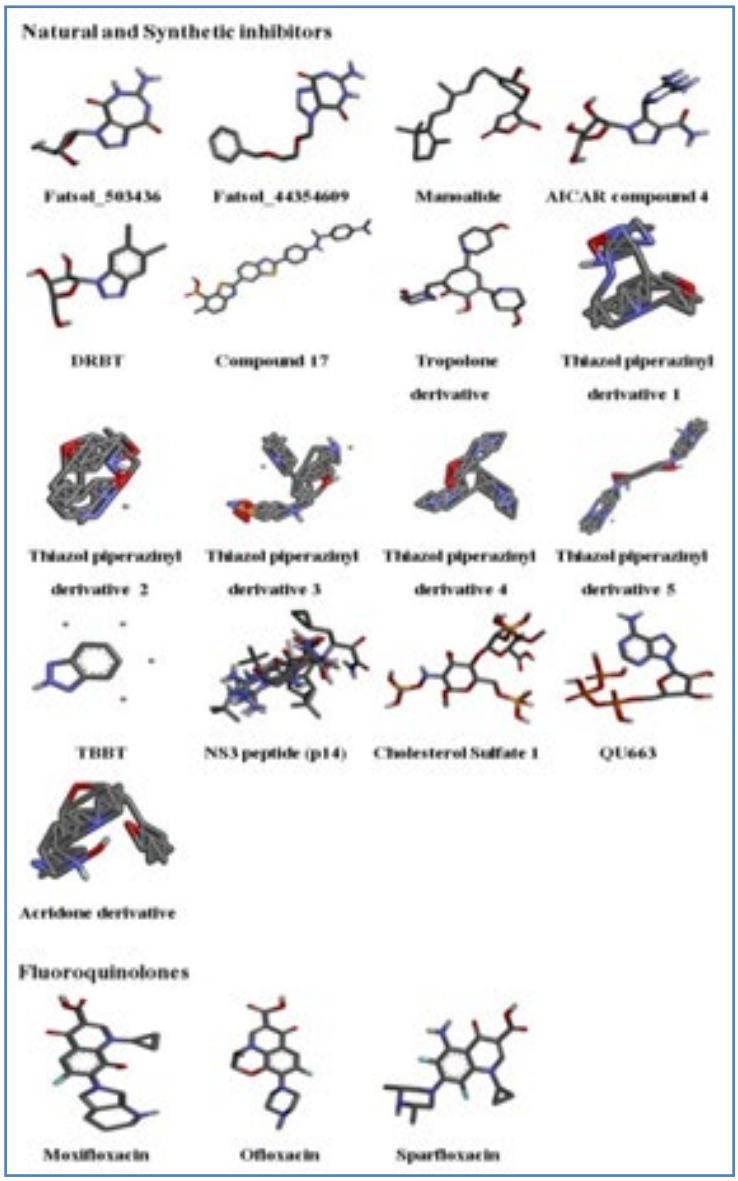

Figure 2: Structures of the ligands used in the study. The structures for potent fluoro-quinolones and other natural and synthetic ligands reported targeted the NS3- helicases are shown.

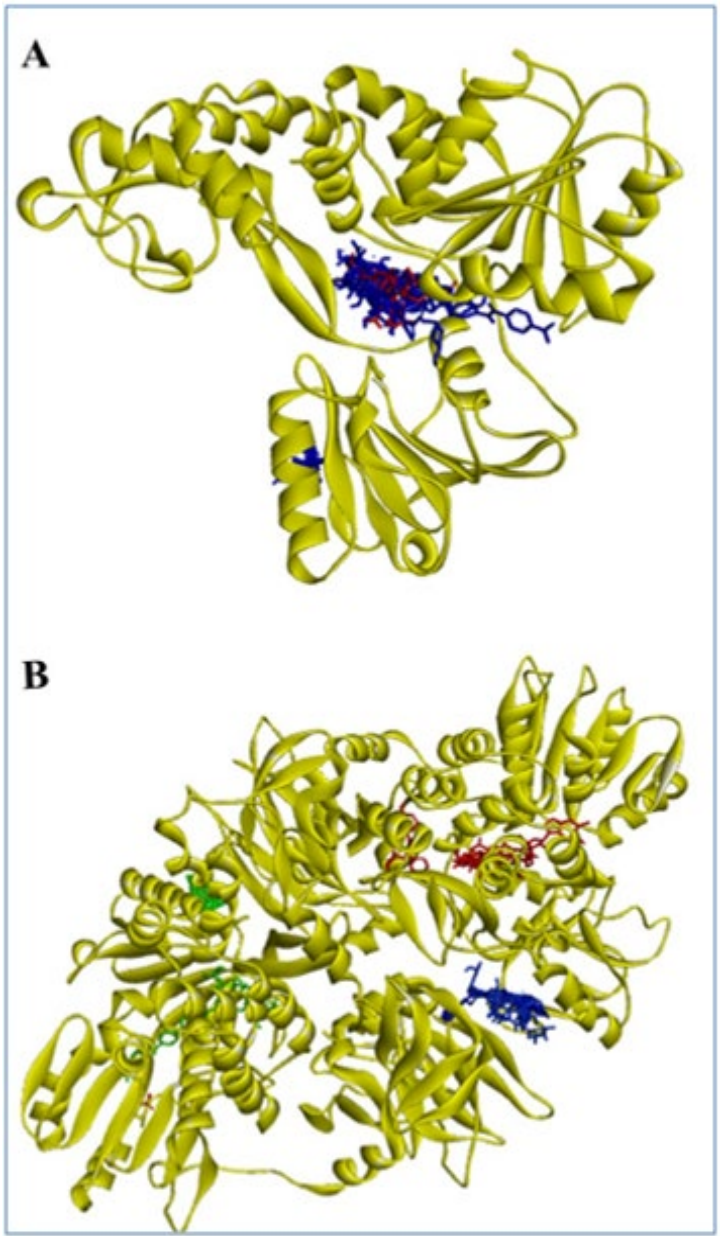

Figure 3: Molecular docking analysis of the drug-protein interactions: HCV helicases (**A)** 1A1V and (**B)** 1CU1 with the putative ligands are shown. (**A)** The fluoroquinolones bound to the catalytic domain of 1A1V are shown in red while other ligands are colored blue. (**B)** The ligands bound to the catalytic domain of 1CU1 are colored blue.

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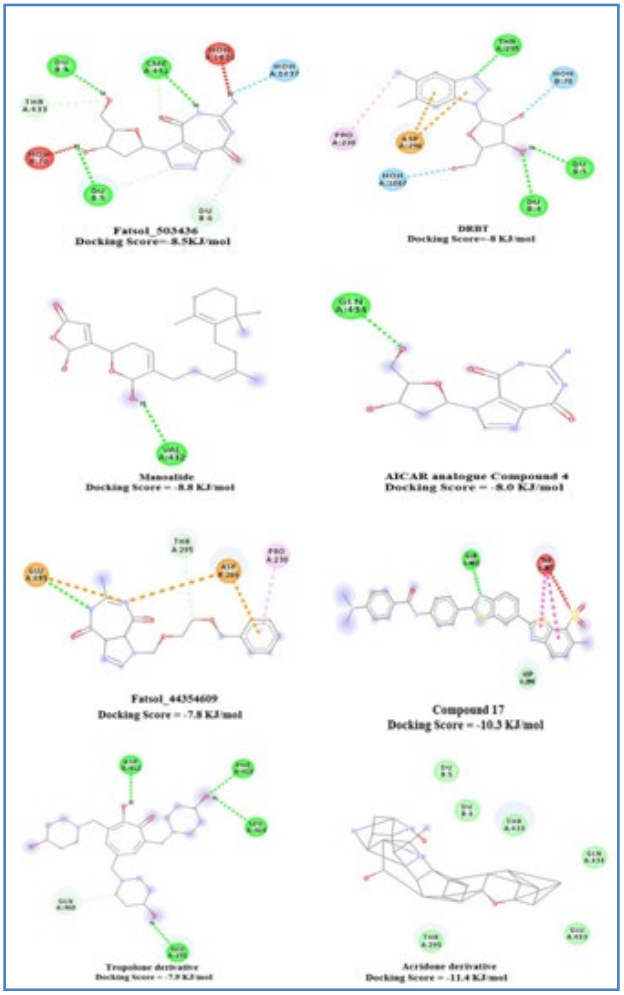

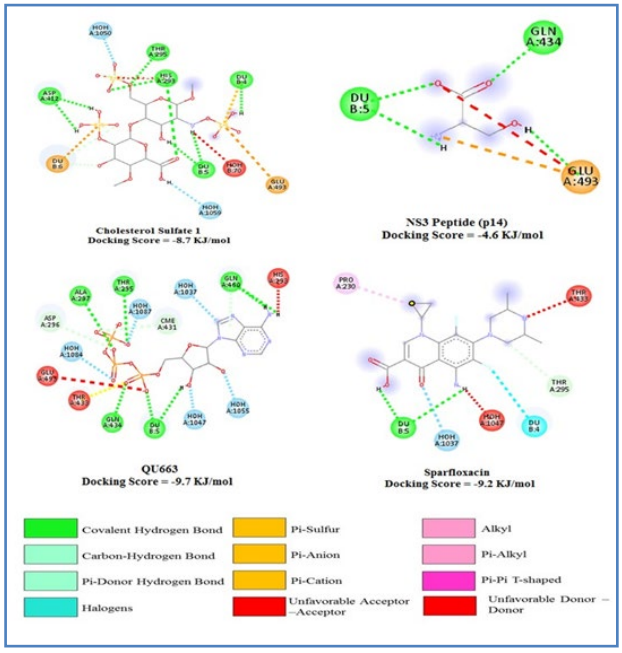

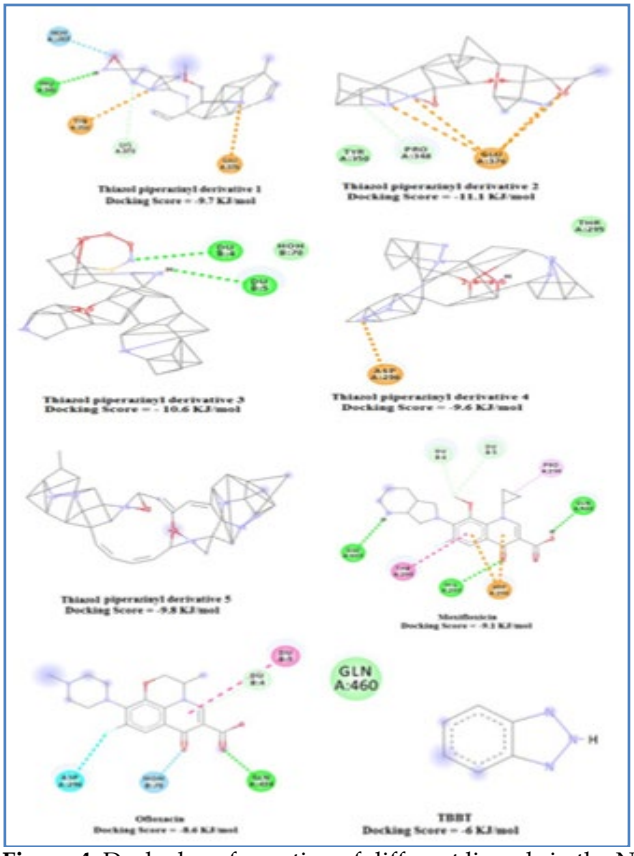

Figure 4: Docked conformation of different ligands in the NS3 cavity. The figure shows 2D representations of the predicted binding modes, type of bonds/interactions, and binding scores for each of the 20 drugs inside the active site of the HCV genotype 1a NS3-helicase (PDB ID:1A1V).

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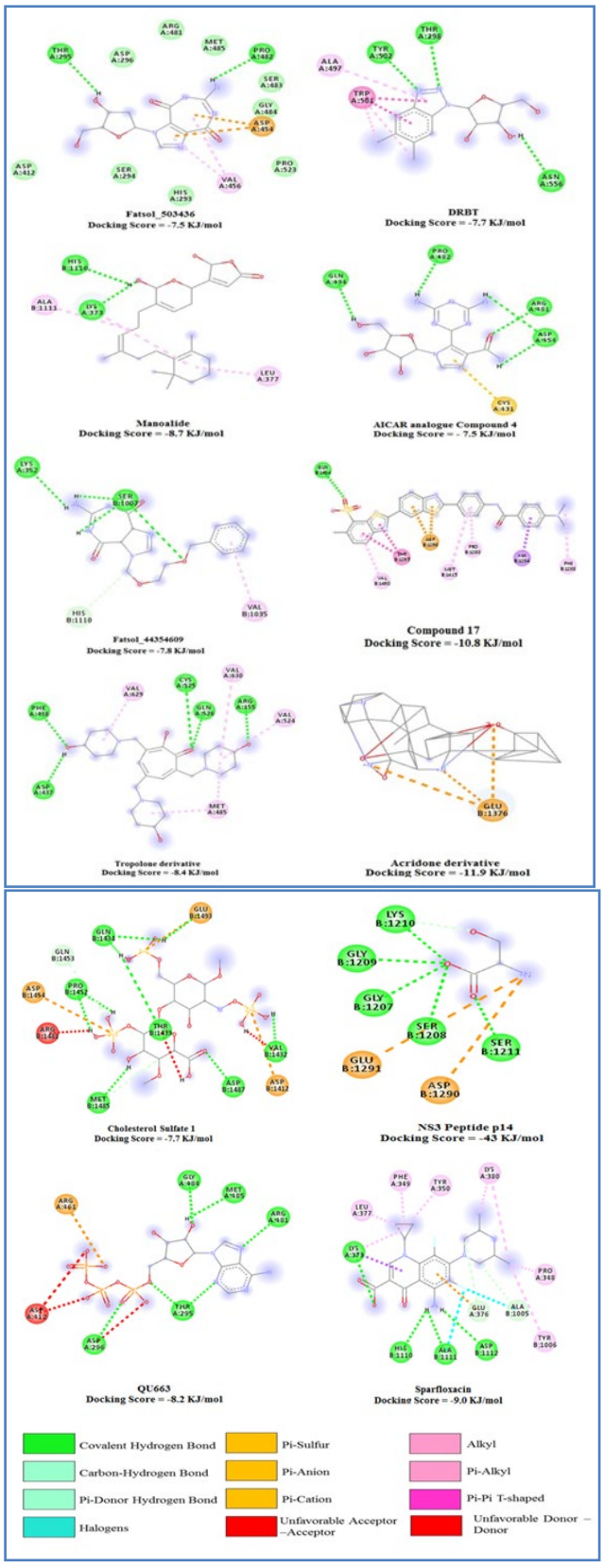

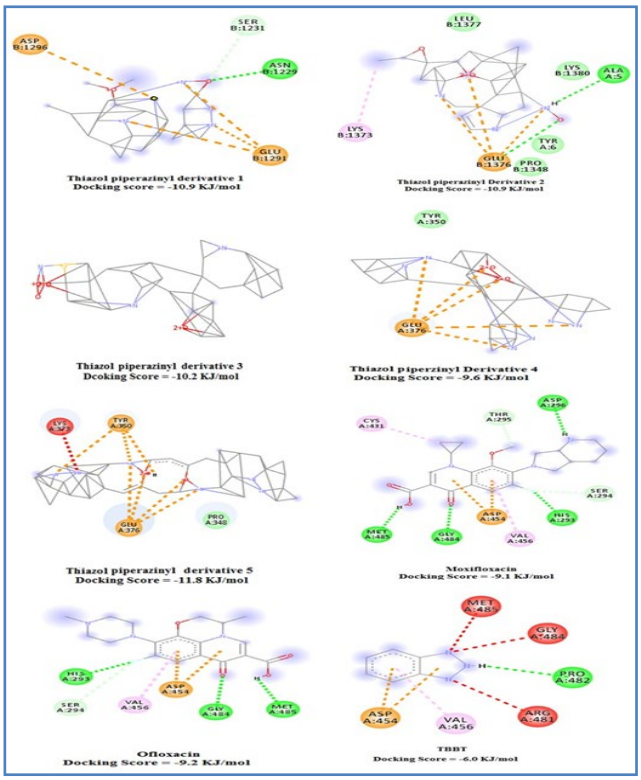

Figure 5: Docked conformation of different ligands in the NS3 cavity. The figure shows 2D representations of the predicted binding modes, type of bonds/interactions, and binding scores for each of the 20 drugs inside the active site of the HCV genotype 1b NS3-helicase (PDB ID:1CU1).

Results and Discussion:

The molecular docking analysis revealed that, in the case of NS3 helicase from genotype 1a, the fluoroquinolones; of loxacin, moxifloxacin, and sparfloxacin, as well as other NS3 inhibitors, formed interactions with the NS3 helicase in the catalytic groove (**Figure 3**). On the contrary, in the case of NS3 helicase from genotype 1b, the ligands formed interactions with NS3 helicase in three different regions, where sparfloxacin, thiazolpiperazinyl derivatives 3 and 5, and acridone derivative (**Figure 3**) bound in the catalytic domain of the helicase, while ofloxacin, TBBT, fatsol, and thiazolpiperazinyl derivatives 1, 2 and 4 (**Figure 3**) bound to the allosteric site of the helicase (**Figure 3**). Analysis of the docking energies revealed a strong binding affinity between NS3 helicase and the acridone derivatives (-11.4KJ/mol), compound 17 (-10.3KJ/mol), and thiazolpiperazinyl derivatives 1-5 (-9.7KJ/mol, -11.1KJ/mol, -10.6KJ/mol, -9.6KJ/mol, -9.8KJ/mol, respectively) (**Figure 3 and Table 1**). The fluoroquinolones namely, moxifloxacin (-9.1KJ/mol) and sparfloxacin (-9.2KJ/mol) also exhibited a high binding affinity with the NS3 helicase, comparable to thiazolpiperazinyl derivatives 1-5, and superior to several of the synthetic and natural derivatives (**Figure 4 and Table 1**). In the case of genotype 1b (1CU1), analysis of docking energies revealed a strong binding affinity between NS3 helicase and the acridone derivatives (-11.9KJ/mol), compound 17 (-10.8KJ/mol), and thiazolpiperazinyl derivatives 1-5 (-10.9KJ/mol, -10.9KJ/mol, -10.2KJ/mol, -9.6KJ/mol, -11.8KJ/mol respectively). Besides this, fluoroquinolones namely, ofloxacin (-9.2KJ/mol) and sparfloxacin (-9.2KJ/mol) also showed a higher affinity towards the catalytic region, which was superior to several of the synthetic and natural derivatives (**Figure 5 and Table 1**). Based on the binding energy values, the order of efficacy of interaction in between the fluoroquinolones and NS3 enzyme is in order, ofloxacin > moxifloxacin > sparfloxacin for genotype 1a.For genotype 1a, analysis of the drug-protein interactions revealed that amino acids, Glutamine-493, Proline-230, Aspartate-296, Threonine-295, Glutamate-460, and Glutamic acid-496 in the groove of the active site, were involved in the binding with all the three fluoroquinolones, whereas other synthetic and natural derivatives made strong hydrogen bonds with Valine-372, Lysine-373, and Tyrosine-350.These amino acids located within these catalytic regions are reported to be crucial for the enzyme activity and the HCV lifecycle, where Threonine-295 and Proline-230 mediate ATPase dependent binding to the active site, and aids in the maintenance of the electrostatic environment needed for ssDNA and RNA binding, thereby controlling the helicase activity **[29, 30]**. In the case of genotype 1b, fluoroquinolones sparfloxacin and ofloxacin showed higher affinity towards the enzyme. The amino acids most commonly targeted by these fluoroquinolones includedThreonine-295 and Proline-230 Glutamine-493 having proximity toTryptophan-501. Glutamine-493 and Tyrosine-501,along with Proline-230 and Threonine-295 have a role in the ATPase-dependent binding of ssDNA and RNA within the DNA binding cleft, essential forhelicase activity **[31]**. Targeting these amino acids by ofloxacin and sparfloxacin in the catalytic region may result in the prevention of DNA binding and thereby ceasing the enzyme activity. The results for molecular docking are in agreement with another study reporting the *in vitro* inhibition of NS3 helicase, where the IC_{50} of ofloxacin was reported to be 0.1 µM, while the IC_{50} of moxifloxacin and sparfloxacin lied within the range of 0.1-10 µM **[24]**, which was superior to some of the most potent NS3 helicase inhibitor tested, such as acridone derivatives, NS3 peptide (p14), QU663 and DRBT reportedly having IC_{50} values in the range of 10-20 µM **[10]**, making fluoroquinolones better *in-vitro* candidates for the inhibition of NS3 activity.

Conclusion:

Data shows fluoroquinolones that because of their established safety profile can be repurposed as a supplemental therapy against HCV, especially against the infections that arenon-responsive to DAAAs.

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Conflict of interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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