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Computational Analysis of *De Novo* Evolution of Hepatitis C Virus NS5B Polymerase Inhibitors

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Abstract. *HCV (Hepatitis C virus) that causes chronic liver disease. HCV NS5B RNA-dependent RNA polymerase (RbRp) and NS3 protease are able to affect virtual replication of genes. Computer-aided drug design (CADD) aims at designing new molecules with pharmacological activity. In this study, we used the Discovery Studio 2.0 program and the scoring function to estimate the Dock Score, piecewise linear potential 1 (PLP1), piecewise linear potential 2 (PLP2), and potential of mean force (PMF) score of novel compounds. In this way, novel compounds with "de novo evolution" can be found. Using the the pharmacophore features that are near the receptor pocket and the score functions to calculate scores for the ligand-receptor interaction, the new ligands were selected, developed and virtually placed in the binding site of the receptor. A new compound, EVO12, gave the best score, indicating that it may be an efficient polymerase inhibitor of HCV NS5B.*

Hepatitis C virus (HCV) infection constitutes a global problem and is the major cause of chronic liver disease (1-2). Previous studies reveal that over the course of 20-30 years, up to 20% of patients develop cirrhosis and hepatic carcinoma. HCV is a small, enveloped, and positive-strand RNA virus that belongs to the Flaviviridae family, which makes up the 9,600-nucleotide genome that encodes a single large polyprotein (3-5). This kind of polyprotein consists of four structures (core, E1, E2, and p7) and six nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The NS3 protease and NS5B RNA-dependent RNA polymerase (NS5B RdRp) are both essential enzymes for viral replication (6-8). Recent drug studies have focused on these enzymes for viral replication of NS3 protease and NS5B RNA-dependent RNA polymerase (NS5B RdRp).

In this study, we focus on NS5B RNA-dependent RNA polymerase inhibitors designed by computer (9-12). Many inhibitors for both NS3 protease and NS5B RNA-dependent RNA polymerase have been used in therapy in recent studies (13-16). For the HCV NS5B RNA-dependent RNA polymerase target, both active sites and allosteric inhibitors been developed in previous research. The NS5B RNA-dependent RNA polymerase inhibitors of 2-C-methyl-3-valine ester cytidine (NM-283) and HCV-796 are shown in Figure 1 (17-18). The nucleoside active site inhibitor 2-C-methyl-3-valine ester cytidine (NM-283) and the non-nucleoside active site inhibitor isothiazoles target the growing RNA chain and have been effective in inhibiting HCV replication.

NS5B is an enzyme (19-20) involved in the synthesis of the negative strand copy of the RNA genome first and subsequently of the positive strand RNA copy which is incorporated into the new progeny virus (21-22). In previous studies, HCV NS5B RNA-dependent polymerase initiated RNA synthesis either by primer-indicated initiation, using a single nucleotide complementary to the base at the 3' end of the viral genome that is also referred to as *de novo* initiation, or by primer-dependent initiation using either DNA or RNA as primers. In other members of the virus, *de novo* initiation of RNA synthesis is thought to be the mechanism used by the polymerase for initiation of viral RNA synthesis in HCV-infected cells.

In this study, we focused on the inhibitors and developed new inhibitors by computer-aided drug design (CADD). By

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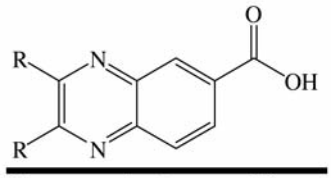
Key Words: HCV NS5B polymerase inhibitors, computational analysis, aromatic substituents.

Table I. *Initial studies on compound 2.*



Compound	R	IC_{50}
2	4-F-Ph-	5.5
3	2-Furyl	17
4	Ph-	79
5	4-Me-Ph	40
6	3-OMe-Ph	>100
7	2-Pyridyl	>100

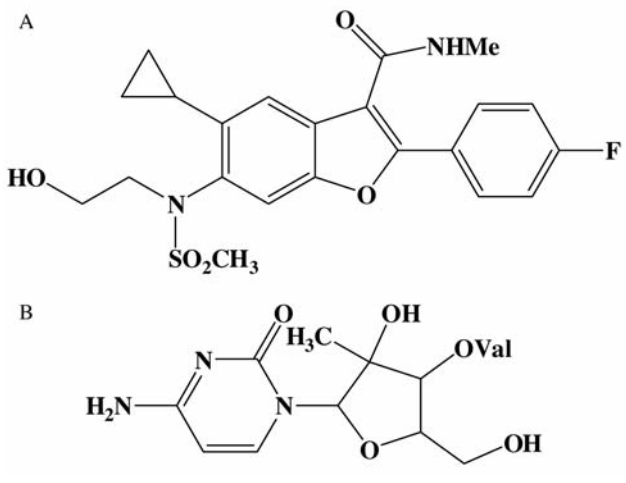


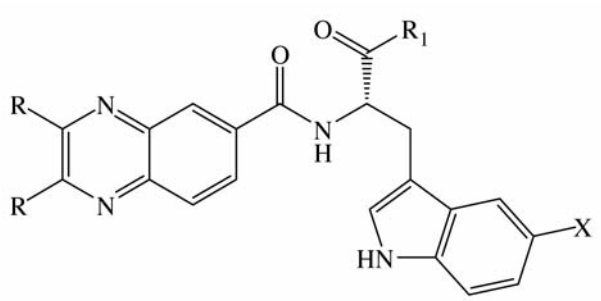
Figure 1. *Structures of the HCV NS5B polymerase inhibitors that have entered clinical trials: (A) HCV-796, and (B) NM-283.*

using the Discovery Studio Program to carry out virtual screening, using the pharmacophore features that are near the protein-binding site and the score functions to calculate the scores for the ligand-receptor interaction, the new ligands from the most potent compound were developed, and virtually placed in the binding site of the target receptor. If the score of the new ligand is higher than that of the potent compound, the new ligand could act as a novel HCV NS5B polymerase inhibitor.

Materials and Methods

Hepatitis C virus NS5B inhibitors. The series of HCV NS5B RNA-dependent polymerase inhibitors used for reference includes

Table II. *IC_{50} values of compounds 8 to 18.*

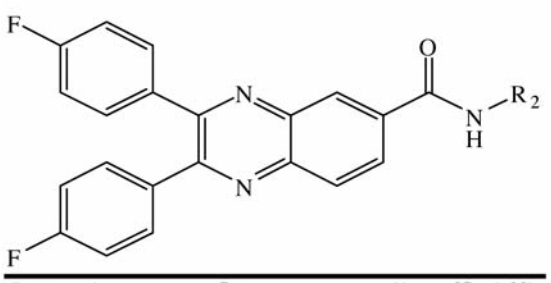


Compound	R	R1	X	IC_{50}
8	2-Furyl	OCH_3	H	>100
8a		OH	H	33
9	Ph-	OCH_3	H	>100
9a		OH	H	15
10	4-F-Ph-	OCH_3	H	>100
10a		OH	H	1.9
11	4_Me-Ph-	OCH_3	H	>100
11a		OH	ND	ND
12	3-OMe-Ph-	OCH_3	H	>100
12a		OH	H	>100
13	2-Pyridyl-	OCH_3	H	>100
13a		OH	H	>100
14	2-Furyl-	OCH_3	OH	26
14a		OH	OH	16
15	Ph-	OCH_3	OH	72
15a		OH	OH	11
16	4-F-Ph-	OCH_3	OH	5.5
16a		OH	OH	1.3
17	4-Me-Ph	OCH_3	OH	49
17a		OH	OH	6.1
18	3-OMe-Ph-	OCH_3	OH	>100
18a		OH	OH	15

aromatic substituents (Tables I-IV). Compound 26a showed the most potent biological activity, so that its IC_{50} of 0.6 μM made it the potent compound. The substituent and the biological activity of compound 11a are not given in the reference (12).

Docking study using Accelrys Software. The HCV NS5B RNA-dependent polymerase inhibitors were screened by being docked into the binding site of the HCV NS5B polymerase, using the program Dock Ligands (Ligandfit) in Discovery Studio 2.0 (Accelrys, San Diego, CA, USA). We took the structure of the virus (PDB ID: 2BRL) from the Protein DataBank (PDB) as the inhibitor receptor.

Table III. *IC_{50} values of compounds 10, 16, and 21 to 24.*



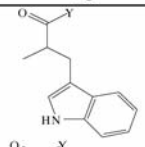
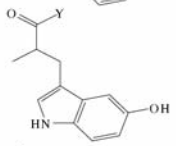
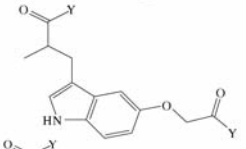
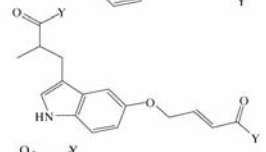
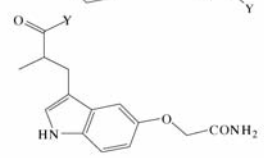
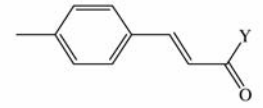
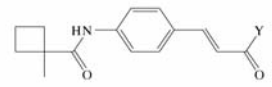
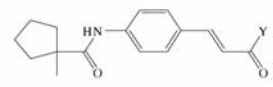
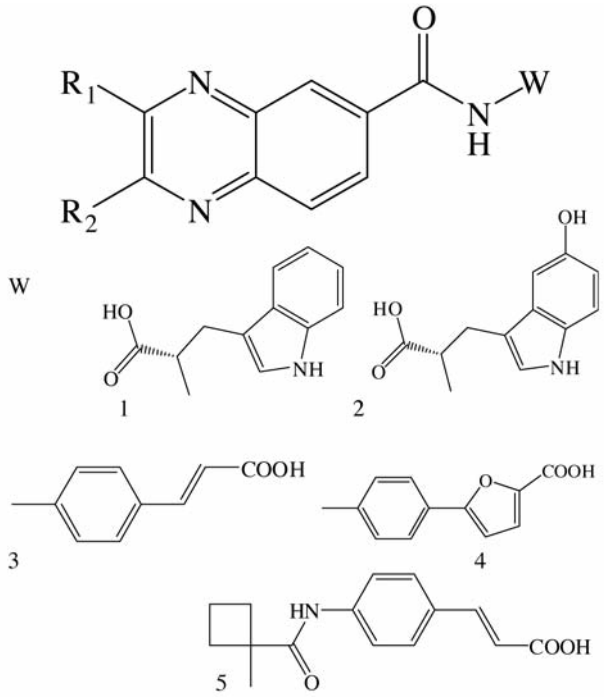
Compound	R_2	Y	IC_{50} (μM)
10		OMe	>100
10a		OH	1.9
16		OMe	5.5
16a		OH	1.3
19		OMe	100
19a		OH	1.7
20		OMe	53
20a		OH	2.2
21		OMe	8.6
21a		OH	3.2
22		OEt	>100
22a		OH	17
23		OEt	37
23a		OH	0.69
24		OEt	>100
24a		OH	1.2

Table IV. *IC_{50} values of compounds 25 to 32.*



Compound	R_1	R_2	W	IC_{50} (μM)
25	Ph-	Cyclohexyl-	1	3.7
25a	Cyclohexyl-	Ph-		1.8
26	Ph-	Cyclohexyl-	2	5
26a	Cyclohexyl-	Ph-		0.6
27	4-F-Ph-	Cyclohexyl-	1	3.4
27a	Cyclohexyl-	4-F-Ph-		1.6
28	4-F-Ph-	Cyclohexyl-	2	4.1
28a	Cyclohexyl-	4-F-Ph-		4.6
29	Ph-	Cyclohexyl-	3	1.3
30	4-F-Ph-	Cyclohexyl-	3	4.1
30a	Cyclohexyl-	4-F-Ph-		4.6
31	Ph-	Cyclohexyl-	4	1.8
31a	Cyclohexyl-	Ph-		1.6
32	4-F-Ph-	Cyclohexyl-	5	5.1
32a	Cyclohexyl-	4-F-Ph-		1.3

The score functions used in the Discovery Studio 2.0 were Dock Score, piecewise linear potential 1 (PLP1), piecewise linear potential 2 (PLP2), and potential of mean force (PMF). In the PLP1 score function, there are four atom types, including hydrogen bond donor only, hydrogen bond acceptor only, both hydrogen bond donor and acceptor, and non-polar (23). When PLP1 is the docking score function, the internal energy is calculated for each ligand conformation in which the ligand is in the binding site. In the PLP2 score function, the atom type remains the same as in the PLP1 score function. In addition, an atomic radius is assigned to each atom except for hydrogen (24).

The PMF score function was developed based on statistical analysis of the 3D structures of the protein ligand complex (25). They were found to be fitted well with protein ligand-binding free energy while also being fast and simple to calculate. The scores were calculated by summing pairwise interaction terms over all

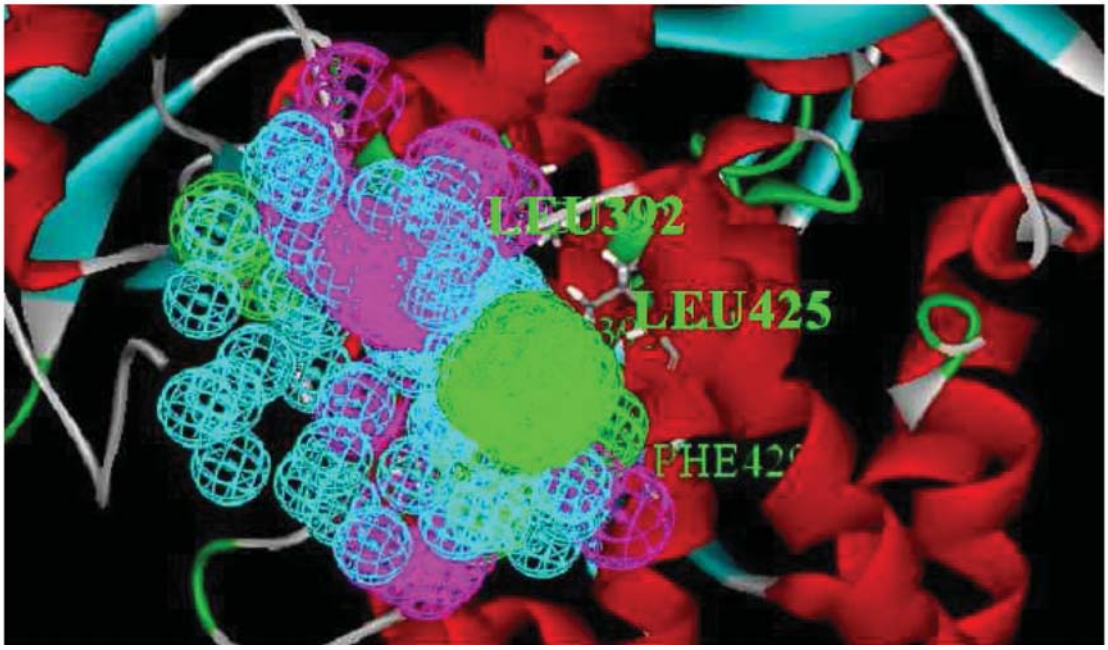


Figure 2. *The pharmacophore features that are near the binding site pocket.*

interatomic pairs of the receptor ligand complex. The score function of Dock Score is the default function in Discovery Studio 2.0.

De novo evolution using Accelrys software. To design new compounds from the potent drug, we used *de novo* evolution in Discovery Studio. The new compounds from the *de novo* evolution were virtually placed into the HCV NS5B polymerase binding site and the scores determined by using Discovery Studio 2.0.

Results and Discussion

The pharmacophore features of the protein structure are shown as follows: the green pharmacophore represents the hydrogen bond acceptor, the purple the hydrogen bond donor, and the blue the hydrophobic feature. There were many blue features near the binding pocket, indicating that the ligand-protein interaction near the binding pocket exhibited many hydrophobic interactions. In Figure 2, many hydrophobic features can be seen near the side chains of Leu 392, Ala 395, Ala 396, Thr 399, Leu 425, and Phe 429.

The score values of the HCV NS5B polymerase inhibitors are shown in Table V. Compound 26a exhibits hydrogen bonds between the compound and the side chains of Val 37, Arg 490, Lys 491, and Gly 493 (Figure 3A). Compound 32 has a Dock Score of 64.769, as shown in Table V, and exhibits hydrogen bond interactions between the ligand and the side chains of Ala 393, Ala 395, Ala 400, and Arg 503. Compound 26a would appear to have the best biological activity (IC_{50}) (Figure 3B). Compound 10 has the highest value of the PLP1 score function, and evokes hydrogen bond interactions with the key residues of Val 37, Ala 396, His 428, Phe 429, Arg 490, Lys 491, and Arg 503. Moreover, compound 10 also has the highest PLP2 score of 101.76 in the series (Figure 3C). In the PMF score function, compound 11 showed the highest value of 128.97.

Compound 32 gave the best Dock Score, since the hydrogen bond interactions are the most important. The side chain of the hydrogen bond interaction to Arg 503 may act as the key residue. To see compound 10 and compound 32, with one or more hydrogen bonds to the side chain of Arg 503, have a higher docking score than the potent compound 26a. As for the pharmacophore features, there were many green features of the hydrogen bond acceptor near the side chain of Arg 503 (Figure 4). Therefore, Arg 503 may be important to the HCV NS5B polymerase inhibitors that are docked into the target receptors.

The twenty new compounds were placed in the design module of the Discovery Studio 2.0, and we took the new compounds that needed to be docked into the receptor binding pocket of the HCV NS5B polymerase (Figure 5). The score values are shown in Table VI. The new compound Evo12 has a Dock Score value that is higher than that of potent compound 26a and that of the best compound, 32. As for the PLP1 and PLP2 score functions, the new compound Evo12 gave better values for both score functions. In the virtual screening, Evo12 has hydrogen bond interactions with the side chains of Ala 395, Ala 400, Arg 503, Ala 393, and Gly 493 (Figure 6A).

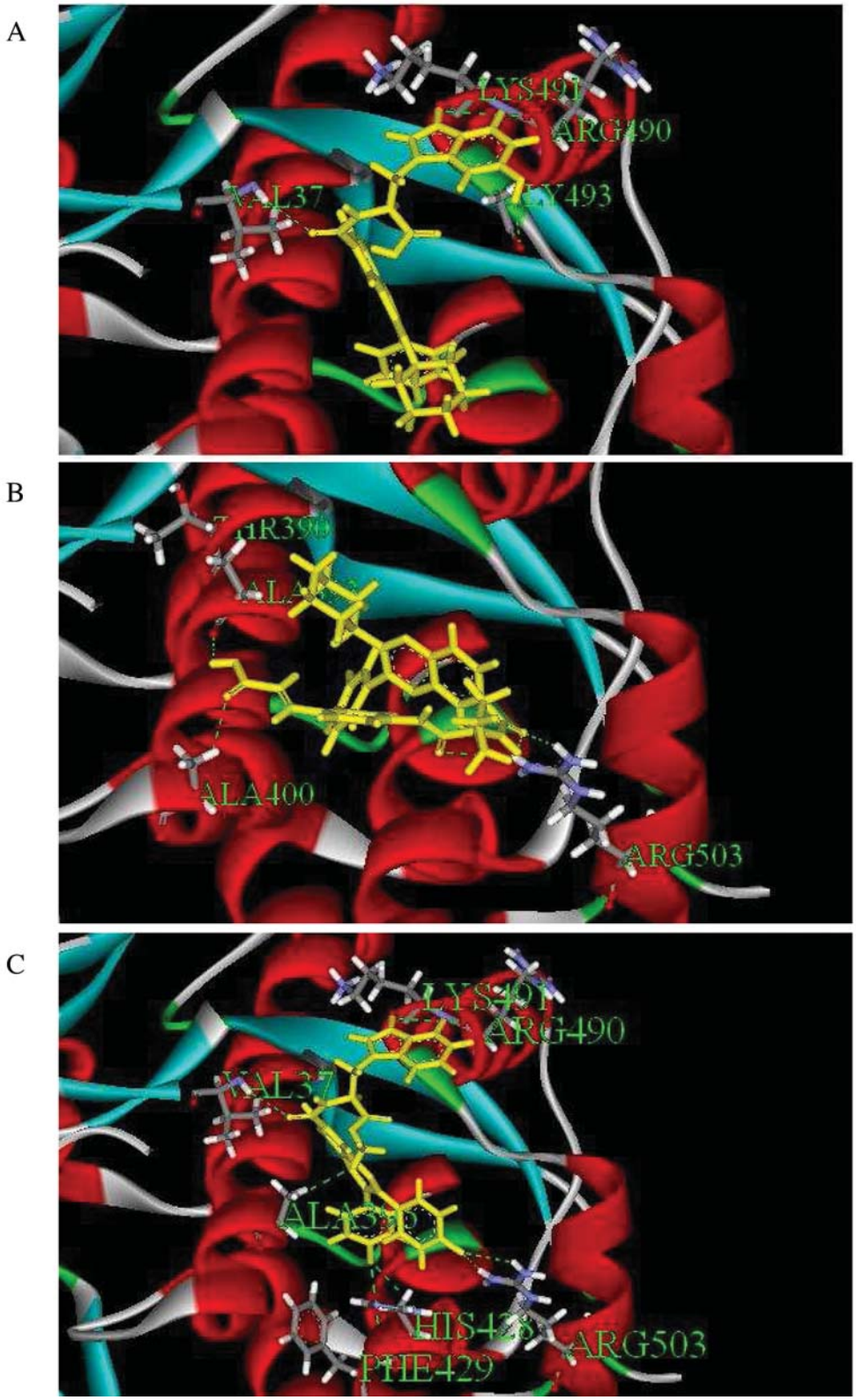


Figure 3. *The conformation of the HCV NS5B polymerase inhibitors in the binding site, and the hydrogen bond interactions that are between the compound and the side chains. A: Compound 26a that has the best biological activity; B: compound 32 that shows the highest Dock Score; and C: compound 10 that has the highest score values in both the PLP1 and PLP2.*

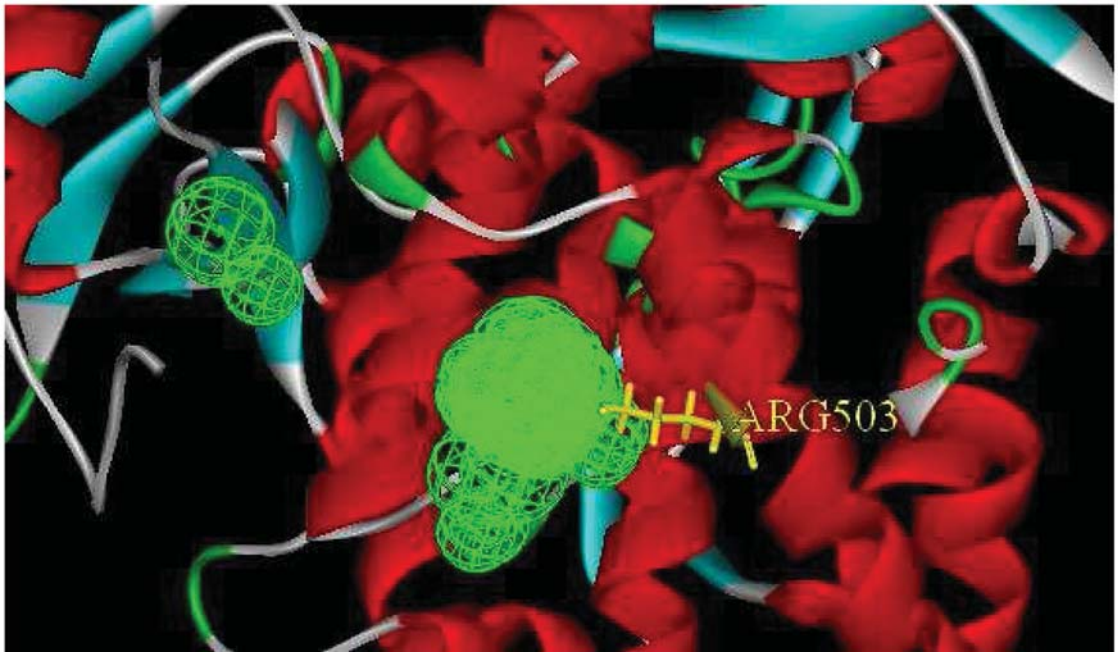


Figure 4. *The key Arg503 residue which shows many hydrogen bond acceptor features near the key residues.*

Table V. *The score values of the HCV NS5B polymerase inhibitors.*

Compound	PLP1	PLP2	PMF	Dock Score	Compound	PLP1	PLP2	PMF	Dock Score
1	80.09	76.48	96.73	50.39	19	82.99	77.90	66.82	53.79
2	83.02	81.09	74.35	51.68	19a	67.76	62.25	69.58	54.54
3	74.66	70.62	75.05	49.59	20	70.69	64.63	78.24	52.97
4	80.45	77.50	81.05	50.51	20a	57.19	57.77	70.06	55.40
5	81.46	77.20	77.52	49.43	21	96.12	88.28	105.85	52.55
6	78.76	72.48	73.63	52.78	21a	89.26	81.68	92.19	49.65
7	80.46	75.74	84.50	51.36	22a	83.96	78.83	67.90	52.10
8	80.04	72.97	100.51	47.49	23	98.40	88.23	75.50	62.55
8a	87.74	81.89	105.55	50.93	23a	91.83	89.27	96.50	62.71
9	82.85	77.58	94.54	49.55	24	64.07	59.88	85.44	64.55
9a	86.74	82.18	107.94	47.02	24a	64.07	59.88	85.44	64.55
10	110.06	101.76	84.31	50.70	25	76.60	75.23	73.43	52.98
10a	82.85	77.51	57.21	49.11	25a	74.92	72.74	57.33	50.56
11	66.32	59.81	128.97	49.01	26	87.11	82.51	67.13	49.32
11a	76.80	72.11	54.83	52.75	26a	97.17	91.26	72.22	50.23
12	64.65	63.28	74.43	50.32	27	75.24	75.05	71.08	54.46
12a	84.10	75.67	43.54	49.35	27a	92.94	87.45	111.14	49.61
13	84.96	77.19	102.50	49.91	28	97.18	92.25	79.54	54.04
13a	88.78	80.71	100.64	52.05	28a	102.44	95.56	72.34	51.69
14	71.56	62.29	57.76	52.91	29	82.36	72.22	95.78	52.14
14a	88.45	83.23	102.81	49.32	30	83.46	76.84	96.53	52.20
15	87.97	82.57	107.83	46.59	30a	89.00	84.44	76.35	52.86
15a	94.76	87.66	83.21	49.00	31	79.54	75.39	64.49	62.15
16	97.68	93.82	115.96	50.35	31a	67.20	65.09	75.88	62.04
16a	92.47	82.88	71.08	58.29	32	84.29	79.22	76.63	64.77
17	84.20	81.49	99.84	49.03	32a	85.99	82.30	92.48	60.86
17a	84.90	82.07	99.09	47.95					
18	82.79	76.54	44.56	52.36					
18a	49.02	47.56	9.35	52.69					

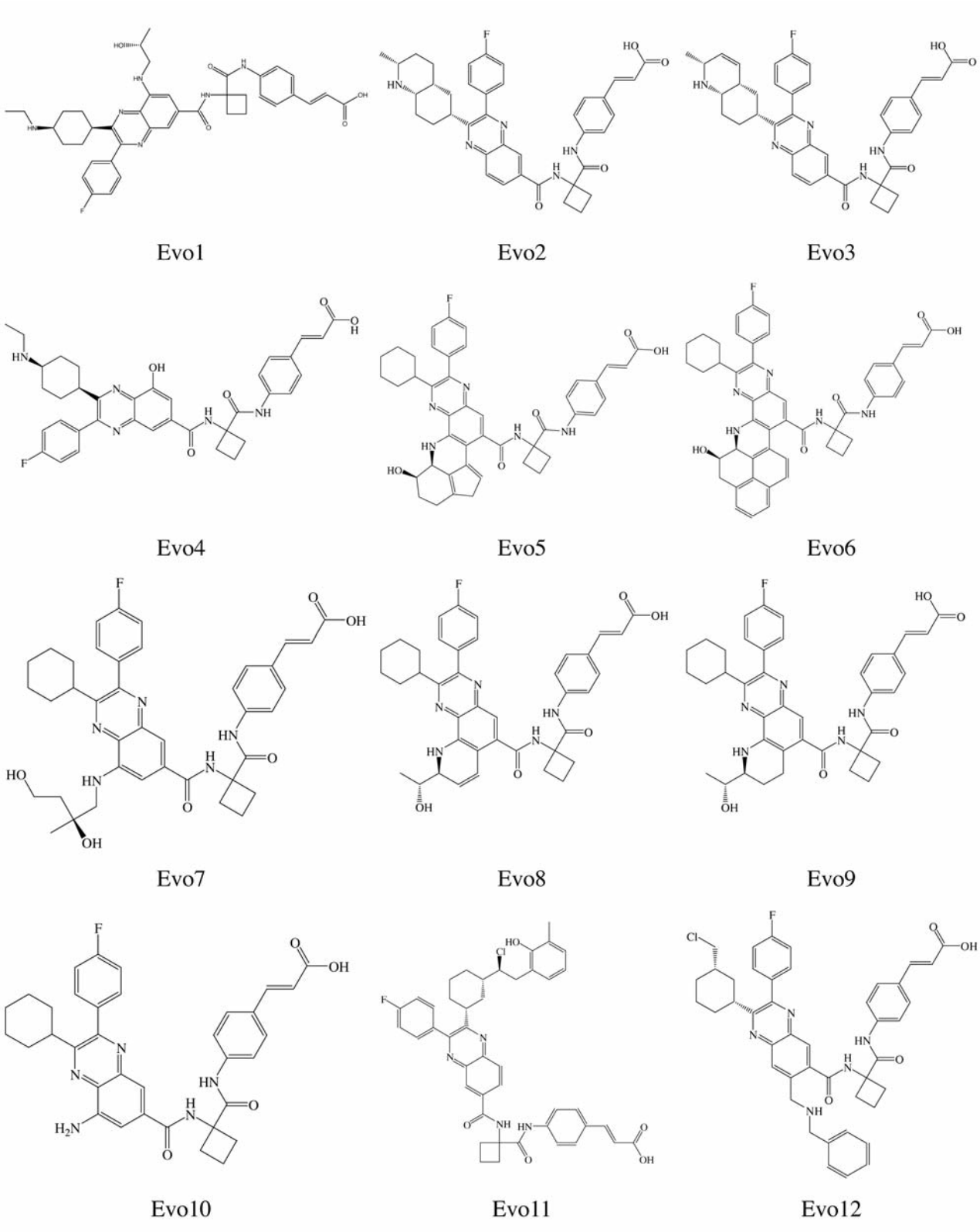


Figure 5 Continued

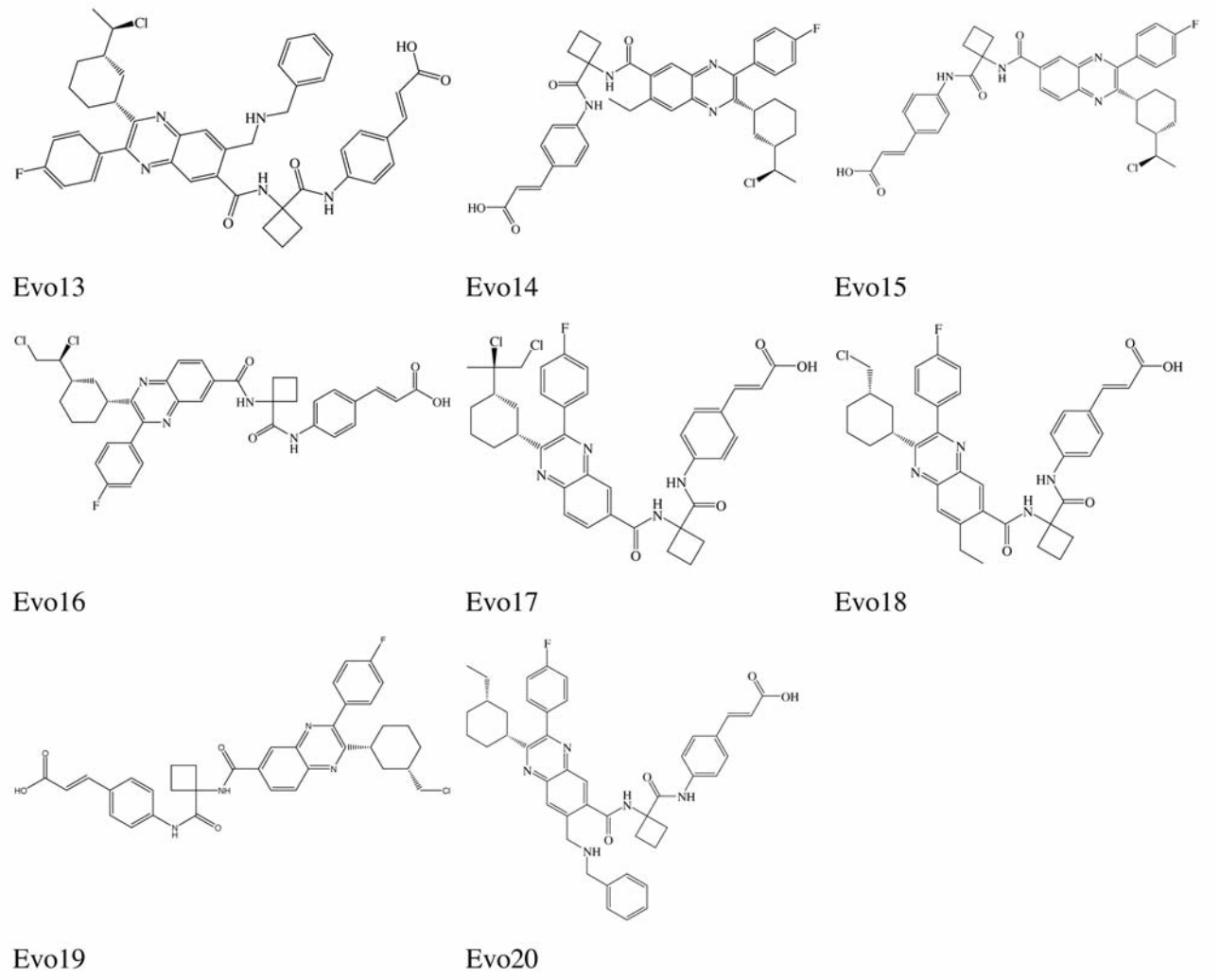


Figure 5. *Structure of the new HCV NS5B polymerase inhibitors.*

As for the pharmacophore, Evo12 has more backbone in the hydrophobic features (Figure 6B). The new compounds have better score values than others. The hydrogen bond interactions in the binding pocket are important features. Many of the new inhibitors in this study had greater ranges of hydrophobic pharmacophore features with good values for the score functions. Therefore, hydrophobic interaction is another important element that increased the score values of the compound.

In summary, we identified the HCV NS5B polymerase interaction and the target protein that has many ligand receptor interactions. For hydrogen bond interaction, the key residue is important. In this study, we attempted to design new inhibitors that have suitable pharmacophore features. The pharmacophore features help us to understand ligand-receptor interactions, and the score value is the index that show how the ligand should be placed in the binding pocket. However, the biological activity of the new inhibitors remains to be determined.

Acknowledgements

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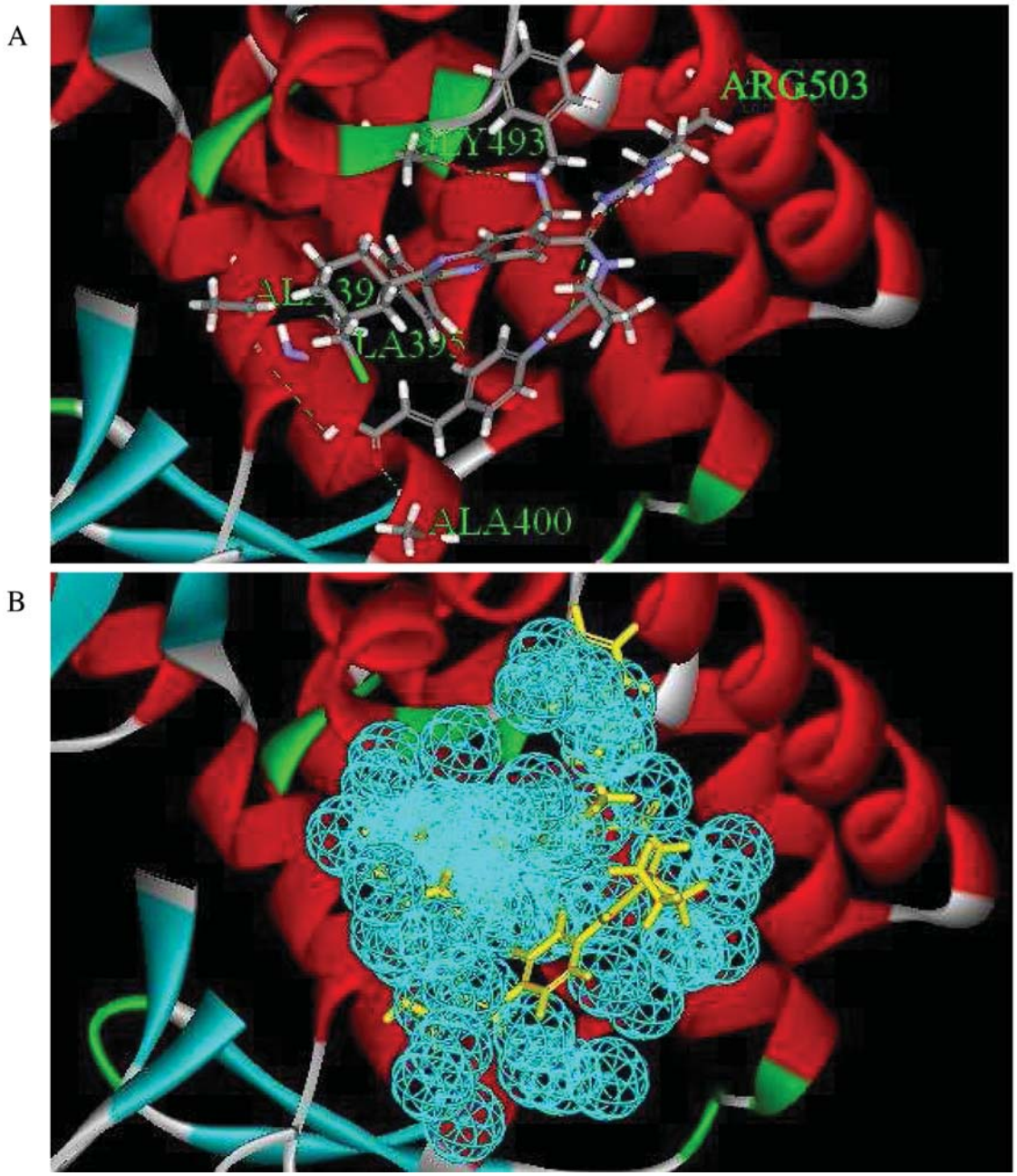

Figure 6. *A: The new HCV NS5B polymerase compound Evo12, showing conformation of the compound in the target protein, and hydrogen bonds with the side chains. B: Compound Evo12, showing pharmacophore hydrophobic features.*

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Table VI. *The score values of the new HCV NS5B polymerase inhibitors.*

Compound	PLP1	PLP2	PMF	Dock Score
Evo1	45.83	41.41	78.84	59.47
Evo2	86.47	84.60	75.47	66.99
Evo3	85.11	83.38	79.06	66.51
Evo4	80.10	76.48	88.91	70.08
Evo5	105.79	103.35	105.91	66.22
Evo6	82.56	79.37	86.02	68.05
Evo7	95.07	95.89	82.94	80.23
Evo8	93.80	91.71	76.15	71.41
Evo9	74.54	72.31	86.72	60.98
Evo10	87.27	83.91	80.21	74.99
Evo11	80.92	80.03	11.46	50.40
Evo12	115.12	112.88	74.80	81.21
Evo13	70.30	64.71	61.56	52.90
Evo14	45.96	51.38	47.66	50.66
Evo15	46.15	41.35	71.30	58.58
Evo16	66.37	64.43	38.24	54.19
Evo17	78.69	73.72	95.13	46.75
Evo18	91.70	87.86	80.54	65.50
Evo19	81.02	74.56	74.18	65.91
Evo20	82.20	80.86	70.79	57.45

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