IN SILICO STUDY REVEALS THAT CASOPITANT, GRAZOPREVIR, REMDESIVIR AND RIMANTADINE ANTIVIRALS SUPPRESS PRE- AND POST-FUSION STAGES OF SARS-COV-2 VIRAL CYCLE

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ABSTRACT: This paper presents the results of an in silico analyze of the action of Casopitant, Stavudine, Grazoprevir, Lamivudine, Remdesivir, Rimantadine and Tenofovir antivirals against the Spike protein (on two conformations), ACE-2 (Angiotensin-converting enzyme 2), 3CLpro protease (3-chymotrypsin like protease) and the RNA-dependent RNA polymerase, that works at pre- and post-fusion stages of viral cycle of the SARS-CoV-2. All the antivirals tested have efficacy and safety certified for other pathologies, mainly against the viruses of Hepatitis B and C, HIV-1 and influenza A. The molecular docking calculation were made using the GOLD program.

Keywords: COVID-19; Viral cycle inhibition; Molecular docking study.

INTRODUCTION

COVID-19 is a pandemic disease of variable consequences that may cause death. It's caused by a coronavirus called SARS-CoV-2 [1]. The particles of this new coronavirus are spherical in shape and have attached to its surface the Spike protein, which is the main responsible for the infection [1,2]. After attaching to host cells, the Spike protein pass by a lot of conformational changes resulting in the fusion of the particle membrane with the cell membrane [2]. Thus, the RNA of the virus is introduced into the cells, where replicates its genome [3]. Initially, Spike protein binds to receptor proteins ACE-2, known as angiotensin-converting enzymes 2, identified as the cause of the outbreak of this respiratory disease, at Wuhan, at the end of 2019 [1]. SARS-CoV-2 is a beta coronavirus that shares about 79% of the genome sequencing of SARS-CoV and 50% of MERS-CoV, having six structures of open reading frames (ORFs), identified as: Replicase (ORF1a/ORF1b), Spike protein (S), Envelope (E), Membrane (M), Nucleocapsid (N) [2,3] However, Spike protein is a protein that controls all biological process of SARS-CoV-2 and is the main responsible for fixing the viral particles, membrane fusing and for the entrance of the virus into the cells. That's why this protein is considered the main therapeutic target of new drugs against COVID-19 [4].

On the other hand, the proteases are extremely important proteins and very studied against coronavirus [5]. They are fundamental on the process of polyproteins that are translated by viral RNA [6]. Thus, the inhibition of these proteases results in the block of action and replication of the virus. Moreover, RNA-dependent RNA polymerase (RdRp) is an enzyme essential for replicate the virus and performs an important roll when processing the post-translation of viral proteins, what makes it another important target to develop therapeutic agents against COVID-19 [7].

Many researchers already have undertaken efforts to combine inhibitors of SARS-CoV-2 proteins, inclusive during the experimental acquisition of the 3-chymotrypsin like protease (3CLpro) that has an es-

sential role during the process of translated proteins because is a potent inhibitor of the virus circle of life [8,9]. And, although there are a lot of research about SARS-CoV-2, the outbreak still producing global effects. The development of therapeutic agents sensitive and effective against the new coronavirus still being a necessity.

A lot of studies are being conducted to track if drugs already known and are used in the treatment of other pathologies are effective against SARS-CoV-2. It was already discovered that a lot of these drugs have potent antiviral activities against some proteins that this pandemic agent uses during its circle of life. Among them: Stavudine (DB00649), Casopitant (DB06634), Grazoprevir (DB11575), Lamivudine (DB00709), Remdesivir (DB14761), Rimantadine (DB00478) and Tenofovir (DB14126) [10-15]. Although there are effects very known in different proteins of SARS-CoV-2, its application against COVID-19 isn't totally clear. The main objective of this paper is to verify, on molecular docking, the bioactivity of these antivirals against different proteins of SARS-CoV-2. For this, the binding affinity were evaluated in 35 models of complex receptor-ligand. The trials of molecular testing were performed against two systems. The first on the Spike Protein on the locked conformation (PDB ID:6VXX), and on ACE-2 (PDB ID:1R42), representative of pre-fusion stage of the virus on the cell. The second on the Spike protein in the opened conformation (PDB ID: 6VYB), about the 3-chymotrypsin like protease, 3CLpro (PDB ID: 6LU7) and on RNA--dependent RNA polymerase (PDB ID:6M71), representative of the post-fusion stage of the viral cycle.

COMPUTATIONAL METHODOLOGY

PREPARING THE LIGANDS AND TARGET-PROTEINS

The tridimensional structures (3D) of the ligands were obtained from the server PubChem [16,17]. On all of them it was made an addition of hydrogen, application of MMFF94 force field to balance the

electrostatic charges, besides the geometry optimization by molecular mechanics MMFF94 using the program Avogadro [17,18]. After this, the most solid-state conformations were selected from a systematic search with MMFF94 Molecular Mechanics [19,20], and this stage was performed by the program Avogadro [17,18].

Different proteins were also obtained from the Protein Data Bank (PDB) [21], including the enzyme ACE-2, which is the receptor of the virus SARS-CoV-2 (PDB ID: 1R42) [3,22], Spike protein on open and closed conformational states (PDB ID: 6VXX and 6VYB) [10,23], 3-chymotrypsin like protease, 3CLpro (PDB ID: 6LU7) [24] and the RNA-dependent RNA polymerase, RdRp (PDB ID: 6M71) [9,25]. The 3D structures of the proteins were verified about its bindings, chain anomalies and absence of hydrogen suing the program PyMOL [26]. Moreover, all the water molecules and other not important cofactors were removed from the experimental structures using the program BIOVIA Discovery Studio 2020 [27]. The optimized structures of proteins and ligands were used in the molecular docking, using the program GOLD [28,29], as it is described on the following section.

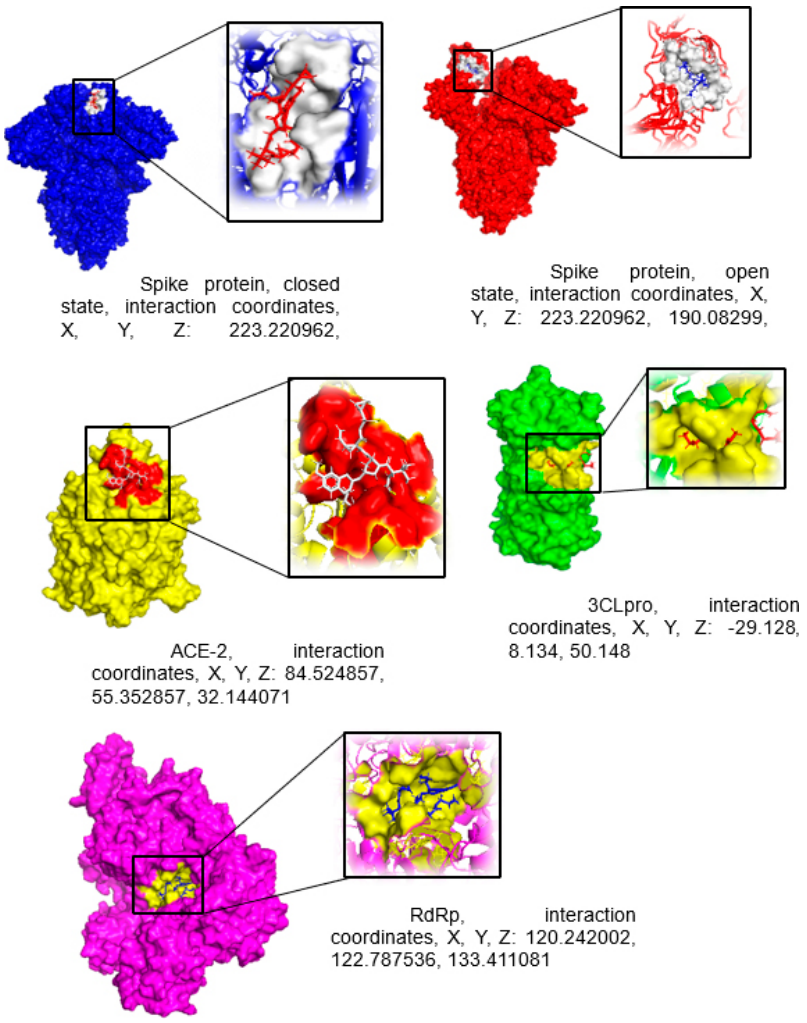
PREDICTION OF THE ACTIVE SITE AND MOLECULAR DOCKING

The active sites of the studied proteins were determined using Computed Atlas of Surface Topography of proteins (CASTp) [30,31] and the program BIOVIA Discovery Studio 2010 [27], as per the procedure described below.

One after another, the proteins were uploaded to the server CASTp 3.0 (http://sts.bioe.uic.edu/castp/index.html?2pk9). After this, a probe radius of 10Å was defined. Finally, after the calculations, the surface pockets, internal cavities, and the resulting crossed channels, after the binding pockets had been chosen, confirmed in the program BIOVIA Discovery Studio 2020 [27], represented at Fig.1. Furthermore, for Spike protein, not only on the closed conformational state (PDB ID: 6 VXX), but also on the open (PDB ID: 6VYB), the active sites were chosen based on the receptor binding domain (RBD) that interacts with ACE-2 [32].

In the case of 3CLpro protein, the binding site selected was the ligand co-crystallized N3 [24].

Figure 1. Tridimensional structures of Spike proteins in the closed conformation (PDB ID: 6VXX) and open (PDB ID: 6VYB), ACE-2 (PDB ID: 1R42), 3CLpro (PDB ID: 6LU7) and RdRp (PDB ID: 6M71). It's represented the binding pocket (amplified) founded after the calculus with the CASTp 3.0 and the interaction coordinates of the program BIOVIA Discovery Studio 2020 [27], [used] in the molecular docking.



The outputs achieved after the molecular docking consisted in ten poses and the ones with higher score were selected. In all cases, an empirical function Chemscore, not only for the fitting, but also for the score, adopting an interaction radius of 10Å [33,34]. The interaction protein-ligand was visualized in 3D at PyMOL [26] and in 2D at BIOIA Discovery Studio 2020 [27]. The visualization in 3D allowed the exact placement of the binding pocket of the target proteins, while the visualization in 2D allowed to identify the different interactions shaped by the ligands with residues of amino acids of the active site [23,24,35].

RESULTS AND DISCUSSIONS

The Table 1 shows the results achieved for the relationship of the antiviral's binding on each complex, not only for the pre-fusion stage but also for the post-fusion stage of the virus in cells. The initial stage of SARS-CoV-2 infection, identified as the pre-fusion stage, involving the Spike protein binding to the receptors of cell's entrance, including the angiotensin-converter enzyme 2 and other proteases [36[. However, after fusion to SARS-CoV-2 cell, express and replicates its RNA to produce new copies, which ones are integrated to the new produced viral particles, involving translation processes, production of polyproteins and production the of viral genome [37]. It's possible to note that for the pre-fusion stage, involving the Spike protein in the closed conformation (PDB ID: 6VXX) and the Angiotensin-converting enzyme 2 (PDB ID: 1R42), the best values to inhibit the action of the virus were for Casopitant, Grazoprevir and Rimantadine. On the other hand, Remdesivir and Lamivudine had results slightly lower. The same happened in the post-fusion stage, involving the Spike protein the open conformation (PDB ID: 6VYB), 3-chymotrypsin like protease (PDB ID: 6LU7) and the RNA-dependent RNA polymerase (PDB ID: 6M71), where this pattern of behavior was also reproduced. The antiviral Tenofovir was not efficient on any stage and, except for relating to RNA-dependent RNA polymerase (6M71), it was inefficient to inhibit the other viral proteins.

Table 1. Binding energies (kcal/mol) of the interactions among the antivirals Casopitant, Stavudine, Grazoprevir, Lamivudine, Remdesivir, Rimantadine and Tenofovir with SARS-CoV-2: S Protein [PDB ID: 6VXX (closed), 6VYB (open)], 3-chymotrypsin like protease (3CLpro) (PDB ID: 6LU7), ACE-2 (PDB ID: 1R42) and the RNA-dependent RNA polymerase (RdRp) (PDB ID: 6M71).

Ligand	Prefusion		Post-fusion		
	6VXX	1R42	6VYB	6LU7	6M71
Casopitant	-23.73	-17.19	-28.86	-48.55	-17.21
Stavudine	-17.48	-18.20	-11.97	-21.69	-18.05
Grazoprevir	-24.03	-20.86	-31.08	-48.96	-27.09
Lamivudine	-20.03	-15.13	-12.94	-17.83	-18.45
Remdesivir	-23.20	-17.13	-16.25	-32.07	-21.72
Rimantadine	-28.72	-19.70	-24.16	-32.52	-21.13
Tenofovir	-10.77	-6.92	-3.69	-3.79	-17.46

As a try to visualize the relation between the two stages of the mentioned infection, it was done a hierarchical cluster analysis (HCA). Using this method, a data set is split into groups organized according to its degrees of similarity [38]. In this case, it was used a metric of complete linkage as a measure of distance among the groups to linkage of Pearson correlation [39]. The Figures 2 and 2 shows the results about the proteins and about the studied antiviral, respectively.

Figure 2. Dendrogram obtained by application the HCA method on the studied proteins. The percentage of similarity were achieved using the metric of complete linkage and the Pearson correlation coefficient.

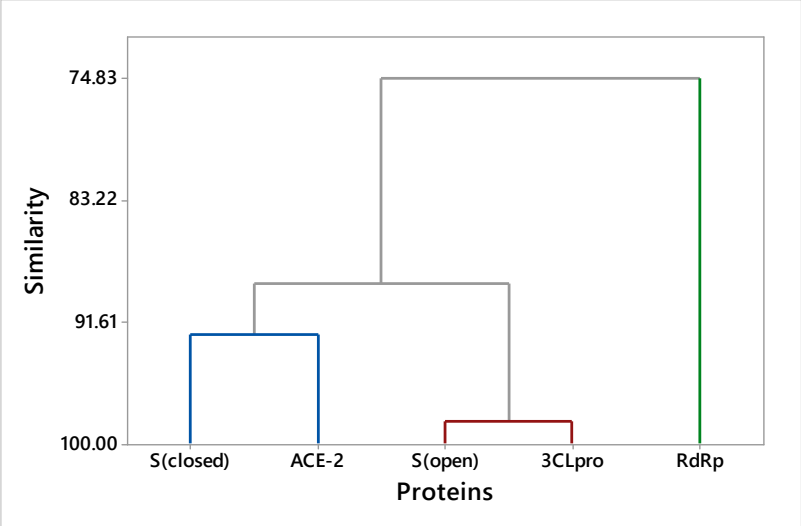
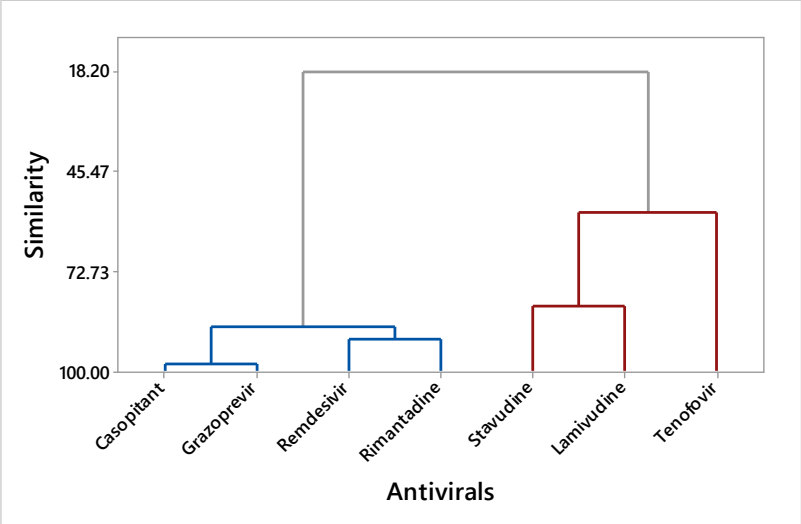


Figure 3. Dendrogram obtained by application the HCA method on the studied antivirals. The percentage of similarity were achieved using the metric of complete linkage and the Pearson correlation coefficient.



The HCA on Fig 2 shows that the viral proteins are allocated on three groups: ACE-2 (1R42) and Spike Protein in the closed conformation (6VXX), grouped with 92.46% of similarity and represents the pre-fusion stage. The 3-chymotrypsin like protease, 3CLpro (6LU7) and the Spike Protein in the open conformation (6VYB), grouped with 98.43% of similarity and represents the post-fusion stage. At last, the RNA-dependent RNA polymerase (6M71), creating an individual group very distinct from the others and related to the post-fusion stage. On the other hand, on Fig 3 it's possible to note the presence of two groups: the group Casopitant, Grazoprevir, Remdesivir e Rimantadine (with 87.92% of similarity), and the group Stavudine, Lamivudine and tenofovir (with 56.59% of similarity).

Thus, the HCA method was able to capture the degree of similarity among the viral proteins that participates of both stages of infection of the new coronavirus through the values of the affinities of calculated binding (Tab.1). Consequently, the treatment strategies against the action of the new coronavirus includes not only the inhibition of the RNA viral synthesis, but also to inhibit its replication, blocking the binding to the cell receptors and inhibit the virus process of self-assembly [40]. Among the non-structural proteins codified by the genome of this pathogen, the main protease (3CLpro) is considered as essential for the proteolytic process of replicase polyproteins, whereas RdRp is a fundamental enzyme for the RNA synthesis on all positive--strand RNA virus [7,40].

With this is mind, HCA provided an analyze more punctual of the interaction's receptor-ligand achieved in the molecular docking, as we will do during the discussions further on.

By the data at Tab.1, in general, the group of the antivirals Stavudine, Lamivudine and Tenofovir presented smaller binding affinities than the antivirals of the group Remdesivir, Grazoprevir, Rimantadine and Casopitant. This may justify the split among them according to its effects on the target proteins. However, it's also possible to note that the binding affinities of Stavudine and Lamivudine are smaller on pre-fusion stage, when compared to post-fusion stage, targeting a higher potential of inhibition during this last stage of the SARS-CoV-2 circle of life, the

reason why we will focus on the four most promising antivirals: Casopitant, Grazoprevir, Remdesivir, Rimantadine.

BINDING PATTERN OF ANTIVIRALS CASOPITANT, GRAZOPREVIR, REMDESIVIR AND RIMANTADINE ON THE PRE-FUSION STAGE OF THE VIRUS, INVOLVING THE SPIKE PROTEIN IN THE CLOSED CONFORMATION (PDB ID: 6VXX) AND ACE-2 (PDB ID: 1R42)

The antivirals Casopitant, Grazoprevir, Remdesivir and Rimantadine create a lot of hydrophobic and hydrophilic bindings with amino acids residues in the active site, not only relating to Spike protein in the closed conformation (6VXX), but also relating to the enzyme ACE-2 (1R42). Moreover, as we can note in the Fig. 4 and 5 (supplementary material) and in the Tab.2 and 3, there is a high number of hydrogen bindings and Van der Waals interactions that contribute for a higher stability of the created sites, making the receptor-ligand interaction even more effective.

The mentioned tables and figures show that the GlyB339 and AsnB343 amino acids from the active site of Spike protein were conserved on all interactions of the studied antivirals could be observed for the SARS-CoV-2 pre-fusion stage. And, excepting for Rimantadine, the SerB371 amino acid was conservated in relation to the complex of the other antivirals. And for the ACE-2 enzyme, the residues that were conservated on all complexes were HisA195 and TyrA196. And, excepting for Rimantadine, the residue AsnA103 was conservated for the other antivirals. The same happens to GlnA101, that only was not conservated for Grazoprevir.

Table 2. Classification and charge of the residues of Spike protein in the closed conformation.

Spike-6VXX	Hydrogen bond					VDW
Ligand	L	P		T	E	
Casopitant	6-ring	C	AlaB372	π-H	1,8	GlyB339, PheB338, SerB371
	O	N	AsnB343	H-acceptor	-3,5	
	F	O	AspB364	Ionic	-3,5	
	6-ring	C	ValB367	π-H	4,2	
	N	O	CysB336	Ionic	2,5	
Grazoprevir	O	N	AsnB343	H-acceptor	-3,5	ArgB509, AsnB437, SerB371, GlyB339
	6-ring	O	SerB373	π-H	-0,8	
	6-ring	O	TrpB436	π-H	-0,9	
Remdesivir	6-ring	C	ValB367	π-H	4,2	AsnB437, SerB438, ArgB509, GlyB339, PheB338, SerB466
	6-ring	C	AsnB370	π-H	-3,5	
	6-ring	C	SerB371	π-H	-0,8	
	N	O	AsnB343	H-donor	-3,5	
	C	5-ring	TrpB436	π-H	-0,9	
	C	C	LeuB441	H-donor	3,8	
	6-ring	C	LeuB368	π-H	3,8	
Rimantadine	C	5-ring	TrpB436	π-H	-0,9	GlyB339, Phe338
	C	6-ring	PheB342	π-H	2,8	
	N	O	AsnB343	H-donor	-3,5	
	C	C	LeuB368	H-donor	3,8	

L: Ligand; **P:** Protein, **T:** Types; **E:** Energy (kcal/mol); **VDW:** Van der Waals interactions

Table 3. Classification and charge of the residues ACE-2 enzymes.

ACE-2	Hydrogen bond					VDW
Ligand	L	P		T	E	
Casopitant	O	N	AsnA103	H-acceptor	-3,5	ProA82, Tyr196
	F	O	GlnA81	Ionic	-3,5	
	C	O	GlnA101	H-donor	-3,5	
	C	O	AsnA194	H-donor	-3,5	
	F	C	MetA82	H-acceptor	1,9	
	6-ring	C	LeuA85	π-H	3,8	
	C	5-ring	HysA195	π-H	-3,2	
Grazoprevir	O	N	AsnA103	H-acceptor	-3,5	TyrA196, AsnA194, GlyA104, Glu87
	N	O	GlnA81	H-donor	-3,5	
	5-ring	C	ProA84	π-H	-1,6	
	6-ring	C	LeuA85	π-H	3,8	
	5-ring	C	HisA195	π-H	-3,2	
Remdesivir	O	O	AsnA194	H-donor	-3,5	ArgA219, GlnA102, TyrA202, GlnA102, GlyA104
	N	O	GlnA81	H-donor	-3,5	
	C	O	GlnA101	H-donor	-3,5	
	O	N	AsnA103	H-acceptor	-3,5	
	6-ring	5-ring	TyrA196	π-π	-1,3	
	O	C	HisA195	H-acceptor	-3,2	
Rimantadine	N	O	GlnA101	H-donor	-3,5	AsnA194, GlnA102, HisA195, GlnA98, TyrA202
	C	C	LeuA85	H-acceptor	3,8	
	C	6-ring	TyrA196	π-H	-1,3	

L: Ligand; **P:** Protein, **T:** Type; **E:** Energy (kcal/mol); **VDW:** Van der Waals interaction

The pre-fusion stage of a virus in human cells is a determinant factor, not only about the viral pathogenesis, but also about its virulence and, for this, this stage is an important target for the action of drugs in sanitary control, of intervention strategies and fighting its spread [41].

And this already suspected that the infection that occurs with high number of viral particles benefits the transformation of the cells into reproduction true factories of new viral copies, elevating the disease gravity and making the clinical condition of the patient even more critical [42]. And this fact was noted on the HCA of Fig. 1, where the correlation between pre- and post-fusion stages was 88.98% of similarity, indicating the pre-fusion events influences the post-fusion events.

The results obtained for this stage shows that all antivirals tested have a high binding affinity not only on Spike protein in the closed conformation, but also on the ACE-2 enzyme, fact that can also be noted by the high number of residues of amino acids "dragged" from active sites of these two proteases. Consequently, it shows able to inhibit SARS-CoV-2 action not only related to the reduction of quantity of viral particles that connects to the protease of cell surface (ACE-2), but also to inhibit the quantity of virus susceptible to the fusion unleashed by transmembrane protease serine 2 (TMPRSS-2), which is responsible by the catalyzation of virus entrance into the cells. This last achievement can also collaborate to minimize the gravity of the clinical condition of the patients.

BINDING PATTERN OF ANTIVIRALS CASOPITANT, GRAZOPREVIR, REMDESIVIR AND RIMANTADINE IN THE POST-FUSION STAGE OF THE VIRUS, INVOLVING SPIKE PROTEIN IN THE OPEN CONFORMATION (PDB ID: 6VYB), 3CLPRO PROTEASE (PDB ID: 6LU7) AND RDRP (PDB ID: 6M71)

In a very similar way comparing to the events occurred in the pre-fusion stage of the Covid-19 virus, in the post-fusion stage there is a great movement of residues of hydrophobic and hydrophilic amino acids, result of the antiviral interactions with the active sites of the proteases that works in the intracell processes of the viral replication (Figs. 6, 7, and 8 – supplementary material, and Tabs. 4, 5 and 6). And, as can be noted, a great volume of hydrogen bindings and Van der Waals interactions is present.

For this stage, the amino acids ValB512, ProB426 and CysB432 of Spike protein active site were conservated on all antivirals studied. On the other hand, excepting to Remdesivir, the amino acid TyrB380 was conservated relating to the other antivirals. And for RdRp protease, the amino acids LysA621, ArgA553 and AspA623 were conservated in all antivirals.

Table 4. Classification and charge of residues of Spike protein in open conformation (PDB ID: 6VYB).

Spike-6VYB	Hydrogen bond					VDW
Ligand	L	P		T	E	
Casopitant	C	O	LysB424	H-donor	-3,9	PheB464, TyrB396, SerB514, CysB432, TyrB380, PheB429, ProB412, AlaB419, GlnB409
	6-ring	C	SerB514	π-H	-0,8	
	6-ring	C	ThrB430	π-H	-0,7	
	6-ring	C	LeuB513	π-H	3,8	
	C	O	LeuB425	H-donor	3,8	
	5-ring	C	ValB512	π-H	4,2	
	C	5-ring	ProB426	π-H	-1,5	
	C	C	IleB410	H-donor	4,5	
	C	C	IleB402	H-acceptor	4,5	
	6-ring	C	IleB418	π-H	4,5	
	F	O	GlyB431	Ionic	-0,4	
	F	O	GluB406	Ionic	-3,5	
	F	O	AspB398	Ionic	-3,5	
Grazoprevir	C	O	GlyB431	H-donor	-0,4	ProB463, PheB429, CysB432, GlyB381, TyrB380, PheB464
	C	5-ring	ProB426	π-H	-1,6	
	6-ring	C	LeuB425	π-H	3,8	
	C	6-ring	ProB412	π-H	-1,6	
	6-ring	C	ValB512	π-H	4,2	
	C	C	ArgB355	H-donor	-4,5	
	C	C	ArgB466	H-donor	-4,5	

Remdesivir	N	O	IleB410	H-donor	4,5	TyrB430, PheB429, AspB398, ValB511, CysB432, LysB378
	C	O	ProB412	H-donor	-1,5	
	6-ring	C	ValB512	π-H	4,2	
	6-ring	C	ProB426	π-H	-1,6	
Rimantadine	6-ring	C	LeuB425	π-H	3,8	ProB426, SerB514, LeuB513, CysB432, GlyB381, CysB379, TyrB380
	C	5-ring	ProB412	π-H	-1,6	
	C	C	AlaB411	H-donor	1,8	
	N	O	LysB378	H-donor	-3,9	
	N	O	GlyB431	H-donor	-0,4	
	C	C	ValB512	H-donor	4,2	

L: Ligand; **P:** Protein, **T:** Type; **E:** Energy (kcal/mol); **VDW:** Van der Waals interaction

Table 5. Classification and charge of residues of 3-chymotrypsin like protease, 3CLpro (PDB ID: 6LU7).

3CLpro	Hydrogen bond					VDW
Ligand	L	P		T	E	
Casopitant	N	6-ring	ArgA298	π-H	-4,5	CysA128, TyrA126, PheA112, ThrA292, AspA153
	N	O	ThrA111	Ionic	-0,7	
	F	O	AsnA151	H-acceptor	-3,5	
	F	C	IleA152	π-π	4,5	
	6-ring	6-ring	PheA291		2,8	
Grazoprevir	O	N	GlnA299	H-acceptor	-3,5	ThrA292, ArgA4, ValA296
	O	C	MetA6	H-acceptor	1,9	
Remdesivir	C	C	LysA5	H-donor	-3,9	AsnA151, ThrA292, PheA8
	5-ring	5-ring	ProA9	π-π	-1,6	
	C	O	GluA290	H-donor	-3,5	
	6-ring	O	ThrA111	π-π	-0,7	

→

Rimantadine	C	O	GluA290	H-donor	-3,5	ValA296, ThrA292, ValA296
	C	C	LysA5	H-acceptor	-3,9	
	C	6-ring	PheA3	π-H	2,8	
	C	S	MetA6	H-donor	1,9	

L: Ligand; **P:** Protein, **T:** Type; **E:** Energy (kcal/mol); **VDW:** Van der Waals interaction

Table 6. Classification and charge of residues of RNA-dependent RNA polymerase, RdRp (PDB ID: 6M71).

RdRp	Hydrogen bond					VDW
Ligand	L	P		T	E	
Casopitant	F	C	AspA618	H-acceptor	-3,5	ArgA624, ArgA555, ValA557
	6-ring	O	AspA760	π-íon	-3,5	
	6-ring	C	LysA621	π-H	-3,9	
	O	O	TyrA455	H-acceptor	-1,3	
	F	O	AspA623	Ionic	-3,5	
	F	O	ThrA556	Ionic	-0,7	
	F	C	ArgA553	H-acceptor	-4,5	
	F	C	ArgA555	H-acceptor	-4,5	
	F	C	SerA682	H-acceptor	-0,8	
	F	N	LysA545	H-acceptor	-3,9	
Grazoprevir	C	O	AspA761	H-donor	-3,5	AlaA554, TyrA548, ArgA624, AspA452, ThrA680, CysA813, LeuA758, AsnA691
	C	C	ArgA555	H-acceptor	-4,5	
	C	C	LysA545	H-acceptor	-4,5	
	C	O	AspA623	H-donor	-3,5	
	N	O	ArgA553	H-donor	-4,5	
	C	O	SerA682	H-donor	-0,8	
	O	O	ThrA687	H-acceptor	-0,7	
	C	6-ring	TyrA455	π-H	-1,3	
	O	N	LysA621	H-acceptor	-3,9	

Remdesivir	C	6-ring	CysA622	π-H	2,5	AspA452, LysA621, ValA557, SerA759
	6-ring	O	AspA623	π-π	-3,5	
	N	N	ArgA624	H-acceptor	-4,5	
	O	O	ThrA556	H-donor	-0,7	
	C	C	AlaA688	H-acceptor	1,8	
	O	N	LysA545	H-acceptor	-3,9	
	O	N	ArgA555	H-acceptor	-4,5	
	O	N	ArgA553	H-acceptor	-4,5	
	N	O	TyrA619	H-donor	-1,3	
Rimantadine	N	O	AspA623	H-donor	3,5	CysA622, TyrA458, ArgA553, AspA452
	C	6-ring	TyrA455	π-H	-1,3	
	C	C	LysA621	H-acceptor	-3,9	
	C	C	ArgA624	H-acceptor	-4,5	

L: Ligand; **P:** Protein, **T:** Type; **E:** Energy (kcal/mol); **VDW:** Van der Waals interaction

The fitting poses of the compounds considered the most important in this paper showed that they strongly interact with the RDB of SARS-CoV-2 viral protein, confirming the perfect fit at the binding pocket RDB S-ACE2. The results of these poses are on Tab.1.

The interaction residues at Figs. 4 and 5, detailed at Tabs. 2 and 3, they agree with the binding residues described on lecture. These residues are crucial for the interaction of the S protein with the receptor ACE-2, among them the residues GlyB339, AsnB343, ValB512, ProB426, CysB432, HisA195 and TyrA196, which were conservated in all SARS--CoV-2 complexes. The related ones to inhibit the synthesis of the viral RNA (RdRp) that were conservated: LysA621, ArgA553 and AspA623.

Usually, catalytic pockets of target enzymes are places even more important for the project and development of new inhibitors of pathogens action. It's possible to observe that the residues of the site of these enzymes are highly conservated and share similarities of sequence with the correspondent CoVs. And once that SARS-CoV-2 shares a high identity of sequence on its proteins RdRp and 3CLpro with SARS-CoV

and MERS-CoV75, so it's believed that the therapeutic molecules used to direct SARS-CoV and MERS-CoV may be useful for COVID-19 treatment too, with a similar efficiency [43,44]. However, the SARS-CoV-2 RDB is significantly different from SARS-CoV, specially at the two sites that interact with the ACE-2 (subdomains S1 and S2).

Shang and collaborators sowed that the main protease of the new coronavirus (3CLpro) has a central role when translating the viral polyproteins, which occurs through RNA-dependent RNA polymerase (RdRp) that is used not only to replicate virus genome, but also for the transcription of its genes [45]. In other words, the interaction effectiveness of an antiviral with the protease 3CLpro inhibits the production of viral proteins relevant for the shaping of the Covid-19 virions due to the crack of translation process performed by the RNA-dependent RNA polymerase [46]. For this case, Casopitant and Grazoprevir presented higher binding affinities for the inhibition of this processing of proteins of the virus cycle, with almost -49 kcal/mol of charge is higher than Remdesivir and Rimantadine.

It's already known that the protease 3CLpro works on about 11 different cleavage sites, especially related to the amino acids Leu-Gln and Ser-Ala-Gly, what takes to the appearance of a lot of non-structural proteins (nsPs), including the RNA-dependent RNA polymerase (RdRp). Thus, the antiviral activity against the protease 3CLpro has the power to inhibit of the nsPs, making hard the replication occurred during the viral cycle [47]. Thus, the HCA present on Fig.2 shows exactly this functional interdependence between Spike protein in active conformation (6VYB) and the 3CLpro protease (6LU7), with almost 98,43% of similarity, certificating the quality of the used protocol.

The *in vitro* and *in vivo* clinical trials have showed many characteristics of action of the studied antivirals that confirm the results achieved in this paper. Consequently, the use of Remdesivir against coronaviruses has already showed that is metabolized inside the cell as a similar to adenosine triphosphate that inhibits the viral polymerase RNA [48,49]. However, among its most important characteristics is the fact that is an inhibitor of RNA-dependent RNA polymerase (RdRp), as per the studies of its efficiency against the coronavirus MERS-CoV and

SARS-CoV [50]. This drug inhibits not only the production, but also the replication of the viral genome, mainly because changes the exonucleases function of the virus and the reading of disturbed proof [51,52]. Moreover, Remdesivir has the effectiveness against epithelial cells of human airways, where there is a higher expression of the receptor of the new coronavirus, the ACE-2 [51,53].

On the other hand, Rimantadine is an antiviral with proved efficiency against infections caused by influenza A, being able to inhibits the uncoating of the virus nucleocapsid of this disease [54]. So, it's possible that it is a good alternative to treat COVID-19, once this drug is efficient against a lot of coronaviruses, including HCoV-OC43 and SARS-CoV, mainly blocking the entrance of SARS-CoV-2 into vulnerable cells, blocking the attachment and the fusion of the virus into the cells [55,56].

Studies of cell's culture already showed that Grazoprevir can inhibit the activity of protease PLpro (papain-like cysteine protease) of the hepatitis C virus (HCV). Moreover, it has been considered on the literature that HCV drugs that are able to inhibit PLpro or 3CLpro (3-chymotripsin like protease) proteases are also able to prevent or reduce the replication of SARS-CoV-2 virus [57,48]. It's right that the experimental studies of higher density on these drugs still short, however, this paper shows that the calculated binding affinity and the volume of amino acids residues, not only through hydrophobic bindings but also the hydrophilic ones point for its potential to reduce the charge of viral particles in the intracell environment.

Casopitant is a neurokinin-1 receptor antagonist drug, used in patients in chemotherapy treatment and presents symptoms of nausea after therapy sessions [59]. However, many computational studies point to the possibility of its use against COVID-19, mainly due to the conservation of many amino acids residues of the catalytic sites of the RNA-dependent RNA polymerase [60,61].

Two aspartate residues, Asp-760 and Asp-761, were already considered as the main catalytic residues of the RdRp protease, that are conservated in all coronaviruses [10,60,61]. However, our results points to the presence of another residue from this type, AspA623 (Fig.8 – supplementary material, and Tab.6). Moreover, the complexes RdRp-li-

gands are stabilized by 6 H-bindings and 3 Van der Waals interactions for Casopitant, and by 8 H-bindings and 8 Van der Waals interactions for Grazoprevir. The ligand perfectly attaches to the protein's catalytic cavity, completely covering both catalytic aspartate residues; on Casopitant, the residues AspA760 and AspA623, on Grazoprevir, AspA761 and AspA623 (Fig.8 – supplementary material, and Tab.6).

CONCLUSIONS

From the seven antivirals studied on this paper, only Casopitant, Grazoprevir, Remdesivir and Rimantadine presented good binding charges in all stages of the SARS-CoV-2 infection, strongly suggesting them as promises to block the virus attachment and the membrane fusion that occurs during the viral pre-fusion stage, as well to inhibits the production of virus proteins and RNA synthesis that occurs during the post-fusion.

The values of the binding charges calculated for each complex receptor-ligand (Tab.4) do not reveal any apparent difference of behavior of the antivirals against any of the studied proteases, but the HCA based on the values of binding charges applied not only to the antivirals, but also to the proteins studied, showed that these four antivirals produce the same effect related to all stages of the infection. This is a supposed fact o Fig.3, where the similarity of this group related to the others (Stavudine, Lamivudine and Tenofovir) is of only 18,20%, what is too tiny to consider the association between these two groups as relevant. However, with each other, they keep a similarity grade of 87.92%, what confirms the behavior information that is attributed to them in the beginning of the discussion.

On the other hand, HCA also showed that the proteases of pre--fusion stage S protein in the closed conformation and ACE-2 shape a group with elevated similarity, with a rate of 92.46% (Fig.2). But, contrary to antivirals, this group presented a similarity rate of 88.98% in relation to proteases of post-fusion stage, what indicates to be highly correlated events. In other words, the events' intensity of pre-fusion stage (membrane attachment and fusion) decisively influences the quantity of intracell processes in the host cell (post-fusion stage).

It's also important to note that during the post-fusion stage, HCA showed that the events involving the group S protein and 3CLpro has almost 100% of similarity (Fig.3), what points to an intense activity involving both proteins. Consequently, except for Remdesivir-S protein complex, all the others have binding charge moderately higher than the complexes shaped with the RNA-dependent RNA polymerase (RdRp), possibly because it is on a group isolated on Fig.2.

So, after the membrane fusion, the virus enters the cells where its content is discharged. After this, the virus is replicated and negative--strand RNA, what is done by the positive-sense single-stranded RNA, pre-existing due to the polymerase RNA (transcription) activity. And this negative-strand RNA recently shaped has as function to produce new positive-sense RNA strands, which will synthesize new proteins in the cell cytoplasm (translation). In this case, RdRp's biological activity is very distinct from other proteases, what can be noted in the HCA on Fig.2.

It's already well characterized that the Spike protein binds to the host cell receptor and induce to a virus cell-membrane fusion, that has crucial role on the process of this pathogen invasion. Moreover, the high affinity between Spike protein and ACE-2 increases SARS-CoV-2 infectivity. Thus, Table 1 shows that there was a decrease on the bioactivity rate of antivirals, ranging from 13.19% (Grazoprevir) to 31.5% (Rimantadine), indicating a catalytic action higher of the virus attachment block than the inhibition of ACE-2 activity.

AUTHORS' CONTRIBUTIONS

Nelson Henrique Morgon designed research, analyzed data and wrote the article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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MATERIAL COMPLEMENTAR

Figure 4. Map of ligand interaction Casopitant, Grazoprevir, Remdesivir and Rimantadine with the residues of Spike protein amino acids in the closed conformation (6VXX). VDW are Van der Waals interactions and C are conventional carbonic bindings.

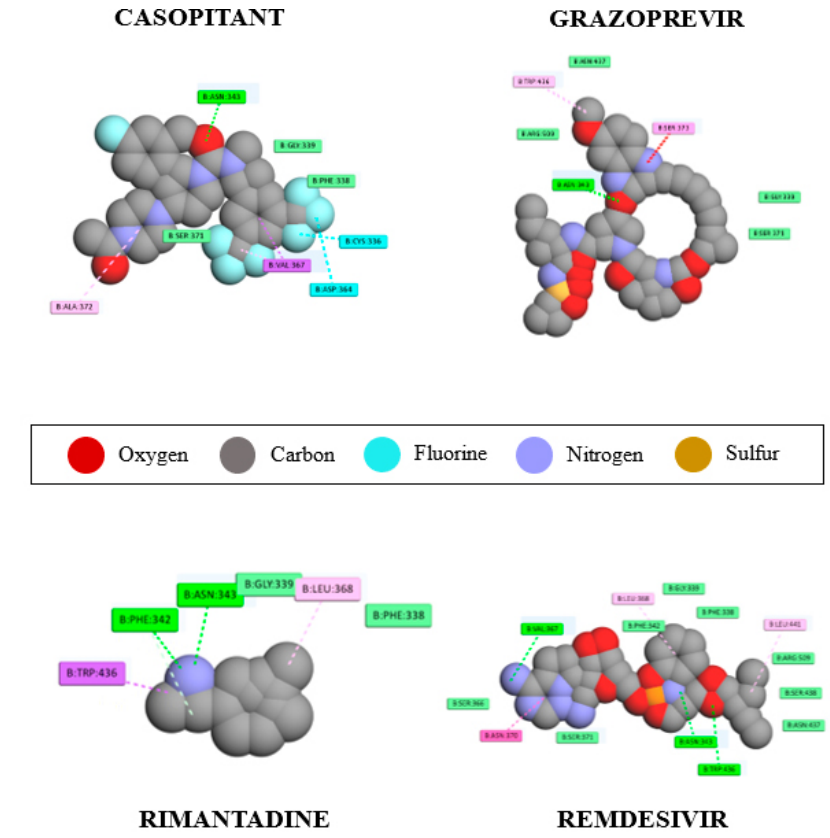

Figure 5. Map of ligands interactions Casopitant, Grazoprevir, Remdesivir and Rimantadine with the residues of angiotensin-converting enzyme 2 (ACE2) amino acids. VDW are Van der Waals interactions and C are conventional carbonic bindings.

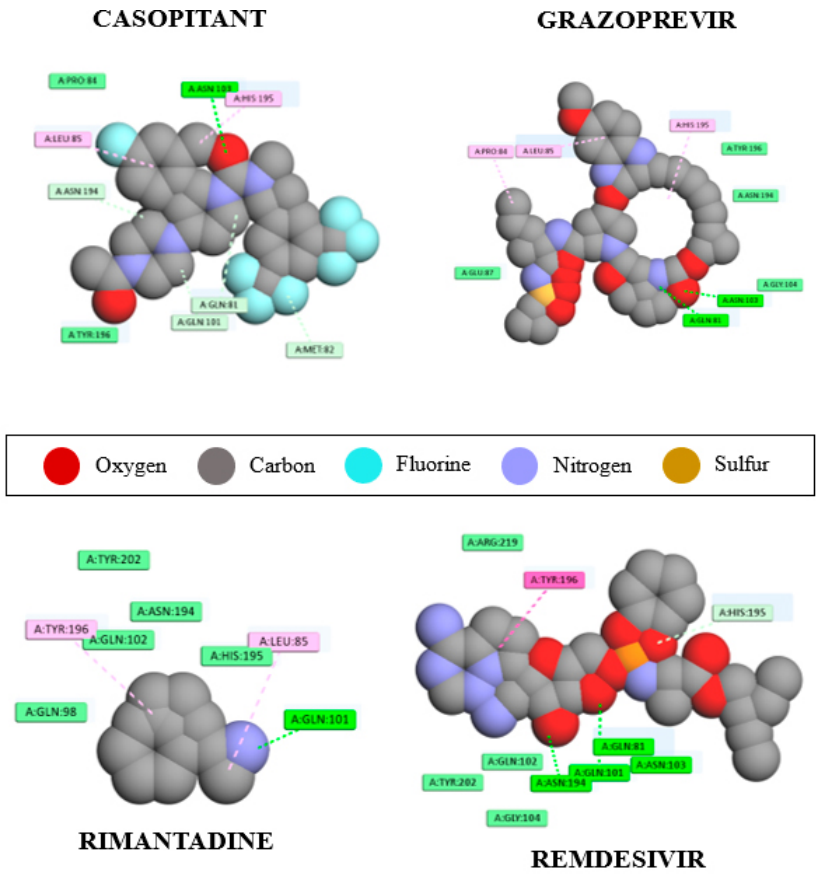

Figure 6. Map of ligands interaction Casopitant, Grazoprevir, Remdesivir and Rimantadine with residues of Spike protein amino acids in the open conformation (6VYB). The binding pocket is indicated in yellow. VDW are Van der Waals interactions and C are conventional carbonic binding.

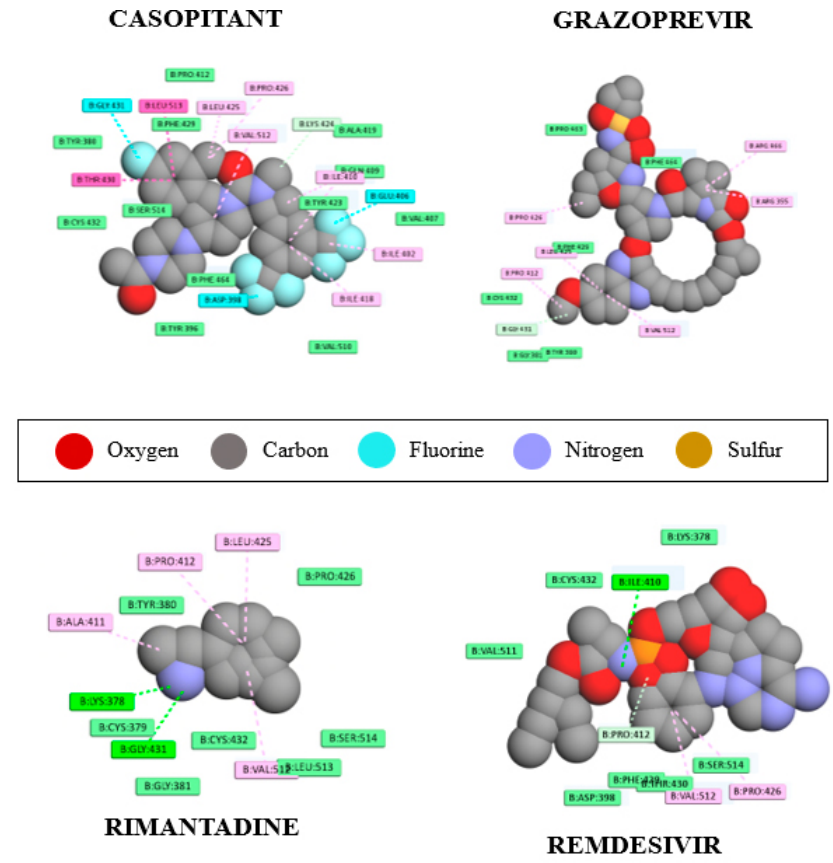

Figure 7. Map of ligands interaction Casopitant, Grazoprevir, Remdesivir and Rimantadine with residues of the 3- chymotrypsin like protease (6LU7). VDW are Van der Waals interactions and C are conventional carbonic bindings.

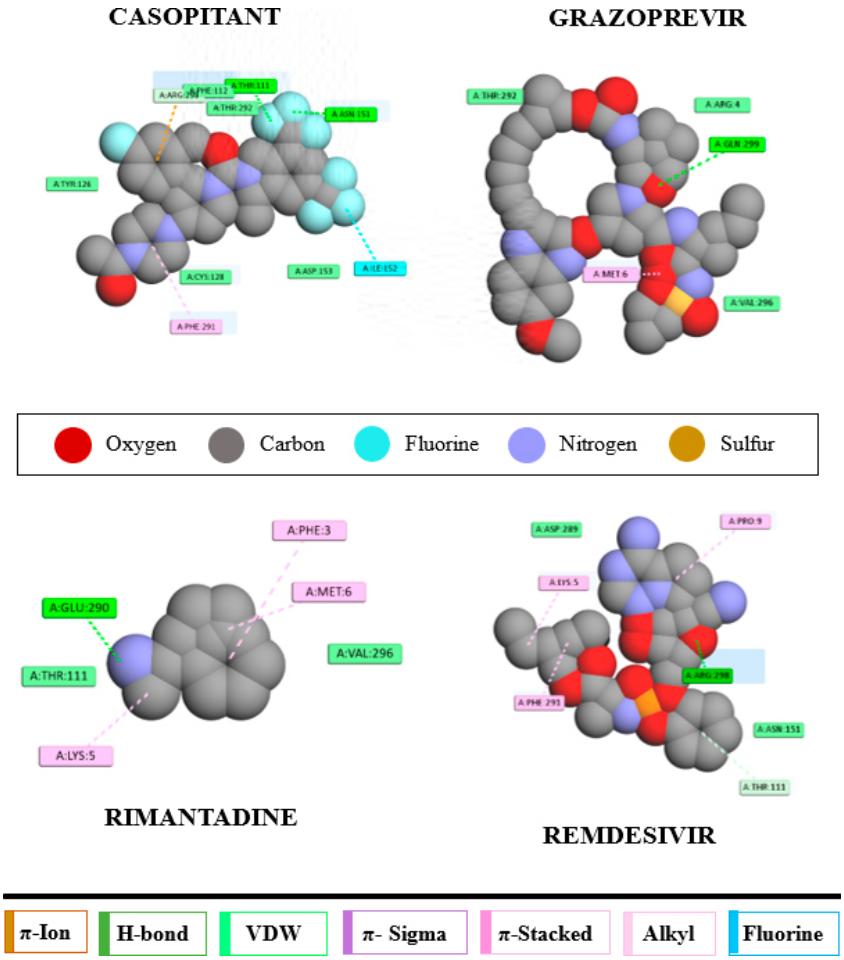

Figure 8. Map of ligands interaction Casopitant, Grazoprevir, Remdesivir and Rimantadine with residues of RNA- dependent RNA polymerase (RdRp). VDW are Van der Waals interactions and C are conventional carbonic bindings.