THE COMPUTATIONAL INVESTIGATION OF SIXTEEN ANTIVIRAL DRUGS AGAINST MAIN PROTEASE (M^{PRO}) AND SPIKE PROTEASE (S^{PRO}) OF SARS-CoV-2

AJOY KUMER [a, b], UNESCO CHAKMA [c], MD. TAWHIDUL ISLAM [c], DEBASHIS HOWLADER [c] AND TOMAL HOSSAIN [c]*

[a]*Department of Chemistry, European University of Bangladesh, Gabtoli, Dhaka-1216, Banglades.*
[b]*Department of Chemistry, Bangladesh University of Engineering and Technology, Dhaka-1000, Bangladesh.*
[c]*Department of Electrical and Electronics Engineering, European University of Bangladesh, Gabtoli, Dhaka-1216, Bangladesh.*

ABSTRACT

In this research, the fourteen commonly used antiviral drugs were investigated through the computational tools against CoV-19 or SARS-2, as well as two small bioactive molecules from the cannabis plant, Tetrahydrocannabinol (THC) and Cannabinol (CBN). Thus, these were selelcted for molecular docking against main protein (5r7y) and spike protein (6xs6) of coronavirus. It was illustrated that the binding energies of M^{pro} for Pimodivir, Baloxavir Marboxil, Lopinavir, Baricitinib, Remdesivir, THC, Darunavir, Galidesivir, Nitazoxanide, CBN, Ritonavir, Penciclovir, Ribavirin, Favipiravir, Umifenovir, and Chloroquine were -8.6, -7.7, -7.6, -7.5, -7.3, -6.8, -6.6, -6.6, -6.6, -6.5, -6.5, -6.3, -6.2, -6.0, -5.7 and -5.4 kcal/mol, respectively, which could be supported for good binding molecules against micropathogens, where it was -9.8, -6.9, -6.9, -7.1, -7.1, -7.1, -7.5, -6.0, -6.2, -7.4, -5.8, -5.9, -5.7, -5.6 and -5.4 kcal/mol, respectively, for S^{pro}. Among these, Pimodivir is a best-bonded molecule with M^{pro} and S^{pro} in view of molecular docking score. Secondly, the ligand interaction was accounted for this protein against required corona virus protein consisting of weak H bonding, hydrophobic bond and Van dar Waal interaction. For justification of molecular docking, the molecular dynamics was calculated for top six scored drugs where the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) were showed that the six drugs for both main protein and spike protein. Additionally, the chemical hardness and softness have calculated, and the lowest value of softness has found in sample 06 and 13 around 0.24. The HOMO-LUMO gap has calculated with a different value for all, but the lowest value has obtained for 01. Finally, the pharmacokinetics and Lipisinki rule were calculated, and all of these molecules had satisfied the Lipisinki rule. Finally, using the admetsar online data base, absorption, distribution, metabolism, excretion and toxicity have calculated.

Keywords: Corona virus, Antivirus drug, Molecular docking, Molecular Dynamics, and ADMET.

1. INTRODUCTION

In the concurrent time, our globe has been stopped their freshness activity and fear from death by an invisible enemy, SARS-2-CoV-19 from December 2019, which has considered the one of the greatest historical world pandemic disease although our globe faced some other outbreaks pandemics, such as SARS-1, ZIKA virus, Soyan flu, Spanish flu, HIV, AIDS, third plague pandemic, Asian flu, Honk Kong flu, third cholerea pandemic and bird flu (1-3). First of all, the SARS-2-CoV-19 was introduced at Wuhan providence in China, December 2019 (4-6) which was caused as lethal endemic diseases, such as Extreme Acute Respiratory Syndrome (SARS) and Endemic Middle East Respiratory Syndrome (MERS) (7-8). Now it was exploited that the main protease(Mpro) strain of SARS-2-CoV-19 is a single-stranded with positive-sense RNA genome, sub-family Coronavirinae in the family Coronaviridae and the order Nidovirales, which is the similar genome of Mouse Hepatitis Virus (MHV) (9). The genomic structures of the COVID-19 is almost similar to human betacoronaviruses, such as SARS-CoV-2, SARS-CoV, and MERS-CoV, but also have a small variation in their genomic and phenotypic structure that can manipulate their pathogenesis (10). Chemically, it could be identified as a spherical or pleomorphic enveloped particles, which contains club-shaped glycoprotein projections, as RNA allied a nucleoprotein within a capsid comprised of matrix protein. Finally, COVID-19 virus composes of at least six open reading frames, such as spike glycoprotein, Envelope, small membrane protein, membrane protein, hem agglutinin- esterase, Nucleoprotein and Genomic protein. In general, there are two types of polypeptides, which are classified according to their length, and consist of hymotrypsin-like protease (3CLpro) or main protease (Mpro). Though three or four type of abundant viral proteins are obtained in COV-19, the membrane (M) glycoprotein is most common whereas the a short unique N-terminal fragment (-NH2) is connected with the spike protein (outside), and a long -COOH terminus (cytoplasmic domain) is added with the virion (inside) (11).

In case of its activity, SARS-CoV-2 (CoV-19) binds to ACE2 (the angiotensin-converting enzyme 2 as cellular ligand) by its spike as virus receptor, and enters to the host cell while the spike protein has to be effected by an enzyme called a protease (TMPRSS2), a type 2 TM serine protease located on the host cell membrane, for finishing the process. During the time of RNA replication in host body, various symptoms, such as cellular immune deficiency, coagulation activation, myocardial injury, hepatic and kidney injury, and secondary bacterial infection were occurred (12). This infection has attacked the brain and spinal cord and especially damaged for weak nervous containing cell. As a result, the neuropath logical changes have occurred for fresh necrosis, neuronal death, glial nodules, and polymorphonuclear infiltrations (13-14). Therefore, more than a few kinds of vaccines and antiviral drugs have been designed and testing on base of spike protein or main protein. The protein of corona viruses was taken for the provided link https://www.rcsb.org/structure/5r7y of protein data bank(pdb) which was uploaded by Fearon, D. et al. (2020) in protein data bank (15). In addition, the spike protein was taken from pdb, linked https://www.rcsb.org/structure/6XS6.

In our work, some traditional drugs, used as antivirus drug, were simulated based on computational tools. Several drugs for the treatment of corona virus (CoV-19) have been used since last one and half years which have not been perfectly prescribed meditation from World Health Organization (WHO). Among of these, the most common drugs are Pimodivir as anti influenza drug (16), Baloxavir Marboxil as also anti influenza drug (17), Lopinavir and Ritonavir as novel protease inhibitors of HIV (18-23), Baricitinib as drug of rheumatoid arthritis (24-25), Remdesivir used for Ebola virus disease (EVD) as treating for RNA virus (26), Darunavir as antiretroviral-naive adults with HIV-1 (27-28), Galidesivir (BCX4430) used as broad-spectrum antiviral drug (29). Moreover, the Nitazoxanide had prescribed as a good candidate for the treatment of chikungunya virus (CHIKV) (30) while the Penciclovir and Ribavirin had been widely used for the treatment of herpesvirus infections and hepatitis C virus (HCV), respectively (31-32). In addition, Favipiravir,Umifenovir and Chloroquine are most three commonly used antivirus drug in all over the world (33). For the vast applications of selected drug for medication, these have been chosen for computational study against the CoV-19 virus stains to evaluate their activity as anti drugs for CoV-19. Once important point is that for evaluating drug activity from natural sources, cannabis is one of the most commons element where THC, CBD, CBG, and CBN have already used as a drug as anticancer, pain killer, increased anxiety, paranoia and impairment of memory (34-36). Although some vaccines have been started for meditation and safe the human being from CoV-19, the demand of drugs is the crucial fact to remove this disease from our globe, which is why this study has designed for searching new drugs.

*Corresponding author email: kumarajoy.cu@gmail.com

Molecular Docking, initially introduced by Kuntz et al. 1982 (37), belongs to a computational method that virtually seeks to predict a complex of two binding partners, such as biological macromolecules and small molecules as drug. Moreover, it predicts how a drug can interact and bind to protein of pathogens as well as give the information about the binding energy as docking score (38-40). Regarding this fact, molecular docking tools have been used against sixteen antiviral drug against main protein of CoV-19 and molecular dynamic has performed for justify the accuracy of the docking method. Moreover, to fill up the computational literature study of sixteen antiviral drugs, the chemical activity indicator and AMDET study have included.

2. COMPUTATIONAL DETAILS

2.1 Preparation of ligand and calculation of Chemical reactivity and descriptors

The eighteen antiviral drugs were taken from the PubChem website in SDF form (41). The Material Studio 8.0 was used for geometry optimization (42). For the optimization, B3LYP of DMol code was used in the this software to calculate the chemical reactivity indicators using frequency calculation by DFT (43). After optimization, the molecular frontier orbital diagram of HOMO and LUMO were taken with its magnitude. It had then saved in PDB form, which was further used for molecular docking as ligand.

2.2 Method for molecular docking

The starting three-dimensional (3D) structure of RNA protein of coronavirus disease (CoV-19) is a new strain that was discovered in December, 2019 from Wuhan, China. It was found in Protein Data Bank (PDB) with ID: 5r7y, following link (https://www.rcsb.org/structure/5r7y), which was considered as one of the initial strain or main protease of CoV-19 virus and established as the RNA strain with all carried getenical characteristics. Moreover, the spike protease (6xs6) was taken from PDB with the link:

https://www.rcsb.org/structure/6XS6 (44). After taking the protein from PDB, and it was viewed by the Pymol software version using PyMOL V2.3 (https://pymol.org/2/) (45). All water molecules and unexpected ligands or heteroatoms were removed to get fresh protein, and it was saved as PDB files. The both of protein and drug PDB files were uploaded in PyRx software for molecular docking as the auto dock vina. After the molecular docking, the docked complex was taken Discovery Studio version 2017 for result analysis and view (46).

2.3 Determination the data of ADMET

The ADMET properties were completed by the online database amdetSar, http://lmmd.ecust.edu.cn/admetsar2, which is the most acceptable database for predicting the AMDET parameters (47-49).

2.4 Molecular Dynamic

To perform MD simulations, NAMD software was used using run interactively with live view or in batch mode on a desktop or laptop computer (50). MD simulation was devoted to underpin the docking results gained for the best antiviral drugs and CoV-19 protein up to 5000 ns for holo-form (drug-protein) applying AMBER14 force field (51). In the presence of a water solvent, the total system was equilibrated with 0.9% NaCl at 298 K temperature. A cubic cell was propagated within 20 Å on every side of process and periodic boundary circumstance during the simulation. After simulation, the RMSD and RMSF were analyzed using the VMD software.

3. RESULTS AND DISCUSSIONS

3.1 HOMO, LUMO and chemical reactivity descriptors

The computed εLUMO, εHOMO and ΔE gap, chemical potential (μ), electronegativity (χ), hardness (η), softness (s) and electrophilicity (ω) of antiviral drugs are presented in table 01. These data have calculated by B3LYP functional. The chemical susceptibility of a molecule has determined by the HOMO-LUMO energy gap and a large HOMO-LUMO gap mentions the high kinetic and low chemical stability (52-58). From the table 1, it is found that the HOMO–LUMO gap is about 6.126 to 8.508 eV for all tested drugs while Pimodivir shows the lowest energy gap as well as having the highest the softness value (34, 59-61).

Table 1: Frontier molecular orbitals and Reactivity descriptor analysis

	εLUMO, eV	εHOMO, eV	εHOMO εLUMO gap, eV	Ionization potential (I), eV	Electron affinity (A), eV	Chemical potential (μ), eV	Hardness (η), eV	Electrons activity (x), eV	Electrophilicity (ω), eV	Softness (S), eV
1	-1.191	-7.317	6.126	7.317	1.191	-4.254	3.063	4.254	-2.954	0.326
2	-1.56	-8.993	7.433	8.993	1.56	-5.277	3.717	5.277	-3.746	0.269
3	-0.552	-8.499	7.947	8.499	0.552	-4.525	3.974	4.525	-2,576	0.251
4	-2.212	-8.761	6.549	8.761	2.212	-5.487	3.275	5.487	-4.597	0.305
5	-1.713	-8.528	6.815	8.528	1.713	-5.120	3.408	5.120	-3.846	0.293
6	-0.031	-8.283	8.252	8.283	0.031	-4.157	4.126	4.157	-2.094	0.242
7	-0.635	-9.143	8.508	9.143	0.635	-4.889	4.254	4.889	2.809	0.235
8	-0.74	-8.388	7.648	8.388	0.74	-4.564	3.824	4.564	-2.723	0.261
9	-1.610	-9.850	8.240	9.850	1.610	-5.730	4.120	5.730	-3.984	0.242
10	-0.623	-8.066	7.443	8.066	0.623	-4.345	3.722	4.345	-2.536	0.269
11	-1.41	-8.818	7.408	8.818	1.41	-5.114	3.704	5.114	-3.530	0.270
12	-0.996	-8.615	7.619	8.615	-0.996	-4.806	3.809	4.806	-3.031	0.263
13	-1.301	-9.469	8.168	9.469	1.301	-5.385	4.084	5.385	-3.550	0.245
14	-2.126	-9.007	6.881	9.007	2.126	-5.567	3.441	5.567	-4.503	0.291
15	-1.235	-8.132	6.897	8.132	1.235	-4.684	3.449	4.684	-3.181	0.290
16	-1.007	-8.137	7.130	8.137	1.007	-4.572	3.566	4.572	-2.931	0.280

The frontier molecular orbital(FMO) has determined the chemical reactivity and active sites where the protein can be banded. The lower magnitude of energy gap contributes to form an interaction with SARS-2 protein with drugs. From the figure 1, the FMO has presented. In case of LUMO, the yellow color indicates the negative node and blue color indicates the positive node of orbitals. On the other hand, the violet color for HOMO indicates positive node of orbital and light greenish color expresses the negative node of orbitals. It must be written that the protein can be attached the part of LUMO.

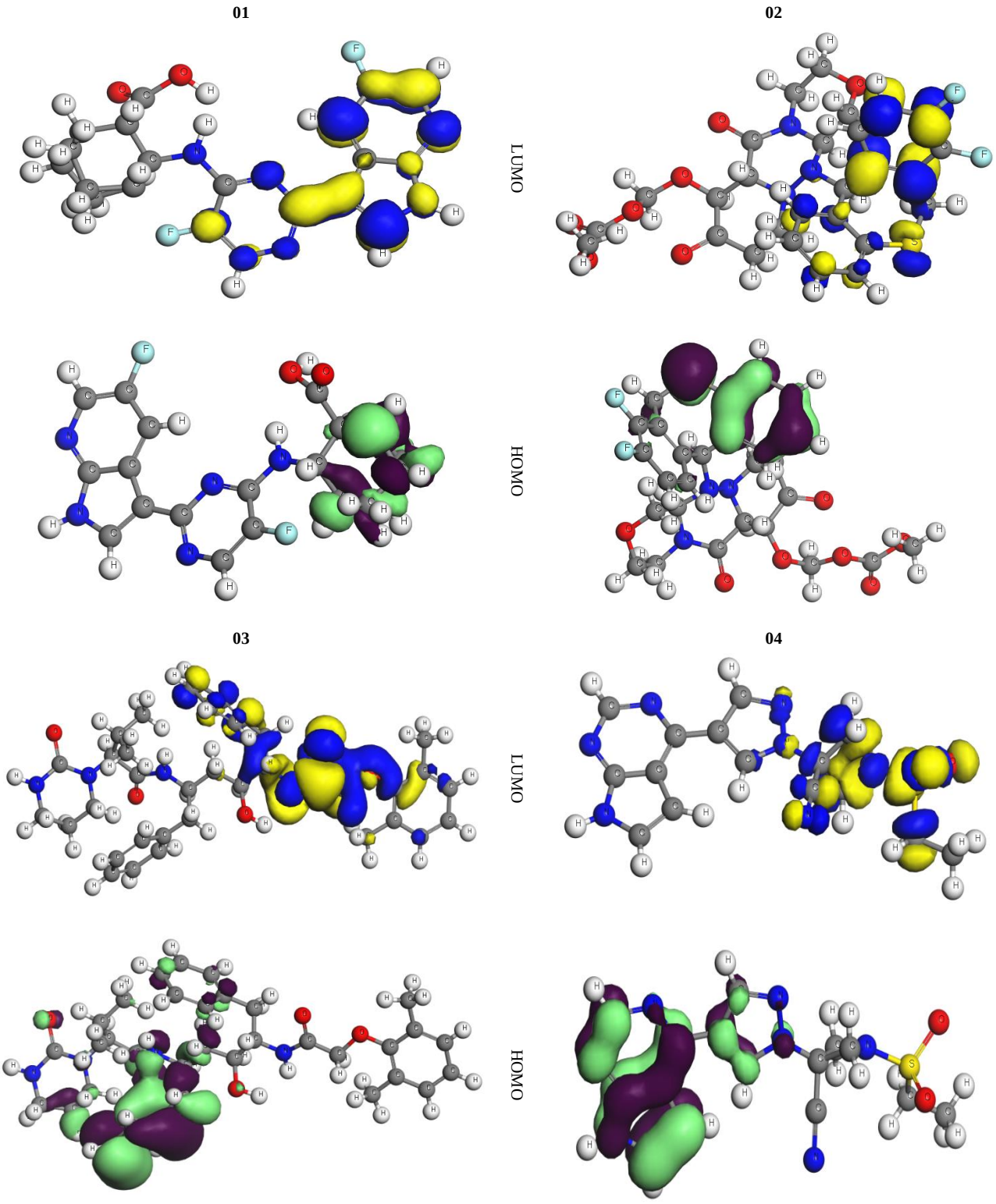


Figure 1. Frontier molecular orbitals diagram for HOMO LUMO

3.2 Molecular docking score

Molecular docking studies were conducted in order to validate the obtained pharmacological data and provide evidence for binding affinity of drug compounds with protein of CoV-19 or SARS-2 (62-63).

As the protein-ligand interaction plays a significant role in structural based drug designing, the H bonding and hydrophobic bonding are the main cause for docking score where the docking score above 6.00 kcal/mol has been considered as standard drug (63-65).

Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand, which would form a complex with overall minimum energy. *In Silico* studies revealed that all drug molecules showed good binding energy toward the target protein ranging from -8.60 to -5.44 kcal/mol shown in table 02 while the Pimodivir, Baloxavir-Marboxil, Lopinavir, Baricitinib, Remdesivir could be considered as the standard drug although THC, natural occurring molecule, has its also standard docking score in term of binding energy.

Table 2. Data of binding energy and name of interacted ligand for main protease (M^{pro})

Ligand	Binding Affinity (kcal/mol)	No of H bond	No of Hydrophobic bond	No of van der Waal bond	Total bonds
Pimodivir	-8.6	02	06	absent	08
Baloxavir-Marboxil	-7.7	08	02	absent	10
Lopinavir	-7.6	08	04	absent	12
Baricitinib	-7.5	05	01	absent	06
Remdesivir	-7.3	08	03	absent	11
THC	-6.8	absent	09	absent	11
Darunavir	-6.6	01	02	absent	03
Galidesivir	-6.6	06	absent	absent	06
Nitazoxanide	-6.6	04	03	absent	07
CBN	-6.5	01	05	absent	06
Ritonavir	-6.5	07	06	absent	13
Penciclovir	-6.3	05	01	absent	06
Ribavirin	-6.2	05	absent	absent	05
Favipiravir	-6.0	07	02	absent	09
Umifenovir	-5.7	04	02	Absent	06
Chloroquine	-5.4	01	06	absent	07

In case of spike protease, the Pimodivir can show the highest docking score, -9.8 kcal/mol which is more than the main protease. The Baricitinib, Remdesivir and THC have shown from the table 3 the similar docking score,-7.1 kcal/mol and Darunavir and CBN have obtained the -7.5 and -7.4 kcal/mol shown in table 3. It could be said that the THC and CBN are highly active inhibitor against S^{pro} than M^{pro} and the activity of Pimodivir has the towering among all drugs although it is higher against S^{pro} than M^{pro}. There are small change in H bonding and hydrophobic bonding between S^{pro} and M^{pro} that more H bonding has created for S^{pro}.

Table 3. Data of binding energy and name of interacted ligand for spike protein (S^{pro})

Ligand	Binding Affinity (kcal/mol)	No of H bond	No of Hydrophobic bond	No of van der Waal bond	Total bonds
Pimodivir	-9.8	04	02	absent	06
Baloxavir-Marboxil	-6.9	07	04	absent	11
Lopinavir	-6.9	02	06	absent	08
Baricitinib	-7.1	05	03	absent	08
Remdesivir	-7.1	01	05	absent	06
THC	-7.1	01	08	absent	09
Darunavir	-7.5	03	12	absent	15
Galidesivir	-6.0	05	02	absent	07
Nitazoxanide	-6.2	05	02	absent	07
CBN	-7.4	absent	11	absent	11
Ritonavir	-7.5	05	08	absent	13
Penciclovir	-5.8	05	02	absent	07
Ribavirin	-5.9	04	absent	absent	04
Favipiravir	-5.7	04	02	absent	06
Umifenovir	-5.6	02	07	absent	09
Chloroquine	-5.4	01	08	absent	09

3.3 Protein - Ligands Interaction

To design a new drug, the main key factor is ligand-protein interaction that provides the information of binding or bonding of drugs with the protein of virus or micro pathogens. The interaction of drug molecule with the main protease, 5r7y, of corona virus, has been investigated with bond distance. From table 04, it is illustrated that there are two types of bonds, H- bond and hydrophobic bond but Van dar Waal bond is not presented for all drugs. For the Pimodivir drug, three H bonds and six hydrophobic bonds are formed with CoV-19 protein whereas the hydrogen bonds distance is lower than hydrophobic bond distance. Similarly, the type of bond interaction with bond distance for all drugs is listed in table 04.

Table 4. Protein- Ligands Interaction with amino acid residues and their bond distance

		PubChem Code	Hydrogen bond		Hydrophobic bond		Van der Waals bond
			Interacting residue of amino acid	Distance, A°	Interacting residue of amino acid	Distance, A°	
1	Pimodivir	67286591	ASP – 289 LEU – 287	2.45 3.18	TYR – 237 TYR – 237 LEU – 272 LEU – 272 LEU – 286 LEU – 286	5.16 5.40 4.79 5.05 4.27 4.83	absent
2	Baloxavir-Marboxil	124081896	ARG – 298 ARG – 298 ARG – 298 ASN – 151 GLN – 110 PHE – 294 ASP – 153 ILE – 106	4.55 3.43 3.01 3.44 3.50 3.53 3.33 3.56	ARG – 298 ARG – 298	3.92 4.55	absent
3	Lopinavir	92727	TYR – 237 TYR – 237 ASP – 289 LEU – 287 LEU – 287 THR - 199 THR - 199 LYS - 236	1.91 2.07 2.68 2.65 4.19 3.13 2.60 5.00	TYR – 237 LYS – 236 LYS – 236 LEU – 286	3.99 4.64 5.00 4.82	absent
4	Baricitinib	44205240	GLN – 189 HIS – 41 PHE – 140 GLU – 166 HIS – 163	2.90 3.00 2.75 3.39 3.32	CYS – 145	4.63	absent
5	Remdesivir	121304016	GLU – 290 GLU – 288 TYR – 239 THR – 199 THR – 199 ARG – 131 ASP – 289 ASP – 289	2.74 2.96 2.84 2.19 3.17 3.18 2.88 3.72	LYS – 137 LEU – 287 MET – 276	4.98 3.88 5.98	absent
6	THC	16078	absent		PRO – 108 PRO – 108 PRO – 108 ILE – 200 PRO – 132 HIS – 246 HIS – 246 PRO – 293 PHE – 294	5.16 4.51 5.01 4.14 4.25 4.58 4.59 4.31 4.45	absent
7	Darunavir	213039	THR – 199	2.71	LYS – 137 LEU – 286	3.96 3.96	absent
8	Galidesivir	10445549	PHE – 140 GLN – 189 HIS – 41 HIS – 164 CYS – 145 HIS – 163	2.91 3.67 2.59 2.17 2.88 3.34	absent		absent
9	Nitazoxanide	41684	LYS – 137 THR – 199 TYR – 239 LEU – 287	2.88 3.09 2.88 2.22	LEU – 272 ASP – 289 LEU – 286	5.34 4.67 5.04	absent
10	CBN	2543	ARG – 298	2.80	PHE – 294 PHE – 294 PHE – 294 PHE – 294 PHE – 294	4.31 3.92 5.78 3.87 5.31	absent

11	Ritonavir	392622	CYS – 145 ASN – 142 GLU – 166 GLU – 166 GLN – 189 PHE - 140	3.06 1.89 2.76 2.59 2.23 3.63	CYS – 145 MET – 165 PRO – 168 PRO – 168 LEU – 167 LEU – 167 MET – 49	4.87 4.65 4.60 4.30 5.18 4.91 4.82	absent
12	Penciclovir	135398748	ASN – 221 ASN – 221 ASN – 221 SER – 267 ASP – 263	2.55 4.35 4.60 2.23 3.36	LEU – 220	5.07	absent
13	Ribavirin	37542	GLU – 166 GLU – 166 GLU – 166 GLN – 189 THR – 190	2.43 1.94 2.97 2.86 3.27	absent		absent
14	Favipiravir	492405	ARG – 279 ASN – 221 ASN – 221 PHE – 219 PHE – 219 (Halogen) LEU – 271 SER – 267 (Halogen)	3.04 3.22 3.87 2.23 3.42 3.17 2.92	TRP – 218 LEU – 271	5.35 5.22	absent
15	Umifenovir	131411	THR – 199 LEU – 287 ASP – 289 ASP – 289	3.01 3.59 3.59 3.56	LEU – 272 LEU – 286	3.80 4.23	absent
16	Chloroquine	2719	PHE – 140	2.26	CYS – 44 MET – 49 HIS – 41 HIS – 41 CYS – 145 LEU – 27	4.97 4.40 4.82 4.59 4.25 4.27	absent

In the view of S^{pro}, the H bonding interaction and hydrophobic bonds are illustrated in the table 5. Overall, the H bonds are more interacted with protein because its bond distance is less than hydrophobic bond and Van dar Waal bonds are absent in all case. For the CBN, no hydrogen bond was formed although its docking score almost near to highest inhibitor. On the other hand, the Ribavirin could not form the hydrophobic bonds as a result its docking score is low. From the protein-drug interaction, it could be difficult to say about the effect of specific bonds on docking score that which bond is directly involved to forming the molecular docking score but it has observed that the bond distance of H bonding is less than hydrophobic bond.

Table 5. Spike Protein- Ligands Interaction with amino acid residues and their bond distance

		PubChem Code	**Hydrogen bond**		**Hydrophobic bond**		**Van der Waals bond**
			Interacting residue of amino acid	Distance, A°	Interacting residue of amino acid	Distance, A°	
1	Pimodivir	67286591	ALA-123 ASN-121 ASN-121 ARG-190 (Halogen)	2.49 3.29 3.17 3.24	ILE-119 ILE-119	4.94 4.70	absent
2	Baloxavir-Marboxil	124081896	THR-791 LYS-790 LYS-814 LYS-814 LYS-814 SER-875 SER-875	3.02 5.28 3.34 3.19 3.63 3.12 3.01	PRO-807 PRO-807 PRO-809 ALA-871	5.28 4.76 5.29 3.76	absent
3	Lopinavir	92727	THR-33 LYS-300	3.08 3.06	PHE-32 PHE-59 VAL-289 VAL-289 LEU-296 LYS-300	5.42 4.82 5.43 5.31 5.47 3.40	absent
4	Baricitinib	44205240	THR-778 ALA-1056 ALA-1056 SER-730 HIS-1058	1.88 2.44 3.49 3.81 3.54	PRO-863 HIS-1058 HIS-1058	4.99 4.82 5.17	absent

5	Remdesivir	121304016	ASP867	2.97	VAL-860 VAL-860 PRO-863 HIS-1058 PHE-823	5.33 4.75 5.42 4.67 4.33	absent
6	THC	16078	ASN-121	3.37	VAL-126 ILE-203 ILE-203 ILE-128 ILE-119 PHE-192 PHE-192 TRP-104	3.76 5.08 4.78 4.34 3.95 5.39 5.39 4.71	absent
7	Darunavir	213039	ASN-121 THR-124 HIS-207	3.07 2.37 2.90	ILE-203 ILE-119 HIS-207 TRY-170 TRY-170 TRP-104 TRP-104 VAL-227 VAL-227 VAL-126 VAL-126 PHE-192	3.50 5.15 5.28 5.24 5.00 5.66 4.59 3.80 5.00 3.80 5.33 4.84	absent
8	Galidesivir	10445549	ARG-1014 GLN-957 GLN-957 ALA-958 SRE-1003	2.99 2.05 3.45 3.06 2.65	THR-961 ALA-958	3.57 4.91	absent
9	Nitazoxanide	41684	SER-205 ARG-190 ASN-99 ASN-121 ASN-121	2.87 3.18 3.18 2.92 3.32	ILE-203 HIS-207	5.30 5.25	absent
10	CBN	2543	absen		TYR-170 LEU-226 HIS-207 ILE-119 ILE-203 PHE-192 PHE-192 PHE-194 VAL-126 VAL-227 TRP-104	3.73 3.89 4.70 4.96 4.42 5.22 4.43 5.26 5.07 4.33 4.63	absent
11	Ritonavir	392622	TRY-170 TRY-170 TRY-170 SER-172 ASN-121	2.25 2.91 3.26 3.14 3.42	ILE-119 ILE-128 LEU-229 LEU-229 VAL-227 VAL-227 VAL-126 PHE-168	5.30 5.27 5.42 5.31 5.35 4.29 4.90 4.83	absent
12	Penciclovir	135398748	THR-778 GLY-1059 ALA-1056 ALA-1056 ASP-867	2.89 2.56 2.46 2.59 3.34	HIS-1058 ILE-870	5.01 5.10	absent
13	Ribavirin	37542	LEU-966 SER-975 SER-975 GLY-744	2.17 3.65 2.80 3.25	absent		absent

14	Favipiravir	492405	MET-731 THR-778 THR-778 SER-730	2.55 2.98 2.12 3.93	PRO-863 HIS-1058	4.95 4.57	absent
15	Umifenovir	131411	GLU-1092 LYS-1038	2.59 3.30	ARG-1107 ARG-1107 ARG-1107 LYS-1038 LYS-1038 TYR-1047 TYR-1047	4.57 4.76 4.66 4.15 3.66 4.78 4.80	absent
16	Chloroquine	2719	LEU-226	2.95	PHE-192 LEU-226 ILE-203 ILE-119 TRP-104 VAL-227 TYR-170 TYR-170	5.24 4.65 4.63 4.51 4.85 4.77 3.92 4.02	absent

[Note: TRP = TRPptophan, ASP = Aspartic acid, GLU = Glutamic acid, LEU = Leucine, THR = Threonine, ASN = Asparagine, GLN = Glutamine, PHE = Phenylalanine, ILE = Isoleucine, ARG = Arginine, VAL = Valine, SER = Serine, PRO = Proline, GLY = Glycine, HIS = Histidine, LYS = Lysine, TRP = TRPosine, CYS = Cysteine, MET = Methionine.]

3.4 Aromaticity

The ability to design and fine-tune non-covalent interactions between organic ligand and proteins is indispensable to rational drug development. Aromatic stacking has long been recognized as one of the key constituents of ligand-protein interfaces providing the π-π interactions. From figure 2, it finds the edge and face of interaction between drugs as ligand and protein of coronavirus as well as the pocket show how the ligand has interacted with protein and where it is formed a bond. Besides, the maps binding pocket by employing a voxel/grid-based 3D pocket represents its flexibility action between drugs and amino acids of protein and is also useful for the visualization of the active binding site by a selection of representative structures for ensemble docking effect.

Pimodivir **Baloxavir-Marboxil** **Lopinavir**

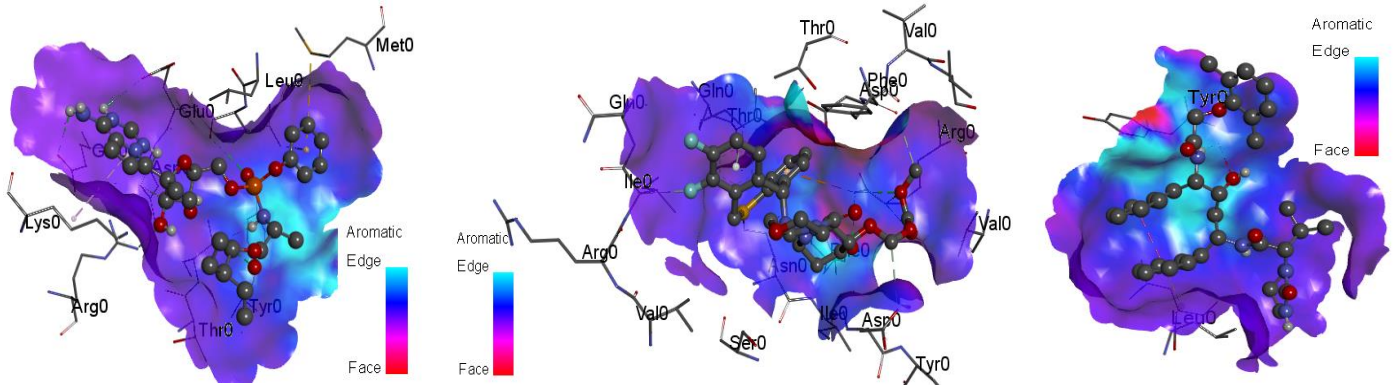
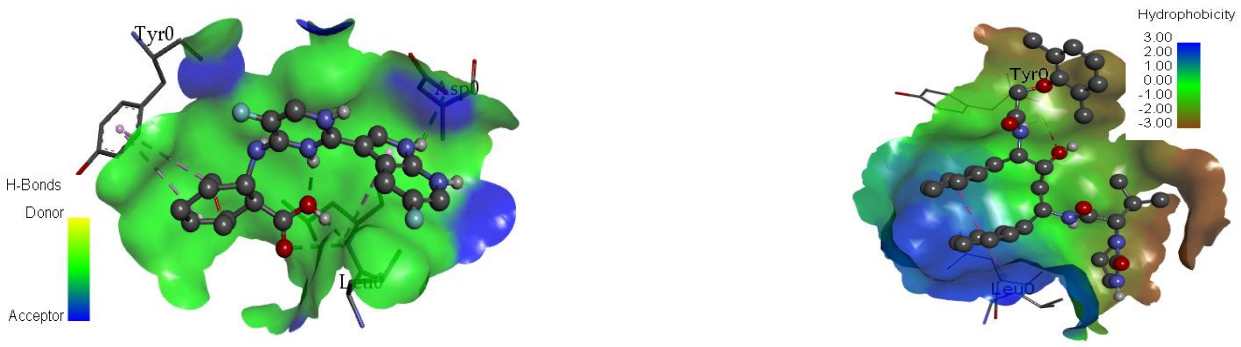


Figure 1. Aromaticity

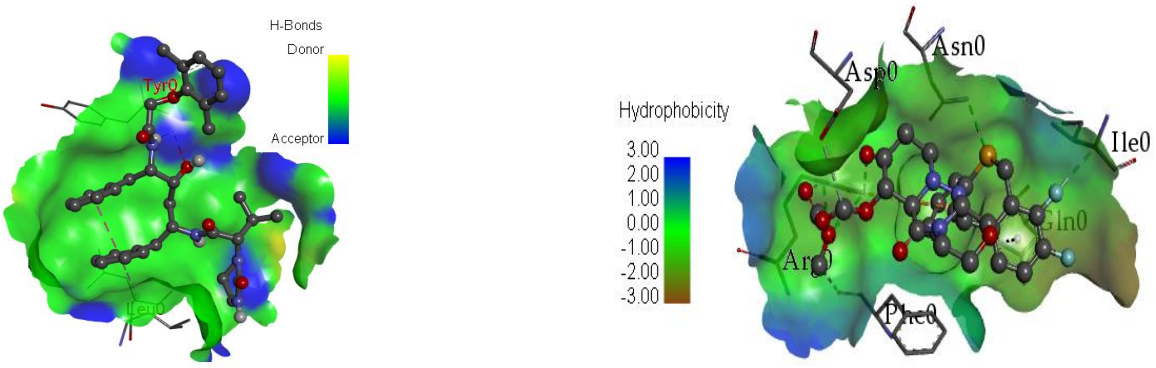
3.5 Hydrogen bonding and Hydrophobicity

Hydrogen bonding is an exchange reaction whereby the hydrogen bond donors and acceptors of the free protein and ligand break their hydrogen bonds with water and form new ones in the protein-ligand complex. We evaluated the Hydrogen bond accepting and donating region in figure 3. In table 5, it was found that there was a strong H bond distance, weak H bond distance, the hydrophobic bond distance for all molecules.

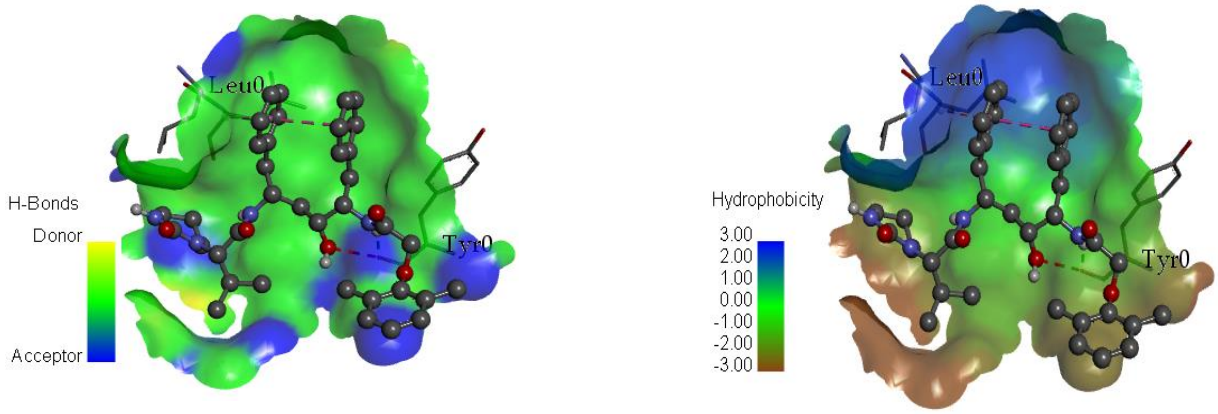
Pimodivir



Galidesivir



Lopinavir



3.6 Pharmacokinetics and Druglikeness study

According to Christopher A. Lipinski rule for drug molecules in 1997 stated that first of all, it has less the 5 hydrogen bond donors and less than 10 hydrogen bond acceptors whereas the no of rotatable bonds is three or more, but the molecular mass is less than 500 Daltons (66). The fifth view is the octanol-water partition coefficient expressed as log P0/w and it is not greater than 5.

Using the Swiss Institute of Bioinformatics online database was used to evaluate the Pharmacokinetics and drug-likeness applying Lipinski rule from the log in the mentioned link https://www.sib.swiss/,and make a comparison study as drug activity (67-68). From the table 06, it demonstrates that all molecules follow the Lipinski rule as a drug.

Table 6. Data of Lipinski rule, Pharmacokinetics and Druglikeness

	NBR	HBA	HBD	TPSA, Å²	Consensus Log Po/w	Log Kp (skin permeation)	Lipinski rule		MW	Bioavailability Score	GI absorption
							Result	Violation			
Pimodivir	4	7	3	103.79	3.18	-6.05	Yes	0	399.39	0.56	High
Baloxavir-Marboxil	6	11	0	123.15	2.59	-7.73	Yes	1	575.58	0.55	High
Lopinavir	17	5	4	120.00	4.37	-5.93	Yes	1	628.80	0.55	High
Baricitinib	4	7	1	128.94	0.42	-8.61	Yes	0	357.39	0.55	High
Remdesivir	14	11	4	189.57	2.03	-8.65	Yes	2	591.59	0.17	Low
THC	4	2	1	29.46	5.28	-3.27	Yes	1	314.46	0.55	High
Darunavir	12	8	3	148.80	1.95	-7.84	Yes	1	533.64	0.55	Low
Galidesivir	2	6	6	140.31	-1.55	-9.38	Yes	1	265.27	0.55	Low
Nitazoxanide	6	6	2	142.35	-0.07	-7.07	Yes	0	308.29	0.55	Low
CBN	4	2	1	20.23	5.21	-3.86	Yes	1	310.43	0.55	High
Ritonavir	22	7	4	225.95	5.18	-6.53	Yes	1	708.98	0.55	Low
Penciclovir	5	5	4	130.05	-0.77	-8.97	Yes	0	253.26	0.55	Low
Ribavirin	3	7	4	143.72	-2.05	-9.10	Yes	0	244.20	0.55	Low
Favipiravir	1	4	3	88.84	-0.93	-7.74	Yes	0	159.12	0.55	High
Umifenovir	8	4	1	80.00	4.26	-6.07	Yes	0	477.41	0.55	High
Chloroquine	8	2	1	27.30	4.15	-4.96	Yes	0	319.87	0.55	High

3.7 In sillico Pharmacokinetics study by ADMET

ADMET stands for absorption, distribution, metabolism, excretion, and toxicity, which are considered as the vital parts of any drug development program and essential for compliance with regulatory guidelines. Both of these conducted for chemical optimization, process development, and pharmacological profile. This study belongs to invariably involved whole-animal models, and it is highly time-consuming and expensive. In order to minimize the cost and time, the ADME study helps to design a new drug for drug preparation or even in the clinic. Such events created a serious disruption of the development process and often resulted in the closure of the project and a lost opportunity. As a result, the situation of drug discovery has been changing rapidly and dramatically. ADME and toxicology technologies have evolved to permit the use of rapid and less expensive methods that have made the early assessment of drug candidates very attractive to the pharmaceutical industry. The absorption, distribution, metabolism, excretion, and toxicity parameters have listed in table 05.

Table 7. Data of pharmacokinetics study by ADME

Drugs/ ADME	Human Intestinal Absorption	Caco-2 Permeability	Blood Brain Barrier	P- I glycoprotein inhibitor	P- II glycoprotein substrate	Renal Organic Cation Transporter	Sub-cellular localization	CYP450 2C9 Substrate	CYP450 1A2 Inhibitor
Pimodivir	0.9853	-6.05	Yes	No	Yes	0.8448	Mitochondrion	No	Yes
Baloxavir-Marboxil	0.9943	-7.73	Yes	Yes	Yes	0.7167	Mitochondrion	No	No
Lopinavir	0.6593	-5.93	Yes	Yes	Yes	0.8578	Mitochondrion	No	No
Baricitinib	1.0000	-8.61	Yes	No	No	0.7387	Lysosomes	No	No
Remdesivir	0.9005	-8.65	Yes	No	No	0.9580	Lysosomes	No	No
THC	0.9949	-3.27	Yes	No	Yes	0.8169	Mitochondrion	No	Yes
Darunavir	0.9287	-7.84	Yes	Yes	No	0.8724	Lysosomes	No	No
Galidesivir	0.9932	-9.38	Yes	No	No	0.8965	Nucleus	No	No
Nitazoxanide	0.8581	0.6231	No	No	No	0.9343	Mitochondrion	No	No
CBN	0.9922	1.7659	Yes	No	Yes	0.8345	Mitochondrion	No	Yes
Ritonavir	0.8344	0.0975	No	Yes	No	0.8140	Lysosomes	Yes	No
Penciclovir	0.9885	0.8545	Yes	No	No	0.7342	Nucleus	No	No
Ribavirin	0.9852	0.2286	Yes	No	No	0.9574	Mitochondrion	No	No
Favipiravir	0.8416	0.5807	Yes	No	No	0.8851	Mitochondrion	No	No
Umifenovir	0.9969	1.4823	Yes	No	Yes	0.5189	Lysosomes	No	Yes
Chloroquine	0.9939	0.8736	Yes	Yes	Yes	0.6046	Lysosomes	Yes	No

The table 8 represents the toxicity of required drugs in case of acute and non acute species, testing on rat and fish, which was obtained by online data base for computational prediction. It is observed that all drugs have more solubility in water medium. As a result, most of drugs are toxic on fish where the LD50 score is about 1.814 to 2.954 mol/kg as non aquatic species, rat. Finally, It can be said that all drugs are non carcinogenic, as well as no responsible for AMES toxicity.

Table 8. Data of pharmacokinetics study by toxicity

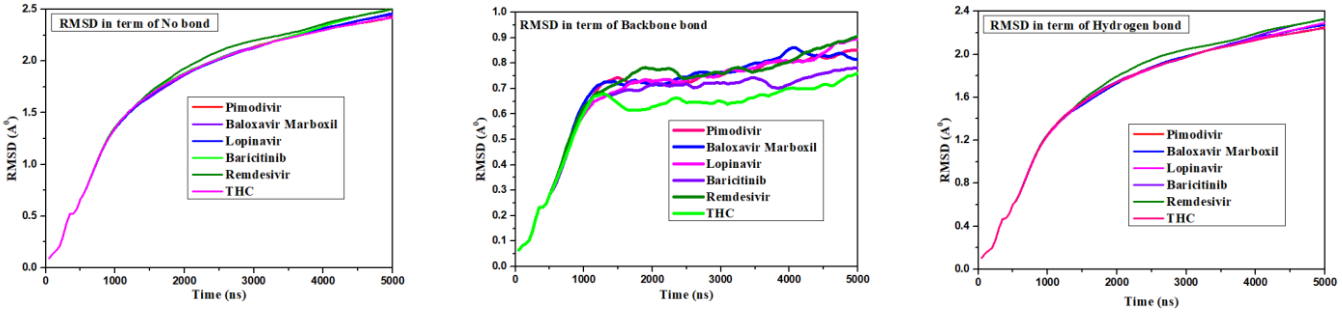
S.L	AMES toxicity	Carcinogenicity (trinary)	Water solubility, Log, S	Plasma protein binding	Fish Toxicity, (Yes/No)	Acute Oral Toxicity, kg/mol	Oral Rat Acute Toxicity (LD50) (mol/kg)	Fish Toxicity pLC50 mg/L	T.Pyriformis toxicity (log ug/L)
Pimodivir	No	No	- 3.986	0.872	Yes	1.72	2.881	1.129	0.557
Baloxavir-Marboxil	No	No	- 3.719	1.100	Yes	3.053	2.582	1.346	0.618
Lopinavir	No	No	- 3.414	1.001	No	3.430	2.250	1.747	0.385
Baricitinib	No	No	- 3.079	0.766	Yes	2.716	2.673	1.690	0.359
Remdesivir	No	No	- 3.469	1.057	Yes	3.939	2.716	1.251	0.548
THC	No	No	- 4.322	1.009	Yes	3.030	2.594	-0.110	1.778
Darunavir	No	No	- 3.512	0.957	Yes	4.044	2.527	1.430	0.431
Galidesivir	No	No	- 1.790	0.494	No	2.360	2.176	1.918	0.019
Nitazoxanide	Yes	No	-1.630	0.660	Yes	2.340	1.814	1.506	0.637
CBN	No	No	- 3.975	0.997	Yes	3.033	2.515	0.368	1.867
Ritonavir	No	No	- 3.256	0.892	Yes	3.095	2.734	1.496	0.541
Penciclovir	No	No	- 2.654	0.179	No	2.381	2.142	1.867	0.180
Ribavirin	No	No	- 1.173	0.237	No	2.774	1.987	1.798	0.150
Favipiravir	No	No	- 2.251	0.319	No	3.395	2.413	2.113	0.254
Umifenovir	No	No	- 4.368	0.980	Yes	2.885	2.245	0.878	0.828
Chloroquine	Yes	No	- 4.348	0.580	Yes	2.827	2.954	0.943	0.808

3.8: Molecular Dynamics

The molecular dynamics is an avenue for testing the accuracy docking procedure in term of the average root-mean square deviation (RMSD) and root-mean square flauctuation (RMSF) which provide information about their binding pose in the respective crystal structures, ligand and protein interaction complex structure (69). It is revealed that the RMSD of docking complex is less than 2 Å for becoming a good fitting pose of ligand in drug pocket and proving that software is able to accurately dock the compounds (70-71). Then simply make parallel both docked pose with that of docked complex by RMSD; lower value indicates the accuracy and stability of the docking method (72-73).

The stability of these six docked complexes was evaluated using protein–ligand RMSD, ligand protein interaction and hydrogen bonding and ligand RMSF among others. In our study, the RMSD was calculated with respect to time (0-5000 ns) and interaction of amino acid residues of protein. Firstly, it is noted that the RMSD illustrates in the figure 4 (a) to (f) in term of time and amino acid residue dependent where an innovative relationship is found for first three figures. The RMSD is obtained less than 2 Å within time, 2000 ns but it has increased 2.4 Å at 5000 ns time for no bond or interaction. But the RMSD has changed after formation of backbone or hydrogen bond. The RMSD has decreased from 2.4 Å to below 0.9 Å in term of backbone bond interaction after docking, indicating high accuracy and stability of docked complexes, but the hydrogen bonding shows the little reducing of RMDS value from on bond. It could be said that hydrogen bonds are little response for molecular docking and stability of docked complex, showing RMSD is at about 2.2 Å, but interaction of protein-ligand leads the major role which shows the value less than 0.9 Å where THC compound has less than 0.7 Å. In case of amino acid residue interaction with ligand, the same phenomenon of RMSD has obtained.

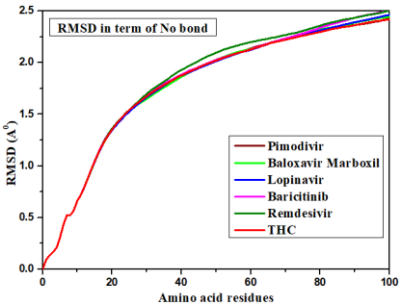
The RMSF of docked complex indicates the stability. Lower value of RMSF mentions the higher stability. From figure (g), it has found that the RMSF lays about 2.4 Å when it has no bonding or interaction as ligand- protein interaction. In case of hydrogen bond, it puts down 2.2 Å which means that hydrogen bond are little response for stability. But it has shifted down 0.8 Å due to backbone interaction while the THC shows the minimum RMSF is about 0.6 Å, meaning the highest stability of docked complex.



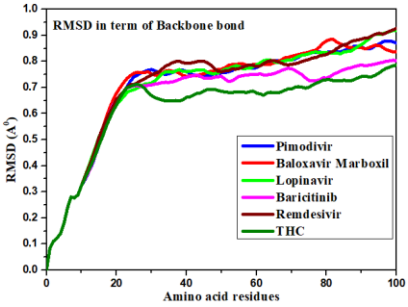
a) RMSD: Time vs no bond

b) RMSD: Time vs back bone

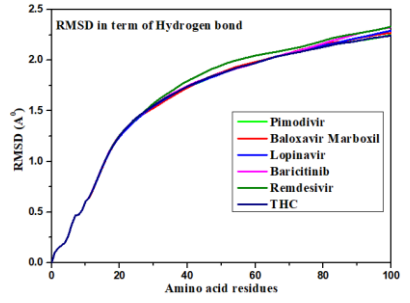
c) RMSD: Time vs Hydrogen bond



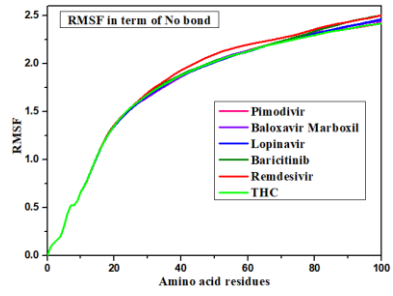
d) RMSD: Amino acid vs No bond



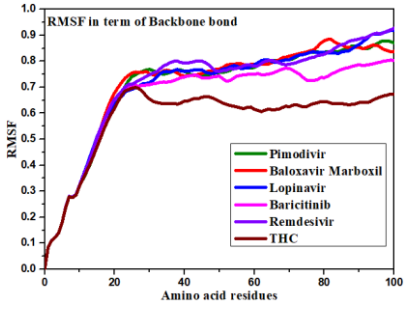
e) RMSD: Amino acid vs backbone



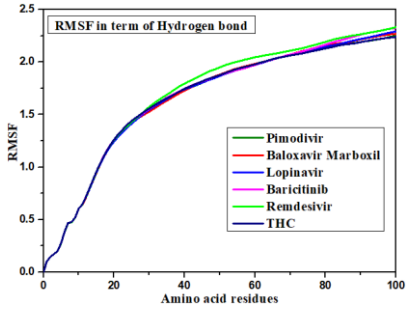
f) RMSD: Amino acid vs H bond



g) RMSF: Amino acid vs No bond



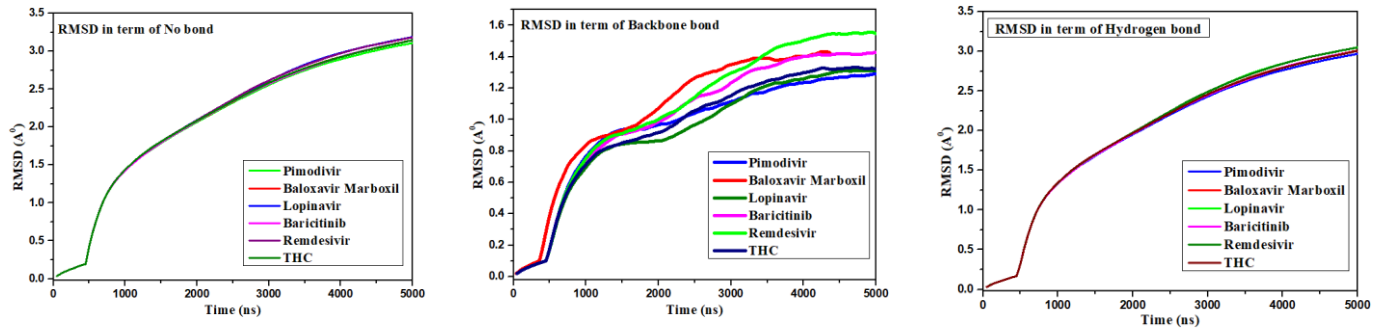
h) RMSF: Amino acid vs backbone



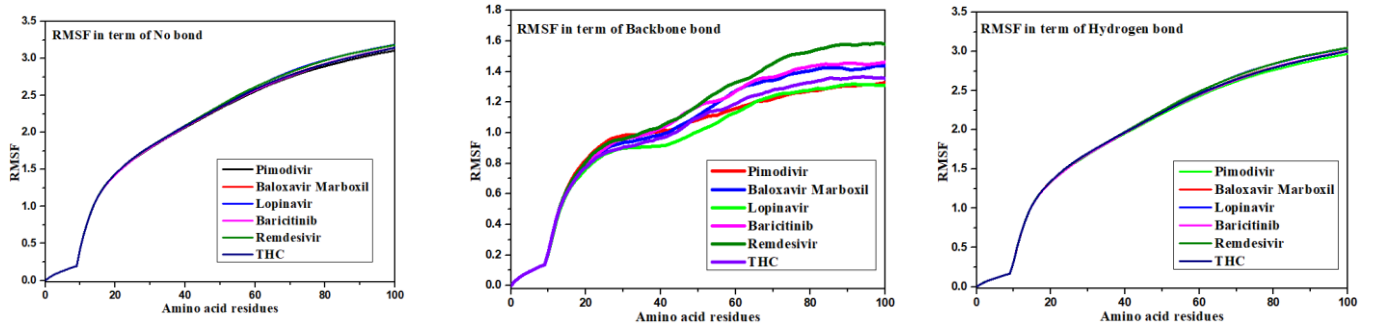
i) RMSF: Amino acid vs H bond

Figure 2. Various picture of RMSD and RMSF for main protein (M^{pro})

In case of S^{pro}, the MD was performed on basis of RMSD and RMSF for the protease and ligand complex after docking. The RMSD value is about 3.0 Å which has occurred without no bond between protein and ligand interaction. It was decreased in 1.3 Å which indicated the standard for drugs discovery. When H bond is created, the RMSD is about 2.9 Å while the RMSF was about 3.0 Å which are not good result for standard drugs. But, when bonds were created as backbone bond with protein residue, the RMSD was in 1.4 to 1.1 Å and RMSF was about 1.5 to 1.2 Å for first six drugs. In case of H bonds, the both of RMSD and RMSF were in about 3.0 Å shown figure 5,(I to VI).



i) RMSD: Time vs no bond ii) RMSD: Time vs back bone iii) RMSD: Time vs Hydrogen bond



iv) RMSF: Amino acid vs No bond v) RMSF: Amino acid vs backbone vi) RMSF: Amino acid vs H bond

Figure 3. Various picture of RMSD and RMSF for spike protein (S^{pro})

CONCLUSIONS

On the basis of molecular docking study, it can be concluded that all drugs show an excellent binding affinity with corona viruses protein. At first, Pimodivir, Baloxavir-Marboxil, Lopinavir, Baricitinib, and Remdesivir showed tha docking score as binding energy at -8.6, -7.7, -7.6, -7.5, -7.5 and -6.8 kcal/mol for Mpro whereas above -6.0 kcal/mol binding energy can be considered as an efficient drug against any micro-pathogens. In case of Spro, the docking score is slightly higher than Mpro. Moreover, for testing the accuracy of docking and stability of docked compound, the molecular dynamic study was performed where the RMSD and RMSF were calculated in term of protein ligand interaction, H bonding and hydrophobic bonding. In case of no bonding,

The RMSD and RMSF were about 2.4 Å where the ligand-protein interaction for Mpro had the vast contribution for stability, showing the value below 0.9 Å, and it was same for Spro. Morover, the H bonding contribution is very poor in docking score which is obtained from both of interaction and RMSD or RMSF value. Finally, it could be said that the docking protocol was highly accurated in term of MD. On the other hand, the HOMO, LUMO and LUMO HOMO gap mention about their chemical reactivity, as well as softness and hardness for becoming a drug. The pharmacokinetic study shows that they have different values, but all drugs can satisfy the Lipinski rule. Lastly, the ADMET data shows the essential information as a drug and its application in a human cell with comparative low toxicity even all of drugs are non carcinogenic materials.

CONFLICTS OF INTEREST

There are no conflicts to declare.

AUTHOR CONTRIBUTIONS

AK designed, optimized and docking of molecules, wrote the manuscript; UC performed the molecular dynamic and analysized the obtained data; DH and MTI analysized the obtained data, and took 2D and 3D picture; and TH gave all technical supports. All authors gave final approval for publication.

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