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Research Article:

Docking, Molecular Dynamics and MMPBSA Study of Selected FDA-Approved Drugs Against SARS-CoV-2 Proteases and Spike Proteins

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Abstract

Background: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), rapidly evolved into a global health emergency. Despite extensive research efforts, the lack of fully effective and specific antiviral therapies during the early stages of the pandemic highlighted the urgent need for alternative therapeutic strategies. Drug repurposing has therefore gained attention as a rapid and cost-effective approach to identify potential antiviral agents from existing FDA-approved drugs. **Aim:** This study aimed to identify promising repurposable FDA-approved drugs with potential inhibitory activity against key SARS-CoV-2 proteins using an integrated computational approach. **Methods:** A total of 28 FDA-approved drugs were computationally screened against three critical SARS-CoV-2 targets: the papain-like protease, main protease, and spike protein. Molecular docking was performed, followed by binding-site optimization and binding free energy estimation using the MM-GBSA method. Based on docking performance, the top eleven compounds were further evaluated using molecular dynamics (MD) simulations to assess the stability of ligand–protein complexes. Binding free energies were recalculated using the MM-PBSA approach. **Results:** Initial docking and MM-GBSA analyses identified hydroxychloroquine, rifampicin, ambroxol, chlorpromazine, clarithromycin, ranitidine, and ofloxacin as high-scoring candidates against SARS-CoV-2 targets. Subsequent MD simulations demonstrated stable binding interactions for ambroxol, rifampicin, famotidine, ranitidine, oseltamivir, ofloxacin, and ciprofloxacin. These compounds exhibited favorable binding free energies and maintained structural stability throughout the simulation period. **Conclusion:** The integrated in silico workflow identified several FDA-approved drugs with stable and energetically favorable interactions with key SARS-CoV-2 proteins. These findings support the potential repurposing of selected compounds, particularly ambroxol and rifampicin, as candidate antiviral agents against SARS-CoV-2. Further experimental validation and clinical investigation are warranted to confirm their therapeutic efficacy.

1. Introduction

Coronavirus Disease-2019 (COVID-19) is a viral pandemic that gave rise to a global crisis with overwhelming consequences (1). In spite of the approval of several types of vaccines worldwide (2), few compounds have been approved for SARS-CoV-2 infection, yet with clinical restrictions. The safe, effective, and available pharmacological interventions remain a priority due to the potential for recurrent spread every six months (3). Furthermore, important questions regarding vaccine efficacy and safety are still under debate (4, 5)

Drug repurposing, or repositioning, involves identifying new indications for existing drugs and represents one of the fastest and most cost-effective strategies for managing emerging infectious diseases, bypassing many challenges associated with de novo drug discovery (6). However, thousands of potential candidates must be screened to identify suitable options, and these candidates often face limitations related to bioavailability, pharmacokinetics, and effective concentrations (7). While experimental techniques are occasionally employed in drug repurposing studies (8), in silico approaches are increasingly diverse, robust, and evolving (9). Molecular docking is commonly used for the initial screening of large compound libraries (10), whereas molecular dynamics simulations and free energy

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calculations based on simulation snapshots provide more refined and focused screening of top candidates (11) (12). A recent involvement of artificial intelligence (AI) in drug repurposing has application in managing infectious and rare diseases (13). The implementation of (AI) is ranged from simple natural language processing of literatures to mechanism-based and bioinformatics-based models (14)
Crystal structures of several SARS-CoV-2 proteins are growingly accessible, including the main protease (Mpro), the papain-like protease (PLpro), and the spike glycoprotein (S-protein). Both Mpro (15)and PLpro (16) are cysteine proteases involved in the processing of viral polyproteins into functional units (17, 18), whereas the S-protein (19) mediates viral attachment to host cells before invasion. Owing to their critical roles in viral infection, these proteins are frequently targeted using in-silico studies for screening phytochemicals (20-26), FDA-approved drugs (27) (28-31), or other compound libraries(32-34).
This study involves screening of 28 FDA-approved drugs that may show activity or have been debated regarding their potential clinical activities against SARS-CoV-2. The drugs are screened against viral PLpro, Mpro, and spike protein. Molecular docking, within-pocket energy minimization and calculation using MMGBSA (Molecular Mechanics Generalized Born Surface Area) approach, molecular dynamic simulation, then snapshots analysis using MMPBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) approach are the steps involved to come out with potential candidates.

2. Methodology

2.1. Molecular docking

The 3D structures for the drugs (compounds) were downloaded from the ZINC database in sdf format, then prepared by LigPrep wizard in Maestro (Schrödinger suites 2024). The receptor proteins used in this study were retrieved from protein drug bank (PDB) using IDs of; 6LU7 for protease in complex with N3 inhibitor (35), 6WUU for Papain-like protease in complex with peptide inhibitor VIR250 (36) and 6LZG for spike receptor-binding domain complexed with its receptor ACE2 (37). The proteins were prepared in the protein preparation wizard in Maestro. For glide docking preparation, the grid boxes were centered at protein binding sites which is centered on crystallized ligand for each of the proteases, while for the spike protein, the box was centered at Gln493 located at the interface of the spike-ACE2 receptor complex. The grid box dimension was set to 80 Å. The docking was performed with Glide using XP mode. The three top-ranked docked poses were retrieved from each drug. The poses were then subjected to prime calculations using MMGBSA. Two sets of MMGBSA calculations were performed that use either a rigid or a flexible receptor (i.e. allowing receptor residues flexibility that are at a distance of not more than 3Å from ligand atoms).

2.2. Molecular dynamics simulation

The protein-drug complexes from MMGBSA calculations that involve a flexible receptor were converted to PDB file format, while the bound drug molecule was converted into SDF file format. The SDF file of the drug molecule is submitted to the SwissParam server (38) to obtain topology and parameter files based on the CHARMM22 force field. The protein and ligand files were used to generate gro files for GROMACS (version 2023.3) calculations (39). The gro coordinate files were manually combined using a text editor to assemble the final complex. This complex was then placed in a triclinic periodic box filled with Simple Point Charge (SPC) water molecules, maintaining a minimum distance of 1 nm between the protein surface and the box boundaries. The system charge was neutralized by the addition of appropriate counter ions. Temperature coupling was applied using two separate coupling groups: one for the protein-ligand complex and another for the surrounding solvent and ions. The system was subjected to energy minimization for 100,000 steps to relax initial steric clashes. This was followed by a 100 ps equilibration under constant volume (NVT) conditions, gradually heating the system to 300 K using the modified Berendsen thermostat. A subsequent 100 ps equilibration under constant pressure (NPT) at 1 bar was performed. Throughout both equilibration phases, position restraints were maintained on the protein and ligand to preserve structural integrity. Following equilibration, a 20-nanosecond production MD simulation was executed under constant pressure and temperature conditions (40). Subsequently, the GMX_MMPBSA script (ref (41)) was used to calculate binding energy using full production MD trajectory

3. Results and Discussion

3.1. Glide docking and MMGBSA calculations

The docking of the compounds was successful against all receptors except for ivermectin against the spike protein, which could be attributed to the large molecular size. For each compound, three poses from the docking stage were selected for MMGBSA calculations to determine binding energy. Glide docking score does not account for the solvent accessible surface area in ligand–protein interaction. Therefore, relying solely on the best XP Glide score for pose selection may overlook potentially favourable binding configurations. To address this limitation, docking was primarily employed to generate a set of top-ranked poses that fit well within the binding pocket, rather than to rank compounds' affinities to receptors. The MMGBSA calculations were then used to score the top-ranked docked poses (for each compound) to identify those with the most favorable binding energies (42,43). The docking and MMGBSA scores for PLpro, Mpro, and spike are summarized in tables 1 to 3. According to the results, the crystallized inhibitors of VIR250, N3, and ACE2 yielded the best score at the end of MMBGSA calculations (-64.32, -68.34, and -108.76 Kcal/Mol, respectively). The binding energy of crystallized ACE2 scores the highest due to the large interaction interface with the spike protein compared to the small peptidomimetics crystallized protease inhibitors, which have almost equivalent binding energy scores.

3.1.1. PLpro

With respect to PLpro, the binding pocket is shown to have a pincer-like structure composed of Tyr268, Cys270,, and Gly271, which represent the upper jaw, and Leu162, which represents the lower jaw (Figure 1). The binding energy calculations showed that the top-ranked drugs of hydroxychloroquine, ambroxol, chlorpromazine, and ranitidine score between -50 to almost -60 Kcal/Mol. While Omeprazole, Ofloxacin, Mebendazole, Metoclopramide, Azithromycin, Clarithromycin, and Famotidine score almost between -46 and -50 Kcal/Mol (**Table 1**).
The interaction diagram with the binding pocket showed that VIR250 established almost 5 H-bonds and an

electrostatic interaction with Arg166. On the other hand, the top-ranked drugs established almost single H-bonds except ambroxol (three H-bonds), while only ranitidine interacts electrostatically through the carboxylic group to Arg166. Solvent-exposed surface area is minimum for the drugs and comparable to VIR250.

During free energy calculations for docked hydroxychloroquine, it partly fit the binding pocket; however, with its aliphatic OH group flipped upward and interacting with the upper jaw amino acid of Tyr268 through H-bond as well as by pi-pi stacking using quinoline rings. Thus, hydroxychloroquine makes three interactions with the catalytic amino acid Tyr268. The protonated tertiary amine is donating H-bond to Tyr264. The MMGBSA calculation, which involved flexible pocket residues, provides the best score of -58.72 Kcal/mol while the amino acids of Gly163 and Tyr268 change conformation during calculations.

The same previously mentioned amino acids are also involved during ambroxol calculation in order to provide complete pocket adaptation to the compound except for the two bromine atoms, which remain solvent exposed. Ambroxol gives two H-bonds using aromatic amine to each of Asp164 (a catalytic amino acid) and Thr301, as well as another two H-bonds using aliphatic amine and hydroxyl groups to each of Tyr273 and Tyr268, respectively. In addition, ambroxol is involved in an electrostatic interaction with the aliphatic amine and Tyr264. The calculated binding energy was −55.42 kcal/mol with the flexible pocket and −46.93 kcal/mol with the rigid pocket, the latter being the most favorable score among all compounds tested, indicating a superior fit to the rigid binding site compared with other ligands.

Chlorpromazine makes pi-pi stacking with Tyr268, as well as a halogen bond (44) with a lone pair of electrons of both aromatic hydroxyl of Tyr273 and backbone amine of Arg166. The protonated tertiary amine interacts with a hydrogen bond to the backbone carbonyl of Gly163 by H-bond. The flexible amino acids include Gly163 and Tyr264 (which form the back of the pocket close to the catalytic amino acids).

For ranitidine, it interacts electrostatically using the negatively charged nitro group with the positively charged side chain of Arg166. Both the imine and the secondary amine of the compound interact through electrostatic interaction with both Asp302 and Asp164, while the secondary amine is involved in an H-bond to Asp164 as well. The furan ring is in pi-pi stacking with Tyr264.

With respect to omeprazole, the pyridine ring established pi-pi stacking with Tyr264 and H-bond using indole amines with the side chain hydroxyl of Thr301. Although omeprazole scores worse considering the rigid pocket (-33.95 Kcal/Mol), the flexibility of Tyr264 improves the pocket fitness and provides a final score of -49.40 Kcal/mol.

According to docking and MMGBSA calculations, none of the compounds were able to extend throughout the binding pocket and between the two jaws as observed for VIR250. The MMGBSA calculated binding energy using a flexible pocket for VIR250 was -64.32 Kcal/Mol, which could not be achieved by any of the compounds; however, considering rigid body calculation, VIR250 scores -47.15 Kcal/Mol, which is close to that of ambroxol. It is noted that none of the compounds is close enough to the Cys111 residue nor suitable for Michael's addition as for VIR250. Unlike all of the docked ligands, the peptidomimetics of VIR250 extend throughout the binding site between the upper and the lower jaws' amino acids. At the same time, most of the docked ligands are similar to VIR250 in occupying the catalytic site, which includes Gly163, Asp164, and Tyr273 (45). Compared to the other proteins, the ligand efficiency (i.e. binding energy per surface area) from MMGBSA calculations for the top 10 ranked compounds was lower than with other proteins, except for ambroxol and ranitidine, which may indicate lower pocket fitness, less efficient mutual use of binding sites in protein and ligands, or higher solvent-exposed surface area for both of the protein and the ligand.

Table 1. The scores of XP Glide docking, MMGBSA using rigid receptor and MMGBSA using flexible receptor binding pocket against PLpro

rank	Drug	XP GScore	MMGBSA ΔG Bind (rigid receptor) Kcal/Mol	MMGBSA ΔG Bind (flexible pocket) Kcal/Mol	Prime MMGBSA ligand efficiency
0	PEPTIDE INHIBITOR VIR250	Crystal	-47.15	-64.32	-1.79
1	Hydroxychloroquine	-5.54	-40.58	-58.72	-1.76
2	Ambroxol	-4.96	-46.93	-55.42	-2.61
3	Chlorpromazine	-4.5	-40.5	-54.46	-1.93
4	Ranitidine	-5.67	-41.29	-51.26	-1.97
5	Omeprazole	-4.74	-33.95	-49.4	-1.41
6	Ofloxacin	-4.06	-44.64	-49.29	-1.72
7	Mebendazole	-3.62	-38.75	-48.32	-1.76
8	Metoclopramide	-5.16	-30.56	-48.21	-1.53
9	Azithromycin	-6.03	-40.76	-47.45	-0.78
10	Clarithromycin	-6.39	-24.58	-45.75	-0.47
11	Famotidine	-5.07	-39.09	-45.63	-1.95
12	Bromhexine	-4.26	-32.65	-43.3	-1.81

13	Rifampicin	-3.07	-8.95	-42.24	-0.15
14	Ciprofloxacin	-4.04	-32.3	-42.08	-1.35
15	Scopolamine	-3.88	-40.35	-41.22	-1.83
16	Oseltamivir	-5.65	-38.1	-40.78	-1.73
17	Nitazoxanide	-2.46	-30.03	-38.21	-1.43
18	Zanamivir	-6.53	-22.79	-32.41	-0.99
19	Fluconazole	-3.86	-28.27	-32.09	-1.28
20	Sulfamethoxazole	-1.09	-17.14	-28.58	-1.01
21	Moroxydine	-5.19	-27.01	-28.49	-2.25
22	Tinidazole	-2.25	-19.44	-27.04	-1.21
23	Metronidazole	-3.05	-19.92	-24.93	-1.66
24	Simethicone	-2.08	-19.83	-24.21	-1.32
25	Ivermectin	-5.33	-20.71	-21.69	-0.33
26	Naproxen	-3.06	-10.44	-19.26	-0.61
27	Clavulanic acid	-3.44	-6.32	-10.71	-0.45
28	Captopril	-2.5	16.26	2.32	1.16

3.1.2. Mpro

Table 2), which could be attributed to better fitness due to the absence of a pincer-like structure in the binding pocket. However, the binding pocket of Mpro appears to be deeper than that of PLpro (**Error! Reference source not found.**). Docking and MMGBSA calculations against the protease enzyme revealed that the highest score was for rifampicin, which occupies the pocket and donates two H-bonds to Glu166 and one to Gln189, and accepts H-bonds from Gln189 and Asn142, while the ionized tertiary amine of piperazine is in electrostatic interaction with Glu166. Due to the smaller size, hydroxychloroquine goes deeper; however, part of the quinoline ring – including the chlorine atom- is solvent exposed. Hydroxychloroquine donates H-bond to the aromatic hydroxyl of Tyr54 and the main chain carbonyl of Phe140 using an aliphatic hydroxyl and the ionized imine of the quinoline ring, respectively. Ranitidine fits the pocket and uses the aliphatic secondary amine to donate H-bond to the main chain carbonyl of Glu166, while using the nitro group to accept H-bond from the main chain amine of Gln192. The ionized tertiary amine of ranitidine is in cation-Pi stacking with the imidazole ring of His41.

Similar to rifampicin, azithromycin also covers the pocket and donates H-bonds to the side chain carboxylic group of Glu166 and the side chain carbonyl of Asn142 using macrocyclic hydroxyls, as well as donates and accepts H-bonds with the side chain\n carbonyl of Gln189 using cladinose sugar hydroxyl and methoxy group, respectively. While the macrocyclic hydroxyl, accepting H-bonds from the amino side chain of Gln189, the ionized tertiary amine is in cation-Pi stacking with His41.

Scopolamine accepts H-bond from Gln189 side chain using the ester group, as well as in cation-Pi stacking with His41 using the phenyl group, and in ionic interaction with Glu166 using the ionized tertiary amine. It is noted that N3 crystal inhibitor interaction with Mpro is stabilized by seven H-bonds with the pocket, including 3 H-bonds with Glu166, while the rest are with Thr190, Gln189, His164, and Phe140, using the amino and hydroxyl groups of N3. Similarly, most of the docked compounds establish from 1 to 3 bonds with the Glu166, especially for rifampicin and azithromycin. It is noted that most of the top 10 ranked compounds showed higher ligand efficiency in comparison with rifampicin, azithromycin, and N3 (the crystal inhibitor), which indicate good fitness within the pocket and minimal solvent exposure.

With respect to Mpro, the calculated binding energies for the compounds are relatively better compared to PLpro (

Table 2. The XP Glide docking score and MMGBSA energy calculated using rigid and flexible receptor pocket for Mpro

rank	Drug	XP GScore	MMGBSA ΔG Bind (rigid receptor) Kcal/Mol	MMGBSA ΔG Bind (flexible pocket) Kcal/Mol	Prime MMGBSA ligand efficiency
0	N3	crystal	-56.21	-68.34	-1.4
1	rifampicin	3.78	-37.58	-67.12	-1.14
2	hydroxychloroquine	-5.23	-52.15	-66.81	-2.9
3	ranitidine	-4.41	-44.17	-57.72	-2.75
4	azithromycin	-3.28	-37.65	-57.08	-1.1

5	scopolamine	-5.72	-50.29	-56.24	-2.56
6	oseltamivir	-5.57	-51.82	-55.75	-2.53
7	metoclopramide	-6.32	-38.79	-53.78	-2.69
8	chlorpromazine	-4.76	-51.41	-53.65	-2.55
9	ambroxol	-5.25	-47.57	-53.52	-2.97
10	omeprazole	-4.82	-43.45	-52.54	-2.19
11	ciprofloxacin	-6.12	-49.94	-52.4	-2.18
12	ivermectin	-4.37	-40.71	-52.2	-0.84
13	ofloxacin	-5.2	-34.93	-48.43	-1.86
14	fluconazole	-4.71	-37.79	-47.98	-2.18
15	bromhexine	-1.48	-37.88	-47.88	-2.66
16	clarithromycin	-3.69	-42.34	-47.11	-0.91
17	famotidine	-4.4	-43.88	-47.07	-2.35
18	mebendazole	-4.4	-41.04	-46.66	-2.12
19	nitrazoxanide	-4.35	-39.1	-45.29	-2.16
20	sulfamethoxazole	-3.82	-36.95	-39	-2.29
21	zanamivir	-6.44	-34.45	-38.21	-1.66
22	moroxydine	-3.11	-31.76	-37.26	-3.11
23	simethicone	-4.72	-30.54	-37.13	-2.48
24	tinidazole	-3.7	-33.53	-35.67	-2.23
25	captopril	-4.14	-29.06	-33.37	-2.38
26	naproxen	-4.31	-21.92	-30.32	-1.78
27	metronidazole	-2.97	-21.89	-27.63	-2.3
28	clavulanic acid	-3.82	-8.07	-20.26	-1.45

3.1.3. Spike protein

For spike protein, large molecules like rifampicin and azithromycin score the highest MMGBSA calculations illustrated in Table3.

The 3D interaction as well as the 2D interaction diagram are shown in. Rifampicin accepts H-bonds using the carbonyl of dihydrofuran and the aliphatic hydroxyl groups and imino group from the side chains of Arg403 and Gln493, main chain amine of Gly496 and side chain phenol of Tyr449, respectively.

While rifampicin donates H-bonds using its aliphatic hydroxyl groups to the side chain phenol of Tyr453 and the main chain carbonyl of Ser494, the piperazine ring is solvent exposed completely. With respect to clarithromycin, only the macrocyclic ring fits the pocket while both sugar moieties are solvent-exposed. It mutually donates and accepts H-bonds with each of Gly496 and Asn105 using its macrocyclic hydroxyl and carbonyl groups. The cladinose methoxy group accepts H-bond from Tyr505. Ofloxacin only accepts H-bonds from Gl498 and Tyr449 using its carboxylic group, and from Tyr453 using the piperazine amine.

Ciprofloxacin binds differently from ofloxacin and donates only a single H-bond to Tyr453 using the carboxylic group. The binding site of hydroxychloroquine is slightly different from the other compounds. It donates H-bond to Ser494 and Leu492 using the aliphatic hydroxyl and amino groups, respectively. In addition, hydroxychloroquine makes an electrostatic interaction with Glu494 using the ionized aromatic heterocyclic amine, where the ring also makes pi-pi stacking with Phe490. While in terms of ligand efficiency, hydroxychloroquine, ciprofloxacin, and ofloxacin showed higher involvement of heteroatoms in receptor binding compared to rifampicin and clarithromycin.

Table 3. The XP Glide docking score and MMGBSA energy calculated using rigid and flexible receptor pocket for spike protein

rank	Drug	XP GScore	MMGBSA ΔG Bind (rigid receptor) Kcal/Mol	MMGBSA ΔG Bind (flexible pocket) Kcal/Mol	Prime MMGBSA ligand efficiency
0	ACE2	crystal	-122.68	-108.76	-0.02
22	rifampicin	-2.923	-42.14	-57	-0.966
7	clarithromycin	-2.834	-51.22	-52.59	-1.011
18	ofloxacin	-2.366	-18.79	-47.06	-1.810
6	ciprofloxacin	-2.388	-33.73	-45.88	-1.912
11	hydroxychloroquine	-3.287	-41.29	-45.65	-1.985
12	mebendazole	-3.508	-36	-44.96	-2.044
19	omeprazole	-3.844	-31.8	-44.84	-1.868
5	chlorpromazine	-2.922	-26.6	-44.7	-2.129
20	oseltamivir	-2.506	-33.94	-43.38	-1.972
21	ranitidine	-3.733	-43.6	-43.34	-2.064
17	nitrazoxanide	-3.046	-34.09	-42.85	-2.040
9	famotidine	-3.634	-26.51	-39.92	-1.996
10	fluconazole	-3.901	-33.06	-38.62	-1.755
1	ambroxol	-3.661	-29.96	-38.02	-2.112
23	scopolamine	-3.359	-35.52	-37.9	-1.723
13	metoclopramide	-3.24	-33.27	-35.92	-1.796
25	sulfamethoxazole	-5.023	-38.69	-35.27	-2.075
3	bromhexine	-2.105	-34.23	-34.44	-1.913
14	metronidazole	-4.03	-29.62	-32.71	-2.726
2	azithromycin	-3.679	-29.16	-31.82	-0.612
8	clavulanic acid	-4.635	-27.03	-31.17	-2.226
16	naproxen	-2.937	-19.54	-28.64	-1.685
27	zanamivir	-6.403	-26.33	-26.83	-1.167
4	captopril	-3.112	-10.72	-25.98	-1.856
24	simethicone	-1.838	-21.23	-22.61	-1.507
26	tinidazole	-2.601	-14.27	-20.73	-1.295
15	moroxydine	-2.911	-17.68	-17.11	-1.426
28	ivermectin	not docked	ND	ND	ND

3.2. Molecular dynamic simulation and MMPBSA calculations

Molecular dynamic (MD) simulation is usually used to verify the stability and the binding energy of docking results over variable ligand-receptor conformations. The average potential and kinetic energies, along with the calculated MM-PBSA terms, are summarized in **Table 4**. The stability of the system during the production MD simulation was judged by the stability of kinetic energy, potential energy, temperature, and protein heavy atoms' RMSD (46). The low standard deviations observed, which were consistent across individual protein simulations, indicate stable simulation systems.While MMGBSA is

considered faster and performs better for ranking binding affinities, MMPBSA is more rigorous and can perform better in predicting absolute binding free energies (47). The binding energies from MMPBSA calculations indicate the highest affinity for VIR250 (-42 Kcal/Mol), N3 (-38 Kcal/Mol), and ACE2 receptor (-31 Kcal/Mol) against PLpro, Mpro and spike protein, respectively. The highest solvation penalty was associated with ACE2 (582 Kcal/Mol), which severely affects binding. The ACE2 receptor is simulated free in solution (i.e., not as an actual membrane-bound), which leads to higher exposure of the non-polar surface (48). In addition, the spike is simulated as a single monomer rather than the full trimeric spike glycoprotein (49). All of the simulations provided ligand RMSD of less than 0.4 Å over 20 ns of simulation, which indicates ligand stability within the pocket. It can be noticed that ligands with higher MMGBSA efficiency during Glide+MMGBSA calculations are frequently top-ranked in MD+MMPBSA calculations. The only exception was for rifampicin in the pockets of Mpro and spike protein, which shows better scoring during MMPBSA calculations. Giving full flexibility and explicit water solvents, rifampicin could convert solvent-exposed groups to be involved in receptor interactions.

Table 4. Energy values calculated from MD simulation followed by MMPBSA analysis are provided as mean ± SD

	MD simulation				MMPBSA (Kcal/Mol)						
Mean energy (Kcal/Mol)	Potential energy (Kcal/Mol)	Kinetic energy (Kcal/Mol)	Temp (Kelvin)	RMSD * (Å)	ΔVDWAALS	ΔEEL	ΔEGB	ΔESURF	ΔGGAS	ΔGSOLV	ΔTOTAL
Papain											
VIR250	-680920.5 ± 1060	139292.8 ± 678.1	299.99 ±1.46	0.27 ± 0.04	-44.94±3.63	-43.99±6.09	53.03± 4.87	-6.10±0.32	-88.93± 6.91	46.93± 4.78	-42.00± 3.83
ambroxol	-679958.5 ± 1049.4	138863.3 ± 661.7	299.99 ±1.43	0.09 ± 0.02	-25.16±3.81	-102.07± 14.14	98.18± 12.14	-3.82±0.35	-127.23 ±13.68	94.36± 12.08	-32.87± 4.57
famotidine	-679958.5 ± 1049.5	137653.9 ± 651.5	300 ±1.42	0.27 ± 0.03	-35.15±3.96	-97.31±3 3.51	106.85 ±28.01	-4.86±0.38	-132.46 ±33.83	102.00 ±27.75	-30.46± 7.27
ranitidine	-684075.4 ± 1053.9	139520 ± 666.2	299.96 ±1.43	0.25 ± 0.03	-28.08±3.19	-179.03± 18.11	182.82 ±17.13	-5.03±0.21	-207.12 ±18.56	177.79 ±17.05	-29.32± 3.36
chlorpromazine	-678961.5 ± 1082.6	138705.8 ± 672	300 ±1.45	0.29 ± 0.04	-27.00±3.15	-87.09±12.87	90.87± 12.70	-3.72±0.35	-114.09 ±13.68	87.14± 12.57	-26.94± 3.14
metoclopramide	-663159.7 ± 1058.6	135773 ± 658.9	299.99 ±1.46	0.28 ± 0.03	-34.32±2.96	-82.44±13.36	97.66± 13.34	-5.08±0.25	-116.75 ±13.63	92.58± 13.33	-24.18± 3.21
hydroxychloroquine	-678780.7 ± 1085.7	138729.1 ± 656.9	300.01 ±1.42	0.28 ± 0.03	-32.15±3.45	-80.17±13.53	95.32± 13.64	-4.48±0.35	-112.32 ±14.24	90.84± 13.51	-21.48± 4.42
clarithromycin	-679958.5 ± 1049.5	138863.3 ± 661.7	300.03 ±1.48	0.31 ± 0.06	-28.25±3.51	-62.52±10.44	74.68± 10.67	-3.82±0.50	-90.77± 11.88	70.86± 10.44	-19.91± 3.22
omeprazole	-670917.6 ± 1057.1	137263.7 ± 652.5	299.94 ±1.43	0.28 ± 0.07	-28.19±2.70	-27.09±8.11	39.55± 6.67	-4.11±0.26	-55.28± 8.50	35.44± 6.54	-19.84± 3.50
azithromycin	-671549.6 ± 1069.7	137653.9 ± 651.5	300 ±1.42	0.28 ± 0.04	-26.21±4.04	-51.07±20.93	62.97± 18.03	-3.93±0.67	-77.28± 22.09	59.04± 17.60	-18.24± 6.40
ofloxacin	-678197.5 ± 1074	138777.1 ± 667.5	299.99 ±1.44	0.32 ± 0.05	-25.93±4.44	-14.62±14.04	25.46± 12.66	-2.96±0.66	-40.55± 17.01	22.50± 12.08	-18.05± 5.50
mebendazole	-678987.5 ± 1100.9	138730.8 ± 666.4	299.97 ±1.44	0.31 ± 0.03	-18.17±8.41	-19.62±15.64	25.32± 15.15	-2.49±1.08	-37.80± 22.41	22.83± 14.21	-14.97± 8.83
Protease											
N3	-609860.7 ± 991.9	124843.3 ± 621.3	300 ± 1.49	0.25 ± 0.03	-49.17±6.76	-20.98±5.55	38.31± 5.21	-6.52±0.94	-70.14± 9.66	31.79± 4.61	-38.36± 6.35
rifampicin	-609743.4 ± 990.3	124757.7 ± 636.5	299.97 ± 1.53	0.28 ± 0.04	-39.23±5.89	-156.45± 14.29	168.41 ±13.93	-5.79±0.79	-195.67 ±16.58	162.62 ±13.57	-33.05± 6.22
oseltamivir	-608913.3 ± 996.6	124738.8 ± 618.8	300.03 ± 1.49	0.22 ± 0.02	-35.11±3	-9.36±4.	20.65± 4.47	-4.61±0.	-44.47±	16.04± 4.32	-28.43±

					.12	64		30	6.15		2.99
ofloxacin	-610320.5 ± 1005.9	124773 ± 635.3	299.98 ± 1.53	0.24 ± 0.03	-32.98±3.81	-13.17±5.14	25.61± 3.86	-4.30±0.46	-46.15± 5.64	21.31± 3.67	-24.83± 3.42
ciprofloxacin	-610380.7 ± 984.1	124783.7 ± 633.4	300.02 ± 1.52	0.23 ± 0.03	-35.51±2.25	-70.42±7.72	85.52± 8.15	-4.05±0.21	-105.93 ±7.91	81.47± 8.10	-24.46± 3.26
chlorpromazine	-610190 ± 968.6	124815.3 ± 632.5	300.01 ± 1.52	0.20 ± 0.02	-31.37±4.42	-89.14±12.48	101.00 ±12.73	-3.40±0.58	-120.52 ±15.07	97.60± 12.38	-22.92± 4.33
ambroxol	-610005.2 ± 990.9	124732.3 ± 611.4	300 ± 1.47	0.24 ± 0.07	-28.82±3.50	-4.93±4.61	14.61± 3.25	-3.28±0.38	-33.75± 6.57	11.33± 3.02	-22.43± 4.19
metoclopramide	-610510.5 ± 985.6	124764 ± 635.8	300.01 ± 1.53	0.19 ± 0.02	-28.11±3.03	-5.47±3.72	16.87± 3.20	-3.82±0.37	-33.58± 5.11	13.05± 3.04	-20.54± 3.26
hydroxychloroquine	-609699.7 ± 974.1	124807.4 ± 632.7	300.05 ± 1.52	0.26 ± 0.04	-29.42±3.82	-93.84±16.54	108.81 ±15.64	-4.13±0.43	-123.26 ±17.18	104.68 ±15.43	-18.58± 3.15
ranitidine	-607485.3 ± 961	124813.9 ± 635.6	300.02 ± 1.53	0.21 ± 0.03	-27.05±4.32	-94.19±18.38	110.59 ±19.18	-3.77±0.56	-121.24 ±20.06	106.82 ±18.89	-14.42± 4.33
scopolamine	-609860.7 ± 991.9	124857 ± 649.9	300.03 ± 1.56	0.21 ± 0.02	-24.58±4.26	-82.49±12.71	98.83± 11.71	-3.20±0.51	-107.07 ±12.41	95.63± 11.71	-11.44± 3.92
azithromycin	-609743.4 ± 990.3	124752.1 ± 623	300 ± 1.5	0.20 ± 0.02	-19.19±7.45	-145.90± 34.34	156.48 ±34.42	-2.68±1.05	-165.10 ±39.19	153.79 ±33.62	-11.30± 6.97
Spike											
ACE	-1173040.2 ± 1248.3	243971.2 ± 909	299.97 ± 1.12	0.20 ± 0.02	-65.5±31.8	-548.3±164.8	592.6± 185.0	-10.0±4.9	-613.8± 195.5	582.6± 180.2	-31.2±15.8
famotidine	-360108.8 ± 772.2	73554.6 ± 486.1	300.01 ± 1.98	0.20 ± 0.02	-20.1±3.8	-62.1±26.7	61.5±18.0	-3.4±0.3	-82.3±25.4	58.1±17.9	-24.2±9.7
rifampicin	-355946.7 ± 775.9	73686.9 ± 490.4	300 ± 2.00	0.17 ± 0.01	-29.5±3.8	-51.5±7.6	64.1±6.8	-3.9±0.6	-81.0±9.3	60.3±6.5	-20.7±4.2
ciprofloxacin	-356417.1 ± 769.9	73564.8 ± 494.7	300.03 ± 2.02	0.17 ± 0.01	-24.1±3.5	10.8±18.2	-2.2±13.0	-3.5±0.4	-13.3±16.6	-5.7±12.8	-19.0±6.1
chlorpromazine	-357657.3 ± 776.4	73567.3 ± 479.5	300.01 ± 1.96	0.20 ± 0.02	-22.8±6.3	38.8±11.7	-30.1±9.7	-2.7±0.7	16.0±10.0	-32.8±10.0	-16.8±5.0
hydroxychloroquine	-357873 ± 761.6	73658.6 ± 485.9	300.07 ± 1.98	0.18 ± 0.02	-11.2±3.7	-59.0±14.9	56.3±12.7	-2.3±0.5	-70.2±14.0	53.9±12.6	-16.3±3.2
omeprazole	-357656.6 ± 787.9	73611.2 ± 479.6	300.12 ± 1.96	0.19 ± 0.02	-9.1±5.0	-63.4±29.8	61.4±28.9	-1.9±0.9	-72.5±33.5	59.6±28.1	-12.9±6.1
ofloxacin	-356658.2 ± 790	73621.4 ± 487.9	300.04 ± 1.99	0.18 ± 0.02	-12.5±5.9	-63.5±31.9	69.2±31.2	-2.0±0.8	-76.0±33.7	67.2±30.7	-8.8±5.0
clarithromycin	-356020.4 ± 796.4	73735.3 ± 485.8	299.99 ± 1.98	0.19 ± 0.02	-12.7±10.8	-3.5±7.1	9.2±8.5	-1.8±1.6	-16.2±15.6	7.5±7.3	-8.7±9.1
nitrazoxanide	-357185.4 ± 780.3	73574 ± 481.5	300.04 ± 1.96	0.18 ± 0.01	-14.7±5.9	-98.5±19.5	106.9± 19.0	-2.3±0.7	-113.2± 20.1	104.6± 18.6	-8.6±3.0
ranitidine	-357782.3 ± 754.7	73577.8 ± 479.8	300.09 ± 1.96	0.17 ± 0.01	-15.1±5.2	18.6±16.9	-7.7±18.0	-2.4±0.8	3.6±19.6	-10.1±17.5	-6.5±3.8
mebendazole	-359082.1 ± 769.4	73553.2 ± 467.8	300.03 ± 1.91	0.19 ± 0.02	-1.6±3.7	-55.8±123.9	56.6±121.9	-0.7±0.7	-57.4±123.9	55.9±121.4	-1.5±3.0

* RMSD is provide for protein heavy atoms.

3.3. Comparison of top-ranked compounds from MD simulation with reported activities

The results from this study may agree or conflict with the results from previous in-silico, enzymatic inhibition and clinical studies, which can provide an integrated perspective about drug repurposing for COVID treatment. Hydroxychloroquine has not demonstrated clinical benefit in either severe (50) or mild (51) COVID-19 infection, with debate about its preventive effect (52). However, hydroxychloroquine shows *in vitro* antiviral activity (53) that is synergistic with azithromycin (54) in addition to its anti-inflammatory effect (53). In our computational analyses, hydroxychloroquine displayed moderate binding energy toward PLpro, Mpro, and spike proteins (-22, -19, and -16 Kcal/Mol, respectively) while azithromycin displayed even weaker binding energies against each of PLpro and Mpro (-18 and -11 Kcal/Mol, respectively).

Although *in-silico* studies suggested that famotidine may interact with viral proteases and the spike protein (55) , experimental evidence demonstrates no measurable inhibition of viral proteases or viral replication (56), and no clear clinical benefit (57). This discrepancy likely reflects a bias in computational predictions, possibly arising from the multiple hydrogen-bond donor and acceptor sites within the famotidine molecule, which can artificially enhance predicted binding scores.

Data on *in-silico* studies of ambroxol activities against SARS-CoV-2 viral proteins remain limited. Experimentally, ambroxol has been reported to inhibit viral titers (58), the TMPRSS2 protease (59), and the viral spike protein (60). However, the reported spike inhibition was derived from a bell-shaped dose–response curve with notable variation between replicates (60). Furthermore, the observed effect on viral attachment may be attributed to the interference with the human ACE2 receptor rather than to the direct spike protein inhibition (61). Consistently, our computational results showed no significant binding of ambroxol to the viral spike protein. However, the activity was observed against viral proteases, with calculated binding energies of −33 kcal/mol for PLpro (compared to −42 kcal/mol for the crystal inhibitor VIR250) and −22 kcal/mol for Mpro (compared to −38 kcal/mol for the crystal inhibitor N3).

In-silico studies have reported the potential activity of rifampicin against SARS-CoV-2 viral proteases (62-64). Consistently, our results indicate a relatively high binding affinity of rifampicin to Mpro and the spike protein, with calculated binding energies of −33 and −21 kcal/mol, respectively. The predominant energetic penalty associated with rifampicin binding arises from desolvation, estimated at 163 kcal/mol for Mpro and 62 kcal/mol for the spike protein. The substantially higher desolvation cost for Mpro reflects the confined nature of its binding pocket, which limits ligand accommodation and leaves a large portion of the ligand surface solvent-exposed, in contrast to the more accessible binding site of the spike protein. To date, no experimental data are available for rifampicin targeting these viral proteins, except for its reported activity on NA-dependent RNA polymerase (65).

Regarding ciprofloxacin, in-silico studies predict interactions with Mpro (66)and the spike protein (67), which are consistent with our calculated binding energies of −25 and −19 kcal/Mol, respectively. Experimentally, ciprofloxacin and related analogues have been reported to reduce SARS-CoV-2 viral titers at sub-micromolar concentrations in cell-based assays (58, 68, 69). However, direct biochemical evidence of ciprofloxacin acting on isolated viral proteins in orthogonal assays remains limited

Chlorpromazine has been experimentally shown to inhibit SARS-CoV-2 replication in cell-based assays (70, 71). However, biochemical enzyme assays providing direct evidence of chlorpromazine binding to individual viral proteins remain limited. In this context, our computational findings suggest that the activity of chlorpromazine could be mediated through interactions with PLpro, Mpro, and the spike protein, exhibiting binding energies of -27, -23, and -17 Kcal/Mol, respectively.

The outcomes of this study are consistent with other computational studies reported for azithromycin and hydroxychloroquine (72), ivermectin (73). All of captopril, ivermectin, clavulanic acid, naproxen, simethicone, metronidazole, tinidazole, moroxydine, sulfamethoxazole, fluconazole, zanamivir, and famotidine showed lower affinity against all of the tested proteins in both molecular docking and molecular dynamics-derived MMGBSA calculations.

4. Conclusion

Molecular docking and MMGBSA calculations of 28 FDA-approved compounds against Mpro, PLpro, and spike protein indicate possible activity of hydroxychloroquine, ambroxol and chlorpromazine against PLpro, while rifampicin, hydroxychloroquine and ranitidine give the best scores on Mpro. On the other hand, rifampicin, clarithromycin, and ofloxacin were mainly active on spike protein. The top eleven ranked compounds from MMGBSA calculations were subjected to MD simulation and MMPBSA calculation. Accordingly, the ranking of compounds is slightly changed since MMPBSA follows rigorous analysis of MD snapshots, thus better precision. The MD simulation involves explicitly represented water molecules as well as thermally controlled flexibility for both of compound's and receptor's atoms that provide potentials for water bridges, better measurement for solvation as well as overcome of local energy minima. The MMPBSA results showed that ambroxol, famotidine and ranitidine were mainly active on PLpro, while rifampicin, oseltamivir, and ofloxacin showed higher affinity for Mpro. On the other hand, famotidine, rifampicin, and ciprofloxacin score best for spike protein. The higher affinity of ambroxol toward PLpro, and rifampicin toward Mpro and spike protein indicate potential inhibitory activity which compel further in vitro and/or ex-vivo experimental validation. The results from this study showed consistency with some previous in-silico studies, ruled out some drugs from potential activity, and explained the potential mechanism for observed experimental activity against SARS-CoV-2 proteins.

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دراسة الالتحام والديناميكيات الجزيئية وطاقة الاتحاد لأدوية معتمدة من إدارة الغذاء والدواء الأمريكية ضد الانزيمات المحللة للبروتينات وبروتين سبايك الخاصة بفايروس كورونا

الخلاصة: سرعان ما تحول مرض فيروس كورونا 2019 (كوفيد-19)، الناجم عن فيروس كورونا 2 المسبب لمتلازمة الالتهاب التنفسي الحاد الوخيم (سارس-كوف-2)، إلى حالة طوارئ صحية عالمية. وعلى الرغم من الجهود البحثية المكثفة، إلا أن غياب علاجات مضادة للفيروسات فعالة ومحددة تمامًا خلال المراحل المبكرة من الجائحة أبرز الحاجة المُلحة إلى استراتيجيات علاجية بديلة. ولذلك، اكتسبت إعادة استخدام الأدوية اهتمامًا متزايدًا كنهج سريع وفعال من حيث التكلفة لتحديد عوامل مضادة للفيروسات محتملة من بين الأدوية المعتمدة من إدارة الغذاء والدواء الأمريكية. **الهدف**: هدفت هذه الدراسة إلى تحديد أدوية واعدة قابلة لإعادة الاستخدام ومعتمدة من إدارة الغذاء والدواء الأمريكية، ذات نشاط تثبيطي محتمل ضد بروتينات رئيسية لفيروس سارس-كوف-2، باستخدام نهج حسابي متكامل. **الطرق**: تم فحص 28 دواءً معتمدًا من إدارة الغذاء والدواء الأمريكية حسابيًا ضد ثلاثة أهداف حيوية لفيروس سارس-كوف-2: البروتياز الشبيه بالباباين، والبروتياز الرئيسي، وبروتين السنبلة. تم إجراء عملية الإرساء الجزيئي، تلتها عملية تحسين مواقع الارتباط وتقدير طاقة الارتباط الحرة باستخدام طريقة MM-GBSA. استنادًا إلى أداء الالتحام، خضعت أفضل أحد عشر مركبًا لمزيد من التقييم باستخدام محاكاة الديناميكا الجزيئية (MD) لتقييم استقرار معقدات الربيطة-البروتين. أُعيد حساب طاقات الارتباط الحرة باستخدام منهجية MM-PBSA. **النتائج:** حددت تحليلات الالتحام الأولية وMM-GBSA هيدروكسي كلوروكين، وريفامبيسين، وأمبروكسول، وكلوربرومازين، وكلاريثروميسين، ورانيتيدين، وأوفلوكساسين كمرشحين ذوي أداء عالٍ ضد أهداف فيروس SARS-CoV-2. أظهرت محاكاة الديناميكا الجزيئية اللاحقة تفاعلات ارتباط مستقرة للأمبروكسول، والريفامبيسين، والفاموتيدين، والرانيتيدين، والأوسيلتاميفير، والأوفلوكساسين، والسيبروفلوكساسين. أظهرت هذه المركبات طاقات ارتباط حرة مواتية وحافظت على استقرارها البنيوي طوال فترة المحاكاة. **الاستنتاج:** حددت عملية المحاكاة الحاسوبية المتكاملة العديد من الأدوية المعتمدة من إدارة الغذاء والدواء الأمريكية (FDA) ذات تفاعلات مستقرة ومواتية من الناحية الطاقية مع بروتينات رئيسية لفيروس SARS-CoV-2. تدعم هذه النتائج إمكانية إعادة استخدام مركبات مختارة، ولا سيما الأمبروكسول والريفامبيسين، كعوامل مضادة للفيروسات محتملة ضد فيروس سارس-كوف-2. ويلزم إجراء المزيد من التجارب والدراسات السريرية لتأكيد فعاليتها العلاجية.

الكلمات المفتاحية: فايروس كورونا انزيم المحلل للبيبتاد بيبتيديز البروتين الشوكي الديناميكا الجزيئية التلاحم الجزيئي ميكانيكا جزيئية مع بواسون بولتزمان للمذيب ومساحة السطح ميكانيكا جزيئية مع بورن العام للمذيب ومساحة السطح