Repurposing Alzheimer's Therapeutics: A Computational Approach To Identifying Potential SARS-Cov-2 Mpro Inhibitors

Calvin R. Wei[1]*, Chenyue Fan[2], Muhammad Osama[3], and Godwin C. Lang'at[4]

Abstract

Introduction: SAR-CoV-2, the virus causing the novel coronavirus disease (Covid-19) and global pandemic, is associated with severe complications and mortality. The WHO has identified five variants of concern (VOCs), with the Alpha, Beta, Gamma, Delta, and Omicron being widespread across the world. SARS-CoV-2 is also capable of random mutations, with its genetic alterations affecting the viral phenotype and influencing transmissibility, virulence, and clinical severity. Exhibiting that the virus is prone to resisting antiviral therapies and vaccines. The uncertainties about the status of SARS-CoV-2 and the future of the pandemic, the inadequacy of the present vaccines and treatments, and the likelihood for further variants of concerns require further investigation on drug repurposing that can be used to treat Covid-19.

Method: In this study, we investigate 30 potential Alzheimer Disease related molecules in targeting SARS-CoV-2 main protease (Mpro) (PDB ID: 6LU7) using molecular docking in Schrodinger Suites and Gromac's molecular dynamic simulation and MMPBSA calculationswere simulated on the top 5 XP Glide compounds of interest to provide novel theoretical insights in possible promising targeting candidates.

Results: The results revealed Pubchem CID: 71059746 (Glide Score: -7.256 kcal/mol and XP Gscore: -6.509 kcal/mol) has the best binding affinity and stable ligand-protein interaction in molecular dynamic simulation from the data set.

Conclusion: While multiple molecules of interest were used to predict promising low binding energies one molecule, CID: 71059746 stood out. Furthermore, molecular dynamic and MMPBSA were carried out revealing favorable conditions. These compounds should be further studied in an effort to repurpose known molecules in counteracting COVID-19.

Keywords:SARS-CoV-2; COVID-19; COVID; Drug Repurposing; Drug Discovery; Molecular Docking; Molecular Dynamic; Virtual Screening; Alzheimer's Disease

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Introduction

Covid-19 represents one of the greatest human health threats in the 21st century, causing 663,640,386 total infections and 6,713,093 deaths as of January 21, 2023 [1]. While most cases of the coronavirus disease are mild to moderate symptoms, the virus is associated with more severe complications and death. The World Health Organization (WHO) has named of the pandemic, with Alpha, Beta, Gamma, Delta, and Omicron variants spreading worldwide [2]. The SARS-CoV-2 genome is similar to other RNA viruses, with the proneness to random mutations affecting structural and nonstructural genes. These genetic alterations affect the viral phenotype and influence the transmissibility, virulence,

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and vaccines. Since Covid-19 is still relevant as a public health concern in 2023, there is a need for new therapeutics despite the available monoclonal antibody treatments and vaccines that have been used so far.

While it seems the worst part of the Covid-19 pandemic is behind us, there still persistuncertainties about the status of the virus and the future of the pandemic. This is especially true for the future of the infection and the disease, with researchers still unsure about what to expect from the booster vaccination of the already-vaccinated population with a fourth dose of the classical vaccines [3]. The WHO maintains Covid-19 as a public health emergency ofinternational concern (PHEIC) in 2023 [4], with the virus still an extraordinary threat to healthowing to the infection and disease rates. The persistent global vaccine inequity contributes to the disparity in health outcomes and the inability to bring the pandemic under control since this gap leaves the potential for further variants of concern.

Since Covid-19 is still relevant as a public health concern in 2023, there is a need for newtherapeutics despite the available monoclonal antibody treatments and vaccines that have beenused so far. Global vaccination faces diverse challenges associated with costs, time, resources to manufacture vaccines, varying levels of vaccine efficacy, limited response to vaccination in immunocompromised patients, and future pandemic coronaviruses unseen yet [5]. This renders it necessary to develop or repurpose drugs to treat SARS CoV-2 to address these limitations and to prepare for future uncertainties.

In the treatment of SARS-CoV-2, the effect of the main protease (Mpro), papain-like protease(PLpro), and RNA-dependent RNA polymerase (RdRp) in virus replication are considered [6].

From the molecular modeling studies and virtual screening of hydroxychloroquine (HCQ) and chloroquine (CQ) action on SARS-CoV-2, it is indicated that the drugs have a high binding energy with the Mpro, PLpro,and RdRp proteins, which are target proteins of interest for the treatment of Covid-19 [7]. HCQparticularly, as indicated in a molecular dynamics (MD) simulation and molecular docking study,binds to the mPro ligand molecule on the surface of the protein structure near the catalytic dyad. Other drugs such as remdesivir, ritonavir, lopinavir, and zanamivir have also been predicted to bind to the mPro, PLpro, and RdRp and have shown efficacy in treating Covid-19 (Figure 1) [8-9].

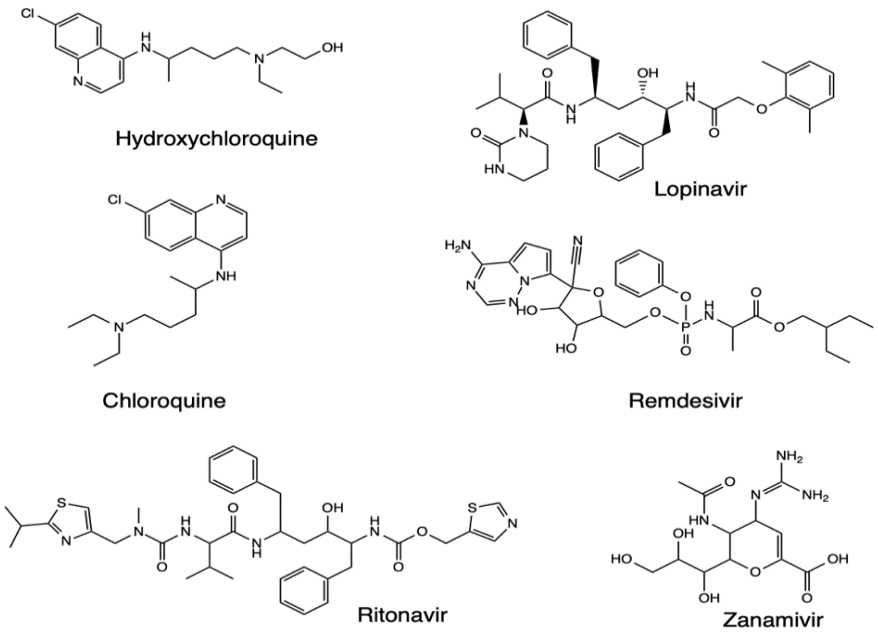

Figure 1: Chemical Structures of Hydroxychloroquine, Lopinavir, Chloroquine, Remdesivir, Ritonavir, and Zanamivir

Drug repurposing is an existing response strategy that is considered effective in treating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In considering the treatment of Covid-19, scientists have observed a similar upstream pathology with that of Alzheimer's disease (AD), which relates to disrupting the intracellular calcium ions (Ca 2+) homeostasis [10]. The calcium dysregulation mechanism in the Covid-19 SARS-CoV-2 infection and replication has been implicated in causing host cell damage. Accordingly, the clinically available treatments capable of inhibiting the disturbed intracellular Ca 2+ homeostasis have been repositioned as Covid-19 therapeutics. The aberrant elevation of Ca 2+ concentrations in the cytosol and endosome as well as the associated amyloid pathology in AD promotes the binding of SARS-CoV-2 to host cells, subsequent infection, and RNA replication in host cells. The excessive inflammation caused by the influx of Ca 2+ is also considered in finding therapeutics for Covid-19, and lithium, having been a primary drug in neurodegenerative diseases like AD, has been shown in a preliminary clinical study to improve inflammatory activity and the immune response.

This study explored 30 different drugs related to Alzheimer's Disease against the Mpro complex as possible inhibitors using molecular docking, molecular dynamic (MD) simulations, and Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) calculations. The purpose of this study was to provide an insight on possible AD designed molecules as possible COVID therapeutics by molecular docking and MD simulations.

2. Materials and Methods

2.1 Preparation of Crystalline Structure

The X-ray crystallography structure of the Covid-19 Main Protease structure (PDB ID: 6LU7) 2.16, was obtained from the Protein Data Bank at the RCSB site (rscb.org) [11]. The structure was then prepared in the Protein Preparation Wizard of the Maestro (Schrödinger Maestro version 2023-3)Schrödinger suite to eliminate defects such as missing hydrogen atoms, incorrect bond order assignment, charge states, alignments issues, and missing side chains with default parameters of optimalization and minimization using the OPLS_4 (Optimized Potential for Liquid Simulations) force field [12-14]. Through a restrained energy minimization, steric hinderance and strained bonds or confirmation were removed. As part of the final ways of preparing the proteins, restrained minimization of the heavy atoms was done to converge them to a root mean square deviation (RMSD) of 0.30 A for the optimal docking study.

2.2 Preparation of Ligands for Virtual Screening

2D structure**s** o**f** 27 different established experimental and related Alzheimer's drugs were compiled in the Pubchem databasealong with the 3 most common Alzheimer's Diseases (AD) medication (Donepezil, Galantamine, and Rivastigmine) (Figure 2)**.** The structures were thendrawn on ChemDraw (PerkinElmer Informatics ChemDraw 22.0.0) and the files were saved as SD files. The files were then loaded onto the BioLuminate workspace (BioLuminate version 2023-**3**)[15-18]. Itwas then prepared using the Ligprep **(**LigPrep version 2023-3)interface in Schrodinger to convert the 2D structures into 3D structures, neutralize charges, stereoisomer generation through an OPLS_4 force field with ionization state of pH 7.0 ± 2.0 using Epik with the condition of generating at most 35 poses per ligand[19].

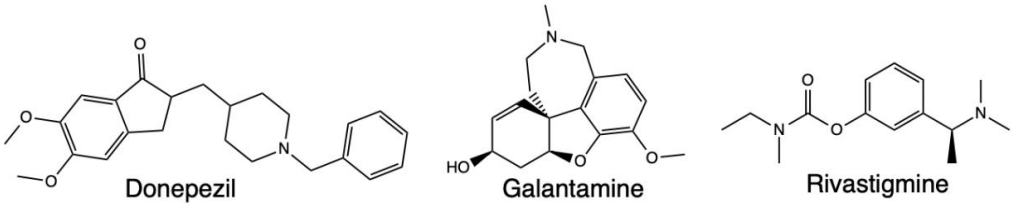

Figure 2: Chemical Structures of Donepezil, Galantamine, and Rivastigmine

2.3 Receptor Grid generation

To facilitate docking into the active site, the receptor grid generation panel of the Glide (Glide version 2023-3) module of Schrodinger was used to generate a grid box around the co-crystallized ligand in the receptor protein [20-23]. The boxes were centered on the centroid of the co-crystallized ligand, and the scaling factor of the van der Waal's radius set to 1.0 A, with a partial charge cutoff maintained at 0.25 without constraints. From this process, there was the creation of a cubic box with coordinates of -11.38 in the *x* direction, 12.409 in the *y* direction, and 68.831 in the *z* direction from the basis of chain A.

2.4 Virtual Screening by Molecular Docking using Glide

In designing drugs or inhibitors, structure based High-Throughput Virtual Screening (HTVS) is crucial for identifying the potent lead molecules since this method is designed to screen for a larger library of compounds. This process can identify the molecules that interact with and fit seamlessly into the active site of the target protein. For this study, HTVS was conducted on all the small molecules in two steps: structure-based virtual screening in standard precision (SP) docking, and extra precision (XP) docking.

Glide contains a workflow for the calculations related to the virtual screening and docking studies. The method involves a grid-based ligand docking to generate a series of ligand poses. Hierarchical filters are then applied to evaluate the ligand interactions with the receptor, with the initial filters testing the spatial fit of the ligand into the active site. Further, the initial filters examine the ligand-receptor interaction complementarity based on the empirical ChemScore function, and the ligand poses that pass the screen enter the algorithm's final stage.

To identify the probable binding compounds from a set of compounds, the study employed the Glide module's SP docking, with more intensive investigation carried out on the Glide XP docking. Crucially, XP docking eliminates false positives let in by SP docking, as well as penalizing the ligands that do not fit well to the receptor. Final scoring focused on energy minimized poses whereby the compounds with the best docked poses and favorable negative score values were studied further.

2.5 Molecular Dynamic Simulation

Molecular dynamics (MD) simulation is a powerful tool to predict and give insights on the stability of protein-ligand complexes in a physiological condition. The top 5 compounds from XP docking were selected for 100 ns molecular dynamic simulation using SiBioLead LLP (https://sibiolead.com), a GROMACS based molecular dynamic server [24-28]. The 5 protein-ligand complex adopted a solvated based triclinic box with water as Simple Point Charges (SPC) and NaCl as counter ions. To maintain physiological conditions, 0.15 M NaCl was added to the simulation system. The CHARMM27 force field was adopted for the simulation. Prior to simulation the system was equilibrated for 100 ps using the NVT/NPT protocol with temperatures at 300K and pressure at 1 bar. Simulation was then carried out using a Leap Frog simulation integrator with 5000 frames saved.

2.6 Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) Analysis

Binding free energies of the 5 simulated molecular dynamic protein-ligand complex were calculated using GROMACS based Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) within SiBioLead LLP (https://sibiolead.com).

3. Results

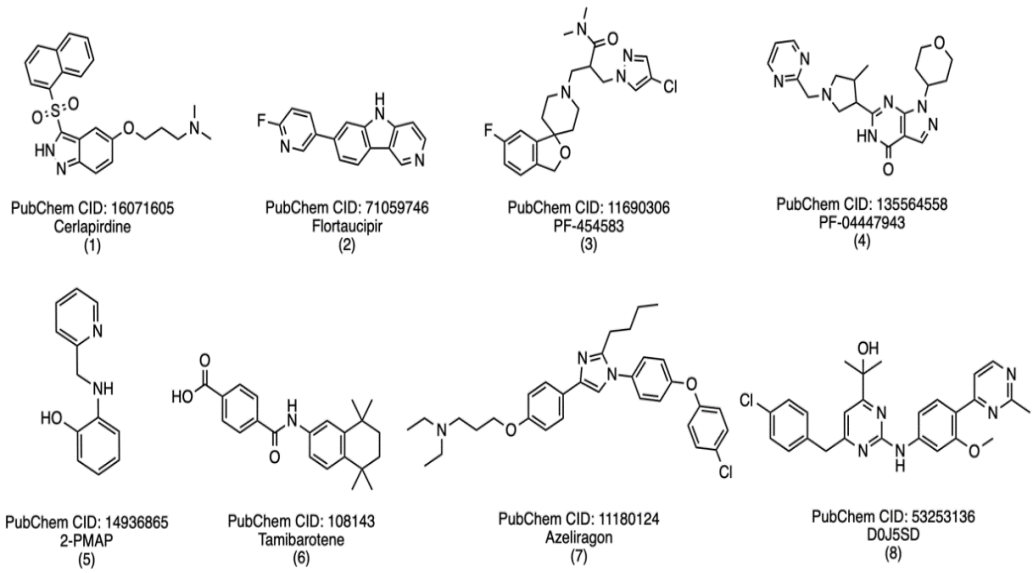

Figure 3: Structures of 8 Compounds of Interest from Docking Results

From our docking analysis, the potential ligand candidates that may inhibit SARS-CoV-2 Mpro are compounds **2** and **3**.The top 5 lowest docking score for standard precision and extra precision are listed in (Figure4) and (Figure 5). Aftersimulating XP docking, compounds **2** and **3** correlated within the top 5 lowest docking scores when compared to SP docking.For comparison, the 3 most popular ADtherapeutics, Donepezil, Galantamine, and Rivastigmine were docked (Figure 6),in order tooffer an insight comparison between the top reported compounds in the studied data set to three commonly available therapeutics used for the treatment of AD.

A

Compound	Docking Score
1	-7.397
2	-7.218
3	-6.762
4	-6.743
5	-6.666

B

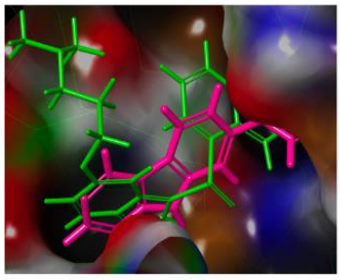

Figure 4. Top 5 Compounds From SP Docking Results (A) Overlap of Compound **1**(Green) and **2**(Pink) in Mpro Complex (B)

A

Compound	XP Docking Score
2	-6.509
6	-6.460
3	-6.434
7	-6.265
8	-6.210

B

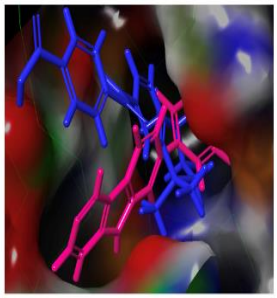

Figure 5. Top 5 Compounds From XP Docking Results (A) Overlap of Compound **2**(Pink) and **6**(Blue) in Mpro Complex (B)

A

Compound	Docking Score	XP Docking Score
Donepezil	-6.662	-5.734
Galantamine	-5.707	-4.916
Rivastigmine	-4.456	-4.456

B

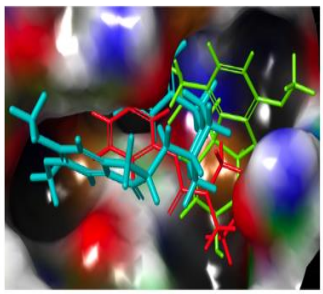

Figure 6. SP and XP Docking Score of Donepezil, Galantamine, and Rivastigmine (A) Overlap of Donepezil (Teal), Galantamine (Yellow-Green), and Rivastigmine (Red) in Mpro Complex (B)

Ligand interaction diagrams (Figure 8) shows polar and hydrophobic residues dominate most of the interactions. All structures besides compounds **7** and **8**formed Hydrogen bond interactions with CYS 145, GLY 143, CYS 145, HIS 164, GLU 166, HIE 166, or GLU1 66. While compounds **1**, **4**, **5**, and **8** formed π- stacking interactions at residue HIS 41. Salt bridge interactions we observed in compound **2**, **4**, and **5** at residues GLU 166 or CYS 145. Halogen bonds were detected at residue HIE 163 and THR 26 for compounds **3** and **7**.

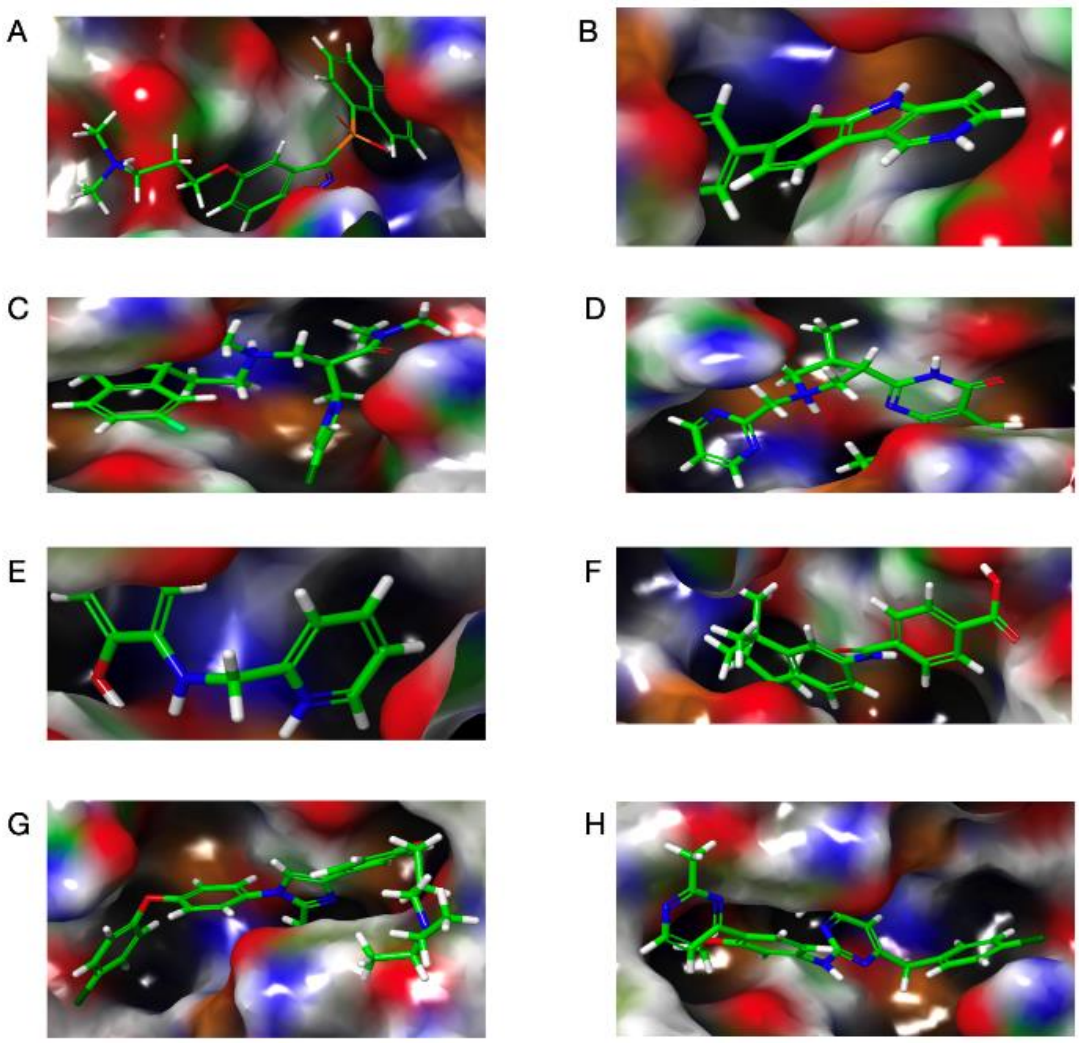

Figure 7.Ligand docked to binding pocket **1** (A), **2** (B), **3** (C), **4** (D), **5** (E), **6** (F), **7** (G), and **8** (H)

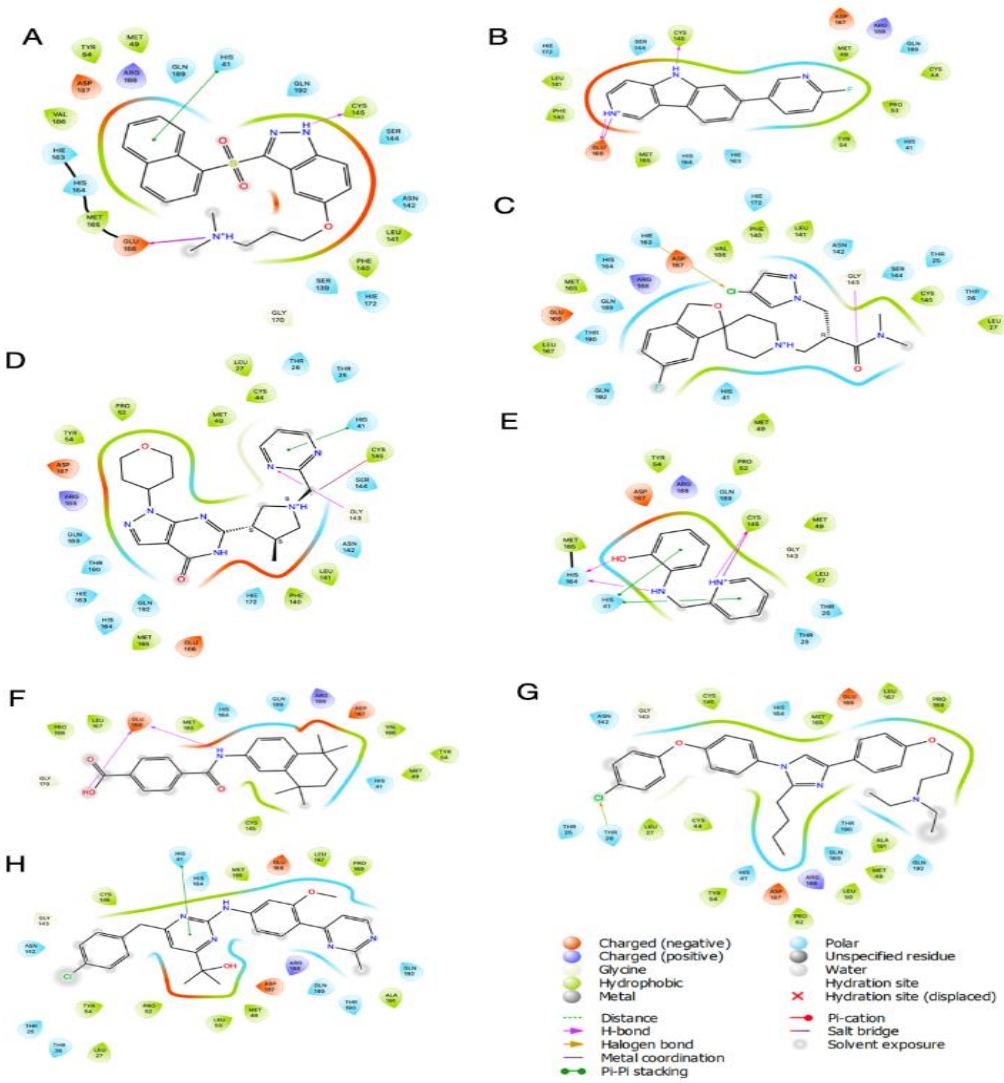

Figure 8. Ligand Interaction Diagram of Compounds 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F), 7 (G), and 8 (H)

Molecular dynamics simulations were conducted to investigate the interactions of five different compounds **2**, **3**, **6**, **7**, and **8** against Mpro. The research aimed to understand the stability and dynamics of these interactions by analyzing several key parameters, including Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), solvent accessible surface area, radius of gyration, and hydrogen bond count. The results revealed significant differences among the compounds in terms of their binding stability and dynamic behavior.

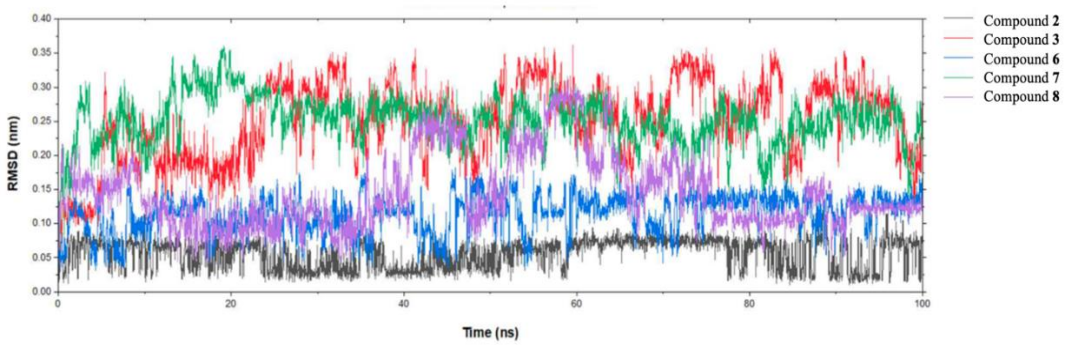

Figure 9. RMSD

Compound 2 showed the least RMSD, i.e. less than 0.1 Å throughout the simulation. This was followed by compound 6, compound 8, compound 7 and finally, compound 3. This suggests that Compound 2 forms a highly stable complex with the protein, while compound 3 displayed the least stability among the compounds. Compound 2 also showed more stable interaction, while fluctuations were noted for other compounds.

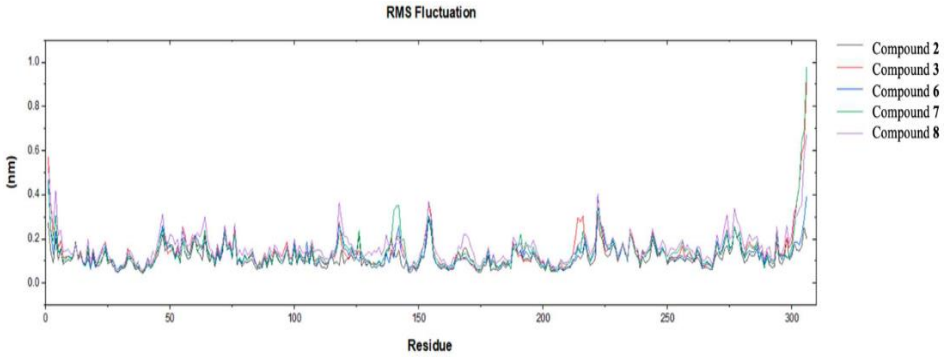

Figure 10. RMSF

RMSF was almost similar for the ligand bound protein for all compounds but overall, compound 8 bound protein showed slightly higher RMSF, indicating greater flexibility in its binding site. Compound 7 showed more fluctuation in region surrounding the residue 140, while compound 6 showed higher RMSF around residue 220. Compound 7 and 8 also showed higher solvent accessible surface area compared to other compounds. This indicates that these compounds may have a larger interface with the solvent environment during the simulation, possibly influencing their interactions with the protein.

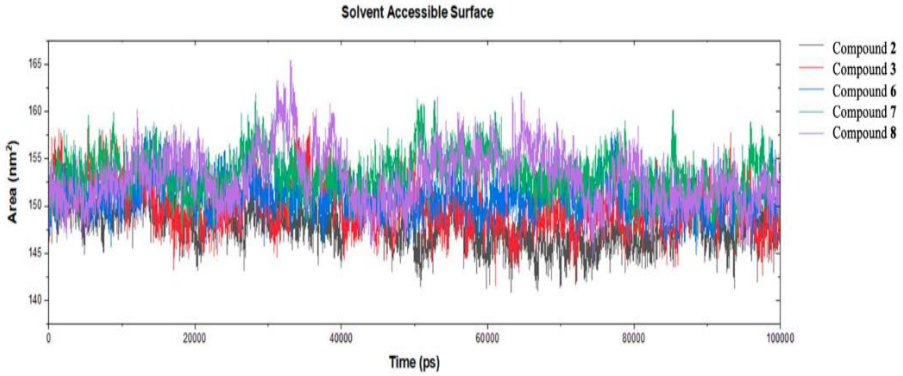

Figure 11. Solvent Accessible Surface

The radius of gyration, a measure of the protein's compactness, remained below 2.28 Å on average for all compounds, indicating that the protein maintained its overall structure during the simulation. However, Compound 8 exceeded a radius of gyration of 2.30 Å around 25 nanoseconds, suggesting a potential disruption in the protein's compactness during this time. The number of hydrogen bonds formed between the compounds and the protein remained relatively stable, ranging from 200 to 240. This suggests that the compounds maintained consistent interactions with the protein throughout the simulation.

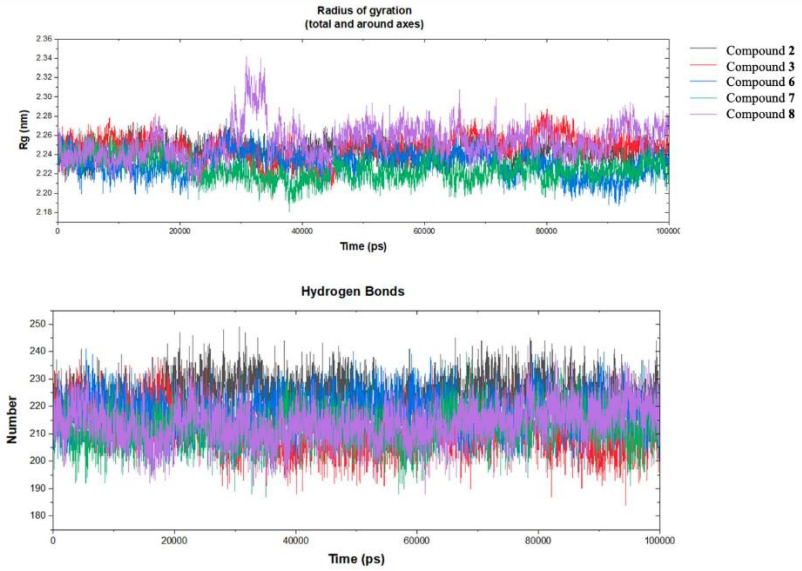

Figure 12. Radius of Gyration and Hydrogen Bonds

MM/PBSA was used to calculate binding free energy of system from 51 selected snapshots, while ΔG was used to depict change in free energy. Total energy of the system and change (Table 1) indicated that compound 2 complex had the most favorable binding energy of the system but ΔG was lowest for compound 7. In summary, compound 2 complex had the most favorable total MM/PBSA energy, indicating strong stability within the system. However, when assessing the thermodynamic favorability of binding or interaction, the ΔG was the lowest for compound 7, implying compound 7 may have a particularly strong binding affinity or interaction with its surroundings, despite compound 2 having the lowest total energy of the system.

Compound	*VDWAALS*	*EEL*	*Epolar*	*Enon-polar*	*Total Energy*	*ΔG*
2	-2428.35	-21540.86	-2795.7	74	-7042.82	-22.74
3	-2409.65	-21686.11	-2866.25	75.38	-6926.79	-23.58
6	-2398.77	-21789.66	-2848.42	76.97	-6949.55	-24.71
7	-2423.79	-21717.09	-2844.8	75.58	-6901.23	-43.58
8	-2413.51	-21602.74	-2911.44	76.23	-6922.8	-29.63

Table 1. MM/PBSA energy table of the analyzed complexes. ΔG depicts energy change bysubtraction of total energy of the receptor and ligand from the complex. Table showsVander Waal's energy, EEL depicts electrostatic energy. All values are in kcal/mol.

4. Discussion

Predicting the binding affinity and mode of small-molecule ligands to receptor binding sites is a crucial step in structure-based drug design process. Schrodinger Glide, a powerful molecular docking and virtual screening tool, has proven to be one of the top predictors of binding modes of small-molecule ligands to receptor binding sites [30]. From analyzing Glide SP and XP results, two compounds of particular interest were identified through as they corresponded in both top 5 lowest docking score.

Compound 2, Flortaucipir is a radioactive tracer primarily used in positron emission tomo-graphy imaging to visualize TAU neurofibrillary tangles in the brain [31]. It is currently being investigated as a potential therapeutic for Alzheimer's disease and other neurode-generative disorders. The binding energy of Flortaucipir in Glide SP and XP was observed at -7.218 and -6.509 kcal/mol. Hydrogen bonding was observed at residues CYS 145 and GLU 166 while a salt bridge interaction formed at GLU 166.

Compound 3, PF-454583 is a novel molecule designed to selectively target and inhibit TAU protein aggregation. PF-454583 has shown promising results during preclinical studied demonstrating the ability to reduce TAU protein aggregation and improve cognitive functions in AD anime models [32]. The

interaction energy between PF-45483 and Mpro was -6.762 and -6.434 kcal/mol for Glide SP and XP. A hydrogen bond was formed at residue GLY 143 and a halogen bond was detected at residue HIE 163.

5. Conclusion

Molecular docking and molecular dynamics simulation were carried out to investigate a data set of 30 different Alzheimer's Disease related molecules. In this study, we presented several insights about SARS-CoV-2 Mpro on possible compounds that can be possible inhibitors. Compound 2 is an approved FDA approved radioactive diagnostic agent used for evaluating patients at risk of developing Alzheimer's Disease. Furthermore, we have identified several other potential molecules as probably inhibitors against SARS-CoV-2 infections. Currently, we have not carried out biological experiments for this study. However, in this study we display the ability for multiple compounds to form stable conformational structure to SARS-CoV-2 Mpro. While also providing possible insight towards drug repurposing for the treatment of SARS-CoV-2.

Data Availability Statement

Not Applicable

Author Contributions

C.R.W. and G.C.L. contributed to the conception and study design. C.R.W., M.S., and G.C.L. contributed to the collection, simulation, and analysis of the data. C.R.W., M.S., and G.C.L. wrote and revised the first draft of the manuscript. All authors contributed to the manuscript revision andapproved the submitted version.

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Conflict of Interest

The authors confirm and declare that the research was conducted without any commercial or financial relations that could be construed as potential conflict of interest

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