***How to Cite:*** Bhimanwar, R., Thomas, A., Kothapalli, L., Godase, A., Gandhi, S., Chandani, S., More, G., Jadhav, G., & Choudhary, S. (2022) Prospective Hybrid Molecules with Dual Anti-Viral and Anti-Thrombotic Activity Against the SARS-CoV-2 Infection and Its Associated Complications Employing *in Silico* Studies. *Bulletin of the University of Karaganda Chemistry*, *108(4)*, *76-88*. https://doi.org/10.31489/2022Ch4/4-22-8

*Article* Received: 19 July 2022 | Revised: 21 October 2022 | Accepted: 31 October 2022 | Published online: 18 November 2022

UDC 530.145:541.27 https://doi.org/10.31489/2022Ch4/4-22-8

R. Bhimanwar[1], A. Thomas[1*], L. Kothapalli[1], A. Godase[1], S. Gandhi[1], S. Chandani[1], G. More[1], G. Jadhav[2], S. Choudhary[3]

*[1]Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, Maharashtra, India (Affiliated to Savitribai Phule Pune University, Pune);*
*[2]School of Medicine, Omaha Campus, Creighton University, NE, USA;*
*[3]RASA Life Science Informatics, Pune, Maharashtra, India*
*(*Corresponding author's e-mail: asha.thomas@dypvp.edu.in)*

# Prospective Hybrid Molecules with Dual Anti-Viral and Anti-Thrombotic Activity Against the SARS-CoV-2 Infection and Its Associated Complications Employing *in Silico* Studies

Covid-19, a SARS-CoV virus-based disease, was identified in Wuhan, China, in December 2019. Initially, it was considered just an infection of the respiratory system, but due to its transmittable nature, it was declared a pandemic. A variety of treatment options were implemented, including antivirals like remdesvir, favipiravir along with vitamins and antioxidants. Further investigations revealed that the Covid-19 infection results in thrombotic cardiovascular complications, which are the major concern for the increased mortality associated with this disease. This study investigates the *in Silico* design of hybrid molecules with antiviral and antithrombotic properties. A docking study was performed using Autodock Vina software, and binding energies of the designed compounds were determined for papain-like protease (PDB: 3E9S) and 3-chymotrypsin-like cysteine protease (PDB: 6LU7). The docked poses and amino acids interactions were verified using Biovia Discovery studio 4.5. The binding energies of all designed compounds were compared with the standards, Compound RL1 (2-(5-(3-carbamoyl-1H-1,2,4-triazol-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)-carbonyl)amino)(hydroxy)methyl)carbamoyl)phenyl acetate) and Compound FL2 (8-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-6-yl(2-(6-flouro-3-oxo-3,4-dihydropyrazine-2-carboxamido)-1-hydroxy-3-phenylpropyl)carbamate) proved to be promising agents with strong binding interactions. Hybrid molecules that inhibit viral replication, possibly as transition state inhibitors, can be investigated further for use in the treatment of SARS-Co-V infection and its associated complications.

*Keywords***:** COVID-19; CL-pro, PL-pro, antiviral, antithrombotic, molecular docking, *in Silico*, hybrid molecule.

## *Introduction*

The Covid-19 pandemic caused by Severe acute respiratory syndrome coronavirus (SARS-CoV-2) virus has affected majority of population around the globe and has resulted in significant mortality and morbidity. It has been initially identified as a respiratory illness, but has now demonstrated extreme individual variability in its symptoms, and severity of infection [1, 2]. This highly infectious virus has undergone rapid mutations with "the double mutant" strain leading to the second wave in almost all countries worldwide. This double mutant Covid strain has been found to be more infectious and lethal and has increased the health risk in patients with high mortality rate [3]. The SARS-CoV-2 belongs to β-coronavirus family and is SS RNA enveloped protein with 9860 amino acids. SARS-CoV-2 gene fragment consists of structural and nonstructural proteins encoded from S, E, M and N gene and ORF region, respectively [4, 5]. Spike glycoprotein (S protein) present on the virus surface is the key component for viral entry into the host cell through recognition and binding with ACE2 (angiotensin-converting enzyme 2) receptor. S1 subunit of S protein recognizes the binding site and binds to the host receptor and S2 subunit forms six-helical bundle with the help of heptad repeat (HR1 and HR2) and mediates fusion cell membrane. Fusion of host and viral membrane is achieved by host protease, which cleaves site at the border of S1 and S2. Sixteen nonstructural proteins perform different function and carry out processing and replication of RNA [6, 7].

Angiotensin-converting enzyme 2 (ACE2) protein, target for coronavirus is found in alveolar epithelial cells of lungs and in small intestines enterocyctes. Breakdown of ACE2 finally causes systemic inflammation in the host cell leading to critical illness and multiorgan dysfunction. Covid-19 patients with cardiovascular

disease have been severely affected and an increase in mortality rate has been observed. Adverse outcomes have been observed due to systemic inflammation, which destabilizes vascular plaques finally demanding increased cardiac activity. Increased levels of IL6, D-dimer and troponins (cardiac specific) direct the patient towards increased risk of pulmonary embolism and thrombosis [8–12].

Currently the treatment line of SARS-CoV-2 infection involves use of anti-virals like remdesivir, favipiravir, ritonavir to address the pulmonary infection phase; while for suppressing the inflammatory/coagulopathy phase, drugs like Tocilizumab, Anakinra, Baricitinib, Eculizumab, Emapalumab and Heparin, including low molecular weight heparins (e.g enoxaprin) are utilized [13, 14]. Several vaccine candidates have received approval for emergency use across the globe [15] and mass immunization drives are under progress [16]. However until a considerable mass of population is vaccinated and herd immunity is achieved, therapeutic interventions will be required to combat the situation.

Latest developments show that SARS-CoV-2 infection precipitates variety of haematological complications associated with increase in D-Dimer and blood thickening. Mortality occurs either due to respiratory failure or thromobotic cardiovascular complications, which requires the management of multiple associated pathways [17–20].

Development of hybrid molecules is an attractive strategy of drug design to achieve multiple targeting, enhance biological activity and improve kinetics [21, 22]. In the past decades, several researchers have utilized this concept to develop agents with antimicrobial [23–25], anti-malarial [26–28] and anti-cancer activity [29, 30]. Researchers have developed multifunctional drugs comprising of two or more pharmacophores with benefits in treatment of multi-factorial diseases [31–35].

So an attempt was made to design hybrids with dual action, namely antiviral and anti-coagulant activity, which would prove advantageous in treatment of the multiple complications occurring during SARS-CoV-2 infection. The strategy involved designing of hybrids of reported anti-viral agents with anti-coagulant molecules through suitable linkers converting them into potentially active molecules, which were studied against suitable anti-viral targets. The hybrids were generated by linking antiviral molecules [36–38], namely ribavirin, favipiravir, oseltimivir and acyclovir with established anti-platelet drugs [39–43] viz. hesperitin, resveratrol and aspirin as test compounds. The selected anti-coagulants are reported to possess dual anti-thrombotic and antiviral action. The criteria for selection of these agents are summarized in Table 1. The linkers selected for the design of molecules included hydrolysable and cleavable linkers like 2-amino 2-hydroxy ethyl amide, malonic acid and succinic acid.

Table 1

**Selection of anti-viral and anti-platelet molecules for design of hybrid molecules**

| Selected Anti-viral molecules | |
|---|---|
| Ribavirin | Broad activity toward conventional and novel viruses of DNA and RNA types; Multiple mechanisms of direct antiviral action; Random mutagenesis of viruses to promote T cell response; Tolerable and well-characterized side effect profile; Mature clinical experience & comprehensive demographic characterization; Accessibility & affordability |
| Favipiravir | Employed for clinical intervention of COVID-19 treatment; Exhibits faster viral clearance and better chest CT changes; Adverse events are rare and tolerable |
| Oseltimivir | Clinical study suggests that Remdesivir treatment among all of antivirals such as Ribavirin, Favipiravir and Oseltamivir proved promising therapeutics in COVID treatment |
| Acyclovir | Similar clinical target as approved drug Remdesivir |
| Selected Anti-platelet agents | |
| Hesperitin | Anti-platelet, anticoagulant, antioxidant, radical scavenging activity and anti-inflammatory activities; Demonstrated antiviral activity by altering the immune system mainly via regulating interferons in the influenza A virus |
| Resveratrol | Inhibits platelet aggregation and platelet membrane-bound fibrinogen (Pfig) induced by adenosine diphosphate (ADP through decreased activity of PLC beta of platelets; Antioxidant-promote nitric oxide production, Cardioprotective agent, Antiinflammatory, Neuroprotective, Antiviral properties |
| Aspirin | Proven anticoagulant action, considerable dose-dependent antiviral activity (CA9, HRV1A, HRV2 and substantial activity against FluA H1N1, HRV14 and HRV39); Possible MOA-involvement of the NF-κB-pathway, Differential regulation of influenza virus RNA synthesis by NF-κB, iNOS expression by down regulating the promoter activity, mRNA and protein expression levels involvement of p38 |

## *Experimental*

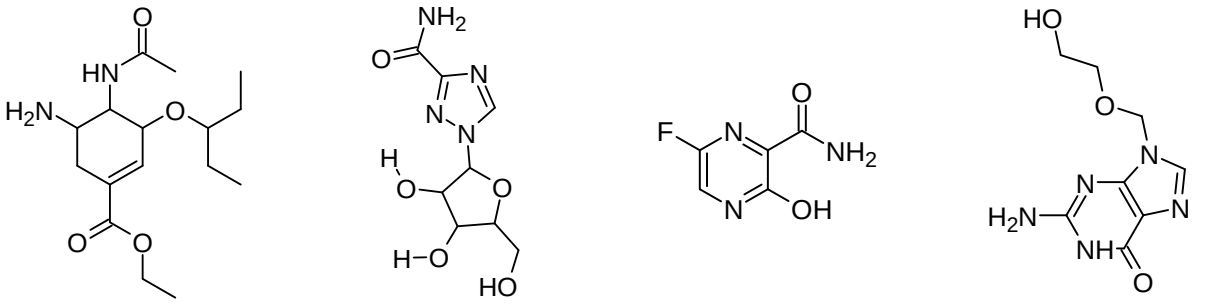
*Selection of Protein*

COVID-19 papain-like protease (PL-pro) (PDB ID- 3E9S) and 3-chymotrypsin-like cysteine protease (CL-pro) (PDB ID- 6LU7) were selected as the protein targets for the present study. The crystal structure of desired proteins was downloaded from RCSB Protein data bank in.pdb format. The native ligand present in protein 6LU7 is n-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-n~1~-((1r,2z)-4-(benzyloxy)-4-oxo-1-{[(3r)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide and in 3E9S is 5-amino-2-methyl-N-[(1R)-1-naphthalen-1-ylethyl]benzamide.

*Selection of Ligands*

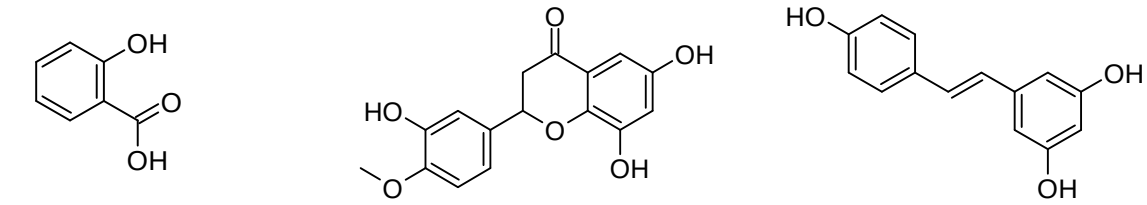
Hybrid ligands that can exhibit dual action, anti-viral activity against the SARS-CoV-2 along with anti-thrombotic activity with improved affinity and efficacy in combination were designed. Promising anti-viral agents that are currently recommended in treatment of the SARS-CoV-2 infection like oseltamavir, ribavirin, fevipiravir and acyclovir (Figure 1A) with molecules like salicylic acid, resveratrol and hespiritin with potent anti-viral and well-established anti-thrombosis profile (Figure 1B) were selected to design the hybrid molecules using appropriate linkers (Figure 1C).

The 3D structures of hybrid type ligands were drawn using Chem Draw in.mol file with all possible combinations and Open Babel (http://openbabel.org/wiki/Main_Page) was used to convert. mol to. pdbqt files. Drug-like properties of the ligands were computed using ADME Schrodinger software QikProp (https://www.schrodinger.com/QikProp).



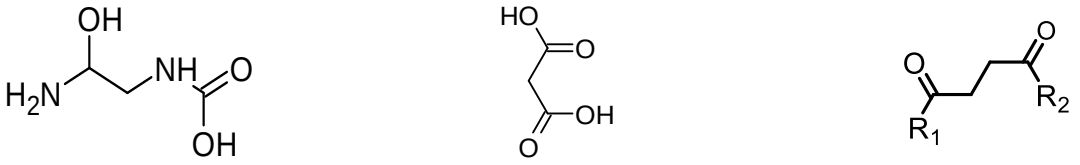
a. Oseltamavir (O) b. Ribavirin (R) c. Fevipiravir (F) d. Acyclovir (A)

Figure 1A. Selected Anti-viral agents



a. Salicylic acid (1) b. Hesperitin (2) c. Resveratrol (3)

Figure 1B. Selected anti-thrombotic agents



2-Amino 2-hydroxy ethyl amide (L) Malonic Acid (M) Succinic acid (S)

Figure 1C. 2-D Structure of the selected linkers

*Molecular Docking studies*

Molecular docking studies were carried out using Autodock Vina software. Optimisation of the ligands and proteins and grid box creation were carried out using Graphical User Interface program Autodock Tools. Target proteins were optimised using Autodock Tools by adding polar hydrogen groups, removing water

molecules, adding kollman and Gasteiger charges and prepared file was saved as.pdbqt file. Ligands were optimised and converted into.pdbqt file using Open Babel software.

The amino acids making up the active site of the target proteins were established by visualization of the binding of native ligands using Biovia Discovery Studio 2016. Grid box was generated by arranging the grid coordinates (X, Y and Z) about the proteins active site. The grid size was set to 40×40×40 xyz points for both targets with grid centre designated at dimensions (x, y and z): –10.891, 16.159 and 66.647 for CL-pro and –30.52, 22.402, 30.288 for PL-pro. During the docking procedure, both the proteins and ligands were considered as rigid structures. The root-mean-square deviation (RMSD) was observed, the pose with the most favourable free binding energy was considered (RMSD value less than 0.1Å). Then with the help of Biovia discovery studio, the pose with lowest energy of binding was aligned with receptor structure for further analysis.

*Validation of Target Proteins*

Target validation was performed to understand the accuracy and reproducibility of the docking process and targets selected for the study. The native ligands n-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-n~1~-((1r,2z)-4-(benzyloxy)-4-oxo-1-{[(3r)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide and 5-amino-2-methyl-N-[(1R)-1-naphthalen-1-ylethyl]benzamide present in target proteins 6LU7 and 3E9S, respectively, were removed from the protein structures and were re-docked into the active sites using Autodock Vina software. The procedure was performed on both the target proteins in Biovia Discovery software; the native ligands were removed from the co-crystallized complexes and saved in PDB file format. Grids were generated about the active sites of the target proteins and the docked complexes were superimposed on their respective reference co-crystallized complexes and the root mean square deviation (RMSD) was computed.

*Prediction of ADME properties*

Along with the biological activity, the pharmacokinetic properties of compounds are critical for selection of good drug candidates. In our study we used ADME Schrodinger online software to predict ADME properties i.e. Absorption, Distribution, Metabolism, and Excretion/Elimination using Lipinski Rule of drug-likeness.

## *Results and Discussion*

*Target validation*

Target validation studies using the selected targets and native co-crystallized ligands indicated low RMSD values within runs confirming the accuracy and repeatability of the docking procedure. The docking results of native ligands with targets are shown in Figure 2.

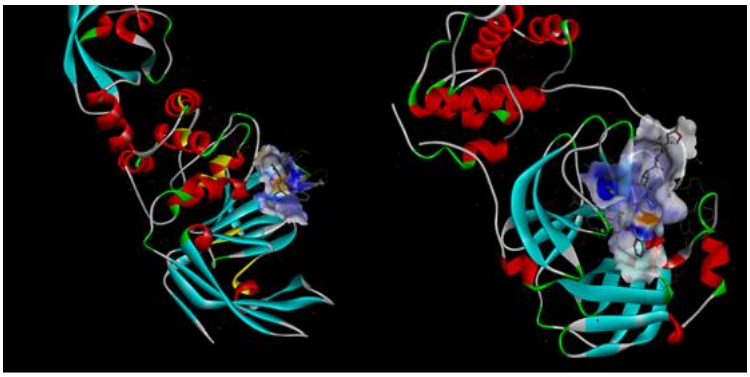



Figure 2. *a* — Papain-like protease with native ligand;
*b* — 3-chymotrypsin-like cysteine protease with native ligand

*Molecular Docking studies*

For the docking studies, 24 hybrid ligands were designed using suitable combinations of the anti-viral and anti-thrombosis agents with selected linkers. Among these hybrids, six ligands demonstrated favourable affinity for the selected target proteins (PL-pro and CL-pro) with low binding energy comparable to the selected standards (Remdesivir, Acyclovir, Ribavirin, Oseltamavir and Fevipiravir). The results of the docking

studies of standards and with interacting amino acid residues and type of interactions are summarized in Table 2.

Table 2

**Docking analysis of Standards with target proteins**

| Compound Name | Binding Energy (kcal/mol) | Interacting Amino acids | Bond type |
|---|---|---|---|
| Target: Papain-like protease (PDB ID- 3E9S) | | | |
| Remdesivir | –6.0 | Tyr 269, Tyr 265<br>Ala 250, Tyr 269 Tyr 274 | H- bond<br>π-π stacking<br>π-π stacking |
| Acyclovir | –6.3 | Gln 270, Gly164<br>Tyr269<br>Tyr274<br>Asp165 | π- π stacking<br>H-bond<br>H-bond<br>H-bond |
| Ribavirin | –6.8 | Tyr 265<br>Asp 165<br>Tyr274<br>Gly164, Gly267 | π-π stacking<br>π-π stacking<br>H-bond<br>H-bond |
| Oseltamavir | –5.8 | Tyr 269<br>Asp165<br>Tyr 265<br>Tyr274 | H-bond<br>H-bond<br>π-alkyl stacking<br>π-alkyl stacking |
| Fevipiravir | –5.7 | Tyr 265<br>Asp 165<br>Tyr274<br>Thr 302, Arg 167<br>Tyr 274 | π-π stacking<br>π-π stacking<br>H-bond<br>H-bond<br>H-bond |
| Target: 3-chymotrypsin-like cysteine protease (PDB ID- 6LU7) | | | |
| Remdesivir | –8.2 | His 163, Phe 140 Gly 143<br>His 41,<br>Met49,165 | H-bond<br>H-bond<br>π-π stacking<br>π-π stacking |
| Acyclovir | –5.8 | Leu 141<br>Ser 144<br>Cys 145, Glu 166<br>His 163 | H-bond<br>H-bond<br>H-bond<br>π-π stacking |
| Ribavirin | –6.3 | Cys 145<br>His 163<br>Thr 26<br>Gly 143 | H-bond,π-π stacking<br>H-bond<br>H-bond<br>H-bond |
| Oseltamavir | –6.0 | Glu 166<br>Met 49, Met165<br>His 41 | H-bond<br>π-alkyl stacking<br>π-alkyl stacking |
| Fevipiravir | –6.3 | Asp 187, Tyr 54<br>His 41<br>Met 165<br>Arg 188 | H-bond<br>H-bond<br>π-π stacking<br>Halogen interaction |

The best six hybrid ligands with low binding energies were selected for further docking interaction analysis. Figure 3 displays the 2-D structure of these hybrid ligands. The best-docked complexes of these ligands with their interacting amino acid residues are shown in Figures 4 and 5, respectively.

Based on the docking results, among the six hybrid ligands, compound RL1 exhibited high binding affinity with both the target proteins (PDB:3E9S and PDB: 6LU7) with dock score of –8.1 and –8.0, respectively. In the interaction study with PL-pro, the hydrogen bonds were observed with Tyr 269, Gln 270, Tyr 274 and Asp 165, π–π interactions with Gly 164, Leu 163 (Figure 4A). With 3CL-pro, compound RL1 formed hydrogen bonds with Thr 24, 25, 26, Thr 45, Ser 46, Ser 144, Gly 143 and π–π interactions with Met 165, 49, His 41(Figure 5A).

Compound FL2 exhibited the highest binding affinity with both the target proteins (PDB:3E9S and PDB: 6LU7) with dock score of –9.1 and –9.0, respectively. With PL-pro, compound FL2 formed hydrogen bonds with Arg 167, Asp 165 and π–π interactions with Tyr 264 and Lys 158 (Figure 4B). In interaction with 3CL-pro, the hydrogen bonds were observed with Thr 24,25,45, Asn 14 and His 164, π–π interactions with His 41, Met 49, Thr 24 (Figure 5B).

Also, ligand FL3 (Figure 4C) showed greater binding affinity (dock score -8.1) to PL-pro compared to the standards, which exhibited dock score between –5.7 to –6.3. However, it exhibited lower affinity (Dock score –7.9) with 3-CLpro protease compared to the other docked ligands, but with greater affinity when compared to the standards (Dock score –5.7 to –6.3) with the exception of Remdisivir, which showed improved affinity with dock score of –8.2. With PL-pro (PDB:3E9S), ligand FL3 formed hydrogen bond interaction with Thr 266 and π–π interactions of phenyl rings with Tyr 265, Thr 302, Tyr 269, Arg 167 and Pro 249. In interaction with 3-CLpro, the hydrogen bonds were observed with Gly 143, Ser 144, Thr 26, Cys 145, Thr 190 and π–π interactions with Met 165, Met 49 (Figure 5C).

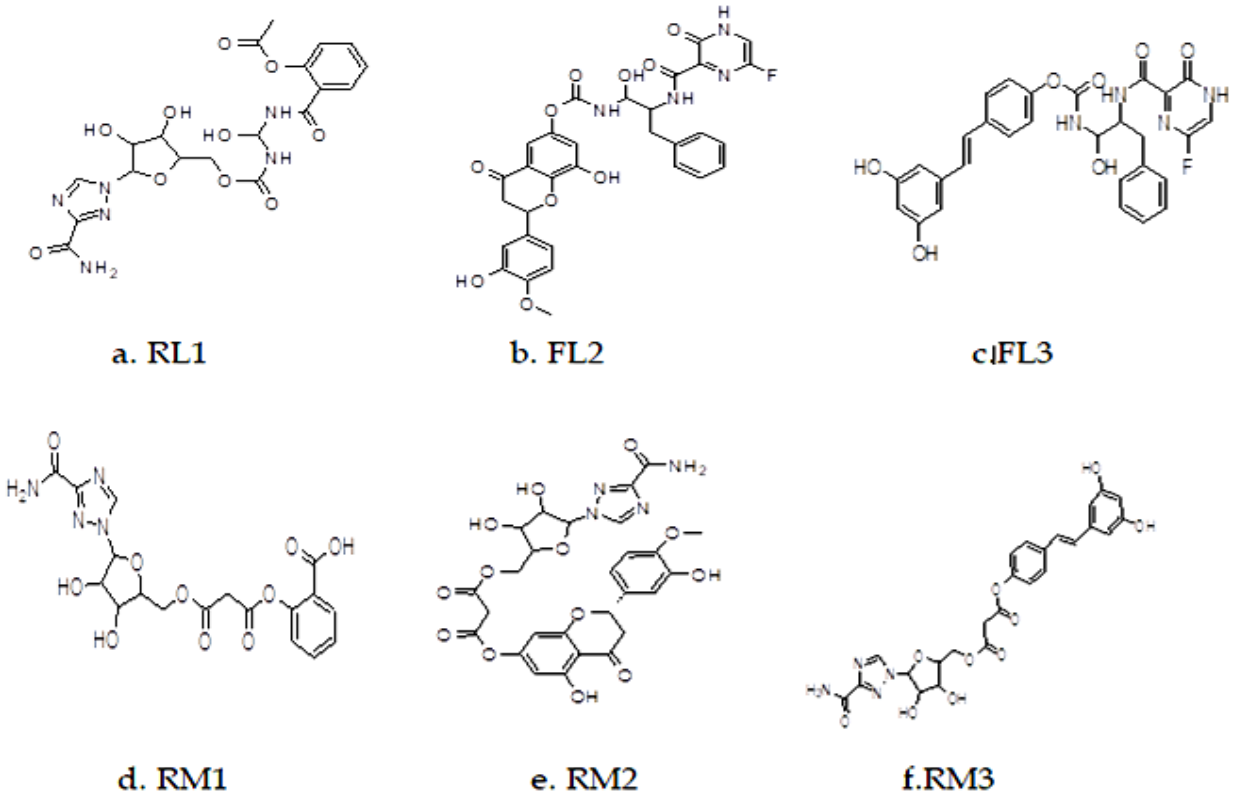



Figure 3. 2-D structures of selected hybrid ligands

Compound RM1 showed the interaction with PL-pro (PDB:3E9S) and formed hydrogen bond interaction with Tyr 269, Asn 268, Gly 267, Tyr 274 and π–π interactions of triazole rings with Asp 165, Tyr 265 (Figure 4D). Figure 5D shows interaction of RM1 with 3-CLpro, the hydrogen bonds were observed with Gly 189, Glu 166, Met 49, Asp 187, Ser 144, Cys 145 and π–π interactions with His 41, Met 49 and Glu 166.

Figure 4E shows the interaction of RM2 with PL-pro (PDB:3E9S) and hydrogen bond interaction with Tyr 274, Gly 164, Tyr 269, Asn 268, Gly 267 and π–π interactions with Asp 165, Tyr 265. In interaction with 3-CLpro, the hydrogen bonds were observed with Gln 189, Met 49, Gly 143, Ser 144, Cys 145 and π–π interactions with Met 49, His 41, Glu 166 (Figure 5E). Among the docked ligands, RM2 showed good affinity to CL-Pro with dock score of –8.6 when compared to the standard and other ligands.

In Figure 4F, compound RM3 showed the interaction with PL-pro (PDB:3E9S) and formed hydrogen bond interaction with Tyr 274, Glu 251 and π–π interactions with Asp 165, Lys 158, Pro 249. Figure 5F shows interaction of RM3 with 3-CLpro, the hydrogen bonds were observed with Thr 24,25,45, Ser 46, Cys 145 and π–π interactions of triazole ring with Met 49 and phenyl ring with Pro 168 AND Met 165.

Some of the common interacting amino acid residues involved in hydrogen bond formation, which play a vital role in binding to the target, identified through our docking studies include residues Tyr 265, 269,

274, Thr 266, Asn 268 with the papain-like protease and residues Thr 24,25,26, Cys 145, Ser 144 with the 3-chymotrypsin-like cysteine protease receptor.

The ADME prediction study of the best six molecules evaluated on QikProp ADME Schrodinger online software, demonstrated relatively satisfactory drug like properties.

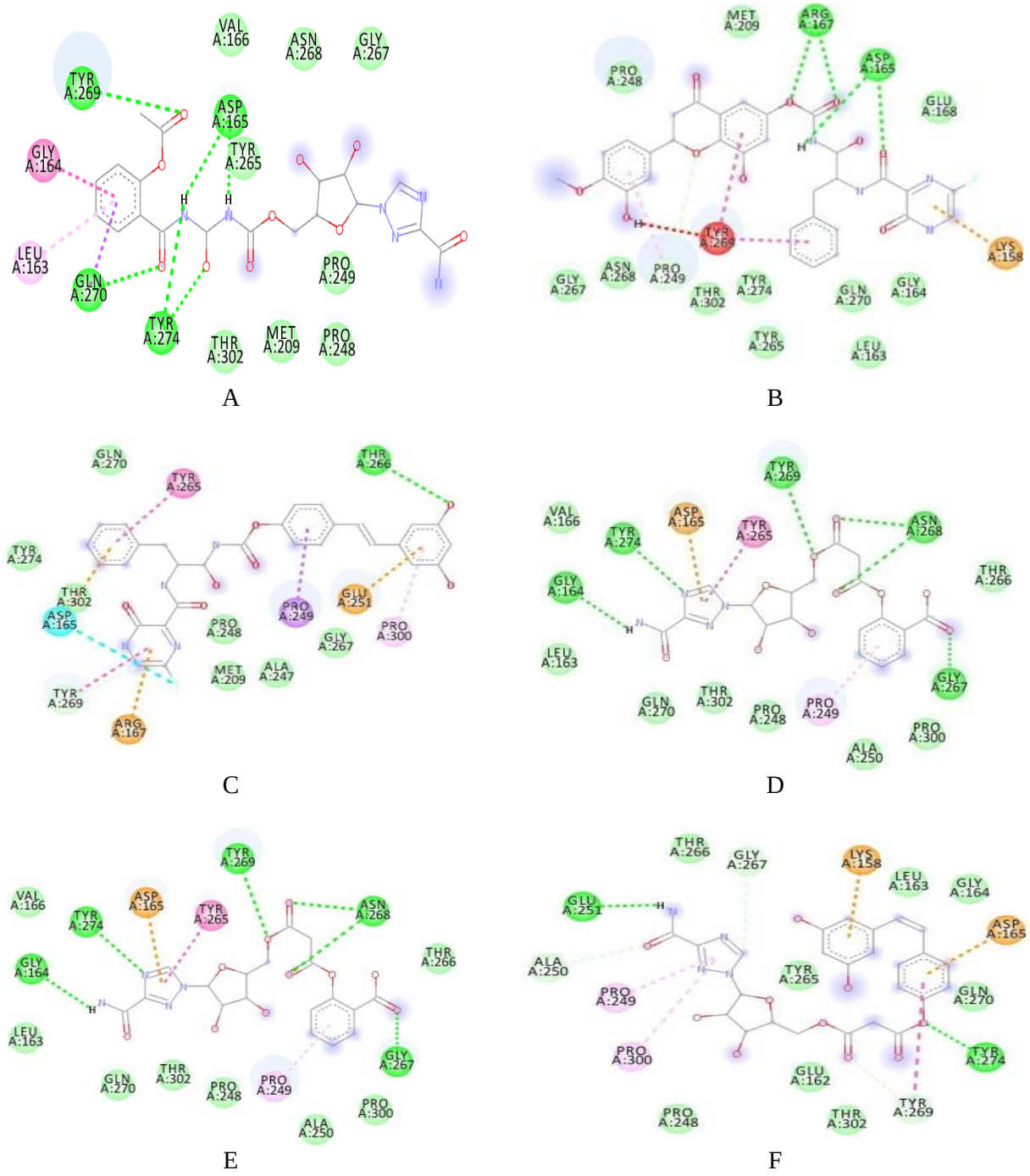



Figure 4. Docked poses in 3E9S receptor binding pocket A) Compound RL1 B) Compound FL2 C) Compound FL3 D) Compound RM1 E) Compound RM2 F) Compound RM3
(The figure shows the ligands docking within the active site. The hydrogen bonds are represented by green dotted lines, π–π interactions with yellow/pink dotted lines

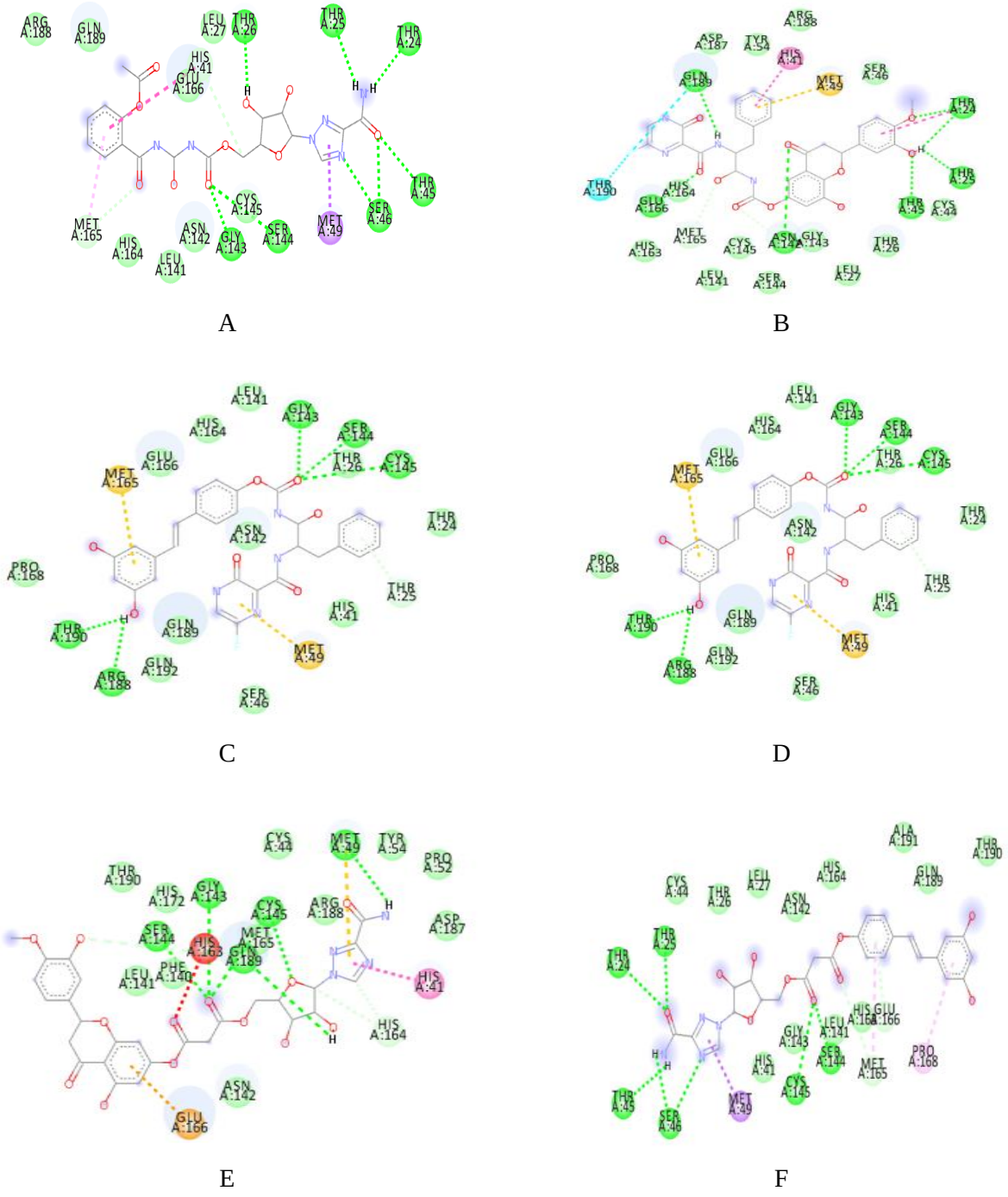



Figure 5. Docked poses in 6LU7 receptor binding pocket A) Compound RL1 B) Compound FL2 C) Compound FL3 D) Compound RM1 E) Compound RM2. F) Compound RM3.
(The figure shows the ligands docking within the active site. The hydrogen bonds are represented by green dotted lines, π–π interactions with yellow/pink dotted lines

Based on the docking and ADME prediction studies on papain-like protease (PL-pro) (PDB ID- 3E9S) and 3-chymotrypsin-like cysteine protease (CL-pro), two cysteine proteases of the SARS-CoV-2 virus that are vital for the replication and transcription of the viral genome, there was observed that FL2, which was a hybrid of favipiravir and hesperitin through 2-amino 2-hydroxy ethyl amide linker seemed to be the most promising hybrid designed to act with almost similar affinity to both the targets.

Also, RL1, a hybrid of ribavarin and salicyclic acid linked with the 2-amino 2-hydroxy ethyl amide chain showed comparable efficacy against both the protease targets. Figure 6 represents the docking poses of the most promising hybrids obtained through this in silico study. Both the identified hybrids contain the

hydoxyethylamine linker that is an important structural component of currently clinically employed HIV protease inhibitors like Nelfinavir, Indinavir and other protease inhibitors used in the treatment of HIV infection. The incorporation of this hydroxyethlyamine linker may help to mimic the transition state of the reactions catalysed by the PL-pro and CL-pro enzymes in the viral replication cycle. These designed inhibitors may serve as transition state inhibitors that may bind with greater affinity to the active site and may be less prone to hydrolysis. Hence these hybrid molecules may represent a new class of anti-viral agents with improved affinity than the individual substrates.

However it is anticipated that the likely hydrolysis of these hybrids may release the individual substrates that may also separately bind to the anti-viral targets and provide synergistic activity. Also as herperitin and salicyclic acid are well established anti-thrombotic agents, they may also elucidate this response, thereby proving to be of great potential in treatment of the rising associated complications of the viral infection.

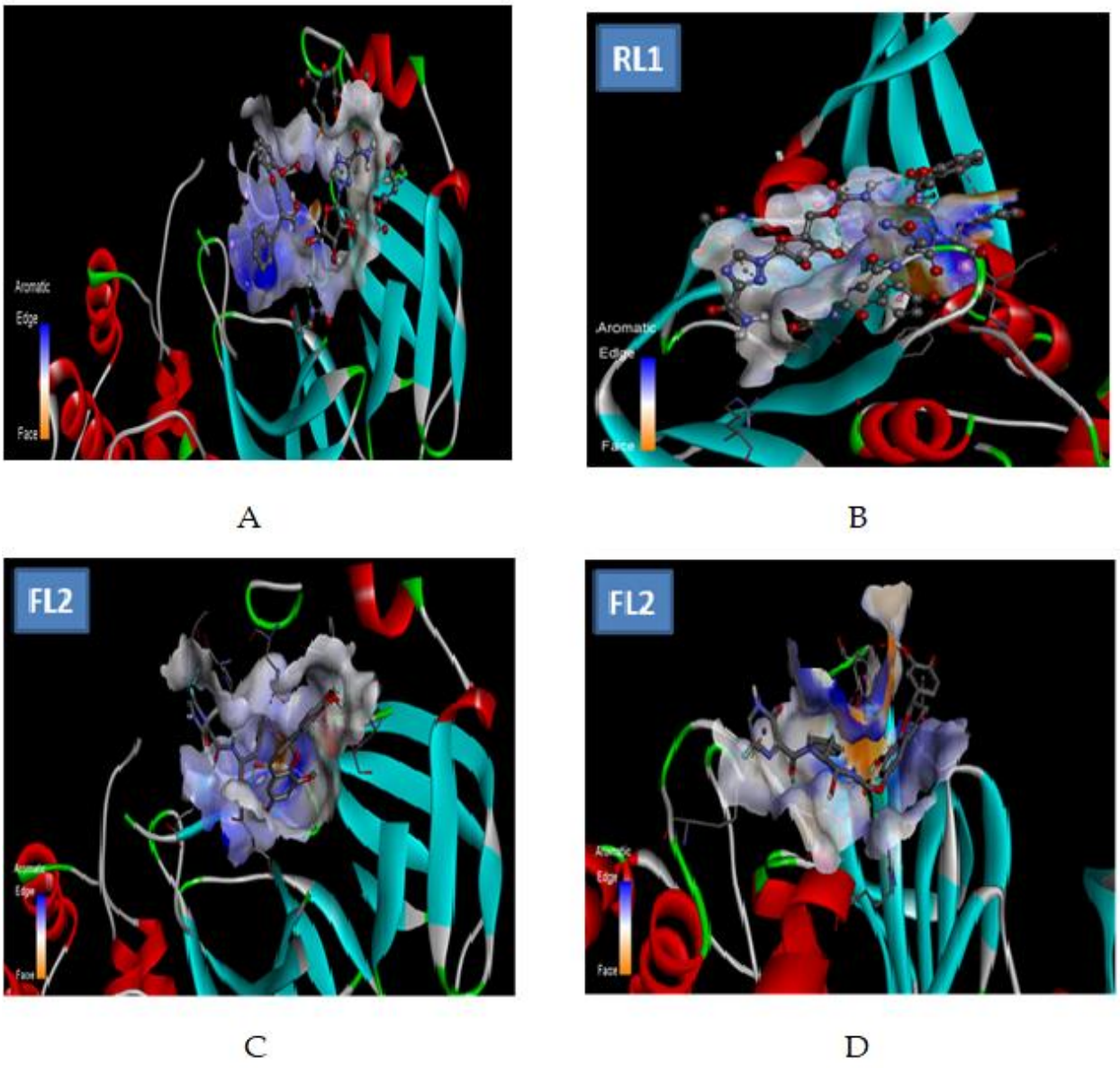



Figure 6. Docking Poses of RL1 in A) CL-pro and B) PL-pro; Docking Poses of FL2 in C) CL-pro and D) PL-pro

*Conclusions*

The present study focuses on the design of novel hybrids of antiviral and antithrombotic agents for synergistic use in the treatment of infections caused by the SARS-CoV-2 virus. Among the 24 compounds

screened using Autodock vina software, Compound FL2 i.e., 8-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-6-yl(2-(6-flouro-3-oxo-3,4-dihydropyrazine-2 carboxamido)-1-hydroxy-3-phenylpropyl)carbamate and Compound RL1 i.e., 2-((((((5-(3-carbamoyl-1H-1,2,4-triazol-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)carbonyl)amino)(hydroxy)methyl)carbamoyl)phenyl acetate prove to be promising agents with good affinity and strong binding interactions with both target proteins, papain-like protease (PDB:3E9S) and 3-chymotrypsin-like cysteine protease (PDB: 6LU7). The results of this study can prove to be useful to medicinal chemists involved in design of newer agents to fight the COVID pandemic. This novel class of hybrid agents may help to address the coronavirus infection and its associated complications and may be further explored for design of novel molecules in this field.

## References


1 World health organization official website https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed on 10th May 2021)

2 Retrived from https://www.medscape.com/answers/2500114-197401/what-is-covid-19 (accessed on 10th May 2021)

3 Retrived from https://www.republicworld.com/world-news/uk-news/uk-strain-spreads-to-30-countries-as-world-enters-2021-with-new-covid-variant.html (accessed on 10th May 2021)

4 Chan Y., Xin-feng X., Wei X., & Shu-wen L. (2020). Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacologica Sinica*, 41, 1141–1149. https://doi.org/10.1038/s41401-020-0485-4

5 Leila M., & Sorayya G. (2021). Genotype and phenotype of COVID-19: Their roles in pathogenesis. *Journal of Microbiology, Immunology and Infection*, 57, 159–163. https://doi.org/10.1016/j.jmii.2020.03.022

6 Mittal A., Manjunath K., Ranjan R., Kaushik S., Kumar S., & Verma V. (2020). COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. *PLoS Pathog.*, 16(8), e1008762. https://doi.org/10.1371/journal.ppat.1008762

7 Wang M., Rong Z., Li-Juan G., Xue-fei G. De-Ping W., & Ji-Min Cao. (2020). SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. *Front. Cell. Infect. Microbiol.*, *10*, 587269. https://doi.org/10.3389/fcimb.2020.587269

8 Brit L., William J. B., Alex K. & Michael G. (2020). Cardiovascular complications in COVID-19. *Am J Emerg Med.*, 38(7), 1504–1507. https://doi.org/10.1016/j.ajem.2020.04.048

9 Samidurai A., & Das A. (2020). Cardiovascular Complications Associated with COVID-19 and Potential Therapeutic Strategies. *Int J Mol Sci.*, 21(18), 6790. https://doi.org/10.3390/ijms21186790

10 Petrovic V., Radenkovic D., Radenkovic G., Djordjevic V., Banach M. (2020). Pathophysiology of Cardiovascular Complications in COVID-19. *Front. Physiol.*, 11, 575600. https://doi.org/10.3389/fphys.2020.575600

11 Page E., & Ariens R. (2021). Mechanisms of thrombosis and cardiovascular complications in COVID-19. *Thromb Res*, 200, 1–8. https://doi.org/%2010.1016/j.thromres.2021.01.005

12 Al-Mohammad A., & Partridge D. (2020). The cardiac complications of Covid-19 many publications, multiple uncertainities. *Vas. Biology*, 2 (1), R105–R114. https://doi.org/10.1530/VB-20-0009

13 Magro G. (2020). COVID-19: Review on latest available drugs and therapies against SARS-CoV-2. Coagulation and inflammation cross-talking. *Virus Res*, 286, 198070. https://doi.org/10.1016/j.virusres.2020.198070

14 Asselah T., Durantel D., Pasmant E., Lau G., & Schinazi R. (2020). COVID-19: discovery, diagnostics and drug development. *J Hepatol*, 74(1), 168–184. https://doi.org/10.1016/j.jhep.2020.09.031

15 RAPS, Regulatory Affairs Professionals Society. Retrived from https://www.raps.org/news-and-articles/news-articles/2020/3/covid-19-vaccine-tracker (accessed May 12, 2021)

16 Our world in Data. Retrived from https://ourworldindata.org/covid-vaccinations (accessed May 12,2021)

17 McGovern R., Conway P., Pekrul I., & Tujjar O. (2020). The Role of Therapeutic Anticoagulation in COVID-19. *Case Rep Crit Care*, 1–7. https://doi.org/10.1155/2020/8835627

18 Liao S., Shao S., Chen Y., Chen Y., & Hung M. (2020). Incidence and mortality of pulmonary embolism in COVID-19: a systematic review and meta-analysis. *Crit Care*, 24(1), 1-5. https://doi.org/10.1186/s13054-020-03175-z

19 Costanzo, L., Palumbo, F., Ardita, G., Antignani, P., Arosio, E, & Failla, G. (2020). Coagulopathy, thromboembolic complications, and the use of heparin in COVID-19 pneumonia. *J Vasc Surg Venous Lymphat Disord*, 8(5), 711–716. https://doi.org/10.1016/j.jvsv.2020.05.018

20 Abdolmaleki A., & Ghasemi J. (2017). Dual-acting of Hybrid Compounds — A New Dawn in the Discovery of Multi-target Drugs: Lead Generation Approaches. *Curr Top Med Chem*, 17(9), 1096-1114. https://doi.org/10.2174/1568026616666160927151144

21 Pawełczyk A., Sowa-Kasprzak K., Olender D., & Zaprutko L. (2018). Molecular Consortia-Various Structural and Synthetic Concepts for More Effective Therapeutics Synthesis. *Int J Mol Sci*., 19(4), 1104. https://doi.org/10.3390/ijms19041104

22 Gupta V., & Datta P. (2019). Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids. *Indian J Med Res*., 149 (2), 97–106. https://doi.org/10.4103/ijmr.IJMR_755_18

23 Pokrovskaya V., & Baasov T. (2010). Dual-acting hybrid antibiotics: a promising strategy to combat bacterial resistance. *Expert Opin Drug Discov*., 5(9), 883–902. https://doi.org/10.1517/17460441.2010.508069

24 Brötz-Oesterhelt H., & Brenner N. (2008). How many modes of action should an antibiotic have? *Curr Opin Pharmacol.*, 8, 564–573. https://doi.org/10.1016/j.coph.2008.06.008

25 Meunier B. (2008). Hybrid molecules with a dual mode of action: dream or reality? *Acc Chem Res.*, 41(1), 69–77. https://pubs.acs.org/doi/10.1021/ar7000843

26 Dana S., Valissery P., Kumar S., Gurung S., Mondal N., Dhar S., & Mukhopadhyay P. (2020). Synthesis of Novel Ciprofloxacin-Based Hybrid Molecules toward Potent Antimalarial Activity. *ACS Med Chem Lett.*, 11(7), 1450–1456. https://doi.org/10.1021/acsmedchemlett.0c00196

27 Pepe D., Toumpa D., André-Barrès C., Menendez C., Mouray E., Baltas M., Grellier P., Papaioannou D., & Athanassopoulos C. (2020). Synthesis of Novel G Factor or Chloroquine-Artemisinin Hybrids and Conjugates with Potent Antiplasmodial Activity. *ACS Med Chem Lett.*, 11(5), 921–927. https://doi.org/10.1021/acsmedchemlett.9b00669

28 Contreras J., & Sippl W. (2208). Homo and heterodimer ligands: The twin drug approach. In: Wermuth CG. (Eds) *The practice of medicinal chemistry*. 3rd edition. Academic Press Elsevier; New York, NY, USA, 380–414. https://dx.doi.org/10.1016 %2Fj.bmc.2015.06.034

29 Hasan M., Leak R., Stratford R., Zlotos D., & Witt-Enderby P. (2018). Drug conjugates-an emerging approach to treat breast cancer. *Pharmacol Res Perspect.*, 6(4), e00417. https://doi.org/10.1002/prp2.417

30 Rodríguez-Franco M., Fernández-Bachiller M., Pérez C., Hernández-Ledesma B., & Bartolomé B. (2006). Novel tacrine–melatonin hybrids as dual-acting drugs for Alzheimer disease, with improved acetylcholinesterase inhibitory and antioxidant properties. *J Med Chem.*, 49(2), 459–462. https://doi.org/10.1021/jm050746d

31 Decker M. (2017). *Design of hybrid molecules for drug development*. New York, NY, USA: Elsevier.

32 Bansal Y., & Silakari O. (2014). Multifunctional compounds: smart molecules for multifactorial diseases. *Eur J Med Chem.*, 76, 31–42. https://doi.org/10.1016/j.ejmech.2014.01.060

33 Przybyłowska M., Dzierzbicka K., Kowalski S., Chmielewska K., & Inkielewicz-Stepniak I. (2020). Therapeutic Potential Of Multifunctional Derivatives Of Cholinesterase Inhibitors. *Curr Neuropharmacol.*, 19(8), 1323–1344. https://doi.org/10.2174/1570159X19666201218103434

34 Starnowska-Sokół J., & Przewłocka B. (2020). Multifunctional Opioid-Derived Hybrids in Neuropathic Pain: Preclinical Evidence, Ideas and Challenges. *Molecules*, 25(23), 5520. https://doi.org/10.3390/molecules25235520

35 Khalili J., Zhu H., Mak N., Yan Y., & Zhu Y. (2020). Novel coronavirus treatment with ribavirin: Groundwork for an evaluation concerning COVID19. *J Med Virol.*, 92(7), 740–746. https://doi.org/10.1002/jmv.25798

36 Cai Q., Yang M., Liu D., Chen J., Shu D., Xia J., Liao X., Gu Y., Cai Q., Yang Y., & Shen C. (2020). Experimental treatment with favipiravir for COVID-19: an open-label control study. *Engineering*. 6(10), 1192–1198. https://doi.org/10.1016/j.eng.2020.03.007

37 Vafaei S., Razmi M., Mansoori M., Asadi-Lari M., & Madjd Z. (2020). Spotlight of Remdesivir in comparison with ribavirin, Favipiravir, Oseltamivir and Umifenovir in coronavirus disease 2019 (COVID-19) pandemic. http://dx.doi.org/10.2139/ssrn.3569866

38 Carvallo H., Hirsch R., & Farinella M. (2020). Safety and Efficacy of the combined use of ivermectin, dexamethasone, enoxaparin and aspirin against COVID-19. medRxiv. https://doi.org/10.1101/2020.09.10.20191619

39 Glatthaar Saalmüller B., Mair K., & Saalmüller A. (2017). Antiviral activity of aspirin against RNA viruses of the respiratory tract — an in vitro study. *Influenza Other Respir Viruses.*, 11(1), 85–92. https://doi.org/10.1111/irv.12421

40 Yang Y., Wang X., Wang S., Wang H., & Chen J. (2008). Suppressive effect in vitro of resveratrol on ADP induced human platelet aggregation and its active mechanism. *Yao Xue Xue Bao.*, 43(4), 356–360. https://doi.org/10.1111/irv.12421

41 Bhat K., Kosmeder J., & Pezzuto J. (2001). Biological effects of resveratrol. *Antioxid Redox Signal.*, 3(6), 1041–1064. https://doi.org/10.1089/152308601317203567

42 Randall R., & Goodbourn S. (2008). Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol.*, 89(1), 1–47. https://doi.org/10.1099/vir.0.83391-0

43 Roohbakhsh A., Parhiz H., Soltani F., Rezaee R., & Iranshahi M. (2015). Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. *Life Sci.*, 124, 64–74. https://doi.org/10.1016/j.lfs.2014.12.030

Р. Бхиманвар, А. Томас, Л. Котхапаллы, А. Годасе,
С. Ганди, С. Чанданы, Г. Мор, Г. Джадхав, С. Чоудхари

## SARS-CoV-2 инфекцияларына және онымен байланысты асқынуларға қарсы қос антивирустық және антитромботикалық потенциалы бар әлеуетті гибридті молекулалар *in silico* зерттеулерін қолдану

COVID, SARS-CoV вирусына негізделген ауру 2019 жылдың желтоқсан айында Қытайдың Ухань қаласында анықталды. Бастапқыда бұл жай ғана тыныс алу жүйесінің инфекциясы болып саналды, бірақ кейін оның таралу сипатына байланысты пандемия жарияланды. Емдеудің әртүрлі нұсқалары жүзеге асырылды, соның ішінде ремдесвир, фавипиравир сияқты вирусқақарсы препараттар, сонымен қатар витаминдер мен антиоксиданттар да бар. Кейінгі зерттеулер COVID-19 инфекциясының тромбоздық жүрек-қантамырлық асқынуларға әкелетінін анықтады, бұл осы инфекциямен байланысты өлім-жітімнің артуына басты алаңдаушылық тудырады. Осы зерттеуде вирусқақарсы және антитромботикалық қасиеттері бар гибридті молекулалардың *in silico* конструкциясы зерттелді. Autodock Vina бағдарламалық құралын пайдалану арқылы докингті зерттеу жүргізілді және жобаланған қосылыстардың байланысу энергиясы папаинтәрізді протеаза (PDB: 3E9S) және 3-химотрипсинтәрізді цистеин протеазасы (PDB: 6LU7) үшін анықталды. Түйіскен позалар мен аминқышқылдарының өзара әрекеттесуі Biovia Discovery studio 4.5 көмегімен тексерілді. Барлық жобаланған қосылыстардың байланыс энергиялары стандарттармен салыстырылды, қосылыс RL1 (2-(5-(3-карбамоил-1H-1,2,4-триазол-1-ил)-3,4-дигидрокситетрагидрофуран-2ил)метокси)карбонил) амин)-(гидрокси)метил)карбамоил)фенилацетат) және қосылыс FL2 (8-гидрокси-2-(3-гидрокси-4-метоксифенил)-4-оксохроман-6-ил-(2-(6-фтор)3-оксо-3,4-дигидропиразин-2-карбоксамидо)-1-гидрокси-3-фенилпропил)карбамат) күшті байланысуы бар перспективалы агенттер болып шықты. Вирустық репликацияны тежейтін гибридті молекулалар, мүмкін өтпелі күй ингибиторлары ретінде, SARA-Co-V инфекциясын және онымен байланысты асқынуларды емдеуде пайдалану үшін әрі қарай зерттелуі мүмкін.

*Кілт сөздер:* COVID-19, CL-pro, PL-pro, вирусқа қарсы, антитромботикалық, молекулалық докинг, гибридті молекула.

Р. Бхиманвар, А. Томас, Л. Котхапаллы, А. Годасе,
С. Ганди, С. Чанданы, Г. Мор, Г. Джадхав, С. Чоудхари

## Потенциальные гибридные молекулы с двойным противовирусным и антитромботическим действием против инфекции SARS–CoV–2 и связанных с ней осложнений с использованием исследования *in silico*

COVID–19, заболевание, вызванное вирусом SARS–CoV, было выявлено в Ухане (Китай) в декабре 2019 г. Первоначально оно считалось просто инфекцией дыхательной системы, но из-за его трансмиссивного характера оно было объявлено пандемией. Были реализованы различные варианты лечения, включая противовирусные препараты, такие как ремдесвир, фавипиравир, а также витамины и антиоксиданты. Дальнейшие исследования показали, что инфекция Covid–19 приводит к тромботическим сердечно-сосудистым осложнениям, что является основной причиной повышенной смертности, связанной с этой инфекцией. В этом исследовании изучена конструкция *in silico* гибридных молекул с противовирусными и антитромботическими свойствами. Исследование докинга проводили с использованием программного обеспечения Autodock Vina, а энергии связывания разработанных соединений определяли для папаиноподобной протеазы (PDB: 3E9S) и 3-химотрипсиноподобной цистеиновой протеазы (PDB: 6LU7). Состыкованные позы и взаимодействия аминокислот были проверены с использованием Biovia Discovery studio 4.5. Энергии связи всех разработанных соединений сравнивали со стандартами, соединение RL1 (2-(5-(3-карбамоил-1H-1,2,4-триазол-1-ил)-3,4-дигидрокситетрагидрофуран-2-ил)метокси)карбонил)амино)(гидрокси)метил)карбамоил)фенилацетат) и соединение FL2 (8-гидрокси-2-(3-гидрокси-4-метоксифенил)-4-оксохроман-6-ил-(2-(6-фтор-3-оксо-3,4-дигидропиразин-2-карбоксамидо)-1-гидрокси-3-фенилпропил)карбамат) оказались многообещающими агентами с сильным связывающим взаимодействием. Гибридные молекулы, которые ингибируют репликацию вируса, возможно, в качестве ингибиторов переходного состояния, могут быть дополнительно исследованы для использования в лечении инфекции SARS–CoV и связанных с ней осложнений.

*Ключевые слова:* COVID–19, CL-pro, PL-pro, противовирусный, антитромботический, молекулярный докинг, гибридная молекула.

Information about authors*

**Bhimanwar, Rachana** — Assistant Professor, Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: rachana.bhimanwar@dypvp.edu.in; https://orcid.org/0000-0001-6392-1526

**Thomas, Asha** (*corresponding author*) — HOD and Professor, Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: asha.thomas@dypvp.edu.in; https://orcid.org/0000-0003-1058-8779

**Kothapalli, Lata** — Associate Professor, Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: lata.kothapalli@dypvp.edu.in; https://orcid.org/0000-0002-7412-5805

**Godase, Anagha** — Assistant Professor, Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: angha.godse@dypvp.edu.in; https://orcid.org/0000-0001-9145-2990

**Gandhi, Sejal** — Assistant Professor, Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: sejal.gandhi@dypvp.edu.in; https://orcid.org/0000-0002-7079-1886

**Chandani, Sneha** — Assistant Professor, Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: sneha.chandani@dypvp.edu.in; https://orcid.org/0000-0003-3891-2841

**More, Ghansham** — Department of Pharmaceutical Chemistry, Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pune- 411018, Maharashtra, India; e-mail: gsmniper@gmail.com; https://orcid.org/0000-0002-9010-2275

**Jadhav, Gopal** — School of Medicine, Omaha Campus, Creighton University, NE; e-mail: gopaljadhav@creighton.edu; https://orcid.org/0000-0002-2883-5574

**Choudhary, Sameer** — RASA Life Science Informatics, Pune, Maharashtra, India; e-mail: aftab@rasalsi.com; https://orcid.org/0000-0001-8056-1374

*The author's name is presented in the order: *Last Name, First and Middle Names*