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Molecular docking study of co-trimoxazole against SARS-CoV-2 main protease and RNA-dependent RNA polymerase: An *in silico* approach

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic, driven by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused significant morbidity and mortality worldwide. Although various therapeutic options are being explored, there is still a need for effective treatments. Co-trimoxazole, a broad-spectrum antibiotic, has shown promising results in clinical studies in patients with COVID-19; however, its direct antiviral activity remains unclear. Thus, this study aimed to evaluate the direct effect of co-trimoxazole on SARS-CoV-2 using computational approaches. The molecular interactions for co-trimoxazole were analyzed against two vital SARS-CoV-2 proteins, the main protease (M^{pro}) and the RNA-dependent RNA polymerase (RdRp), using AutoDock Vina. Our findings reveal that both components of co-trimoxazole, sulfamethoxazole, and trimethoprim, exhibit good binding affinities with M^{pro} and RdRp, implying their potential inhibitory effects on viral replication with binding energies of < - 6 kcal/mol, which were close to reference drugs. This suggests that co-trimoxazole may offer therapeutic benefits for COVID-19 patients, beyond its ability to reduce inflammation and secondary infections. More clinical studies are warranted to investigate its safety and potential as a treatment option for COVID-19.

Keywords: COVID-19; SARS-CoV-2; co-trimoxazole; main protease; RNA-dependent RNA polymerase; molecular docking

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1. INTRODUCTION

Coronavirus diseases 2019 (COVID-19), a viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first diagnosed in December 2019 in Wuhan, Hubei, China. The symptoms were similar to those seen in cases of severe acute respiratory syndrome (SARS) (Naibkhil and Naibkhil, 2020). The disease quickly spread across borders, leading to a pervasive pandemic that inflicted significant morbidity and mortality. As of 18 October 2023, recorded figures indicated a staggering 771,407,825 confirmed cases, a tragic loss of 6,972,152 lives, and a total of 13,516,282,548 vaccine doses administered up to 7 October 2023 (WHO, 2023).

Numerous universities and pharmaceutical firms are actively engaged in the advancement of both vaccines and therapeutic remedies. Various authorized medications, including antiviral and antimalarial compounds, are undergoing rigorous clinical evaluations involving patients with COVID-19. However, the absence of dedicated pharmaceutical interventions to treat COVID-19 remains evident. Different nations have resorted to various medicinal approaches within their medical facilities. However, research endeavors persist as

scientists explore diverse medicinal options, striving to discover effective treatments.

Co-trimoxazole is an antimicrobial agent with a broad-spectrum impact on both aerobic gram-positive and gram-negative bacteria, as well as protozoa and fungi. This compound is effective when composed of fixed amounts of sulfamethoxazole and trimethoprim. For years, co-trimoxazole prophylaxis has been standard of care in low-income countries (Al-Kuraishy et al., 2020). It has been administered to children at risk of or living with human immunodeficiency virus (HIV), adolescents, and adults with the same condition. Similarly, co-trimoxazole is recommended as a prophylactic measure in economically disadvantaged regions, to reduce mortality and morbidity among HIV-infected individuals (WHO, 2006). Several studies have demonstrated the effectiveness of co-trimoxazole in patients, indicating its potential to reduce mortality, hospitalization, and duration of stay in critical care units (Quadery et al., 2020, 2022; Singh et al., 2021). This may be due to the drug's anti-inflammatory effects (Mermin et al., 2004; Al-Kuraishy et al., 2020). However, to our knowledge, no studies have evaluated the direct antiviral activity of co-trimoxazole. COVID-19 spreads to the lungs via air droplets, where the virus binds to the angiotensin-converting enzyme (ACE2) and releases its RNA into the cells. This RNA is then translated to produce polyproteins necessary for viral pathogenesis. RNA polymerases plays an important role in the replication of viral RNA to produce new virus particles that infect neighboring cells. The viral polyproteins are processed into active components by the viral protease, specifically main protease, which is a potential target for anti-COVID-19 drugs (Vijayakumar et al., 2022). Developing pharmaceutical compounds that directly interactions with conserved enzymes such as the main protease or 3C-like protease (M^{pro}), papain-like protease, nonstructural protein 12, and RNA-dependent RNA polymerase (RdRp) could exhibit a board range of activity and efficacy; Consequently, many computational studies target these enzymes (Mengist et al., 2021; Lv et al., 2022).

Computational biology-driven drug development models facilitate the discovery of novel pharmaceuticals and enhancing understanding of the molecular characteristics of protein targets and their interactions with ligands. Additionally, repurposing Food and Drug Administration (FDA) approved drugs can be a time-saving approach for developing treatments targeting COVID-19, as these drugs have already undergone comprehensive assessments for their toxicity and safety in humans (Vijayakumar et al., 2022). In this study, we evaluated the molecular docking of co-trimoxazole's against two proteins of SARS-CoV-2. The results were compared with those of three recently clinically evaluated medicines for COVID-19 patients: nirmatrelvir, ritonavir, and remdesivir.

2. MATERIALS AND METHODS

An *in silico* method was used to evaluate the binding affinity between co-trimoxazole and the main protease (M^{pro}, PDB: 6LU7) and RNA-dependent RNA polymerase (RdRp, PDB: 6M71) of SARS-CoV-2, extracted from Wuhan-Hu-1 variant. The results were compared with the affinities of nirmatrelvir, ritonavir, and remdesivir for these specified SARS-CoV-2 proteins.

Discovery Studio Visualiser 3.0, Open Babel, PyRx, and AutoDock Vina were used for the docking study. The 3D structures of SARS-CoV-2 proteins, including the main protease (M^{pro}, PDB: 6LU7) and RNA-dependent RNA polymerase (RdRp, PDB: 6M71), were downloaded from the Protein Data Bank. The chemical structures of co-trimoxazole components (sulfamethoxazole and trimethoprim), nirmatrelvir, ritonavir, and remdesivir were retrieved from PubChem.

2.1 Ligand and protein preparation

The 3D structures of co-trimoxazole components (sulfamethoxazole and trimethoprim), nirmatrelvir, ritonavir, and remdesivir were retrieved from PubChem and saved in .sdf format. The 3D structures of SARS-CoV-2 proteins, including the main protease (M^{pro}, PDB: 6LU7) and RNA-dependent RNA polymerase (RdRp, PDB: 6M71), were downloaded from the Protein Data Bank. Proteins were checked for missing atoms and corrected. Water molecules and heteroatoms were removed, and polar hydrogens were added and charges were assigned to each protein. The proteins were then saved in .pdb format for docking study.

2.2 Docking studies

Co-trimoxazole and proteins were uploaded to PyRx 0.8, which utilize Autodock Vina and Autodock 4.2 for virtual screening. The energy of the co-trimoxazole structure was minimized using the conjugate gradient optimization algorithm with 200 steps and the UFF forcefield. Both proteins and ligands were then converted to the pdbqt format and designated as macromolecules and ligands, respectively. Docking was performed as blind docking, with all the other software parameters set to default. Visualization was done using Discovery Studio Visualizer 3.0, and the conformer with the minimum docking score was selected for further analysis. Nirmatrelvir, ritonavir, and remdesivir were used as reference molecules, with remdesivir also serving for method validation.

3. RESULTS

In this study, the molecular interactions of co-trimoxazole with different SARS-CoV-2 proteins were analyzed. The validation of the docking methods for 6LU7 and 6M71 involved iterative docking with Remdesivir. The consistent output of these repeated docking trials indicates that the interaction occurred at the same binding site, involving identical residues. Furthermore, the root mean square deviation values for the resulting conformations were found to be below 2 Å. Both sulfamethoxazole and trimethoprim showed molecular interaction with the viral protein, with binding energies of -6.8 kcal/mol and -6.0 kcal/mol, respectively. As shown in Figure 1, the sulfamethoxazole complex with 6LU7 formed three hydrogen bonds with CYS145, HIS164, and ASP187 and three Pi-alkyl bonds with HIS163, CYS145, and MET49. On the other hand, trimethoprim formed four hydrogen bonds with PHE140, LEU141, and GLY143 and five alkyl bonds with CYS145, LEU27, and MET49. When compared to the reference drugs, ritonavir, remdesivir, and nirmatrelvir showed interactions with binding energies of -7.7 kcal/mol, -7.8 kcal/mol, and -7.0 kcal/mol, respectively, which were slightly higher than the co-

trimoxazole complex with the viral protein. Figure 2 illustrates the molecular interaction of ritonavir, remdesivir, and nirmatrelvir with viral 6LU7 protein.

Both sulfamethoxazole and trimethoprim also interacted with the 6M71 viral protein, with binding energies of -6.7 kcal/mol and -6.5 kcal/mol, respectively. As shown in Figure 3, sulfamethoxazole formed three hydrogen bonds with PHE219, ARG116, and GLU86, three Pi-alkyl bonds with ILE114 and TYR87, one Pi-Pi with HIS99, one Pi-sulfur bond with HIS99 and one C-H with HIS99. Trimethoprim formed five hydrogen bonds with SER772, ALA771, ASN781, and VAL776, one alkyl bond with LYS780, and one C-H bond with SER784. For the reference drugs, the binding energies of ritonavir, remdesivir, and nirmatrelvir with the viral protein were recorded at -8.3 kcal/mol, -8.6 kcal/mol, and -8.0 kcal/mol, respectively. Figure 4 illustrated the molecular interactions of ritonavir, remdesivir, and nirmatrelvir with the viral 6M71 protein. Table 1 summarizes the interactions of co-trimoxazole with 6LU7 and 6M71.

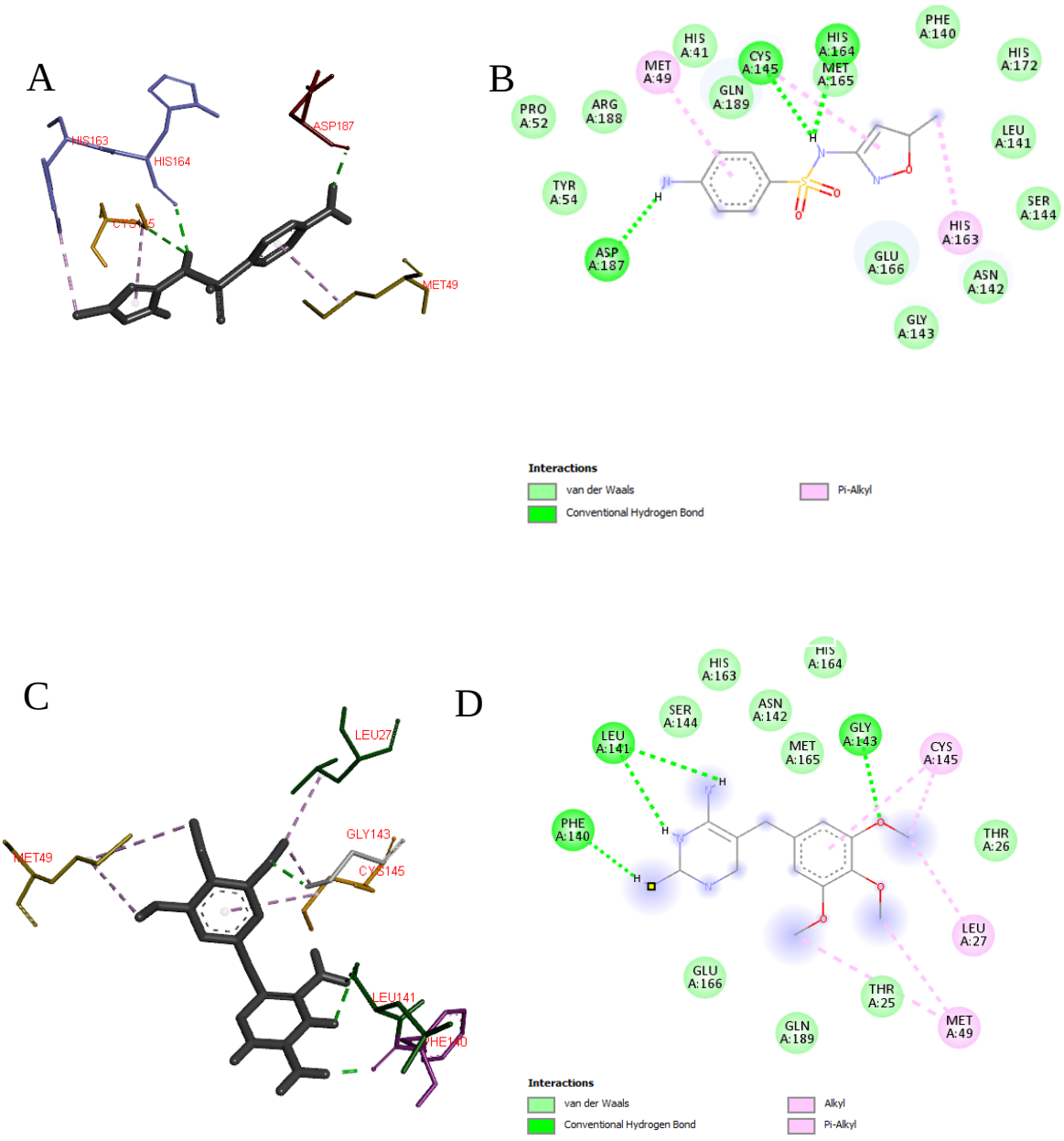

Figure 1. Docking with 6LU1; (A) 3D docking pose of sulfamethoxazole on the binding surface, (B) 2D interaction of sulfamethoxazole, (C) 3D docking pose of trimethoprim on the binding surface, and (D) 2D interaction of trimethoprim

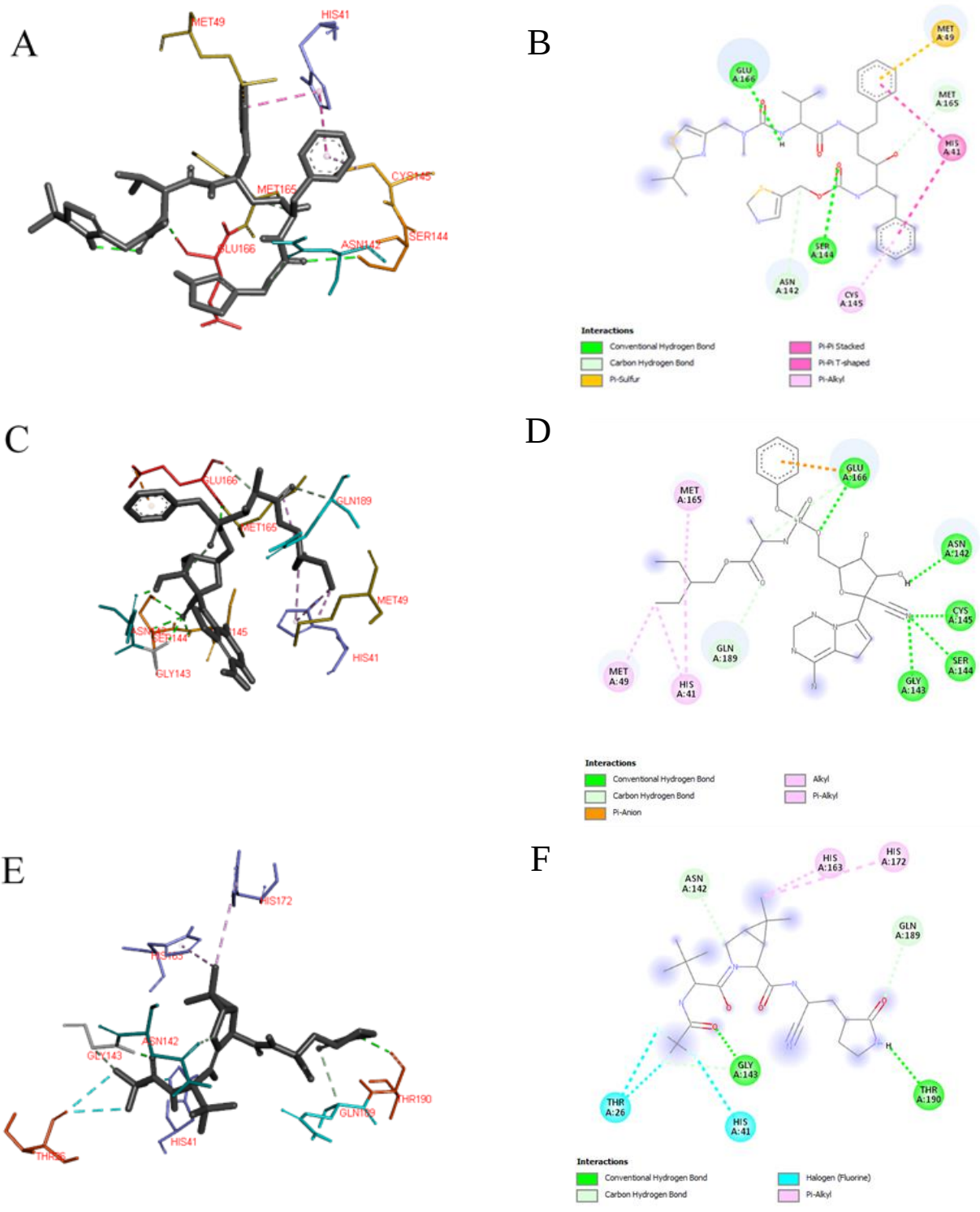

Figure 2. Docking with 6LU1; (A) 3D docking pose of ritonavir on the binding surface, (B) 2D interaction of ritonavir, (C) 3D docking pose of remdesivir on the binding surface, (D) 2D interaction of remdesivir, (E) 3D docking pose of nirmatrelvir on the binding surface, and (F) 2D interaction of nirmatrelvir

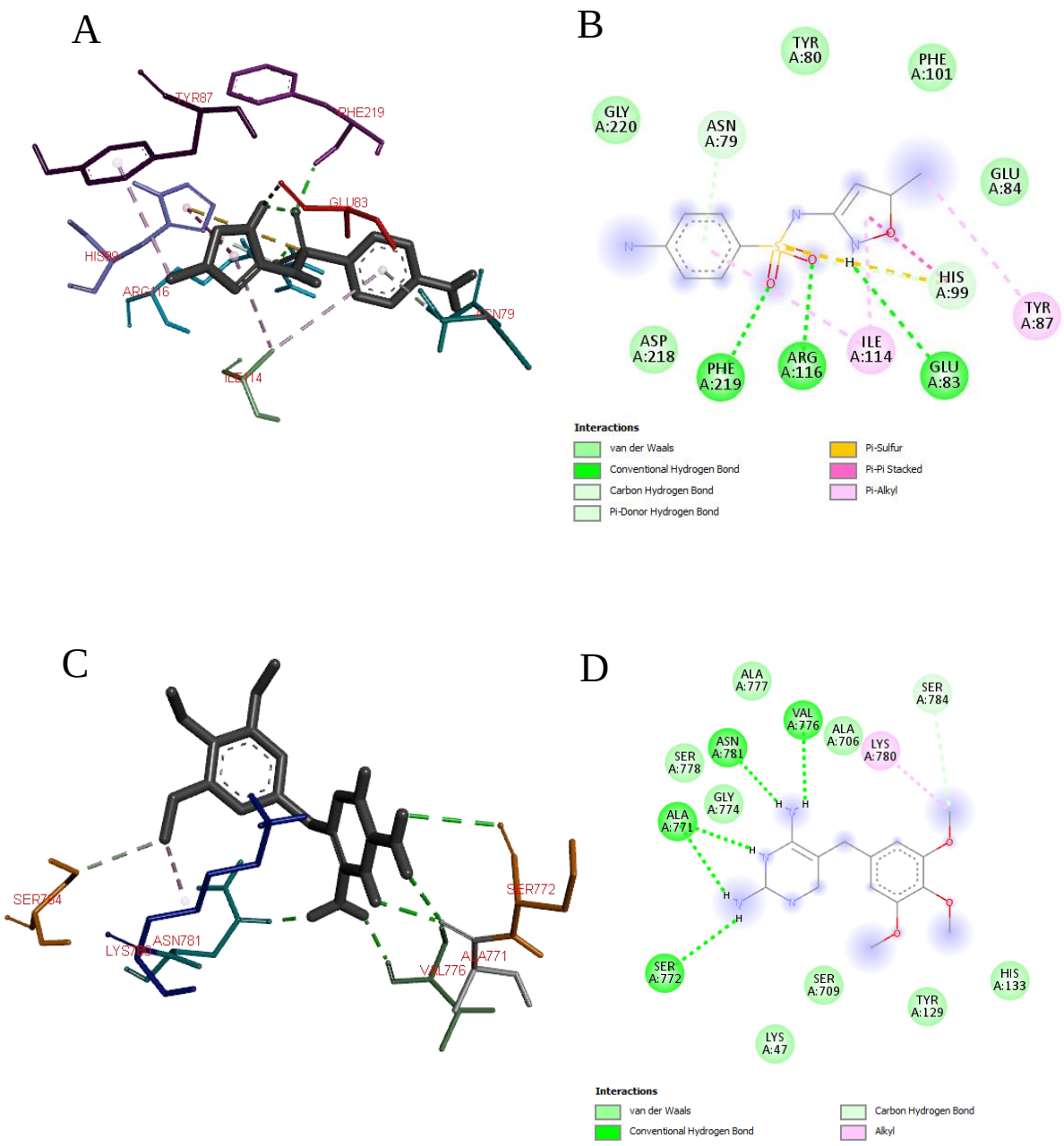

Figure 3. Docking with 6M71; (A) 3D docking pose of sulfamethoxazole on the binding surface, (B) 2D interaction of sulfamethoxazole, (C) 3D docking pose of trimethoprim on the binding surface, and (D) 2D interaction of trimethoprim

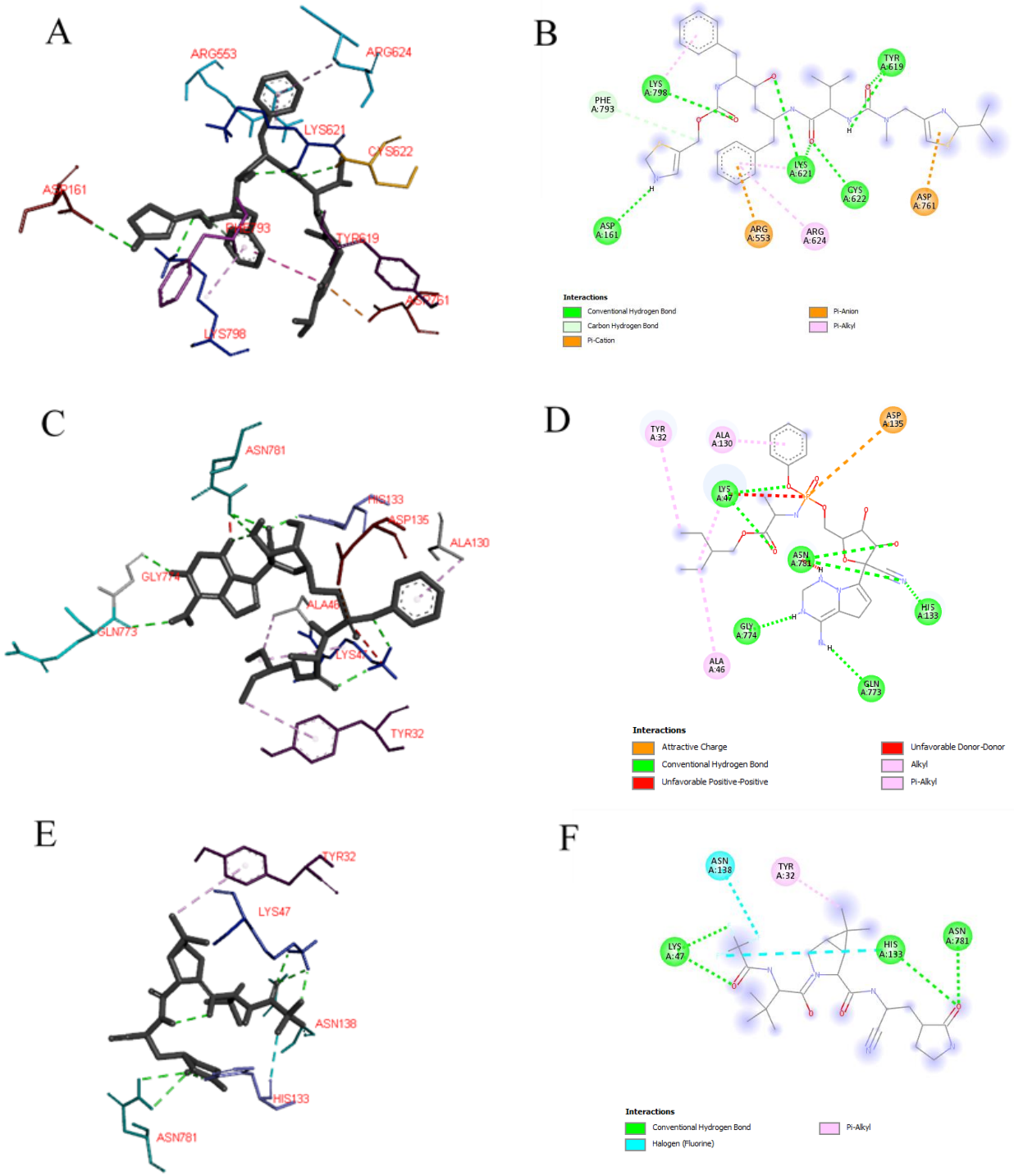

Figure 4. Docking with 6M71; (A) 3D docking pose of ritonavir on the binding surface, (B) 2D interaction of ritonavir, (C) 3D docking pose of remdesivir on the binding surface, (D) 2D interaction of remdesivir, and (E) 3D docking pose of nirmatrelvir on the binding

Table 1. Binding energy and interactions of co-trimoxazole with 6LU7 and 6M71

Protein	Compound	Binding energy (Kcal/mol)	Amino acids involved in the interaction	
			Hydrogen bonds	Other bonds
6LU7	Sulfamethoxazole	-6.8	CYS145, HIS164, ASP187	CYS145, LEU27, MET49
	Trimethoprim	-6.0	PHE140, LEU141, GLY143	
	Ritonavir	-7.7	GLU166, SER144	MET49, HIS163, HIS41, MET165, ASN142, CYS145
	Remdesivir	-7.8	GLU166, ASN142, CYS145, SER 144, GLY143	MET165, MET49, HIS41
	Nirmatrelvir	-7.0	THR190, GLY143	HIS163, HIS172, GLN189, HIS41, THR26, ASN142
6M71	Sulfamethoxazole	-6.7	PHE219, ARG116, GLU86	HIS99, ILE114, TRY87
	Trimethoprim	-6.5	SER772, ALA771, ASN781, VAL776	LYS780, SER784,
	Ritonavir	-8.3	TYR619, TYR619, LYS789, LYS621, LYS621, CYS622, ASP161	ARG628, ASP761, PHE793, ASP558
	Remdesivir	-8.6	LYS47, LYS47, ASN781, ASN781, HIS133, GLN773, GLY774	ASP135, ALA130, TYR32, ALA46
	Nirmatrelvir	-8.0	HIS133, ASN781, LYS47, LYS47	ASN138, TYR32, HIS133

4. DISCUSSION

The present study aimed to evaluate the molecular interaction of co-trimoxazole with two important proteins, M^{pro} and RdRP, which are essential for virus replication and maturation. RdRP is the main polymerase responsible for virus multiplication and the production of viral polyproteins, while M^{pro} is vital for processing these polyproteins into active constituents. Our results showed that the co-trimoxazole components, sulfamethoxazole, and trimethoprim showed good binding affinities of less than -6 kcal/mol to both viral proteins. A binding energy value of 6 kcal/mol is considered the upper limit for the ligand-receptor interaction studies, and an energy of less than -6 kcal/mol is considered indicatives of a good binding affinity between ligands and receptors (Vijayakumar et al., 2022).

SARS-CoV-2 M^{pro}, a cysteine protease, exhibits widespread conservation across coronaviruses. The active form of M^{pro} is a homodimer, with each protomer comprising three distinct domains: domains I, II, and III. Domains I (residues 8 – 101) and II (residues 102 – 184) consist of antiparallel ß bars, and together form a structure similar to chymotrypsin. The substrate-binding pocket is located within domains I and II. The active site encompasses four distinct pockets (S1', S1, S2, and S3), with the S1 pocket containing catalytic dyad consisting of the residues of CYS145 and HIS41 (Lv et al., 2020). Our study showed that sulfamethoxazole binds between domains I and II, making one H-bond and one Pi-alkyl bond with CYS145, one of the two main residues of the M^{pro} active site, with a binding affinity of -6.8 Kcal/mol. On the other hand, trimethoprim binds to domain II of the viral protease, making two Pi-alkyl bonds with CYS145. However, the reference drugs, Ritonavir, Remdesivir, and Nirmatrelvir, also showed molecular interactions with M^{pro} in domains I and II, with a binding affinity relatively higher than those of co-trimoxazole components. The binding of co-trimexazole in the active site of viral M^{pro} with good binding affinities may indicate a potential inhibitory effect of the drug on the viral protein.

The synthesis of viral RNA is facilitated by RdRp, playing a pivotal role in the replication and transcription of the COVID-19 virus. Consequently, it is a significant target for nucleotide analogue antiviral inhibitors like Remdesivir (Gao et al., 2020). The protein resembles a concave right hand, divided into subdomains: the finger domain (amino acid residues 398 – 581, 628 – 687), the palm domain (582 – 627, 688 – 815), and the thumb domain (816 – 919). To maintain the structural stability of the RdRp, two additional Zn ions are necessary. The amino acid residues crucially involved in the interaction include TYR618, CYS622, ASN691, ASN695, MET755, ILE756, LEU757, LEU758, SER759, ASP760, ASP761, ALA762, VAL763, GLU811, PHE812, CSY813 and SER814 (Ahmad et al., 2020). According to our docking study, both co-trimoxazole components effectively bind to the viral RdRp with a good binding affinity; however, only trimethoprim was able to bind in the active site region of the protein. The reference drugs bind with a high binding affinity compared to co-trimoxazole components, mainly in the active site domains of the protein.

Co-trimoxazole comprises sulfamethoxazole and trimethoprim, which act synergistically. Trimethoprim effectively inhibits dihydrofolate reductase (DHFR), demonstrating a stronger affinity for bacterial and protozoal enzymes compared to mammalian enzymes (Tonelli et al., 2017). It serves as a potent enhancer of sulfonamides (Manyando et al., 2013). Although no studies have yet explored the antiviral potential of co-trimoxazole, existing research indicates that substances with DHFR inhibitory properties exhibit efficacy against influenza viruses. For example, compounds like 1-aryl-4,6-diamino-1,2-dihydrotriazines, structurally similar to cycloguanil (an antimalarial drug), demonstrate inhibitory effects on influenza A (H1N1) and B, as well as respiratory syncytial viruses. These compounds target DHFR, exerting direct inhibitory effects (Tonelli et al., 2017). In addition, investigations have shown that co-trimoxazole prophylaxis leads to a reduction in HIV load among infected individuals. Before the use of co-trimoxazole, HIV patients experienced a median viral load increase of 0.90 (range:

0.5 – 1.3) log10 copies per mL per year, whereas during co-trimoxazole prophylaxis, the increase reduced to 0.08 (range: -0.02 – 0.19) log10 copies per mL per year (Mermin et al., 2004). Co-trimoxazole exhibits notable therapeutic efficacy and contributes to a reduction in mortality rates when administered along with antiretroviral drugs in HIV patients. In addition, it enhances the therapeutic effects of acyclovir in cases of initial episodes of genital herpes (Kinghorn et al., 1986).

Few studies have been conducted to evaluate the efficacy of co-trimoxazole on COVID-19. In a study by Quadery et al. (2020), COVID-19 patients who received oral trimethoprim 200 mg twice a day or co-trimoxazole 960 mg twice a day for five days, in addition to standard therapy, exhibited significantly superior outcomes compared to those who received only the standard therapy. These benefits included a reduction in hospital mortality (from 32% to 5%), a shorter hospital stay (mean of 22 days reduced to 9 days), and a decrease in the need for ventilatory support (from 16 patients to 3 patients). Furthermore, patients who received the additional treatment showed improvement in their clinical parameters within 48 h of starting the treatment (Quadery et al., 2020). In a similar study, individuals who received co-trimoxazole 960 mg every 8 h for 7 to 10 days, in addition to standard therapy, exhibited markedly improved outcomes. These included a reduction in inpatient mortality rates (13% compared to 40%, p<0.001), a shorter hospital stay (mean of 11 days compared to 15 days, p<0.001), a shorter length of stay in the critical care unit (mean of 6 days compared to 11 days, p<0.001), and a decreased requirement of mechanical ventilation (16% compared to 42%, p<0.001). Furthermore, there was a notable reduction in C-reactive protein (CRP) levels on day 7, with a mean of 38 mg/L compared to 62 mg/L, p = 0.001 (Singh et al., 2021). In a recent study involving 111 COVID-19 patients, the findings suggest that the mortality rate during hospitalization was 11% in the co-trimoxazole cohort compared to 29% in the standard therapy cohort (p = 0.020). The co-trimoxazole group had 9% of its patients receive mechanical ventilation, while the standard therapy group had 13% of its patients undergo this intervention. The recovery duration was six days for the co-trimoxazole group, compared to 7 days for the standard therapy group (p = 0.466) (Quadery et al., 2022).

Furthermore, co-trimoxazole exerts inhibitory effects on pro-inflammatory cytokines, directly mitigating systemic and intestinal inflammation by modulating immune and epithelial cell activation. It also indirectly influences the microbiome through its antibiotic action. HIV-infected individuals who continue to use co-trimoxazole exhibit significantly lower levels of plasma CRP and IL-6 compared to those who discontinue use. Adverse clinical outcomes are more prevalent in individuals who stop co-trimoxazole usage. Similarly, HIV-infected children who persist with co-trimoxazole treatment show elevated serum albumin levels. Reduced systemic inflammation is independent of HIV disease progression and antibiotic action (Bourke et al., 2019).

The role of pro-inflammatory cytokines in the severity of COVID-19 is crucial (Q. Q. Liu et al., 2020). Asymptomatic individuals show lower levels of these cytokines, experience fewer CD4+T lymphocyte consumption during treatment, and demonstrate greater recovery improvement (Yang et al., 2020). Patients with elevated pro-inflammatory cytokines face a higher risk of severe COVID-19 (Gao et al., 2020). Non-surviving patients exhibit elevated IL-6 upon admission, which further increases during disease deterioration (Zhou et al., 2020; Ruan et al., 2020), inversely correlated with lymphocyte count (Zhang et al., 2020). Although induced immune activation is triggered before the inflection point of CD4+T cells and induction of serum virus load in HIV (Salaza-Gonzalez et al., 1998), a similar pattern has not yet been fully established in COVID-19 patients. Numerous studies, including Cheng et al. (2015), Mermin et al. (2004), and Badri et al. (2001), offer evidence of the positive impact on declining CD4+cell counts. Several lines of evidence suggested that co-trimoxazole has nonspecific anti-inflammatory and immunomodulatory properties, and hence, it is suggested in the rheumatoid arthritis treatment protocol (Rozin et al., 2002).

CRP is a positive acute phase reactant protein, with higher levels in the severe group of COVID-19 patients than in the mild group, even on the day of admission (Gao et al., 2020), and non-survivors have higher CRP levels than discharged patients (Ruan et al., 2020). CRP is strongly related to the Murray score for lung injury (Y. Liu et al., 2020). Age, comorbidities, secondary infections, and higher inflammatory markers in the blood are all associated with fatal outcomes in COVID-19 patients (Ruan et al., 2020). Co-trimoxazole is used to reduce secondary infection and is recommended as a prophylaxis by WHO in HIV patients with CD4+cell counts below 350 cells per mic L (WHO, 2006). Co-trimoxazole prophylaxis lowers CRP levels in HIV patients (Bourke et al., 2019). In the subset of HIV-infected patients who underwent antiretroviral therapy, the cessation of co-trimoxazole led to elevated levels of inflammatory cytokines such as CRP, IL-6, and TNF-α (Kyosiimire-Lugemwa et al., 2020).

Lymphopenia stands out as a prominent laboratory finding shared by patients afflicted with HIV (Seig et al., 2002) or COVID-19 (Tan et al., 2020; Zhang et al., 2020). Furthermore, this condition serves as a reliable indicator of disease progression (Zhang et al., 2020; Tan et al., 2020; Zhao et al., 2020). The percentage of lymphocytes is inversely linked to the severity of COVID-19 (Tan et al., 2020; Zhao et al., 2020; Huang and Pranata, 2020), with severe cases exhibiting lymphocyte percentages below 20% within 10-12 days from the onset of symptoms and these percentages plummeting to less than 5% in fatal cases (Tan et al., 2020). Despite its impact on the decrease in various blood parameters such as total white blood count, absolute neutrophil count, absolute lymphocyte count, and platelet count compared to values recorded during the placebo period, co-trimoxazole managed to lower the occurrence of infections in children diagnosed with lymphocytic leukaemia (Woods et al., 1984).

Notably, no studies have directly explored the antiviral effect of co-trimoxazole. However, research indicates that some substances with inhibitory effects on the DHFR enzyme also demonstrate inhibitory effects on influenza viruses. For example, 1-aryl-4,6-diamino-1,2-dihydrotriazines, which are structurally related to the antimalarial drug cycloguanil, have been shown to inhibit influenza A (H1N1) and influenza B and respiratory syncytial viruses by targeting host DHFR, thereby exerting a direct inhibitory effect (Tonelli et al., 2017). Co-trimoxazole also demonstrated a significant impact on viral load among HIV patients (Mermin et al., 2004).

As discussed earlier, co-trimoxazole reduces pro-inflammatory cytokines and the risk of secondary infections in HIV patients with weakened immune systems. Given these properties, the use of co-trimoxazole in COVID-19 patients could be particularly effective, especially in low-income countries where secondary infections are more common. This hypothesis warrants further investigation through clinical studies.

5. CONCLUSION

This study employed *in silico* methods to evaluate the binding affinity of co-trimoxazole to two crucial SARS-CoV-2 proteins, M^{pro} and RdRp, and compared these interactions with those of established reference drugs. The findings indicate that components of co-trimoxazole, sulfamethoxazole and trimethoprim, exhibit promising binding affinities to these viral proteins, indicating their potential inhibitory effects on viral replication.

Additionally, co-trimoxazole's well-documented anti-inflammatory properties and its ability to reduce pro-inflammatory cytokines and secondary infections in diseases, like HIV, further underscore its potential therapeutic benefits for COVID-19 patients. Although the binding affinities observed in this study were slightly lower than those of reference drugs, the established safety profile and multifaceted mechanism of action of co-trimoxazole make it a strong candidate for further clinical investigation.

The findings of this *in silico* study highlight the potential of co-trimoxazole as a treatment option for COVID-19. Before any clinical use, we recommend conducting clinical studies to explore its safety and efficacy in reducing the severity of the disease and improving patient outcomes, especially in regions with limited healthcare resources.

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