New Materials, Compounds and Applications
Vol.7, No.3, 2023, pp.226-239

IN SILICO EVALUATION OF THE INHIBITORY POTENTIAL OF FOUR COMPOUNDS, ANAKINRA, AVACOPAN, TOFACITINIB AND USTEKINUMAB, AGAINST THE ENTIRE CRYSTAL STRUCTURE SPIKE PROTEIN OF SIX VARIANTS OF SARS-CoV-2

Yosra Modafer*

Department of Biology, College of Science, Jazan University, Jazan 45142, Saudi Arabia

Abstract. The SARS-CoV-2 pandemic is a major concern, prompting research into therapies to combat its spread and mitigate its impacts. This study assessed the potential of four anti-inflammatory drugs (Anakinra, Avacopan, Tofacitinib, Ustekinumab) as inhibitors against six SARS-CoV-2 spike protein variants using computational tools. Sequence alignment revealed high similarity among all S protein variants, with some mutations. Phylogenetic analysis showed that Omicron and Delta variants were more closely related than the wild-type variant. Avacopan emerged as the most potent drug against all COVID-19 variants, with binding energies ranging from -9.2 to -10.7 kcal/mol. Molecular dynamic simulations showed Avacopan formed strong and stable complexes with the Omicron variant compared to the wild-type variant. The study also revealed that Avacopan engaged with more active site residues in the Omicron complex than in the wild-type complex. These findings highlight Avacopan as a promising anti-COVID-19 agent, warranting further investigation for potential clinical use.

Keywords: *COVID-19, spike protein, sequences alignment, Avacopan, molecular docking, MD simulation.*

****Corresponding Author:*** *Yosra Modafer, Department of Biology, College of Science, JAZU, KSA, Tel.: +966 53 515 4213, e-mail: ymodafer@jazanu.edu.sa, yosramodafer9@gmail.com*

Received: *23 September 2023;* ***Accepted:*** *3 December 2023;* ***Published:*** *18 December 2023.*

1. Introduction

The outbreak of COVID-19 has resulted in a worldwide healthcare emergency, with more than six million lives lost and over 514 million confirmed cases by May 24, 2022. Although the overall number of newly diagnosed cases and reported deaths have been declining since March 2022, the virus will possibly continue to circulate and future waves of infections caused by SARS-CoV-2 subvariants are expected in different regions of the planet (Sánchez *et al*., 2022). At present, no appropriate vaccines or antiviral medicines are prescribed for SARS-CoV-2 (Organization, 2022). To reduce infectious diseases, pandemics and epidemics, early warning represents an essential strategy (Rao & Jayabaskaran, 2020). During the spread of the epidemic, one of the viable strategies to combat it is by finding an effective treatment and developing vaccines that target several essential proteins of the SARS-CoV-2 virus. Currently, research efforts are focused on finding potential inhibitors of the proteins involved in the viral life cycle, including proteins implicated in viral entry (S protein, ACE2 and host cell proteases), viral

How to cite (APA):
Modafer, Y. (2023). *In Silico* evaluation of the inhibitory potential of four compounds, Anakinra, Avacopan, Tofacitinib and Ustekinumab, against the entire crystal structure spike protein of six variants of SARS-CoV-2. *New Materials, Compounds and Applications*, *7*(3), 226-239.

replication (3CLpro, PLpro, RdRp and helicase), as well as assembly and release of virions (N protein and 3a protein). However, many other inhibitors could act by multi-target mechanisms of action and might be repurposed to prevent and treat COVID-19 (Wei *et al*., 2021; Raj *et al*., 2021; Abdalla *et al*., 2021).

Since the SARS-CoV-2 virus has been spreading globally, variants (mutations) have emerged and been identified, which may alter the virus's pathogenicity, infectivity, transmissibility and/or antigenicity. As of July 6, 2021, the World Health Organization (WHO) categorized the SARS-CoV-2 virus into four Variants of Concern (VOCs), four Variants of Interest (VOIs) and twelve Alerts for ongoing surveillance. These classifications are based on the virus's clinical and public health impact. These classifications are summarized in Table 1. The four VOCs comprise different versions of the Alpha, Beta, Gamma and Delta variants. From neutralizing antibodies, the largest magnitude of immune escaping was displayed by the Beta variant, while the variant Delta showed intensely higher transmission and infectivity and it remains the most widely spread strain. Moreover, the Omicron variant is a more recently identified VOC that is characterized by an unusual mutation number. On the other hand, there are currently four Variants of Interest (VOIs) that the authorities are keeping an eye on. These are Eta, Iota, Kappa, and Lambda. Additionally, there are 12 alerts that require advanced monitoring; they are listed in Table 1 (Chan *et al*., 2022; Kumar *et al*., 2021). By comparing with the original WT variant of the SARS-CoV-2 virus, phylogenetic tree analysis presented that the Omicron variant evolved and mutated independently of four VOCs (Yin *et al*., 2022). In addition, the omicron variant had 30 mutations dispersed among the three domains of the spike (S) protein (Koley *et al*., 2022).

Table 1. SARS-CoV-2 virus classification based on the World Health Organization (July 6, 2021)

	WHO label	Lineage+ additional mutations
VOCs	Alpha	PANGO lineage B.1.1.7
	Beta	B.1.351/B.1.351.2/B.1.351.3
	Gamma	P.1/P.1.1/P.1.2
	Delta	B.1.617.2/AY.1/AY.2
VOIs	Eta,	B.1.525
	Iota	B.1.526
	Kappa	B.1.617.1
	Lambda	C.37
VOIs	Epsilon	B.1.427/B.1.429
	Zeta,	P.2
	Theta	P.3
Alerts for further monitoring		R.1/R.2
		B.1.466.2
		B.1.621
		AV.1
		B.1.1.318
		B.1.1.519
		AT.1
		C.36.3/C.36.3.1
		B.1.214.2.

Many research endeavors focused on uncovering and creating drugs or vaccines to combat COVID-19, primarily targeting the SARS-CoV-2 S proteins. As is the case with all coronaviruses, these proteins play a crucial role in recognizing receptors and

merging with the host cell membrane. The S-proteins consist of two primary subunits, each with its unique functions: S1 and S2. (Abdalla *et al*., 2022, 2021). The receptor-binding domain (RBD) of the S1 subunit is responsible for identifying and attaching itself to ACE2, a protein found on the host receptor (Sánchez *et al*., 2022; Abdalla *et al*., 2021). In contrast, the S2 subunit forms a crucial six-helix bundle responsible for facilitating the fusion of viral and cellular membranes.

The SARS-CoV-2 S proteins are made up of several domains. It starts with the extracellular N-terminus, followed by a transmembrane (TM) domain that is connected to the viral membrane. Finally, a small terminal segment is located inside the cell called the C-terminus. The S protein comprises three primary components: an initial signal peptide consisting of 1-13 amino acids, followed by the S1 subunit spanning residues 14 to 685 and finally, the S2 subunit encompassing residues 686 to 1273. In addition, each of the two functional subunits of the S has a specific arrangement of amino acid residues and specific functions. For instance, the S1 subunit has two functional regions that bind to the host cell receptor: the N-terminal domain (NTD) and RBD. Whereas the S2 subunit is composed of three functional regions: a domain of 788-806 residues termed Fusion peptide (FP), followed by 912-984 residues named heptad repeat 1 (HR1) then 1163-1213 residues termed heptad repeat 2 (HR2), the next 1213-1237 residues formed the TM domain, and lastly the cytoplasmic domain with 1237-1273 residues; all comprise the S2 subunit and involved in cell membrane fusion. Based on the position of the RBD, The S protein can assume two distinct states depending on the location of the RBD: a closed state that blocks receptor access or an open state that allows it (Huang *et al*., 2020, Tang *et al*., 2020; Gur *et al*., 2020; Jackson *et al*., 2020).

Recent scientific evolutions and developments towards identifying effective antiviral medicines were focused mainly on the S protein's function and structure as crucial in the fight against SARS-CoV-2 (Huang *et al*., 2020; Arbeitman *et al*., 2021). However, several mutations in the SARS-CoV-2 S proteins rearrange its structure, resulting in changes to its function and its response to drugs that target a specific protein region, which represents a significant obstacle to the development of a new drug against SARS-CoV-2 (Boufissiou *et al*., 2022; Shu *et al*., 2020; De Leeuw *et al*., 2022). Unfortunately, its genome, like all viruses, has always experienced numerous mutations. Most of these mutations are related to the S protein that is responsible for this higher affinity for ACE2, so it is not surprising that new versions of variants are emerging (Araf *et al*., 2022; Kulasekararaj *et al*., 2020)

Medical experts suggested several small molecules as blockers of inflammatory signals to control various diseases complicated by inflammation. In viral infections, especially COVID-19, several forms of pulmonary, vascular and renal inflammations were detected as dangerous complications. Therefore, four compounds were selected in the current study as candidate inhibitors of COVID-19 progression and consequently controlled the associated inflammatory complications.

The first candidate, Anakinra, is a safe human interleukin (IL)-1receptor antagonist that promotes beneficial effects in several forms of inflammation, including virus- infected cases. It is involved in the inflammasome pathway to treat severe conditions of COVID-19 (Huet *et al.*, 2020; Zelek *et al*., 2020). Tofacitinib is the second compound that has been used clinically in COVID-19 management to control the dangerous complications related to cytokine-releasing syndrome (Maslennikov *et al.*, 2021). Additionally, Ustekinumab is an approved human monoclonal antibody for treating moderate-to-severe inflammatory diseases that block the binding of IL-12 and IL-23 to the IL-12Rβ1

chain of their receptors (Miyoshi *et al*., 2022). Lastly, Avacopan has received approval from the Food and Drug Administration (FDA) as a competitive antagonist that blocks a complement 5a receptor (C5aR) from binding to anaphylatoxin C5a and hence prevents C5a-mediated neutrophil activation, which subsequently mitigates the inflammatory syndrome in the patient (Osman *et al*., 2021; Laurence *et al*., 2020). Since the influence of S protein's mutations on both the host's receptor and drug binding affinity has not been fully elucidated, using in-silico methods, this study aims to assess the inhibitory possibility of four compounds: Anakinra, Avacopan, Tofacitinib, and Ustekinumab against the entire crystal structure S protein of wild-type (WT), Beta, Alpha, Delta, Gamma and Omicron variants of SARS-CoV-2. Additionally, to determine the stability of the highest-binding complexes between the S proteins of both the WT and Omicron versions with the Avacopan candidate, molecular docking simulations and MD calculations were run.

2. Materials and methods

2.1. Ligand preparation

The 3D-dimensional structure of the four compounds under study, Anakinra, Avacopan, Tofacitinib and Ustekinumab, was collected from PubChem. Moreover, using the MGLTools version 1.5.7, these four compounds went through geometrical optimization and were then saved in ".pdbqt" format (Sanner, 1999).

2.2. Preparation of target

To characterize the exact position of the mutations sought, we compared the sequence of five variants of SARS-CoV-2 with that of the wild-type variants (7CWU) obtained from the protein sequence bank. This was done on the EMBL-EBI tool "Clustal Omega" via a web portal (https://www.ebi.ac.uk/Tools/msa/clustalo/; Sievers *et al*., 2011). Furthermore, Jalview software was used to view and edit sequence alignments, whereas the constitution of the phylogenetic tree of the SARS-CoV-2 variants under examination allowed us to classify and position them into groups of 3 sequences of different known variants of SARS-CoV-2. The first group included Delta and Omicron variants, while the second group contained Alpha and wild-type variants and in the last group, we found Beta and Gamma variants.

The Protein Data Bank (PDB) (https://www.rcsb.org/) provided the 3D crystal structure of the wild-type version (7CWU) of the SARS-CoV-2 (COVID-19) S proteins. However, the SWISS-MODEL server (Kiefer *et al*., 2009) as utilized to build the S proteins' 3D model for the five variants. Then, most small molecules and all water molecules were detached from their crystal structures using the BIOVIA Discovery Studio software. In addition, the proteins were visualized using the Discovery Studio Visualizer 2020, and energy minimization to repair empty atomic gaps and crystallographic perturbations was performed using MGLTools version 1.5.7. Finally, a "pdbqt" file containing the optimized protein structure was created.

2.3. Molecular docking of candidate ligands with S protein targets

Blind docking research was conducted using Autodock-Vina software, specifically version 4, developed by the Scripps Research Institute in La Jolla, CA, USA. The PDB website was utilized to get the variants of SARS-CoV-2. The docking of ligands (the four compounds) was then carried out on the whole surface of the target

S proteins of the six variations. Subsequently, the Discovery Studio Visualizer 2020 was employed to visually illustrate the interactions between the candidate ligands and the amino acid residues within the target proteins (Visualizer, 2020). Then, to select the best poses, the minimum binding energy between candidate ligands and the target proteins was calculated.

2.4. Molecular dynamics (MD) simulation

After performing a molecular docking analysis, we assessed the stability of the most strongly binding complexes using Schrodinger's Desmond module. The simulation of biomolecules involved the application of Gromos 9643a1 and OPLS 2005 force fields. To properly solvate the entire protein-ligand system, we placed two complexes within a cubic water box and ensured a 12-unit buffer space for system neutralization. To mimic physiological conditions, 0.15 M NaCl counterions were introduced, and the cubic water box employed the Simple Point Charge (SPC) model. We conducted energy minimization over two phases, NVT and NPT, spanning 50,000 ps until the process was completed. Throughout this procedure, the system maintained an ambient pressure of 1.013 bar at a temperature of 310 K for a duration of 100 ns (Faisal *et al.,* 2022). The two leading docked complexes, Avacopan-WT with a binding energy (BE) of -10.7 kcal/mol and Avacopan- Omicron with a BE of -9.8 kcal/mol, were subsequently subjected to 100 nanosecond molecular dynamics (MD) simulations to evaluate their structural stability and flexibility. During this analysis, we examined various parameters, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bond counts and Gibbs free energy, from which we derived our findings and conclusions.

3. Results and discussion

3.1. Phylogenetic tree and multiple sequence alignment

The virus responsible for COVID-19 is a member of the Coronaviridae family and falls into the beta-coronavirus category. It possesses a single-stranded RNA as its genetic material. Within the coronavirus genome, four main proteins are encoded: the envelope (E), membrane (M), nucleocapsid (N) and S proteins. Among these, the spike proteins play a pivotal role in facilitating the virus's entry into host cells by attaching to specific receptors, such as the angiotensin-converting enzyme 2 receptors (ACE-2) located on the host's epithelial cells. This binding event leads to viral fusion and entry (Rowaiye *et al*., 2021; Kyrou *et al*., 2021).

The comparison of the S protein sequences from the original SARS-CoV-2 strain, as well as its Beta, Alpha, Delta, Gamma and Omicron variants (as provided in the Supplementary data), reveals a significant level of sequence similarity, exceeding 97.14%. The conserved motifs and amino acids common in the six S proteins are highlighted; however, one or two differences in the amino acid sequence might disturb protein function. The dashes have been detected in the sequences of five variants (Alpha, Beta, Delta, Gamma and Omicron), where - represents GAPs or in Del, which indicates that the protein structures at these points have undergone mutations by deletion during which the amino acids MFVFLVLLPLVS sequences were lost (Mahadani *et al.,* 2022). The regions in Dels of five S protein sequences were predicted by comparing their sequences with the sequence of known wild-type variants (7CWU) and consensus >50 sequences.

The phylogenetic analysis of the sequence of six S proteins (Figure 1) revealed the highest identity rate between the sequences of the Omicron's S protein and the Delta variants. Similarly, there is a close relation between the Alpha's S protein sequence and wild-type variants. Likewise, a similar close relation in the sequence of Alpha and wild-type S protein was detected. Moreover, the EMBL-EBI tool "Clustal Omega" revealed that the Omicron variant's S protein is phylogenetically distant from the wild-type variant (7CWU).

Figure 1. The phylogenetic analysis of the sequence of six S proteins

3.2. Molecular docking analysis

All S proteins were examined to find candidate drugs against COVID-19. We used molecular docking to predict the interaction binding between the S protein trimer of six SARS-CoV-2 variants and the chemical structures of the four compounds being investigated.

Table 2. The binding energy obtained from the blind molecular docking study of four compounds against six S protein variants of SARS-CoV-2

Variants / Compounds	WT	Beta	Alpha	Delta	Gamma	Omicron
Anakinra	-8.6	-7.8	-8.1	-8.0	-8.4	-7.9
Avacopan	-10.7	-10.2	-10.2	-9.9	-9.2	-9.8
Tofacitinib	-7.3	-7.3	-8.6	-6.8	-7.4	-7.9
Ustekinumab	-7.7	-7.4	-7.6	-7.3	-7.5	-7.6

By comparing the results obtained from the blind molecular docking study represented in Table 2, Avacopan was the best candidate drug since it exhibited the highest binding energy values with the S proteins trimer of the six variants (−9.2 to −10.7 kcal/mol). Whereas the remaining compounds, Anakinra, Tofacitinib and Ustekinumab, each displayed a lower binding energy, value with all variants studied ranging from −6.8 to −8.6 kcal/mol as presented in Table 2. The Avacopan-Alpha complex, as in Figure 2 and Figure 3, exhibited an amide (π-H) interaction of the 6-ring of the Avacopan ligand with the backbone of ASN 957 (chain C) of S protein target with a distance of 3.69Å. However, a conventional H-bond between OH of atoms C28 and C41 from the benzene rings of the Avacopan ligand and residue TYR 377 (chain A) of the S protein of the BETA variant was identified in the Avacopan-Beta complex.

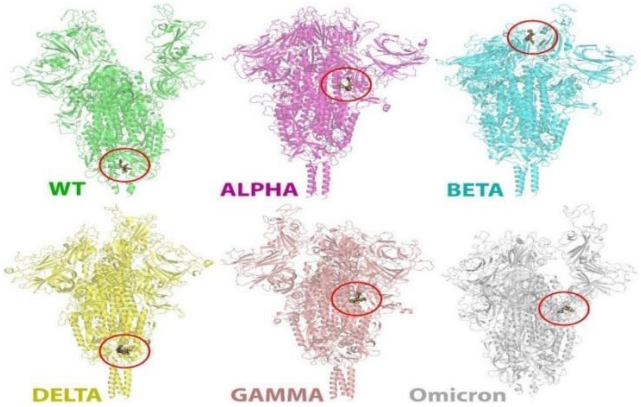

Figure 2. S protein of six variants in complex with the Avacopan compound obtained by blind molecular docking

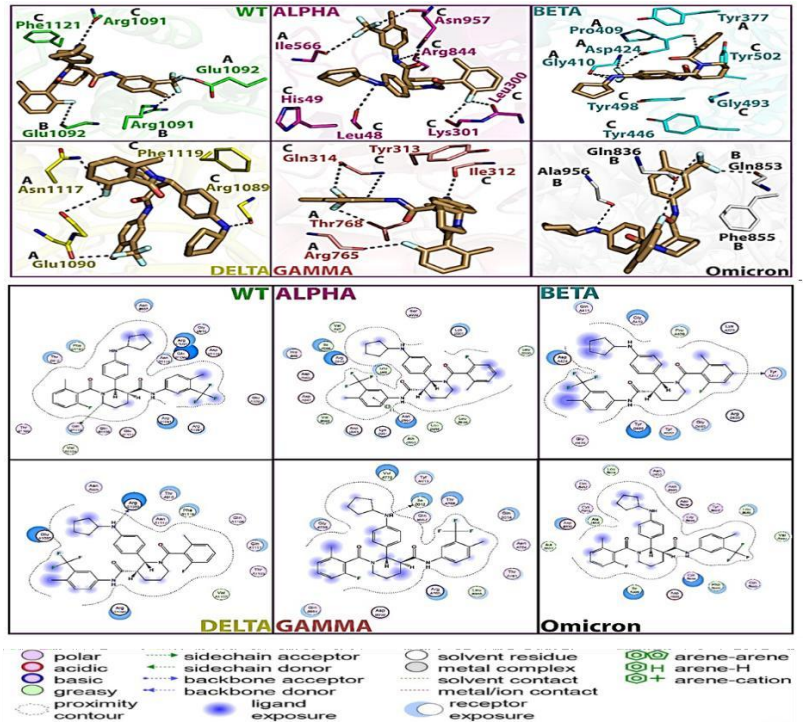

Figure 3. 3D & 2D ligand-receptor interactions of Avacopan against six S protein variants of SARS-CoV-2

Moreover, a H-bond that formed between the oxygen of the residue ARG 1089 (chain C) of the Delta's S protein variant and the hydrogen atoms N 2-30 of the Avacopan ligand could account for the higher binding energy score that the Avacopan-Delta complex displayed (10.2 kcal/mol). Furthermore, a hydrogen bond ensuring the stability of the Avacopan-Gamma complex was observed between the hydrogen of atoms N 2-30 in the Avacopan ligand and the oxygen of the residue ILE 312 (chain C) of the S protein target. As shown in Figure 3, another weak amide (π-H) bond with a distance of 2.69 Å was observed in the Avacopan-Omicron complex, specifically between the hydroxyl functional group of the 6-ring of Avacopan ligand and the

backbone of Phe 855 (chain B). On the other hand, the Avacopan-WT complex showed a docking score of −10.7 kcal/mol; the binding energy resulted from the hydrogen bond interaction between the amino acid GLN 1113 from chain B of WT S protein with atoms C5-7 of the Avacopan ligand. According to the molecular docking results, in addition to the promising results of various complement inhibition strategies reports (Kulasekararaj *et al*., 2020; Zelek *et al*., 2020; Laurence *et al*., 2020; De Leeuw *et al.*, 2022), we made molecular dynamics of the Avacopan with the S protein trimer of both WT and Omicron variants.

3.3. MD simulation

MD simulations were used during 100 ns trajectory periods to evaluate the two protein-ligand complexes' fluctuation analysis and conformational stability (Avacopan-WT and Avacopan-Omicron). The parameters used to estimate the overall stability were RMSD and RMSF during the virtual simulation trajectories.

3.1.1. Stability prediction using RMSD analysis

The protein-ligand complexes' stability can be assessed through two key qualitative parameters: root mean square fluctuation (RMSF) and RMSD. Particular ligand–protein complexes' RMSD values indicate the greatest stability of the candidate compound(s); however, the values of RMSF determine the fluctuations and compactness of the complex(s). For the first complex, early in the first 10 ns, Avacopan-WT, the MD simulation showed that ligand RMSD changed from 4 to 6 Å and became stable for the remaining 100 ns without much variation. In Parallel, the protein RMSD increased from 6 to 14 Å for the early 30 ns. They were steady for the entire period of simulation (Figure 4A). For the second complex Avacopan-Omicron, the results showed a similarity in RMSD changes of both ligand and protein in the first 70 ns around 3 to 5.5 Å, followed by stability in RMSD for Avacopan, whereas an increase in protein RMSD, which arrived 7Å after 100 ns of simulation (Figure 4C). These findings suggested that Avacapon can bind to the Omicron variant very quickly and confirmed the strong binding of the Avacopan-Omicron complex in comparison with Avacopan-WT interactions, which appeared less stable.

3.1.2. Flexibility prediction using RMSF analysis

To assess conformational changes and determine the mobility of protein residues, RMSF analysis is a crucial metric. The complete protein structure, as well as the combined ligand, were measured, as well as the average distance the atom traveled from its starting point. The residual fluctuation of both complexes' RMSF plots exhibited a considerable degree of variance, indicating the structural complexity of the macromolecular system. The Avacopan-WT complex's RMSF values revealed some variations in the 0-500, 1200-1600 and 2200-2700 residue indexes, respectively, then fluctuated within acceptable ranges of 1 to 3 Å, which indicates high stability (Figure 4B). In addition, the Avacopan-Omicron complex's RMSF analysis revealed a variation range from 1 to 3.5 Å for the majority of residual indices, including a few disruptions that seemed to extend beyond the study range and suggested a significant instability (Figure 4D).

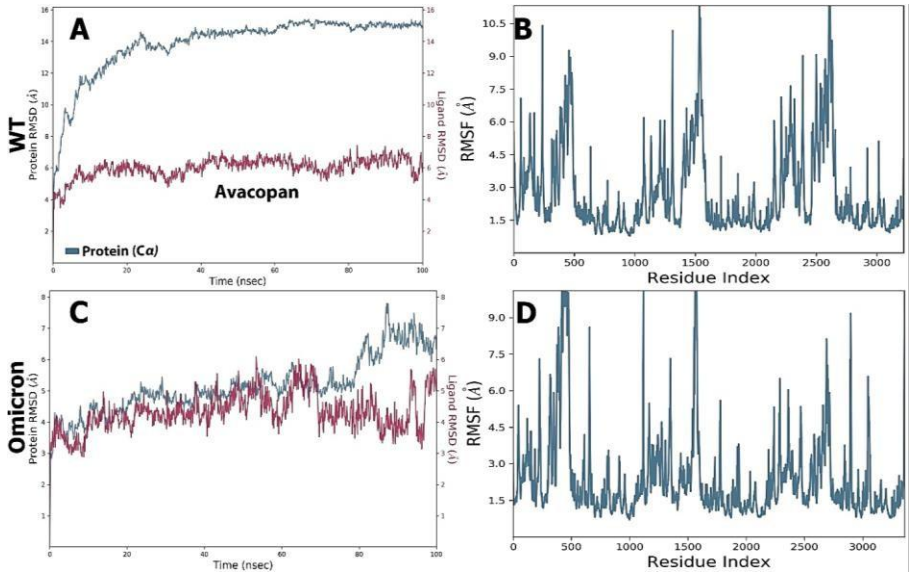

Figure 4. RMSD & RMSF plots of Avacopan against two S protein variants, Omicron and WT complexes
(Protein Cα and ligand RMSD showed in blue and red color, respectively)

3.3.3. Protein-ligand interaction analysis

In addition to RMSD and RMSF, the Protein-ligand interaction between the S protein of two variants and the Avacopan ligand during the 100 ns of the receptor-ligand MD simulations is also measured. For instance, Figure 5 revealed the plots of H-bonds that formed between the Avacopan ligand and respective S protein ligand during the simulation trajectory. The complex contact analysis showed the involvement of more than 18 active site residues in the Avacopan-WT complex interaction and 28 active site residues in the Avacopan-Omicron complex interaction. Moreover, a variety of interacting bonds was formed, encompassing H-bonds, hydrophobic interactions, ionic attractions and water bridges. The histogram for both complexes illustrate the occurrence of hydrophobic interactions, such as π–cation, π–π stacking, and other less specific binding forces, as depicted in Figure 6. The amino acid residues ILE 569 (A), ALA 570 (A), VAL 47 (B), LEU 828 (B), ALA 831 (B), LEU 841 (B), LEU 849 (B), ALA 852 (B), PHE 855 (B), LEU 858 (B) VAL 952 (B) ALA 956 (B) LEU 959 (B) and VAL 963 (B) in the S protein of Omicron variant show hydrophobic interaction (Figure 5). Alongside, only VAL 1104 (B) and PHE 1121 (C) residues in the WT variant show hydrophobic interaction with the Avacopan ligand. The majority of residues in both complexes' Avacopan-WT and Avacopan-Omicron interacted by water bridge bonds (Figure 5).

Generally, the H-bond is formed between the hydrogen atom of the ligand and the highly electronegatively charged group of the residual atom, which is essentially involved in the strength of binding to a ligand and drug specificity (https://www.rcsb.org/). The histograms identified ASP 568 (A), APS 830 (B), ALA 831 (B), GLN 836 (B) and ASP 839 (B) interacting with a strong hydrogen bond with the S protein of the Omicron variant. The amino acids in the S protein of the WT variant that showed hydrogen bond with the Avacopan ligand included ASN 907 (B), GLN 913 (B), GLU 1092 (B), ASN 1119 (B), ARG 1091 (C) and GLU 1092 (C).

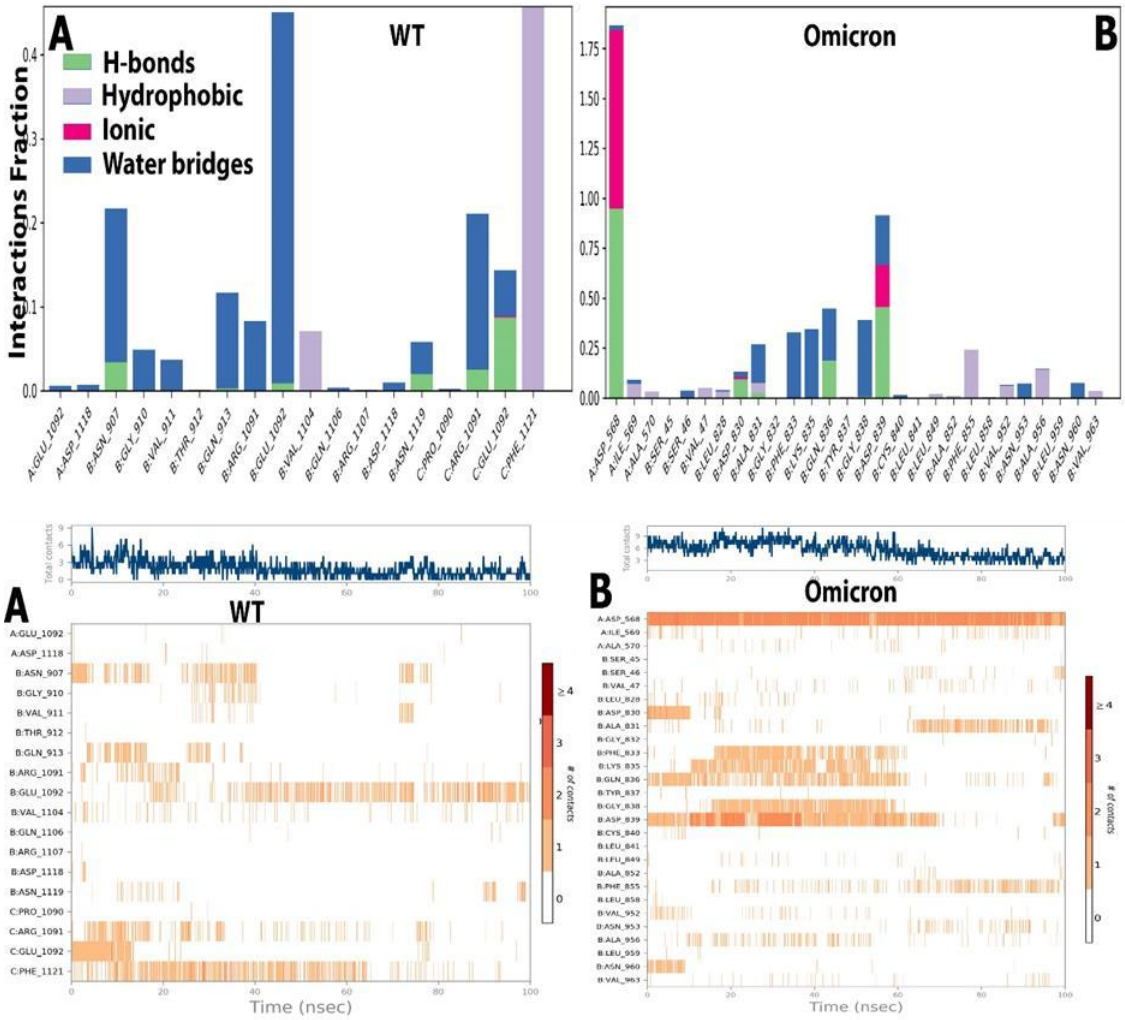

Figure 5. Protein-ligand contact plots and interaction residues of Avacopan against two S protein variants, Omicron and WT complexes

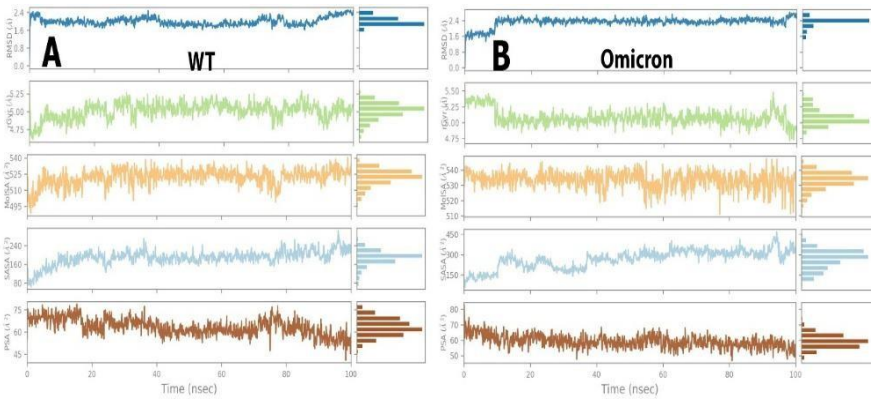

Figure 6. Ligand property trajectory of Avacopan against two S protein variants, Omicron and WT complexes

3.3.4. Ligand properties

The ligand properties assessed in the current study include the values of the following parameters: RMSD, solvent accessible surface area (SASA), molecular surface area (MolSA), polar surface area (PSA) and radius of gyration (rGyr) as presented in Figure 6. The RMSD values exhibited minimal fluctuation ranging from around 1.6 to 2.4Å (equilibrium ~ 1.8Å) throughout the MD simulation for Avacopan with the S protein of the WT variant. On the other hand, the RMSD values of the ligand Avacopan, with the S protein of the Omicron variant, showed slight fluctuation until 10 ns from 1 to 1.6Å, then gradually steadied equilibrium at 2.4Å and persisted constantly through the simulation course. Overall, RMSD values of both complexes suggest that they possessed good stability in the active site of both S protein variants.

The rGyr values for the complex Avacopan-S protein of the WT variant ranged from 4.75 to 5.25Å (equilibrium at 5Å). The rGyr values for ligand Avacopan with S protein of Omicron variant were mostly stable at 5.25Å from 0 ns to 10ns and after 10ns, the values fluctuated between 5.0 and 5.25Å. Whereas, the MolSA values for ligand Avacopan with the S protein of the WT variant ranged from 495 to 540Å2 and attained equilibrium at 525Å2 throughout most of the simulation time, while the same ligand in its complex with the Omicron variant showed constant fluctuations of MolSA that were around 510 to 540Å2 (equilibrium at 535Å2). However, the obtained results of SASA values during the majority of the simulation time demonstrated that the Avacopan-WT complex and Avacopan-Omicron complex exhibited SASA values in the range of 80-140 and 150-400Å, respectively. Moreover, The PSA values for both complexes displayed variations ranging from 50 to 75Å2 but got to equilibrium at 60 Å2 for the most period of the simulation.

4. Conclusion

The blind molecular docking study measured the interactions of four compounds, Anakinra, Avacopan, Tofacitinib and Ustekinumab, against the entire S protein of six SARS-CoV-2 variants, and it revealed strong binding contacts with the lowest binding energies for Avacopan Ligand; thus, it was suggested as the best candidate drug among others. Avacopan ligand did not interact with the same active pocket in the six S protein variants of SARS-CoV-2. The different sites of protein pockets in the six variants of SARS-CoV-2 with the Avacopan ligand can be exploited in drug discovery procedures, in particular, to select the best therapeutic target protein and most suitable pocket during the process of designing and developing antibodies, vaccines, and medications against SARS-CoV-2 variants. Furthermore, The MD simulation profile during the 100 ns demonstrated a stable interaction between the Avacopan ligand with the entire S protein WT and Omicron variants. This in silico study needs experimental validation to identify further the suitability of the four candidates' drugs, such as Avacopan as SARS-CoV-2 inhibitors.

Funding

Deanship of scientific Research, Jazan University funded this research work through the Research Units Support Program, Support Number: RUP2-02.

Acknowledgment

The author extends her appreciation to the Deanship of scientific Research, Jazan University for supporting this research work through the Research Units Support Program, Support Number: RUP2-02.

References

Abdalla, M., El-Arabey, A.A. & Jiang, X. (2021). Are the new SARS-CoV-2 variants resistant against the vaccine? *Human Vaccines & Immunotherapeutics*, *17*(10), 3489-3490. https://doi.org/10.1080/21645515.2021.1925503

Abdalla, M., El-Arabey, A.A. & Jiang, X. (2021). Progress in research on the S protein as the target of COVID-19 vaccines. *Expert Review of Vaccines*, *20*(7), 769-772. https://doi.org/10.1080/14760584.2021.1918003

Abdalla, M., El-Arabey, A.A. & Jiang, X. (2022). What are the challenges faced by COVID-19 vaccines? *Expert Review of Vaccines*, *21*(1), 5-7. https://doi.org/10.1080/14760584.2022.2008245

Abdalla, M., Eltayb, W.A., El-Arabey, A.A., Singh, K. & Jiang, X. (2022). Molecular dynamic study of SARS-CoV-2 with various S protein mutations and their effect on thermodynamic properties. *Computers in Biology and Medicine*, *141*, 105025. https://doi.org/10.1016/j.compbiomed.2021.105025

Araf, Y., Akter, F., Tang, Y.D., Fatemi, R., Parvez, M.S.A., Zheng, C. & Hossain, M.G. (2022). Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. *Journal of Medical Virology*, *94*(5), 1825-1832. https://doi.org/10.1002/jmv.27588

Arbeitman, C.R., Rojas, P., Ojeda-May, P. & Garcia, M.E. (2021). The SARS-CoV-2 spike protein is vulnerable to moderate electric fields. *Nature Communications*, *12*(1), 5407. https://doi.org/10.1038/s41467-021-25478-7

Boufissiou, A., Abdalla, M., Sharaf, M., Al-Resayes, S.I., Imededdine, K., Alam, M. & Yousfi, M. (2022). In-silico investigation of phenolic compounds from leaves of Phillyrea angustifolia L. as a potential inhibitor against the SARS-CoV-2 main protease (Mpro PDB ID: 5R83) using a virtual screening method. *Journal of Saudi Chemical Society*, *26*(3), 101473. https://doi.org/10.1016/j.jscs.2022.101473

Chan, W.S., Lam, Y.M., Law, J.H.Y., Chan, T.L., Ma, E.S.K. & Tang, B.S.F. (2022). Geographical prevalence of SARS-CoV-2 variants, August 2020 to July 2021. Scientific Reports, 12(1), 4704. https://doi.org/10.1038/s41598-022-08684-1

Chen, W., Wang, Z., Wang, Y. & Li, Y. (2021). Natural bioactive molecules as potential agents against SARS-CoV-2. *Frontiers in Pharmacology*, 12, 702472. https://doi.org/10.3389/fphar.2021.702472

De Leeuw, E., Van Damme, K.F., Declercq, J., Bosteels, C., Maes, B., Tavernier, S.J. & Lambrecht, B.N. (2022). Efficacy and safety of the investigational complement C5 inhibitor zilucoplan in patients hospitalized with COVID-19: an open-label randomized controlled trial. *Respiratory Research*, *23*(1), 202. https://doi.org/10.1186/s12931-022-02126-2

Faisal, S., Badshah, S.L., Kubra, B., Sharaf, M., Emwas, A.H., Jaremko, M. & Abdalla, M. (2021). Computational study of SARS-CoV-2 rna dependent rna polymerase allosteric site inhibition. *Molecules*, *27*(1), 223. https://doi.org/10.3390/molecules27010223

Gur, M., Taka, E., Yilmaz, S.Z., Kilinc, C., Aktas, U. & Golcuk, M. (2020). Conformational transition of SARS-CoV-2 spike glycoprotein between its closed and open states. *The Journal of Chemical Physics*, *153*(7). https://doi.org/10.1101/2020.04.17.047324

https://www.ebi.ac.uk/Tools/msa/clustalo/

https://www.rcsb.org/

Huang, Y., Yang, C., Xu, X.F., Xu, W. & Liu, S.W. (2020). Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacologica Sinica*, *41*(9), 1141-1149. https://doi.org/10.1038/s41401-020-0485-4

Huet, T., Beaussier, H., Voisin, O., Jouveshomme, S., Dauriat, G., Lazareth, I. & Hayem, G. (2020). Anakinra for severe forms of COVID-19: a cohort study. *The Lancet Rheumatology*, *2*(7), e393-e400. https://doi.org/10.1016/S2665-9913(20)30164-8

Jackson, C.B., Farzan, M., Chen, B. & Choe, H. (2022). Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology*, *23*(1), 3-20. https://doi.org/10.1038/s41580-021-00418-x

Kiefer, F., Arnold, K., Künzli, M., Bordoli, L. & Schwede, T. (2009). The SWISS-MODEL Repository and associated resources. *Nucleic Acids Research*, *37*(1), 387-392. https://doi.org/10.1093/nar/gkn750

Koley, T., Kumar, M., Goswami, A., Ethayathulla, A.S. & Hariprasad, G. (2022). Structural modeling of Omicron spike protein and its complex with human ACE-2 receptor: Molecular basis for high transmissibility of the virus. *Biochemical and Biophysical Research Communications*, 592, 51-53. https://doi.org/10.1016/j.bbrc.2021.12.082

Kulasekararaj, A.G., Lazana, I., Large, J., Posadas, K., Eagleton, H., Villajin, J.L. & Marsh, J.C. (2020). Terminal complement inhibition dampens the inflammation during COVID-19. *British Journal of Haematology*, *190*(3), e141. https://doi.org/10.1111/bjh.16916

Kumar, A., Narayan, R.K., Prasoon, P., Kumari, C., Kaur, G., Kumar, S. & Kumar, S. (2021). COVID-19 mechanisms in the human body-What we know so far? *Frontiers in Immunology*, 12, 693938. https://doi.org/10.3389/fimmu.2021.693938

Kyrou, I., Randeva, H.S., Spandidos, D.A. & Karteris, E. (2021). Not only ACE2-the quest for additional host cell mediators of SARS-CoV-2 infection: Neuropilin-1 (NRP1) as a novel SARS-CoV-2 host cell entry mediator implicated in COVID-19. Signal Transduction and Targeted Therapy, 6(1), 21. https://doi.org/10.1038/s41392-020-00460-9

Laurence, J., Mulvey, J.J., Seshadri, M., Racanelli, A., Harp, J., Schenck, E.J. & Magro, C.M. (2020). Anti-complement C5 therapy with eculizumab in three cases of critical COVID-19. *Clinical Immunology*, 219, 108555. https://doi.org/10.1016/j.clim.2020.108555

Mahadani, A.K., Awasthi, S., Sanyal, G., Bhattacharjee, P. & Pippal, S. (2022). Indel-K2P: a modified Kimura 2 Parameters (K2P) model to incorporate insertion and deletion (Indel) information in phylogenetic analysis. *Cyber-Physical Systems*, *8*(1), 32-44. https://doi.org/10.1080/23335777.2021.1879274

Maslennikov, R., Ivashkin, V., Vasilieva, E., Chipurik, M., Semikova, P., Semenets, V. & Dzhakhaya, N. (2021). Tofacitinib reduces mortality in coronavirus disease 2019 Tofacitinib in COVID-19. *Pulmonary Pharmacology & Therapeutics*, 69, 102039. https://doi.org/10.1016/j.pupt.2021.102039

Miyoshi, J., Matsuura, M. & Hisamatsu, T. (2022). Safety evaluation of ustekinumab for moderate-to-severe ulcerative colitis. *Expert Opinion on Drug Safety*, *21*(1), 1-8. https://doi.org/10.1080/14740338.2021.1980536

Organization WH (2022). COVID-19 weekly epidemiological update, ed. 84, March 22.

Osman, M., Cohen Tervaert, J.W. & Pagnoux, C. (2021). Avacopan for the treatment of ANCA-associated vasculitis. *Expert Review of Clinical Immunology*, *17*(7), 717-726. https://doi.org/10.1080/1744666X.2021.1932466

Raj, K., Kaur, K., Gupta, G. D., & Singh, S. (2021). Current understanding on molecular drug targets and emerging treatment strategy for novel coronavirus-19. *Naunyn-schmiedeberg's Archives of Pharmacology*, *394*(7), 1383-1402. https://doi.org/10.1007/s00210-021-02091-5

Rowaiye, A.B., Okpalefe, O.A., Onuh Adejoke, O., Ogidigo, J.O., Hannah Oladipo, O., Ogu, A.C. & Haque, M. (2021). Attenuating the effects of novel COVID-19 (SARS-CoV-2) infection-induced cytokine storm and the implications. *Journal of Inflammation Research*, 1487-1510. https://doi.org/10.2147/JIR.S301784

Sánchez, F.J.M., Martínez-Sellés, M., García, J.M.M., Guillén, S.M., Rodríguez-Artalejo, F., Ruiz-Galiana, J. & Bouza, E. (2023). Insights for COVID-19 in 2023. *Revista Española de Quimioterapia, 36*(2), 114. https://doi.org/10.37201/req/122.2022

Sanner, M.F. (1999). Python: a programming language for software integration and development. *J. Mol. Graph Model., 17*(1), 57-61.

Shu, C.J., Huang, X., Tang, H.H., Mo, D.D., Zhou, J.W. & Deng, C. (2021). Mutations in spike protein and allele variations in ACE2 impact targeted therapy strategies against SARS-CoV-2. *Zoological Research, 42*(2), 170-181. https://doi.org/10.24272/j.issn.2095-8137.2020.301

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W. & Higgins, D.G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular systems biology, 7*(1), 539. https://doi.org/10.1038/msb.2011.75

Tang, T., Bidon, M., Jaimes, J.A., Whittaker, G.R. & Daniel, S. (2020). Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antiviral Research*, 178, 104792. https://doi.org/10.1016/j.antiviral.2020.104792

Visualizer, D.S. (2020). *Biovia*. Dassault Systèmes, San Diego.

Yashavantha Rao, H.C., Jayabaskaran, C. (2020). The emergence of a novel coronavirus (SARS- CoV-2) disease and their neuroinvasive propensity may affect in COVID-19 patients. *Journal of Medical Virology, 92*(7), 786-790. https://doi.org/10.1002/jmv.25918

Yin, W., Xu, Y., Xu, P., Cao, X., Wu, C., Gu, C. & Xu, H.E. (2022). Structures of the Omicron spike trimer with ACE2 and an anti-Omicron antibody. *Science, 375*(6584), 1048-1053. https://doi.org/10.1126/science.abn8863

Zelek, W.M., Cole, J., Ponsford, M.J., Harrison, R.A., Schroeder, B.E., Webb, N. & Morgan, B.P. (2020). Complement inhibition with the C5 blocker LFG316 in severe COVID-19. *American Journal of Respiratory and Critical Care Medicine, 202*(9), 1304-1308. https://doi.org/10.1164/rccm.202007-2778LE