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RESEARCH ARTICLE

In Silico Evaluation of ERQ Bioactive Tripeptide as an Anticancer Agent and an Inhibitor of SARS-CoV-2 Enzymes

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ABSTRACT

Objective: Short peptides play a significant role in exploring drugs with higher selectivity and fewer side effects in cancer and COVID-19 therapies. This study evaluated the anticancer and anti-COVID-19 activities of Glu-Arg-Gln (ERQ) tripeptide for the first time. To discover the potentiality of the tripeptide as an anticancer and as a SARS-CoV-2 inhibitor, molecular docking analysis of ERQ tripeptide with DNA (PDB ID: 1BNA) and a variety of SARS-CoV-2 enzymes, namely. Main protease (PDB IDs: 6M03, 6LU7) and Spike glycoprotein (PDB ID: 6VXX) were performed.

Materials and Methods: To determine the binding efficiency of ERQ to target DNA and proteins, molecular docking processes were carried out using the Autodock Vina program. The sorts of bonds and interacting residues in ERQ/DNA and ERQ/protein complexes were determined.

Results: Molecular docking simulations of ERQ tripeptide against 1BNA, 6M03, 6LU7, and 6VXX were performed, and the interactions between the docked ligand and target residues were determined. The binding mechanisms of ERQ with the receptors were clarified. The binding affinities of ERQ towards the targets were predicted to be between -6.3 and -6.7 kcal/mol. ERQ showed the highest binding affinity to Spike glycoprotein (6VXX), with an estimated binding energy of -6.7 kcal/mol.

Conclusion: Molecular docking simulations revealed the potential of ERQ tripeptide as an anticancer and anti-COVID-19 agent. High binding affinity against 1BNA (-6.4 kcal/mol), 6M03 (-6.3 kcal/mol), 6LU7 (-6.6 kcal/mol), and 6VXX (-6.7 kcal/mol) indicated that ERQ could be an excellent new natural therapy for the treatment of cancer and COVID-19.

Keywords: *In silico,* Molecular docking, Glu-Arg-Gln, ERQ, Tripeptide

INTRODUCTION

Cancer is a study topic susceptible to novel techniques in developing drugs because it is a lethal disease.[1] The medications employed in conventional cancer treatment damage both healthy and cancerous cells.[2] During chemotherapy treatment, patients may have adverse effects, including depression, hair loss, and nausea.[2] As anticancer medicines, peptides with low toxicity against these side effects are essential.[3,4] Special drug carriers with peptide structures are currently being studied and developed as an alternative to conventional chemotherapy treatment approaches.[1-4] Activator protein 1 (AP-1) regulates the expression of essential oncogenes in cancer and many other cellular processes.[4,5] AP-1, which has a vital role in cancer, also plays an important role in diseases such as psoriasis, asthma, and rheumatoid arthritis[5] and has been the subject of research in drug production due to its active roles.[5,6] Kumar et al. performed molecular docking calculations to evaluate the activity of the Glu-Glu-Arg tripeptide on the AP-1 (c-Jun:c-Fos: DNA) complex.[6] It was found that Glu-Glu-Arg has a strong affinity towards AP-1 (-9.1 kcal/mol). Thus, the Glu-Glu-Pro tripeptide was determined to be a potent anticancer candidate and can prevent the division of cancer cells.

The molecular docking method used in drug design research investigates the interaction between receptor and ligand[7], the formation of hydrogen bonds, Coulomb interactions, and van der Waals interactions that affect the ligand-receptor binding potential.[8]

DNA plays a significant role in controlling cellular functions; for this reason, it is considered an excellent target for treating genetic diseases, particularly cancer. Moreover, due to the rapid

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proliferation of cancer cells, most anticancer drugs target the cell cycle. The structural changes that occur by binding of a ligand to DNA affect the biological functions of DNA, including the inhibition of transcription, replication, and DNA repair processes, thus making the ligand a potential antitumor agent.[9]

On the other hand, SARS-COV-2, main protease (M^{pro}), and spike proteins are essential targets for promising anti-COVID-19 agents. In a study to determine the anthraquinone derivative's ability to prevent SARS-CoV-2 infection, Celik et al. carried out molecular docking analyses against the apo- and holo-forms of the significant protease (Mpro) and the spike glycoprotein of SARS-CoV-2 targets.[10] Molecular structure and molecular docking have yet to be published for the ERQ tripeptide. This work used molecular docking models to simulate the action of the ERQ tripeptide against DNA(1BNA), SARS-CoV-2 proteases (6M03, 6LU7), and spike glycoprotein (6VXX) to evaluate its anticancer and anti-COVID-19 properties.

MATERIALS AND METHODS

Compound Selection and Preparation

The 3D molecular structures of the SARS-CoV-2 main protease (6M03,6LU7), Spike glycoprotein (6VXX) and DNA (1BNA) were taken from the Protein Data Bank.[11]

Conformational Analysis

Using quantum chemical computations and molecular mechanics, Spartan is an effective computational modeling method for investigating organic, bioorganic, inorganic, and organometallic chemistry in academic and research fields. The Molecular Mechanics Force Field (MMFF) method[12] with Spartan06[13] program was used to determine the conformational features of ERQ (Glu-Arg-Gln) tripeptide. The determined optimized lowest energy conformer of ERQ in the gas phase by the MMFF method was taken as the initial ligand structure for molecular docking. During the molecular docking simulations, the ligand was taken as flexible.

Molecular Docking

In this study, docking simulations of ERQ tripeptide were performed using the AutoDock-Vina software program.[14] A semi-flexible docking study was carried out, where ERQ was treated as a flexible ligand by modifying its rotatable torsions, but the target DNA or protein was considered a rigid receptor. The crystal structure of DNA (PDB ID: 1BNA)[15], the crystal structures of COVID-19 main protease, in apo form (PDB ID: 6M03)[16] and in complex with N3 (PDB ID: 6LU7)[17], and the crystal structure of SARS-CoV-2 spike glycoprotein (closed state) (PDB ID: 6VXX)[18] were retrieved from protein data bank. The DNA and protein targets were conformed to the docking by removing water molecules and adding polar hydrogens. The optimized structure of the ERQ ligand molecule in the gas phase was adapted for the docking. The partial charges of the ERQ molecule were calculated using the Geistenger method. The active sites of receptors were screened using the CAVER program[19], and then the active sites of the targets were defined within the grid size of 40Åx40Åx40Å.

RESULTS

Three-Dimensional Structure of the ERQ Tripeptide

In the first step of the study, the conformational features of the ligand were searched by the MMFF method, and all the conformers were optimized. Among the 2601 optimized conformers, the lowest energy conformer was taken as the initial structure for docking. The optimized structure of the tripeptide is shown in Figure 1. The selected bond lengths and angles from the obtained structural parameters of the ERQ tripeptide are given in Table 1.

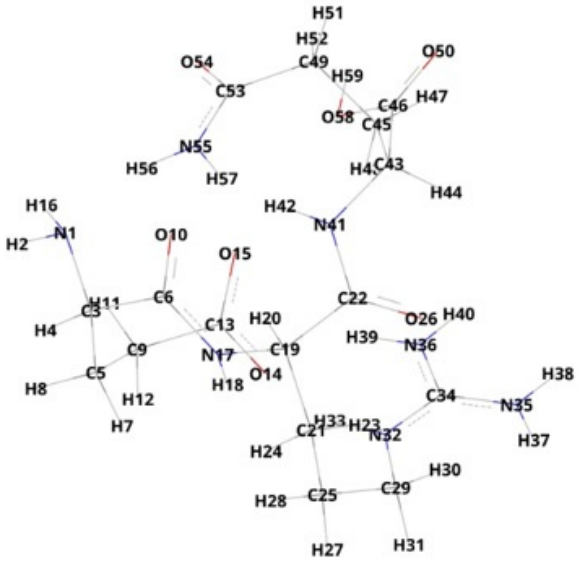

Figure 1. Optimized structure of ERQ tripeptide.

Molecular Docking Calculations

Molecular docking, an effective drug design and discovery method, reveals bimolecular interactions between the target protein and the docked ligand. To investigate anticancer and anti-COVID-19 properties of the investigated tripeptide (ERQ), it was docked in the determined binding site of DNA (1BNA), SARS-CoV-2 Mpro (6M03, 6LU7) and Spike glycoprotein (6VXX). The molecular docking results and molecular interactions are shown in Figures 2-5.

Table 1. The selected geometry parameters of ERQ tripeptide (Glu-Arg-Gln).*

Atoms		Atoms	
C6-O10	1.24	C6-N17-C19-C21	150.6
C13-O14	1.26	N17-C19-C21-C25	48.7
C13-O15	1.27	C19-C21-C25-C29	74.7
C22-O26	1.23	C21-C25-C29-N32	-82.4
C46-O50	1.22	C25-C29-N32-C34	154.8
C46-O58	1.35	C6-N17-C19-C22	-79.2
C53-O54	1.14	N17-C19-C22-N41	51.8
H2-N1-C3-C5	48.5	C19-C22-N41-C43	165.5
H2-N1-C3-C6	-179.1	C22-N41-C43-C46	-102.3
N1-C3-C5-C9	46.5	C22-N41-C43-C45	131.1
C3-C5-C9-C13	58.3	N41-C43-C45-C49	79.7
C3-C6-N17-C19	-175.7	C43-C45-C49-C53	-59.7

***** Bond lengths in A and angles in degree (°). For atom numbering, see Figure 1.

ERQ tripeptide was docked to DNA to reveal its anticancer property. Figure 2 shows the docking results and the interactions of the tripeptide with DNA. The binding affinity (ΔG) was predicted as -6.4 kcal/mol. H- bonding is crucial in the interaction between the tripeptide and DNA bases. The ERQ tripeptide formed nine conventional hydrogen bonds with the DNA bases. These are hydrogen bond of 2.53 Å with DNA, DG2 nucleobase; hydrogen bond of 2.39 Å with DG4; hydrogen bond of 2.47 Å, with DA5; hydrogen bond of 1.91 Å with DC21; hydrogen bonds of 2.17, 2.26 and 2.46 Å with DG22; hydrogen bonds of 2.54 Å and 2.68 Å with DA6. There is also an unfavorable donor-donor interaction and a carbon-hydrogen bonding interaction with DA5 and DA6 bases with 2.03 Å and 3.74 Å longs, respectively.

The docking results of ERQ tripeptide into 6M03 are shown in Figure 3. Tripeptide forms seven conventional hydrogen bonds with the target residues. The bond lengths predicted for the hydrogen bonds between the ERQ ligand and the THR24, LEU141, ASN142, and GLY143 residues of 6M03 are 2.32, 2.59, 2.46, and 2.84 Å, respectively. Additionally, ERQ forms three conventional hydrogen bonds with GLU166 residue of 6M03, with the predicted bond lengths 2.12, 2.16, and 2.76 Å. ERQ tripeptide also involves 1.58 Å long unfavorable donor-donor interaction with HIS163, 3.53 Å long carbon-hydrogen bonding interaction with MET165, and 3.26 Å long unfavorable negative-negative interaction with GLU166.

Figure 4 displays the ERQ tripeptide's docking results into 6LU7. As seen from Figure 4, the predicted lengths of the hydrogen bonds formed between the docked ligand ERQ and the 6LU7 target residues HIS41, TYR54, ASN142, GLY143, HIS164, GLU166 are 2.49, 2.55, 2.55, 2.13, 2.8, 2.59 Å, respectively. Moreover, docking results demonstrated that ERQ forms two hydrogen bonds with both PHE140 and CYS145 residues with bond lengths of 2.34Å, 2.49 Å, and 2.93Å, 3.62Å, respectively. In addition, ERQ forms a 2.72 Å long salt bridge interaction with GLU166 and an unfavorable donor-donor and attractive charge interactions with HIS41, at 1.77 Å and 5.58 Å bond lengths, respectively.

Figure 5 displays the results of the docking calculation for the ERQ-6VXX ligand-receptor system in which hydrogen bonding is predicted between the ERQ ligand and the GLU780, ASN1023, THR1027, LYS1028, VAL1040 residues; the corresponding hydrogen bond lengths were calculated as 2.9 Å, 2.46 Å for GLU780; 2.28 Å, 2.31 Å for ASN1023; 2.18 Å, 2.31 Å for THR1027; 2.28 Å for LYS1028; 2.71, 3.04 Å for VAL1040. Also, docking results indicate carbon-hydrogen bonds of 3.42 Å and 3.53 Å are formed through ASP1041 and PHE1042 residues. In comparison, an attractive charge interaction in a distance of 4.16 Å and an unfavorable negative-negative interaction in a distance of 4.78 Å exist between the ligand and GLU780 residue.

DISCUSSION

As we noted in the result part, the interactions resulting from DNA docking with the ERQ tripeptide were compared with similar studies in the literature. As a result of these comparisons, it was determined that the DNA nucleobases with which the ERQ tripeptide interacts in this study were the same as those in the study conducted by Demirag et al., docking analysis of the Pemetrexed molecule with DNA.[20]

A comparison of the hydrogen bonding interactions previously reported for the Pemetrexed-DNA system with those reported in this study for the ERQ-DNA system has been given here as items. i) A hydrogen bond of 2.05 Å length was reported between the Pemetrexed ligand and DG2 residue, and here we report a hydrogen bond of 2.53 Å length for the ligand ERQ and DG2 residue. ii) A hydrogen bond of 2.48 and 2.97 Å length was reported between the Pemetrexed ligand and DG4 residue,

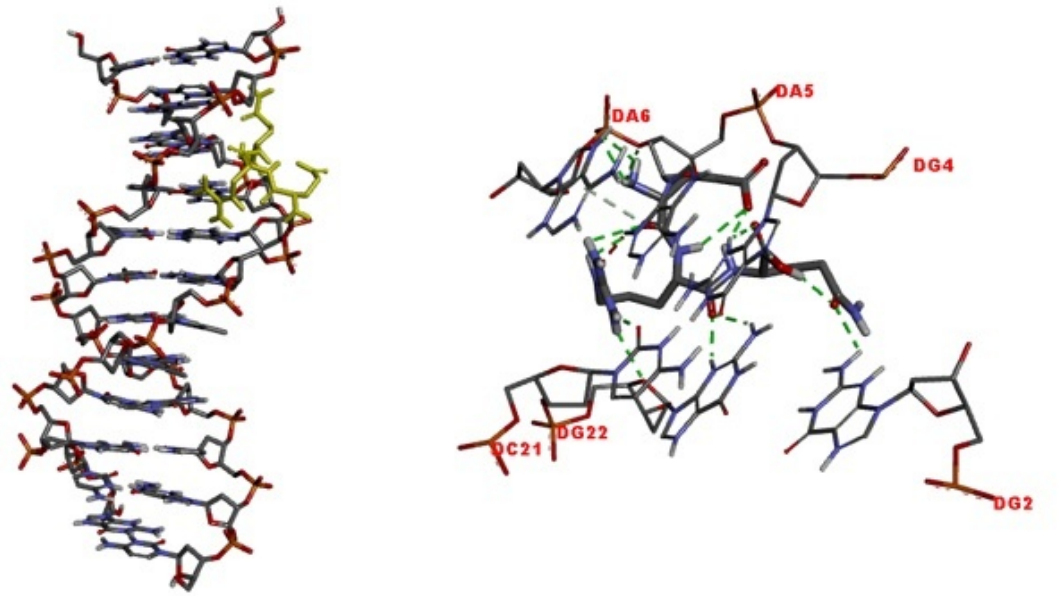

Figure 2. Molecular docking results were obtained for the ERQ-1BNA ligand-receptor system; the corresponding binding affinity is -6.4 kcal/mol.

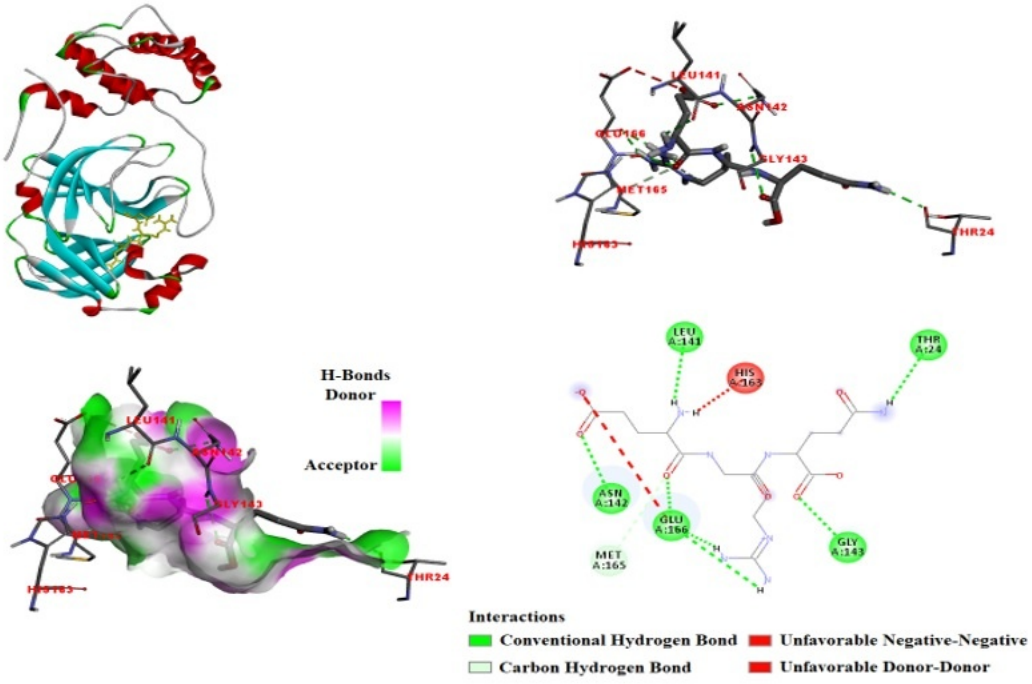

Figure 3. Molecular docking results were obtained for the ERQ-6m03 ligand-receptor system; the corresponding binding affinity is -6.3 kcal/mol.

and we report a hydrogen bond of 2.39 Å length for the ligand ERQ and DG4 residue. iii) A hydrogen bond of 2.16 Å length was reported between the Pemetrexed ligand and DA5 residue, and here we report a hydrogen bond of 2.47 Å length for the ligand ERQ and DA5 residue. iv) Hydrogen bonds of 1.91 Å and 3.03 Å length were reported between the Pemetrexed ligand and the DT20, and DC21 residues respectively, and we report a hydrogen bond of 1.91 Å length between the ligand ERQ and DC21 residue. v) Hydrogen bonds of 2.49 Å and 3.09 Å length were reported between the Pemetrexed ligand and DG22 residue, and here we report hydrogen bonds of 2.17 Å, 2.26 Å and 2.46 Å length for the ligand ERQ and DG22 residue. The

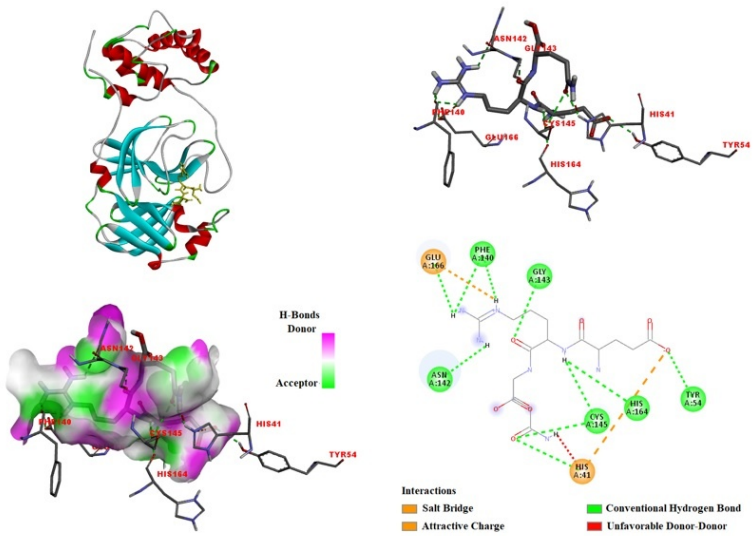

Figure 4. Molecular docking results were obtained for the ERQ-6lu7 ligand-receptor system; the corresponding binding affinity is -6.6 kcal/mol.

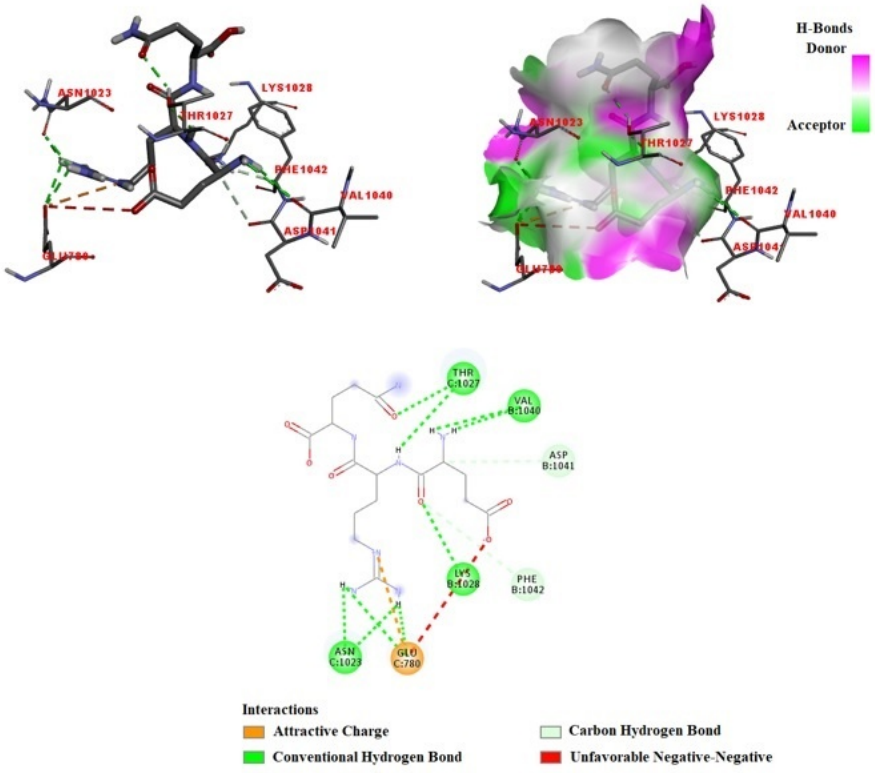

Figure 5. Molecular docking results were obtained for the ERQ-6vxx ligand-receptor system; the corresponding binding affinity is -6.7 kcal/mol.

other hydrogen bonds reported for the Pemetrexed (drug)-DNA system are as follows; The H-bond contact length between the DC23 residue and the drug is 2.93 Å, the carbon hydrogen bond interaction length between the DG4 residue and the drug is 3.49 Å, the unfavorable donor-donor interaction length is 1.72 Å, and the Pi Donor hydrogen bond interaction length is 2.76 Å between the DG4 residue and the drug.[20] This similarity between the results previously reported for the Pemetrexed-DNA system

and those reported here for the ERQ-DNA system supports the reliability of our docking simulations.

Molecular docking simulations of ERQ tripeptide against 6M03 were performed and compared with similar studies. In the study conducted by Sagaama et al., a molecular docking simulation of Succinic acid (SA) with 6m03 was performed, and found that SA interacted with Ser144, Cys145, and Glu166 through hydrogen bonding interactions.[21] In another study conducted by Begum et al., tetrandrine, a bis-benzylisoquinoline alkaloid, was docked with a 6m03 receptor and binding free energy -8.91 kcal/mol was determined. Tetrandrine was highly stabilized by many non-bonded interactions.[22] Tetrandrine, bonded to the His-165 residue by hydrogen bonding interaction. The hydrophobic contacts of the tetrandrine molecule were demonstrated by Thr-25, Leu-27, His-41, Ser-46, Met-49, Asn-142, Cys-145, Glu-166, and Gln-189.

Another investigation on the relationship between aminopterin and 6M03 was carried out.[23] The binding affinity was recorded as -6.7 kcal/mol and aminopterin was bonded with THR24, THR26, ASN119, Leu141, Gly143, Cys145, and Gln189 with eight conventional hydrogen bonds, Asn142 (It was determined that it forms a carbon-hydrogen bond with a length of 3.44 Å). Additionally, pi-sigma and pi-alkyl interactions with Thr27 and Cys145, respectively, were present.[23]

The docking results on the 6m03 receptor have confirmed that the ERQ tripeptide interacts with certain residues identical to those previously reported for the 6m03 receptor in literature. This consistency supports the reliability of our docking simulations for the ERQ-6m03 ligand-receptor system. In this respect, the determined hydrogen bond lengths (2.32 Å for Thr24; 2.59 Å for Leu141; 2.46 Å for Asn142; 2.84 Å for Gly143; 2.12, 2.16 and 2.76 Å for Glu166) are remarkable. In addition, it should be noted that in the same ligand-receptor system, ERQ tripeptide forms a carbon-hydrogen bond of 3.53 Å length with the residue Met165 and involves in an unfavorable donor-donor interaction with the residue His163 (in a distance of 1.58 Å) and in an unfavorable negative-negative interaction with Glu166 (in a distance of 3.26 Å). It is believed that the main protease (M^{Pro}) amino acids HIS41, CYS44, MET49, SER144, CYS145, and GLU166 are crucial for interactions between drugs and receptors.[24]

According to the results of the comparison of ERQ tripeptide docking into 6LU7 results with similar studies in the literature: In a study conducted by Özdemir et al. in 2020, the different interactions between coumarins and the receptor's (6LU7) active site residues are shown and tabulated.[25] It is shown that one of the fluorines and the hydroxy group at position C-7 created a hydrogen bond with TYR54 and PHE140, respectively. Furthermore, a π-alkyl bond was established by one of the fluorines with HIS41. TYR54 and PHE140 have hydrogen bond lengths of 2.725 Å and 2.201 Å, respectively. And Hatada et al., (B), it was discovered that the pharmacophore's hydrogen bonding with neighboring residues was significant for each of the five pieces.[26] The ligand's fourth portion was found to be the key component in interactions with His163, His164, and Glu166. It was also shown that dispersion interactions—like the CH/π contact with His41—provided further ligand stability. It was proposed that the ligand's fifth component may be further optimized for ligand binding to facilitate interactions with Thr25, Thr26, and Asn142 in addition to His41. Additionally, the impact of the covalent connection between the ligand and Cys145 was examined.[26] According to this study, hydrogen bonding is the primary mechanism by which His41, His163, His164, and Glu166, amino acid residues of Mpro interact with the inhibitor are shown.[26] In this study, the primary protease (MPRO) amino acids Tyr54, Asn142, Gly143, His164, Glu166, Phe140, Cys145, and His41 are the interacting amino acids. This is also compatible with the literature.

Molecular docking simulations of ERQ tripeptide against spikeglycoprotein (6VXX) were performed and compared with similar studies. In the following, these comparisons over some similar studies in the current literature are seen. In the study, in which the molecular docking analysis of cepharanthine with the residue 6vxx is given, the authors report that the cepharanthine molecule interaction with the residues Glu725 (2.69Å), Gln784 (3.52 Å), Ala1026 (4.16 Å), Asp1041 (3.11 Å), Phe1042 (3.44 Å), Lys1045 (2.64 Å) and its binding affinity is -9.7 kcal/mol.[27]

The residues Val1040, Asp1041, Leu1049, Val1068, Asn907, and Lys1038 have a significant attraction for the ligand, and they may serve as anchoring residues, as shown by Holanda et al.[28] In docked complex analysis, two residues, Arg (1039) and Asn (1023), show hydrogen bond formation with Amentoflavone with a range of 2.0 to 3.5 A, according to Joshi et al. (C), with 6VXX (Spike protein).[29] In the molecular docking simulations performed in this study, the ERQ tripeptide is involved in interaction against the receptor 6VXX through the Glu780, Asn1023, Thr1027, Val1040, Lys1028, Asp1041, and Phe1042 amino acids.

Based on the molecular docking data, we obtained in this study and also considering those previously published in relevant studies in the literature, we concluded that the amino acids through which the ligand ERQ interacts with DNA (1BNA), SARS-CoV-2 Mpro (6M03, 6LU7) and Spike glycoprotein (6VXX) are almost similar (shown in bold).

CONCLUSION

In this molecular docking study, in which the ERQ tripeptide was docked with DNA and several SARS-CoV-2 enzymes, including the Main protease and Spike glycoprotein, the ERQ tripeptide's potential as an anticancer and SARS-CoV-2 inhibitor was investigated. ERQ docked with 6vxx compound showed the highest binding affinity, demonstrating a binding energy value of -6.7 kcal/mol against the active site of Spike

glycoprotein (6VXX). It showed nine H-bonds with GLU780, ASN1023, THR1027, LYS1028, and VAL1040, two Carbon-hydrogen interactions with ASP1041 and PHE1042, as well as an attractive charge interaction, and an unfavorable negative-negative interaction with GLU780 residues. Considering these promising docking simulation outcomes with the SARS CoV-2 Spike glycoprotein (6VXX), we recommend that Glu-Arg-Gln (ERQ) tripeptide be tested to discover new natural therapies for the treatment of COVID-19. This study is a novel, effective, and time-saving in silico study of short peptides against both COVID-19 and cancer drug discovery.

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