Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 13(2), 839-850, 2023
Journal of the Institute of Science and Technology, 13(2), 839-850, 2023
ISSN: 2146-0574, eISSN: 2536-4618
Biology **DOI: 10.21597/jist.1179752**
Research Article
Received: 24.09.2022 **Accepted: 04.02.2023**
To Cite: Ilhan, S. (2023). *In silico* Screening of the Potential Anti-SARS-CoV-2 Activities of Peptides from *Vipera ammodytes ammodytes* Venom by Molecular Docking. *Journal of the Institute of Science and Technology*, 13(2), 839-850.

In silico Screening of the Potential Anti-SARS-CoV-2 Activities of Peptides from *Vipera ammodytes ammodytes* Venom by Molecular Docking

Süleyman İLHAN[1]

Highlights:

- Anti-SARS-CoV-2 activities of VA peptides were analyzed
- Adamalysin II, Ammodytoxin A, Ammodytin L, L-amino acid oxidase were active against ACE2 and 3CLpro

Keywords:

- *Vipera ammodytes ammodytes*
- SARS-CoV-2
- ACE2
- 3CLpro
- Molecular docking
- Venom

ABSTRACT:

The coronavirus disease 2019 (COVID-19) is induced by the SARS-CoV-2 virus, which caused the global pandemic, infecting approximately 608.328.548 confirmed cases and bringing about 6.501.469 deaths worldwide, as WHO stated in September 2022. The disease is more deadly due to the lack of specific drug molecules or a treatment plan. Therefore, the development of potent pharmacological compounds is urgently required to combat COVID-19. Due to their biological actions, snake venoms constitute a source of potentially beneficial medicinal compounds. *Vipera ammodytes ammodytes* (VA) is a viper species whose venom has been shown to have anti-proliferative, anti-metastatic, anti-cancer, and anti-microbial activities. This *in silico* study was conducted to evaluate the efficacy of selected VA venom proteins (Adamalysin II, Ammodytoxin A, Ammodytin L, L-amino acid oxidase) against molecular targets; Main protease (3CLpro) and Angiotensin-Converting Enzyme 2 (ACE2) by molecular docking study. Molecular docking investigations were performed by using AutoDock Vina software. All compounds displayed negative binding energy values to 3CLpro and ACE2, suggesting that their interactions with the active sites were favourable. L-amino acid oxidase had the highest binding affinity with both 3CLpro and ACE2. This study revealed for the first time that VA venom proteins are functional inhibitors of 3CLpro and ACE2 activities, and the components of VA venom can be considered potential SARS-CoV-2 inhibitors. However, more studies are needed to validate these compounds *in vitro* and *in vivo*.

[1*] Suleyman ILHAN (**Orcid ID:** 0000-0002-6584-3979), Manisa Celal Bayar University, Faculty of Arts and Sciences, Department of Biology, Manisa, Türkiye

Corresponding Author: Suleyman ILHAN, e-mail: suleyman.ilhan@cbu.edu.tr

Ethics Committee Approval: This study does not require ethics committee permission or any special permission

INTRODUCTION

The outbreak and rapid spread of the coronavirus disease 2019 (COVID-19) epidemic caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) have caused many deaths. The disease has become more complex with the emergence of new variants. According to the data from the World Health Organization, it has been reported that more than 600 million people in many countries were infected with SARS-CoV2, and more than 6 million people died due to this epidemic (WHO, 2022). Therefore, a worldwide struggle against this epidemic has begun. Despite many vaccines and drug treatment attempts, the COVID-19 epidemic still poses a significant threat to humanity due to the weak immune response.

The SARS-CoV2 virus binds spike protein (glycoprotein S) to angiotensin-converting enzyme (ACE2) expressed on the surface of host cells and specifically targets lung cells (Sheahan et al., 2020). Two open reading frames, ORF1a and ORF1b, found in the SARS-CoV2 genome are translated into two similar viral polyproteins, pp1a and pp1ab, by host ribosomes. Two cysteine proteases, a 3C-like protease (3CLpro) and a papain-like protease (PLpro), are encoded by ORF1a. Since autoclaving is essential for the replication of viruses, 3CLpro has been implicated as a therapeutic target for the suppression of virus replication (Zumla et al., 2016).

Studies are ongoing to search for new vaccines/drugs and new compounds that target the SARS-CoV2 receptor or that can inhibit the replication of the virus. In this context, compounds of natural origin (herbs, spices, and therapeutic substances derived from animals) are extensively studied (Ahmad et al., 2021; Jin et al., 2020; Joshi et al., 2020; Siniavin et al., 2021). In the literature, it is seen that plants from China, India, and the Mediterranean regions are investigated because of their high antioxidant and immune-enhancing effects, and animal venoms are also investigated because they contain active compounds with different mechanisms of action.

Due to their biological actions, snake venoms constitute a source of potentially beneficial medicinal compounds (El-Aziz et al., 2019). Therefore, they have great potential for the discovery of new compounds. They may include compounds suitable for drug design or development, enabling the discovery of new proteins and protein families. Venomous snakes commonly found in our country are members of the Viperidae family. *Vipera ammodytes ammodytes* (VA) is a viper species living in southern Europe, almost exclusively restricted to the Balkans (Latinović et al., 2016). Venom proteins of this species were revealed by electrophoretic methods in the literature (Gopcevic et al., 2021; Latinović et al., 2016). Metalloproteinases, disintegrins, L-amino acid oxidases, and cysteine-rich secretory proteins were the prominent protein families (Göçmen et al., 2015). Although anti-proliferative, anti-metastatic, anti-cancer, and anti-microbial activities of VA venom have been investigated previously (Gopcevic et al., 2021; Karabuva et al., 2017), the effects of venom peptides on SARS-CoV2 have not yet been investigated. Adamalysin II is a 203-amino acid metalloendopeptidase, ammodytoxin A is the subgroup IIA of the phospholipase A2 family of enzymes, ammodytin L is a Ser48 phospholipase A2-homologue and L-amino acid oxidase is a flavoenzyme that catalyzes the oxidative deamination of an L-amino acid (Gopcevic et al., 2021).

Molecular docking is a drug design method based on the receptor property and the active compound/drug-receptor interaction mode (Pinzi & Rastelli, 2019). It is a cutting-edge research technique that combines physical and chemical principles with sophisticated computational algorithms to offer a practical tool for examining the origin and mechanism of potential novel compounds (Salmaso & Moro, 2018).

In this study, the interactions of some proteins (Adamalysin II, Ammodytoxin A, Ammodytin L, L-amino acid oxidase) identified in the VA venom with 3CLpro and ACE2 were investigated to reveal the potential anti-viral molecules to target SARS-CoV2.

MATERIALS AND METHODS

Receptor preparation

The SARS-CoV-2 main protease 3CLpro (PDB ID: 6LU7) and ACE2 (PDB ID: 1R42) were chosen as receptors. The three-dimensional (3D) structures of 3CLpro and ACE2 proteins were downloaded from the protein databank in PDB format (https://www.rcsb.org/). Autodock Vina 4.2.5.1 software was used for water removal, hydrogenation, and load distribution adjustment.

Ligand preparation

3D structures of selected snake venom proteins (Adamalysin II; PDB ID: 1IAG, ammodytoxin A; PDB ID: 3G8G, ammodytin L; PDB ID: 3DIH, L-amino acid oxidase; PDB ID: 3KVE) were downloaded from PubChem in PDB format. Autodock Vina software was used for water removal, hydrogenation, and load distribution adjustment.

Docking

High throughput molecular docking was carried out by Autodock Vina. The grid centre for 3CLpro was set as X= 21.41, Y=3.62 and Z=21.94 with dimensions of the grid box 60 A˚× 60 A˚× 60 A˚. The grid centre for ACE2 was set as X=19.81, Y=-5.57 and Z=14.73 with the grid box 60 A˚× 60 A˚× 60 A˚. The same grid box size and other parameters were utilized for docking experiments of all four proteins after calibration and optimization, and the complete setup was run to produce various docked conformations. The PyMOL software was used to visualize the secondary structures of molecules.

RESULTS AND DISCUSSION

It takes many years to test the pharmacokinetic and pharmacodynamic properties of components for the discovery or design of new drugs. Existing ingredients have gone through many stages since their discovery and have well-defined profiles, so they do not require lengthy preclinical studies. These properties make them excellent candidates for use in new purposes. A new window has been opened for the theoretical investigation of SARS-CoV2, with the determination of the crystal structure of 3CLpro and ACE2 (Jin et al., 2020). A non-structural protein called 3CLpro divides two replicase polyproteins into mature proteins needed to facilitate viral transcription and replication. Inhibiting the Mpro in this way prevents viral replication, whereas inhibiting the ACE2 catalytic pocket by small molecules alters the shape of ACE2 to prevent SARS-CoV-2 entrance into host cells through ACE2. Much of this theoretical research is conducted to discover potential natural or synthetic inhibitors for COVID-19. Based on *in silico* studies, the researchers showed that drugs such as ivermectin, rutin, curcumin, quercetin, hesperidin, naringin, paritaprevir, vitexin, orientin, berberine, thymoquinone which are currently in use, inhibit ACE2 or 3CLpro (Jin et al., 2020).

Despite rapidly continuing *in vitro* and *in silico* studies, an effective antiviral drug has still not been found. In this context, natural products have gained importance as potential antiviral agents in recent years. Of the 175 drugs approved by the Food and Drug Administration (FDA), 49 are derived from natural compounds. Of these, Captopril® (Enalapril), Integrilin® (Eptifibatide) and Aggrastat® (Tirofiban) are obtained from snake venom (Waheed et al., 2017). Apart from these, preclinical and clinical studies of many therapeutic substances obtained from venoms are still in progress. According

to several studies, the antiviral properties of snake venom include action against measles, Sendai, dengue, yellow fever, and human immunodeficiency viruses (Borkow & Ovadia, 1999; Meenakshisundaram et al., 2009; Shimizu et al., 2017). The properties of snake venom proteins may create promising treatment options for breaking the defence mechanisms developed by viruses. VA venom proteins are well defined, structured, and demonstrated many biological activities, but no data are available on their potential anti-COVID activities.

Considering the urgent need for therapeutic and preventive drugs against COVID-19, in our study, the binding mechanism of natural compounds (Ammodytoxin A, Ammodytin L, Adamalysin II, L-amino acid oxidase) from VA venom with two distinct targets from COVID-19 (3CLpro and ACE2) (Figure 1) was predicted using computational docking.

Docking of ligands into 3CLPro main protease active site

The best compounds for inhibiting the target receptor were those with the lowest binding energy of docking scores because lower binding energy corresponds to increased binding affinity (Simon et al., 2017). Docking scores corresponding to the binding energies of the tested compounds with 3CLpro are presented in Table 1.

Table 1. Interaction of the SARS-CoV2 Main Protease 3CLpro with VA proteins

Compound	Docking Score (Binding Energy, Kcal/mol)	H Bond	Amino acid Residue
Ammodytoxin A	-106.3	-4.80	Asp48, Tyr27, Tyr21, Cys44, Phe5
Ammodytin L	-129.2	-10.92	Ser48, Cys44, His27, Tyr21, Gly29, His47
Adamalysin II	-129.3	-6.97	Glu35, Arg32, Asn133, Val33, Val136, IIe36, Thr31
L-amino acid oxidase	-143.9	-5.55	Glu457, Cys293, Arg71, Pro88, Gly42

All compounds displayed negative binding energy values to 3CLpro, suggesting that their interactions with the 3CLpro active sites were favourable. The most outstanding binding energy value was calculated for L-amino acid oxidase which was -143.9 kcal/mol. It formed hydrogen interactions with Glu457, Cys293, and steric interactions with Arg71, Pro88, and Gly42 (Figure 2A-B). L-amino acid oxidase is a flavoenzyme that catalyzes the production of ammonia and hydrogen peroxide and oxidatively deaminates an L-amino acid to a -keto acid. Antimicrobial and anti-viral activities have been demonstrated (Kasai et al., 2021; Sant'Ana et al., 2008). Anti-cancer and pro-inflammatory activities of L-amino acid oxidase obtained from *Bungarus fasciatus* snake venom have also been shown in the literature (Wei et al., 2009). However, there is no study on the biological activity of L-amino acid oxidase obtained from VA venom. Therefore, this *in silico* study is the first to demonstrate the potential anti-COVID-19 activity of L-amino acid oxidase.

Adamalysin II was the second molecule with the highest binding energy (-129.3 kcal/mol). When it was analyzed by docking tools it successfully docked against the inhibitor region of the main protease of SARS-CoV-2. Active site residues were Glu35, Arg32, and Asn133, and Val33, Val136, IIe36, and Thr31 participated in hydrogen bond interactions with Adamalysin II (Figure 2C-D). The results showed that the binding energy of Ammodytin L to 3CLpro was -129.2 kcal/mol. It formed hydrogen bond interactions with Ser48, Cys44, His27, Tyr21, Gly29, and steric interactions with His47 (Figure 4).

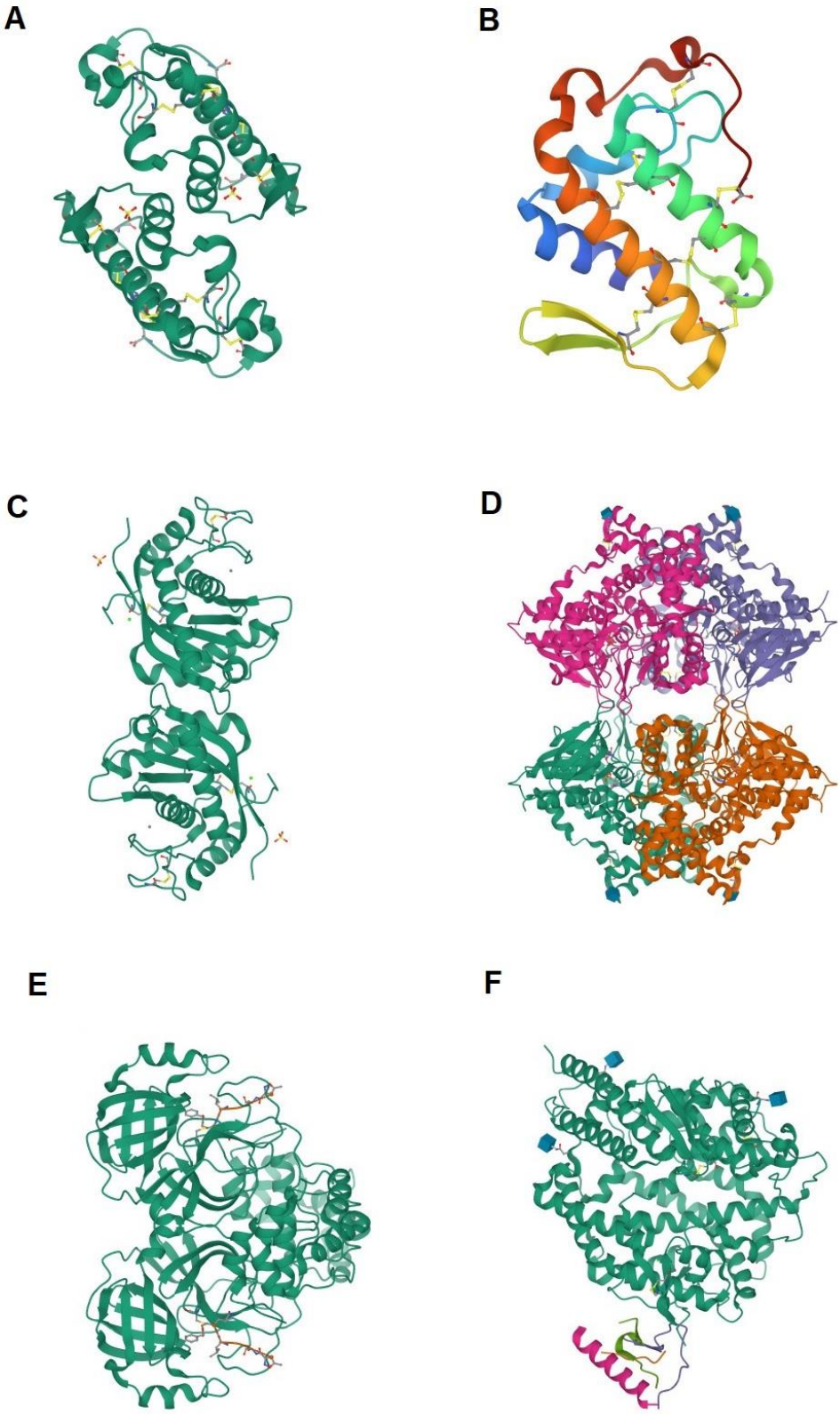

Figure.1. 3D- structures of VA venom components investigated for potential anti-covid effects. A) Ammodytoxin A, B) Ammodytin L, C) Adamalysin II, D) L-amino acid oxidase. 3D- structures of the SARS-CoV-2 main protease E) 3CLpro and F) ACE2

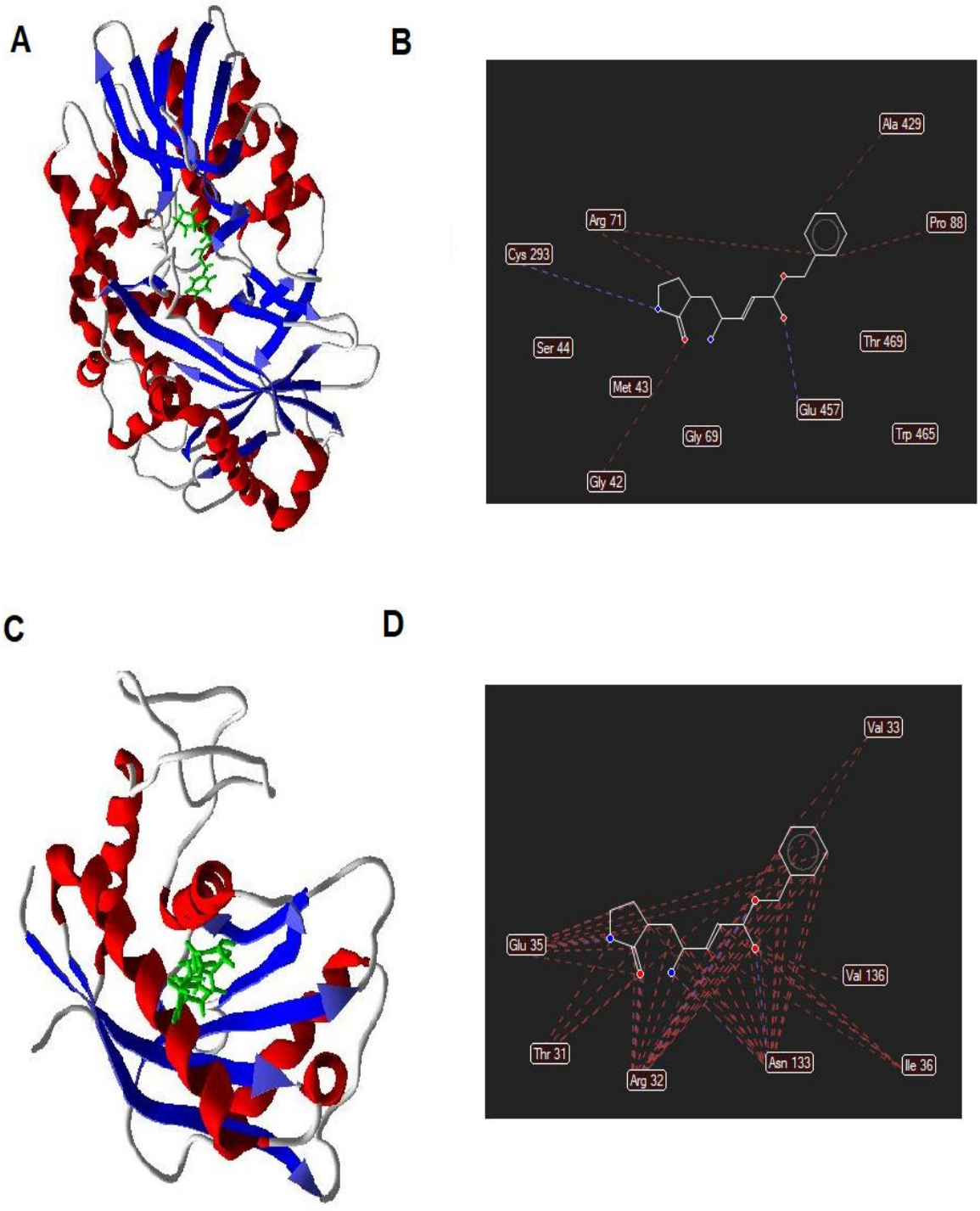

Figure.2. (A) Molecular docking of SARS CoV-2 main protease (3CLpro) and L-amino acid oxidase and (B) interactions with critical residues. (C) Molecular docking of SARS CoV-2 main protease (3CLpro) and Adamalysin II and (D) interactions with critical residues (Red dashes show steric interactions, and blue dashes show hydrogen bonds)

Adamalysin II is a 24 kDa zinc endopeptidase, a member of a large family of metalloproteinases (Gomis-Ruth et al., 1993). Adamalysins are known to play a role in inflammation and various other regulatory roles in the cell. Some metalloproteinases have been shown to exhibit broad-spectrum antiviral activities against vesicular stomatitis virus (VSV), influenza A virus (H1N1) and human herpes virus 1 (HSV-1). It is known that they show anti-viral activity by changing their place from the cytoplasm to the cell nucleus upon virus infection and influencing NF-κB activities (Feng et al., 2022).

Another VA protein, a phospholipase-like snake venom toxin that effectively docked against both 3CLpro, was Ammodytin L. It has been reported that Ammodytin L is non-neurotoxic, weakly toxic and has low PLA2 activity (Logonder et al., 2008). Results showed that the binding energy of Ammodytin L to 3CLpro was -129.2 kcal/mol indicating a good binding affinity. It formed hydrogen bond interactions with Ser48, Cys44, His27, Tyr21, Gly29, and steric interactions with His47 (Figure 3A-B).

The binding energy of Ammodytoxin A, a neurotoxic secretory phospholipase A2, was the VA protein with the weakest binding affinity with 3CLpro (-106.3 kcal/mol). Active site residues were Asp48, Tyr27 and Tyr21. It also formed steric interactions with Cys44 and Phe5 (Figure 3C-D). Its biological activity in neuronal cells has been demonstrated. It has been shown to block the cell cycle in the G2 phase, but there is no study investigating its anti-viral effects (Pražnikar et al., 2009).

Docking of ligands into ACE2 active site

When venoms of the Viperidae family are analyzed, it has been determined that they contain proteins, including snake venom metalloproteinases (SVMPs), disintegrin, snake venom serine proteinases (SVSPs), hyaluronidases, 5'-nucleotidases, phospholipase A2 (PLA2), C-type lectin proteins (CLPs), cysteine-rich secretory proteins (CRISPs). Natriuretic peptides, bradykinin enhancing peptides (BPPs), nerve growth factors (NGFs), snake venom vascular endothelial growth factors (VEGF-Fs), Kunitz type proteinase inhibitors (Nalbantsoy et al., 2017). To investigate their anti-covid effects, we selected Ammodytoxin A, Ammodytin L, Adamalysin II and L-amino acid oxidase from VA venom proteins whose molecular structures are well defined in the literature. The docking scores (binding energies) of selected VA venom proteins with ACE2 are given in Table 2.

Table 2. Interaction of the ACE2 with selected VA proteins

Compound	Docking Score (Binding Energy, Kcal/mol)	H Bond	Amino acid Residue
Ammodytoxin A	-75.3	-7.46	Tyr2, Asp48, His47, Cys44
Ammodytin L	-84.8	-4.98	Ser22, IIe9, Cys44, Phe5
Adamalysin II	-97.4	-11.65	Thr139, Glu143, Pro168, Arg167, IIe165, Gly169, Val138, Leu170
L-amino acid oxidase	-146.6	-4.67	Ala429, Tyr372

Among the VA proteins tested, L-amino acid oxidase had the highest binding energy, with a binding energy of -146.6 kcal/mol. The active site residues were Ala429 and Tyr372 (Figure 4A-B). The binding energy of Adamalysin II was -97.4 kcal/mol, and the active site residues were Thr139, Glu143, Pro168, Arg167, and IIe165. It also established steric interactions with Gly169, Val138 and Leu170 (Figure 4C-D). The docking results revealed that the binding energy of Ammodytin L was -84.8 kcal/mol. It formed hydrogen interactions with Ser22 and steric interactions with IIe9, Cys44 and Phe5 (Figure 5A-B). The binding energy of Ammodytoxin A was -75.3 kcal/mol, and the active site residues were Tyr2, Asp48 and His47 and steric interactions with Cys44 (Figure 5C-D). Since the beginning of the pandemic, researchers have targeted the ACE2 enzyme and reported the reuse purpose of previously used drugs. The high binding energies of VA proteins with ACE2 suggest these molecules are potential anti-viral candidates.

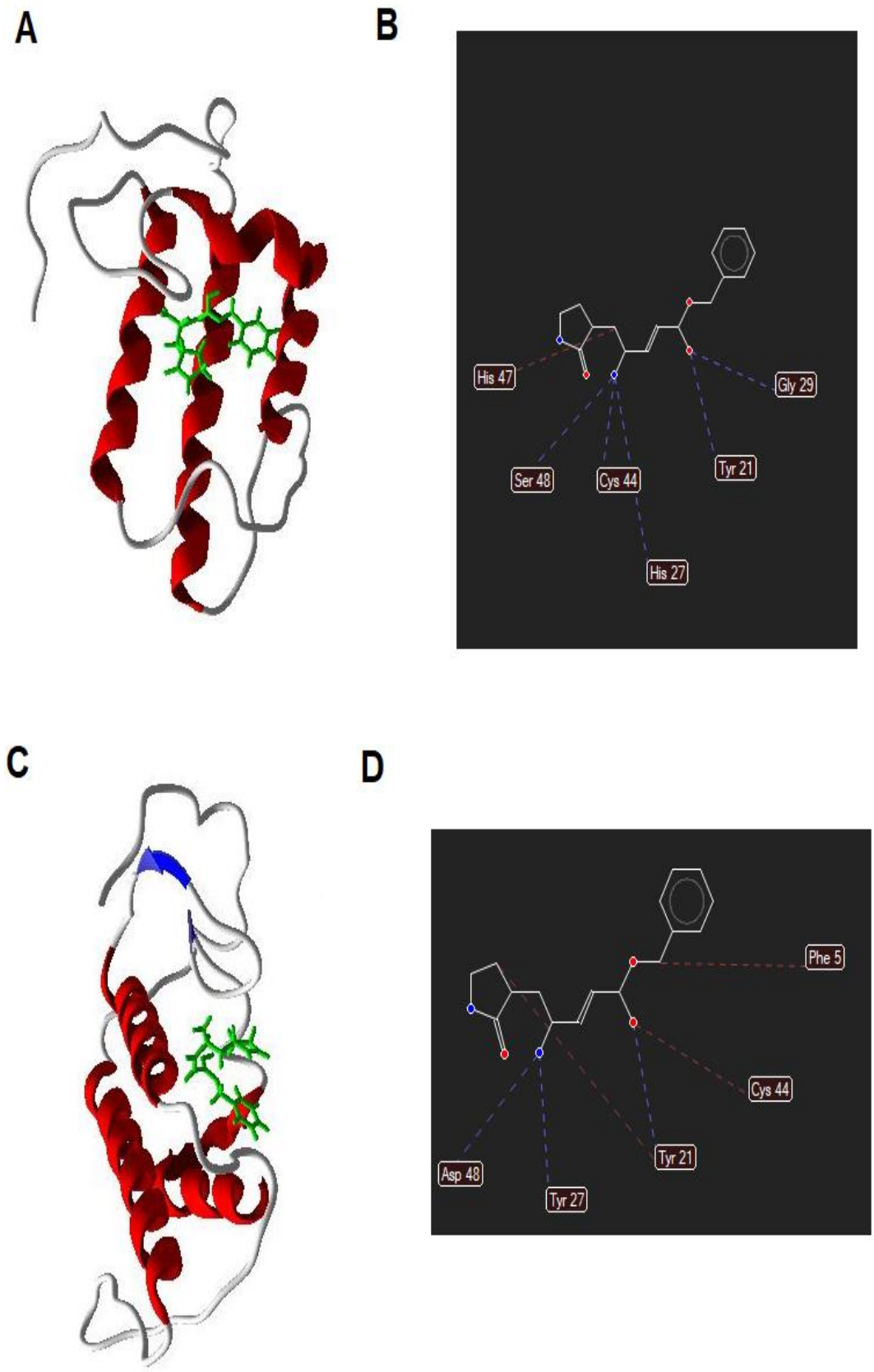

Figure.3. (A) Molecular docking of SARS CoV-2 main protease (3CLpro) and Ammodytin L and (B) interactions with critical residues. (C) Molecular docking of SARS CoV-2 main protease (3CLpro) and Ammodytoxin A and (D) interactions with critical residues. (Red dashes show steric interactions, and blue dashes show hydrogen bonds)

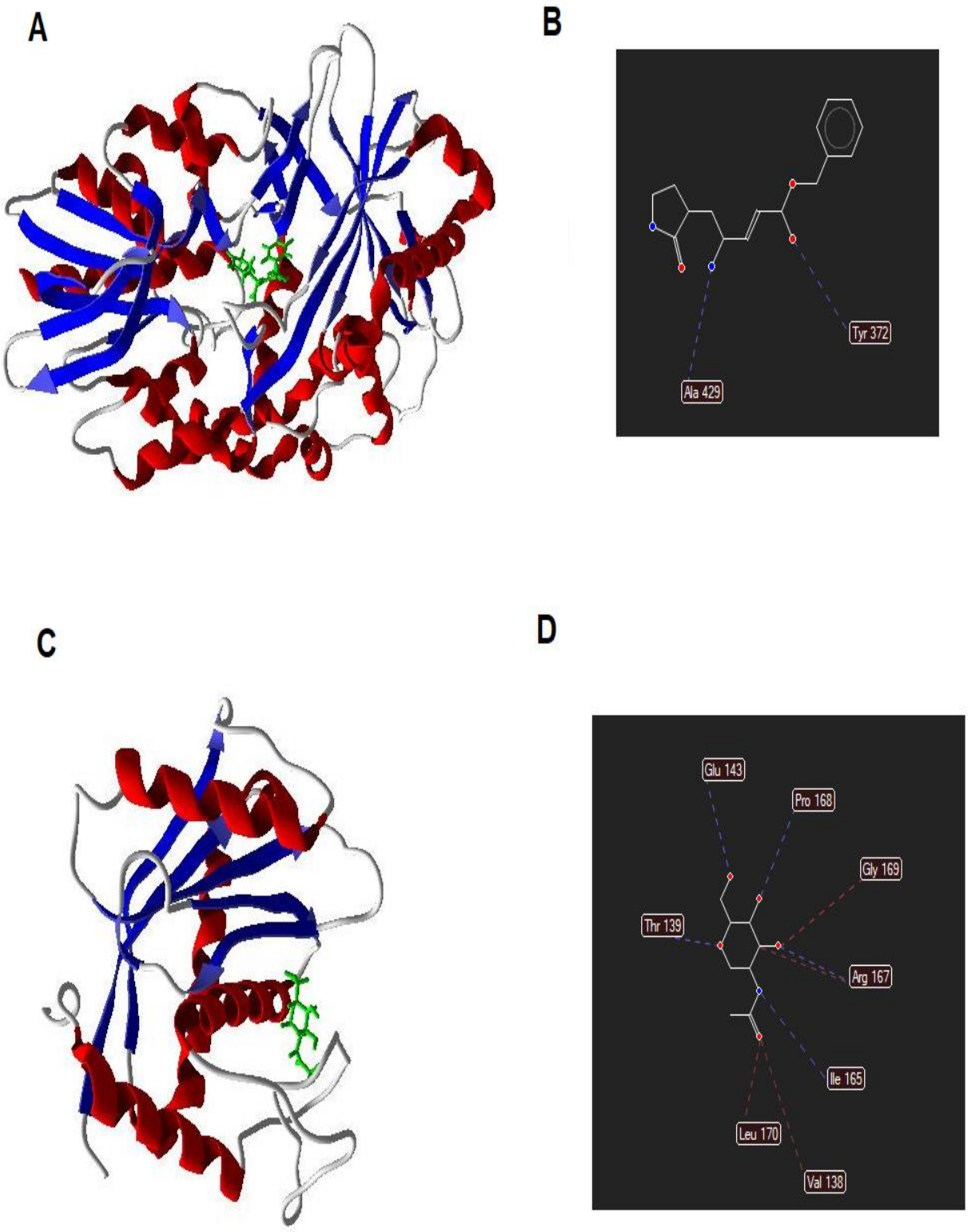

Figure.4. (A) Molecular docking of ACE2 and L-amino acid oxidase and (B) interactions with critical residues. (C) Molecular docking of ACE2 and Adamalysin II and (D) interactions with critical residues (Red dashes show steric interactions, and blue dashes show hydrogen bonds)

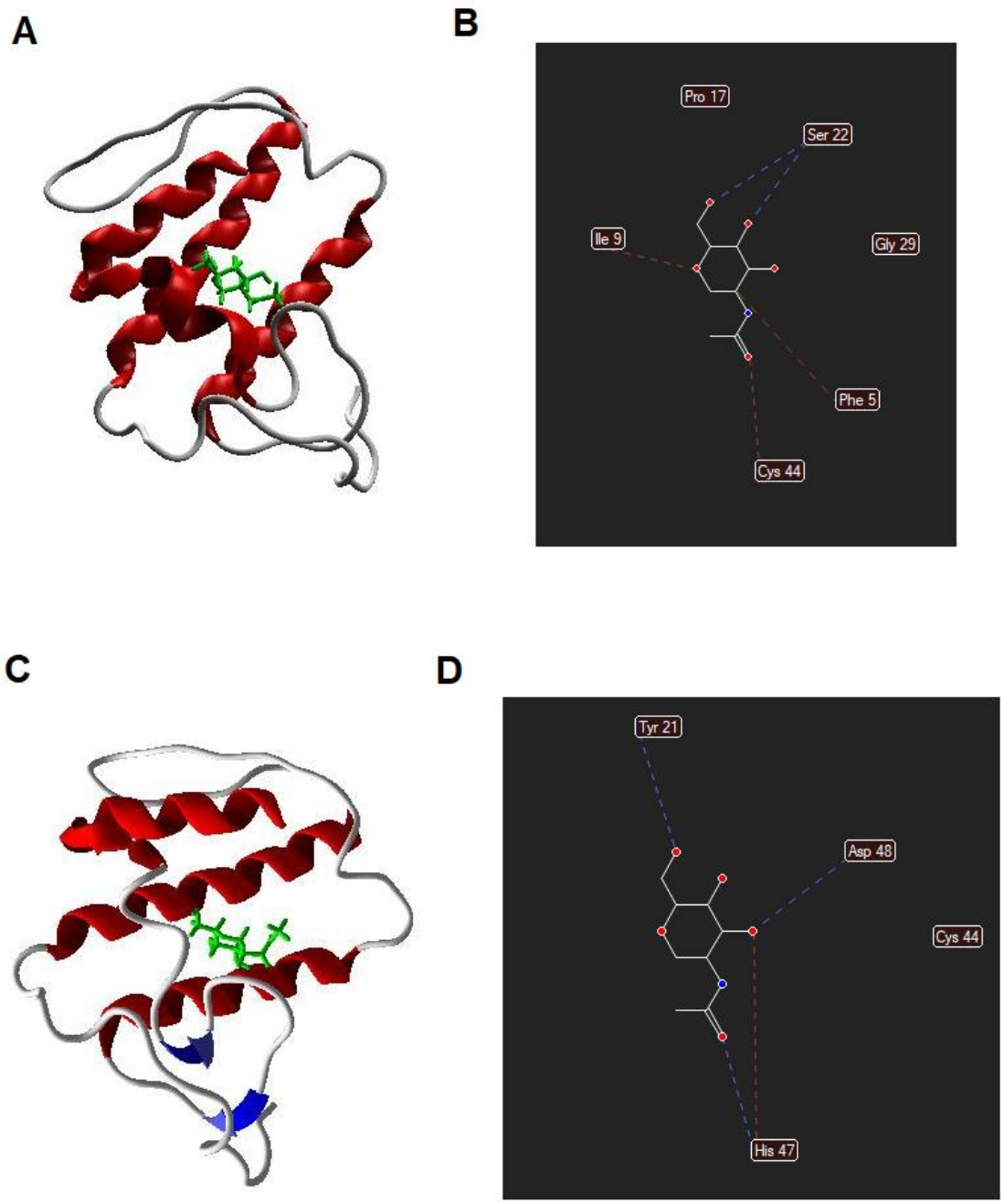

Figure.5. (A) Molecular docking of ACE2 and Ammodytin L and (B) interactions with critical residues. (C) Molecular docking of ACE2 and Ammodytoxin A and (D) interactions with critical residues (Red dashes show steric interactions, and blue dashes show hydrogen bonds)

CONCLUSION

Interspecies comparison of other snake venom enzymes showed that the toxic sites were not the same in all subsets of presynaptically active toxins (Križaj et al., 1991). Therefore, the activities of similar toxin groups in different species can be very different from each other. In this study, the potential anti-COVID activities of the elucidated proteins in the VA venom were investigated by *in silico* methods. As a result, it was revealed that the investigated VA venom proteins could be potentially active against COVID-19 by interacting with both 3CLpro and ACE2. Snake venoms may offer a valuable source of therapeutic compounds in managing the pandemic. However, more studies are needed to validate these compounds *in vitro* and *in vivo*.

Conflict of Interest

The article author declares that there is no conflict of interest between them.

Author's Contributions

The author declares that contributed solely to the article.

REFERENCES

Ahmad, I., Pawara, R., Surana, S., & Patel, H. (2021). The Repurposed ACE2 Inhibitors: SARS-CoV-2 Entry Blockers of Covid-19. In *Topics in Current Chemistry* (Vol. 379, Issue 6). https://doi.org/10.1007/s41061-021-00353-7

Borkow, G., & Ovadia, M. (1999). Selective lysis of virus-infected cells by cobra snake cytotoxins: A Sendai virus, human erythrocytes, and cytotoxin model. *Biochemical and Biophysical Research Communications*, *264*(1). https://doi.org/10.1006/bbrc.1999.1483

El-Aziz, T. M. A., Soares, A. G., & Stockand, J. D. (2019). Snake venoms in drug discovery: Valuable therapeutic tools for life-saving. In *Toxins* (Vol. 11, Issue 10). https://doi.org/10.3390/toxins11100564

Feng, T., Tong, H., Ming, Z., Deng, L., Liu, J., Wu, J., Chen, Z., Yan, Y., & Dai, J. (2022). Matrix metalloproteinase 3 restricts viral infection by enhancing host antiviral immunity. *Antiviral Research*, *206*, 105388. https://doi.org/10.1016/J.ANTIVIRAL.2022.105388

Göçmen, B., Heiss, P., Petras, D., Nalbantsoy, A., & Süssmuth, R. D. (2015). Mass spectrometry guided venom profiling and bioactivity screening of the Anatolian Meadow Viper, Vipera anatolica. *Toxicon*, *107*. https://doi.org/10.1016/j.toxicon.2015.09.013

Gomis-Ruth, F. X., Kress, L. F., & Bode, W. (1993). First structure of a snake venom metalloproteinase: A prototype for matrix metalloproteinases/collagenases. *EMBO Journal*, *12*(11). https://doi.org/10.1002/j.1460-2075.1993.tb06099.x

Gopcevic, K., Karadzic, I., Izrael-Zivkovic, L., Medic, A., Isakovic, A., Popović, M., Kekic, D., Stanojkovic, T., Hozic, A., & Cindric, M. (2021). Study of the venom proteome of Vipera ammodytes ammodytes (Linnaeus, 1758): A qualitative overview, biochemical and biological profiling. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *37*, 100776. https://doi.org/10.1016/j.cbd.2020.100776

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, X., You, T., Liu, X., … Yang, H. (2020). Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature*, *582*(7811). https://doi.org/10.1038/s41586-020-2223-y

Joshi, T., Joshi, T., Sharma, P., Mathpal, S., Pundir, H., Bhatt, V., & Chandra, S. (2020). In silico screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking. *European Review for Medical and Pharmacological Sciences*, *24*(8). https://doi.org/10.26355/eurrev_202004_21036

Karabuva, S., Lukšić, B., Brizić, I., Latinović, Z., Leonardi, A., & Križaj, I. (2017). Ammodytin L is the main cardiotoxic component of the Vipera ammodytes ammodytes venom. *Toxicon*, *139*. https://doi.org/10.1016/j.toxicon.2017.10.003

Kasai, K., Nakano, M., Ohishi, M., Nakamura, T., & Miura, T. (2021). Antimicrobial properties of L-amino acid oxidase: biochemical features and biomedical applications. In *Applied Microbiology and Biotechnology* (Vol. 105, Issue 12). https://doi.org/10.1007/s00253-021-11381-0

Križaj, I., Bieber, A. L., Ritonja, A., & Gubenšek, F. (1991). The primary structure of ammodytin L, a myotoxic phospholipase A2 homologue from Vipera ammodytes venom. *European Journal of Biochemistry*, *202*(3). https://doi.org/10.1111/j.1432-1033.1991.tb16485.x

Latinović, Z., Leonardi, A., Šribar, J., Sajevic, T., Žužek, M. C., Frangež, R., Halassy, B., Trampuš-Bakija, A., Pungerčar, J., & Križaj, I. (2016). Venomics of Vipera berus berus to explain differences in pathology elicited by Vipera ammodytes ammodytes envenomation: Therapeutic implications. *Journal of Proteomics*, *146*. https://doi.org/10.1016/j.jprot.2016.06.020

Logonder, U., Križaj, I., Rowan, E. G., & Harris, J. B. (2008). Neurotoxicity of ammodytoxin A in the envenoming bites of Vipera ammodytes ammodytes. *Journal of Neuropathology and Experimental Neurology*, *67*(10). https://doi.org/10.1097/NEN.0b013e318188c2d7

Meenakshisundaram, R., Sweni, S., & Thirumalaikolundusubramanian, P. (2009). Hypothesis of snake and insect venoms against Human Immunodeficiency Virus: A review. In *AIDS Research and Therapy* (Vol. 6). https://doi.org/10.1186/1742-6405-6-25

Nalbantsoy, A., Hempel, B.-F., Petras, D., Heiss, P., Göçmen, B., Iğci, N., Yildiz, M. Z., & Süssmuth, R. D. (2017). Combined venom profiling and cytotoxicity screening of the Radde's mountain viper (Montivipera raddei) and Mount Bulgar Viper (Montivipera bulgardaghica) with potent cytotoxicity against human A549 lung carcinoma cells. *Toxicon*, *135*, 71–83. https://doi.org/10.1016/j.toxicon.2017.06.008

Pinzi, L., & Rastelli, G. (2019). Molecular docking: Shifting paradigms in drug discovery. In *International Journal of Molecular Sciences* (Vol. 20, Issue 18). https://doi.org/10.3390/ijms20184331

Pražnikar, Z. J., Petan, T., & Pungerčar, J. (2009). A neurotoxic secretory phospholipase A2 induces apoptosis in motoneuron-like cells. *Annals of the New York Academy of Sciences*, *1152*. https://doi.org/10.1111/j.1749-6632.2008.03999.x

Salmaso, V., & Moro, S. (2018). Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: An overview. In *Frontiers in Pharmacology* (Vol. 9, Issue AUG). https://doi.org/10.3389/fphar.2018.00923

Sant'Ana, C. D., Menaldo, D. L., Costa, T. R., Godoy, H., Muller, V. D. M., Aquino, V. H., Albuquerque, S., Sampaio, S. V., Monteiro, M. C., Stábeli, R. G., & Soares, A. M. (2008). Antiviral and antiparasite properties of an l-amino acid oxidase from the Snake Bothrops jararaca: Cloning and identification of a complete cDNA sequence. *Biochemical Pharmacology*, *76*(2). https://doi.org/10.1016/j.bcp.2008.05.003

Sheahan, T. P., Sims, A. C., Zhou, S., Graham, R. L., Pruijssers, A. J., Agostini, M. L., Leist, S. R., Schafer, A., Dinnon, K. H., Stevens, L. J., Chappell, J. D., Lu, X., Hughes, T. M., George, A. S., Hill, C. S., Montgomery, S. A., Brown, A. J., Bluemling, G. R., Natchus, M. G., … Baric, R. S. (2020). An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. *Science Translational Medicine*, *12*(541). https://doi.org/10.1126/SCITRANSLMED.ABB5883

Shimizu, J. F., Pereira, C. M., Bittar, C., Batista, M. N., Campos, G. R. F., Da Silva, S., Cintra, A. C. O., Zothner, C., Harris, M., Sampaio, S. V., Aquino, V. H., Rahal, P., & Jardim, A. C. G. (2017). Multiple effects of toxins isolated from Crotalus durissus terrificus on the hepatitis C virus life cycle. *PLoS ONE*, *12*(11). https://doi.org/10.1371/journal.pone.0187857

Simon, L., Imane, A., Srinivasan, K. K., Pathak, L., & Daoud, I. (2017). In Silico Drug-Designing Studies on Flavanoids as Anticolon Cancer Agents: Pharmacophore Mapping, Molecular Docking, and Monte Carlo Method-Based QSAR Modeling. *Interdisciplinary Sciences – Computational Life Sciences*, *9*(3). https://doi.org/10.1007/s12539-016-0169-4

Siniavin, A. E., Streltsova, M. A., Nikiforova, M. A., Kudryavtsev, D. S., Grinkina, S. D., Gushchin, V. A., Mozhaeva, V. A., Starkov, V. G., Osipov, A. V., Lummis, S. C. R., Tsetlin, V. I., & Utkin, Y. N. (2021). Snake venom phospholipase A2s exhibit strong virucidal activity against SARS-CoV-2 and inhibit the viral spike glycoprotein interaction with ACE2. *Cellular and Molecular Life Sciences*, *78*(23). https://doi.org/10.1007/s00018-021-03985-6

Waheed, H., Moin, S. F., & Choudhary, M. I. (2017). Snake Venom: From Deadly Toxins to Life-saving Therapeutics. *Current Medicinal Chemistry*, *24*(17). https://doi.org/10.2174/0929867324666170605091546

Wei, J. F., Yang, H. W., Wei, X. L., Qiao, L. Y., Wang, W. Y., & He, S. H. (2009). Purification, characterization and biological activities of the l-amino acid oxidase from Bungarus fasciatus snake venom. *Toxicon*, *54*(3). https://doi.org/10.1016/j.toxicon.2009.04.017

Zumla, A., Chan, J. F. W., Azhar, E. I., Hui, D. S. C., & Yuen, K. Y. (2016). Coronaviruses-drug discovery and therapeutic options. In *Nature Reviews Drug Discovery* (Vol. 15, Issue 5). https://doi.org/10.1038/nrd.2015.37