Research Article

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Venom Peptides of *Echis carinatus* against SARS-CoV-2: Effective Inhibition of Human ACE2 and Mpro

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ABSTRACT

The search for effective inhibitors against SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has led to re-screening of existing potential molecules. Molecular docking and virtual screening techniques have been employed to identify potential drug candidates. Natural products, known for their wide variety and reduced toxicity, have gained significant attention in these screenings. Snake venom proteins, characterized by their diverse biological activities and unique molecular structures, offer a promising avenue for the discovery of new antiviral molecules. In this study, we focused on the investigation of snake venom proteins isolated from *Echis carinatus*, specifically Schistatin (SCH), Phospholipase A2 (PLA2), Disintegrin (DS), and Echistatin (ECH) for their potential as inhibitors against SARS-CoV-2. Through molecular docking analysis, the binding interactions between these venom proteins and key SARS-CoV-2 targets, the main protease (Mpro), and the ACE2 receptor were examined. Results revealed that PLA2 exhibited the most favorable binding affinity to both Mpro and ACE2, surpassing the reference drug ritonavir (RTV). SCH, DS, and ECH also demonstrated promising binding affinities with both targets. This study sheds light on the unexplored potential of snake venom proteins, specifically PLA2, SCH, DS, and ECH from *E. carinatus* venom, as inhibitors against SARS-CoV-2. The exploration of snake venom proteins presents an intriguing avenue for the discovery of novel drug candidates with broad applications in the treatment of various diseases, including viral infections such as COVID-19.

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Introduction

Echis carinatus, commonly known as the Indian saw-scaled viper, is a venomous snake species found in various parts of the Indian subcontinent, Middle East, and Central Asia. It is one of several species in the genus , which includes several small to medium-sized venomous snake species from the Viperidae family [1]. Adult *E. carinatus* typically range in length from 40 to 70 centimeters, although some individuals can grow up to 90 centimeters. They have a slender body with a distinctive triangular-shaped head, covered in small scales. The scales on their back possess keels, which give them a rough or "saw-

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like" appearance. They inhabit a variety of ecosystems, including grasslands, scrublands, rocky areas, and semi-desert regions. They also can adapt to different altitudes, ranging from sea level to elevations of around 2,500 [2].

Like other vipers, *E. carinatus* possesses venom that is primarily hemotoxic, meaning it affects the blood and circulatory system [3]. The venom contains a mixture of enzymes, peptides, and other compounds that disrupt blood clotting, cause tissue damage, and can lead to systemic effects in envenomed individuals. It contains several enzymes such as metalloproteinases, phospholipases, and serine proteases, which play a crucial role in the venom's effects on the human body. While many biological activities of snake venom, including anti-viral, have been demonstrated, its potential SARS-CoV-2 inhibitory effects have not been studied [4]–[6].

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has had a significant global impact since its emergence in late 2019. Globally, there have been 767.750.853 confirmed cases of COVID-19, including 6.941.095 deaths, reported to the World Health Organization (WHO) to date [7].

Angiotensin-converting enzyme 2 (ACE2) serves as the entry receptor for the SARS-CoV-2 virus, allowing it to enter and infect human cells. The spike protein on the surface of SARS-CoV-2 binds to ACE2 receptors on host cells, facilitating viral entry, particularly in the respiratory system [8]. ACE2 is widely expressed in various tissues, including the respiratory tract, lungs, heart, kidneys, gastrointestinal tract, and blood vessels. Its presence in the respiratory epithelium makes the lungs a primary target for SARS-CoV-2 infection. Considering the crucial role of ACE2 in the progression of COVID-19, researchers have been actively investigating potential therapeutic strategies that specifically target ACE2 [9]. These strategies encompass various approaches, such as modulating ACE2 expression, enhancing its activity, or developing molecules that can compete with SARS-CoV-2 for binding to ACE2 receptors.

The 3-chymotrypsin-like protease (Mpro), also known as the main protease, is a cysteine protease that plays a vital role in the replication of coronaviruses, including SARS-CoV-2. It plays a crucial role in processing viral replicase polyproteins into functional non-structural proteins (NSPs), which are essential for viral replication and transcription [10]. 3CLpro has been identified as a promising target for the development of antiviral drugs against coronaviruses. Inhibiting 3CLpro activity can disrupt viral replication and

potentially halt the spread of the virus. Researchers have been actively studying the structure and function of 3CLpro to identify potential inhibitors. Structure-based drug design approaches, such as virtual screening and molecular docking, have been utilized to identify small molecules that can bind to and inhibit 3CLpro activity [11].

Molecular docking is considered a valuable tool in the early stages of drug discovery, as it provides information about the potential binding modes of small molecules with the target protein. It assists researchers in the selection and optimization of drug candidates, guiding further development and experimental testing to identify potential therapeutics for a variety of diseases [9].

In the current study, the research focused on examining the interactions between venom peptides derived from *E. carinatus* (ECVPs), namely Schistatin (SCH), Phospholipase A2 (PLA2), Disintegrin (DS), and Echistatin (ECH), with ACE2 and Mpro receptors. The objective was to investigate the potential of these peptides as anti-viral molecules targeting SARS-CoV-2.

Material and Methods

Receptor preparation

The receptor proteins chosen for this study were the SARS-CoV-2 main protease 3CLpro with the PDB ID 6LU7 and ACE2 with the PDB ID 1R42. The three-dimensional (3D) structures of Mpro and ACE2 were downloaded from the Protein Data Bank (PDB) using the PDB format (https://www.rcsb.org/). Preprocessing steps, including load distribution, hydrogenation, and removal of water molecules, were performed using the PyMOL software. Hydrogen atoms were added to the receptor molecule using the MG Tools plugin in the AutoDock Vina program. The final structures were saved in PDB format for further analysis.

Ligand preparation

The three-dimensional structures of the identified snake venom proteins, including SCH (PDB ID: 1RMR), PLA2 (PDB ID: 1OZ6), DS (PDB ID: 1Z1X), and ECH (PDB ID: 2ECH)

were retrieved in PDB format from PubChem. The ligand structures underwent water removal, hydrogenation, and load distribution adjustments using AutoDock Vina 4.2.5.1 software.

Molecular docking

AutoDock Vina was employed for high-throughput molecular docking. The grid center for Mpro was set at X=21.41, Y=3.62, and Z=21.94, with a grid box size of 60 Å×60 Å×60 Å. For ACE2, the grid center was set at X=19.81, Y=-5.57, and Z=14.73, with the same grid box size. These parameters were used for all 4 proteins. Multiple docked conformations were generated, and the setup was optimized and calibrated accordingly. The secondary structures of the docked molecules were visualized using PyMOL for further analysis.

Results

Docking of Mpro protease with ECVPs

Molecular docking scores of SCH, PLA2, ECH, and DS with Mpro were- 92.998, -116.513, -27.197 and -81.601 Kcal/mol, respectively (Table 1). Among ECVPs, PLA2 showed the best binding affinity (-116.513 Kcal/mol) with Mpro as compared to RTV with the docking score of-73.550 Kcal/mol. SCH and DS showed similar binding affinities with Mpro with docking scores of-92.998 and-81.601 Kcal/mol, respectively. The lowest docking score was ECH with-27.197 Kcal/mol (Table 1).

PLA2 also formed hydrogen bonds with His48, and Asp49, and steric interactions with Leu106, Tyr52, and Asp49 (Figures 1A and B). SCH formed hydrogen bonds with Gly43, Asn46, and steric interactions with Ala41, Asp44, Asn46, Asp48 residues while interacting with MPro (Figures 2A and B). DS formed steric interactions with Arg59, Asp48, Lys39, Ala41, Leu46 residues (Figures 3A and B). ECH formed only hydrogen bonds with Arg24, Ala23, Arg22, Asp29, and steric interactions with Arg22 and Arg24 with MPro (Figures 4A and B).

Docking of ACE2 receptor with ECVPs

Molecular docking scores of SCH, PLA2, ECH, and DS with ACE2 were -58.260, -78.067, -39.800 and -61.540 Kcal/mol, respectively (Table 1). PLA2 showed a higher docking score (-78.067 Kcal/mol) than RTV (-108.731 Kcal/mol), indicating a better binding than the reference drug. DS also showed a similar binding energy with RTV with a docking score of -61.540 Kcal/mol. Although the binding energies of SCH and ECH were lower than the reference drug, they were favorable and were -58.260 and -39.800 Kcal/mol, respectively.

Table 1 Molecular docking analysis of the interaction of ECVPs with ACE2 and MPro.

	ACE2			Mpro		
	Docking Score (Kcal/mol)	**Honda**	**Steric interactions**	**Docking Score (Kcal/mol)**	**Hbonds**	**Steric interactions**
Schistatin (SCH)	-92.998	Gly43, Asn46	Ala41, Asp44, Asn46, Asp48	-58.260	Lys39, Asp48, Asn46, Asn63	Gln47, Ala41, Arg59
Phospholipase A2 (PLA2)	-116.513	His48, Asp49	Leu106, Tyr52, Asp49	-78.067	Gly30, Cys29	His48, Cys45, Cys44, Cys29
Echistatin (ECH)	-27.197	Arg24, Ala23, Arg22, Asp29	Arg22, Arg24	-39.800	Arg22, Arg24, Asp29	Arg24, Ala23
Disintegrin (DS)	-81.601	-	Arg59, Asp48, Lys39, Ala41, Leu46	-61.540	Lys39, Leu46, Asp48, Asn63	Ala41, His47
Ritonavir (RTV)	-73.550	Asn98, Ile3, Thr96, Pro1	-	-108.731	Gln2	Asn98, Ile3, Thr96,

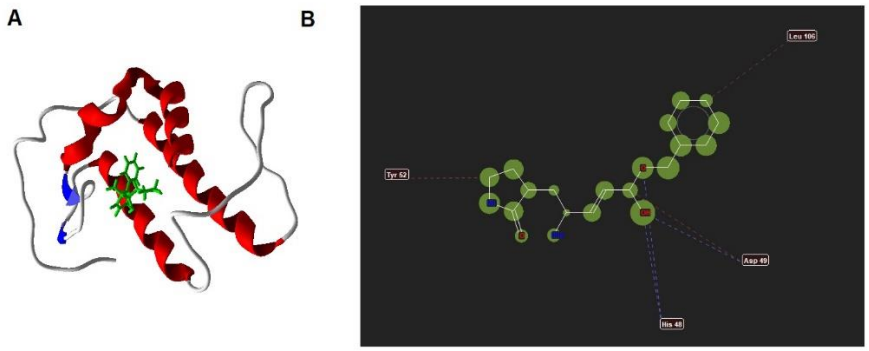

Fig 1 (A) 3D-Molecular docking images of PLA2 and MPro. (B) Amino acid residues with which PLA2 interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

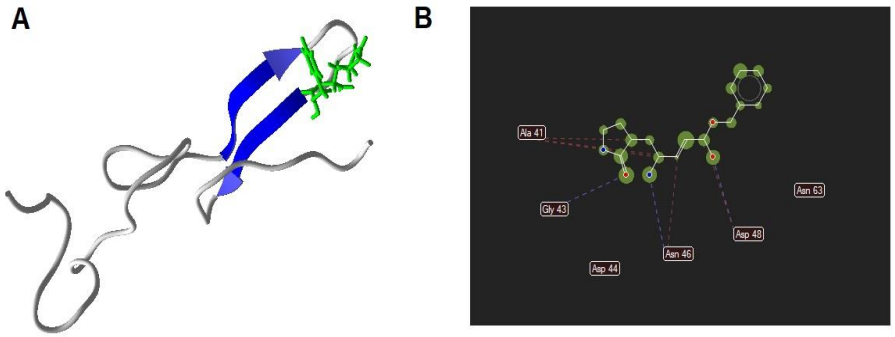

Fig 2 (A) 3D-Molecular docking images of SCH and MPro. (B) Amino acid residues with which SC interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

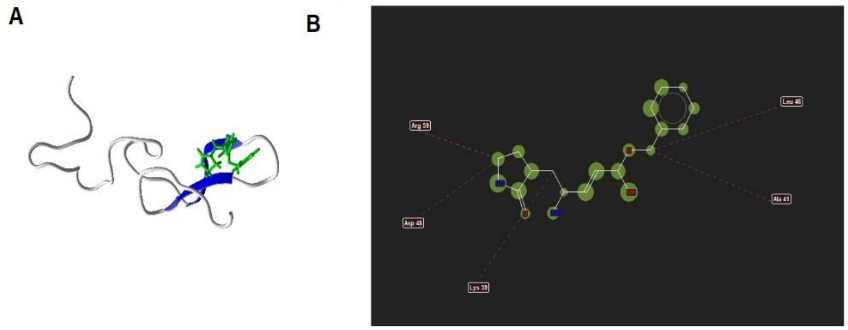

Fig 3 (A) 3D-Molecular docking images of DS and MPro. (B) Amino acid residues with which DS interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

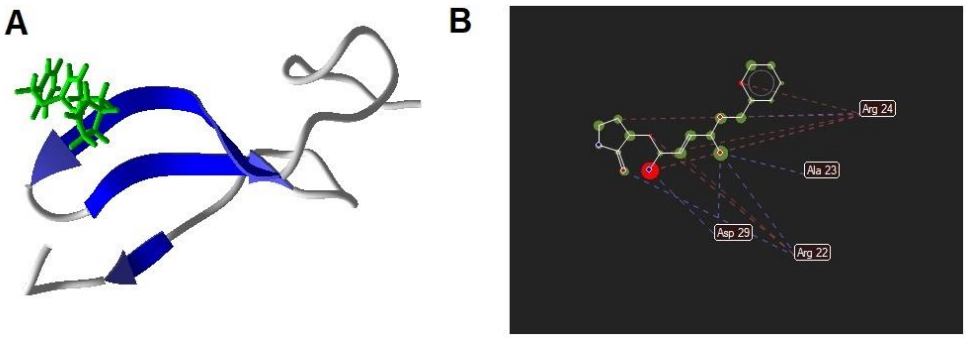

Fig 4 (A) 3D-Molecular docking images of ECH and MPro. (B) Amino acid residues with which ECH interacts with MPro. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

PLA2 formed hydrogen bonds with Gly30, and Cys29 residues and steric interactions with His48, Cys45, Cys44, Cys29 residues while interacting with ACE2 (Figures 5A and B). It was the molecule with the lowest binding energy due to its high steric interactions. DS formed hydrogen bonds with Lys39, Leu46, Asp48, and Asn63 residues and steric

interactions with Ala41 and His47residues (Figures 6A and B). The number of hydrogen bonds formed by SCH was high and these bonds were formed with Lys39, Asp48, Asn46, and Asn63 residues. SCH also formed steric interactions with Gln47, Ala41, and Arg59 residues (Figures 7A and B). ECH formed hydrogen bonds with Arg22, Arg24, Asp29 and steric interactions with Arg22, Arg24 and Asp29 residues while interacting with ACE2 (Figures 8A and B).

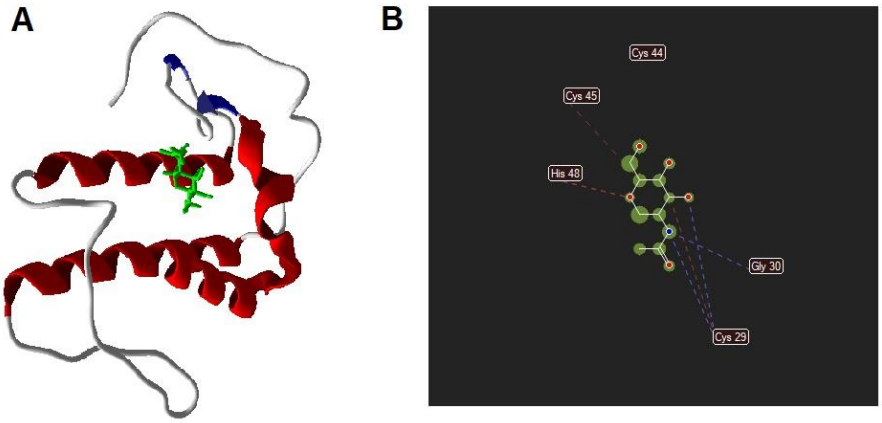

Fig 5 (A) 3D-Molecular docking images of PLA2 and ACE2. (B) Amino acid residues with which PLA2 interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

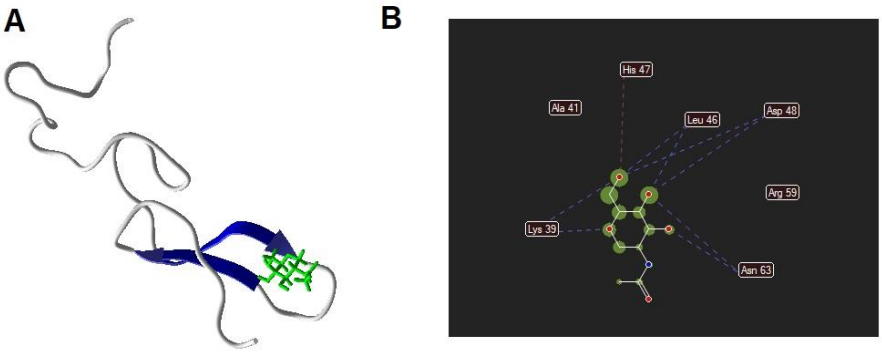

Fig 6 (A) 3D-Molecular docking images of DS and ACE2. (B) Amino acid residues with which DS interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

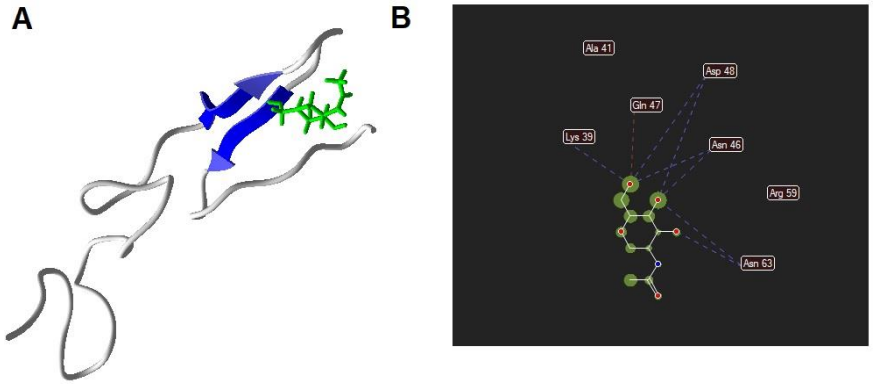

Fig 7 (A) 3D-Molecular docking images of SCH and ACE2. (B) Amino acid residues with which SCH interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

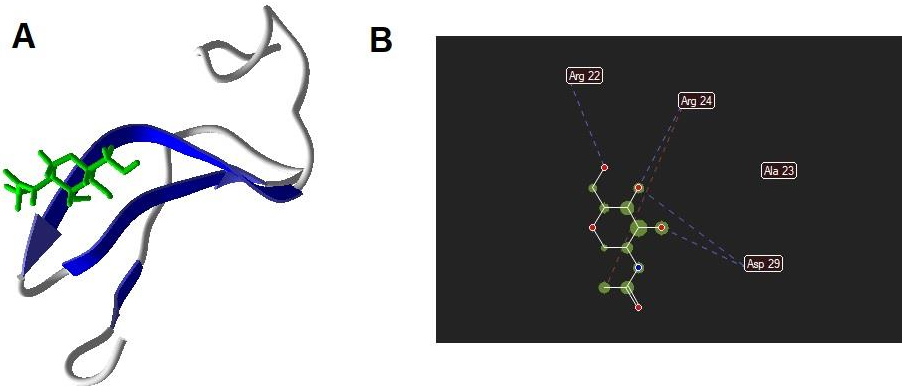

Fig 8 (A) 3D-Molecular docking images of ECH and ACE2. (B) Amino acid residues with which ECH interacts with ACE2. (Blue dashes indicate hydrogen bonds and red dashes indicate steric interactions)

Discussion

Many studies use molecular docking to screen large compound libraries and identify potential SARS-CoV-2 inhibitors [9]–[11]. These studies usually include the virtual screening of databases of small molecules, in which compounds with the best insertion scores and binding interactions are selected as potential inhibitors, and these detected compounds are then subjected to experimental assays to confirm their inhibitory activities.

The majority of these screenings are for natural products. Natural products are considered the best drug candidates because of their wide variety, abundance in nature, and less toxicity [12]. Snake venom proteins have gained attention as potential drug candidates due to their diverse biological activities and unique molecular structures. Several

components of snake venom have been investigated for their therapeutic potential in various areas of medicine, including pain management, cardiovascular disorders, cancer, and neurological conditions [13]–[15]. Tirofiban is an antiplatelet drug that received approval from the US Food and Drug Administration (FDA) in 1998 and from the European Medicines Agency (EMA) in 1999. Tirofiban is derived from echistatin, a disintegrin toxin found in *E. carinatus* venom, and is used in the treatment of acute coronary syndrome [16]. It blocks platelet aggregation via bonding with the fibrinogen receptor. Overall, snake venom proteins represent a fascinating source of bioactive molecules that offer potential therapeutic applications. Continued research and exploration in this field may uncover novel drug candidates and contribute to the development of new treatments for various diseases such as COVID-19.

The antiviral proteolytic fraction was isolated from the venom of *E. carinatus sochureki* and investigated for its activity against the Sendai virus [4]. However, the potential effects of ECVPs on SARS-Cov-2 have not been studied. In the current study, we used the molecular docking technique to determine the possible interactions between ECVPs and Mpro and also the ACE2 receptor. Among the tested ECVPs, PLA2 had the most negative value, indicating the best binding affinity with ACE2 than the reference drug RTV. PLA2 can be isolated from *E. carinatus* venom by chromatography and electrophoresis combination [17]. In the literature, PLA2s obtained from different snakes have been characterized and their antiviral activities have been demonstrated [4], [18]. However, the antiviral activity of PLA2 from *E. carinatus* has not been studied. In this study, it was determined that the PLA2 contained in *E. carinatus* may show better activity than the reference drug. SCH, ECH, and DS showed similar binding affinities with RTV. SCH is a disintegrin homodimer with 64 amino acids. Observations indicate that disintegrins, acting as antagonists, disrupt the functioning of integrins, thereby serving as agents with anticancer and antithrombotic properties. Some of the disintegrins obtained from snake venoms have been shown to have antiviral activity, but there is no study yet on SCH [19]. SCH showed -92.998 and -58.260 Kcal/mol binding scores with ACE2 and MPro, respectively, indicating a favorable interaction with both receptors. ECH is a 49 amino acid protein, a potent inhibitor of platelet aggregation [20]. There is no study in the literature investigating the antiviral activity of ECH. Thus, the present study was the first to investigate the potential anti-Sars-Cov2 activity of ECH.

Conclusion

The findings of this study highlight the potential of snake venom proteins, specifically PLA2 SCH, DS and ECH from *E. carinatus* venom, as promising candidates for the development of antiviral agents against SARS-CoV-2. Through molecular docking analysis, these venom proteins demonstrated favorable binding affinities with key targets involved in SARS-CoV-2 infection. Further experimental investigations are needed to validate their antiviral activities and evaluate their efficacy in combating COVID-19. The exploration of snake venom proteins as a source of bioactive molecules offers a fascinating avenue for the discovery of novel therapeutic options against viral infections and may contribute to the development of effective treatments for diseases such as COVID-19.

Abbreviations
SARS CoV 2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; SCH: Schistatin; PLA2: Phospholipase A2; DS: Disintegrin; ECH: Echistatin; Mpro: Main protease; RTV: Ritonavir; WHO: World Health Organization; ACE2: Angiotensin-converting enzyme 2; NSPs: Non-structural proteins; ECVPs: *Echis carinatus* venom peptides; 3D: three-dimensional; PDB: Protein Data Bank; FDA: US Food and Drug Administration; EMA: European Medicines Agency

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Data Availability statement
The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
All authors contributed equally to the study.

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