Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.42A, No.1,
January-June 2023: P.149-156

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: 10.48165/bpas.2023.42A.1.13

Original Research Article *Content Available online at: https://www.bpasjournals.com/zoology*

Screening of Spider Venom Peptides and Molecular Docking of FLT3 and LCK

Durgesh M. Agase

Author's Affiliation:
Assistant Professor, Department of Zoology, Govt Jatashankar Trivedi College, Balaghat, Madhya Pradesh 481101, India

***Corresponding author:**
Dr. Durgesh M. Agase,
Assistant Professor, Department of Zoology, Govt Jatashankar Trivedi College, Balaghat, Madhya Pradesh 481101, India
E-mail: sbt.durgesh@gmail.com

Article Info:
Received on 27.02.2023
Revised on 05.05.2023
Approved on 19.05.2023
Accepted on 26.05.2023
Published on 16.06.2023

ABSTRACT:
The drawbacks of traditional chemotherapy include its inability to dissolve in water, lack of selectivity, and multidrug resistance. The use of anticancer peptides is a unique therapeutic approach against cancer cells. In this In-silico work, the kinase inhibition activity for both chosen target molecules (Flt3 and Lck protein) was evaluated in order to find a possible anti-leukemic spider venom peptide. Out of the 11 spider venom peptides, Lycosin-I peptide (from *Lycosa singoriensis*) for Lck and Latarcin 2a peptide (from *Lachesana tarabaevi*) for Flt3 were suggested as the best lead peptides for the creation of anti-leukemic drugs.

Keywords: Anticancer Peptides, Chemotherapy, Spider Venom

How to cite this article: Agase D.M. (2023). Screening of Spider Venom Peptides and Molecular Docking of FLT3 and LCK. *Bulletin of Pure and Applied Sciences-Zoology*, 42A (1), 149-156.

INTRODUCTION

One of the main therapeutic modalities for the treatment of cancer, including leukaemia, is chemotherapy, which may be applied alone or in conjunction with other forms of therapy. However, water solubility, selectivity, and multidrug resistance are problems with traditional treatment. Chemotherapy for cancer damages quickly reproducing normal cells because it only targets non-specific targets (Wallace, Laskowski, & Thornton, 1995). In order to effectively treat slow-growing and dormant cells without triggering chemoresistance mechanisms, medicines that may selectively kill cancer cells are therefore in considerable demand (Bhujbal, Keretsu, & Cho, 2023). The use of anticancer peptides is, an unique therapeutic approach against cancer cells (ACPs). As an alternative to traditional chemotherapy, the use of ACPs that can specifically target cancer cells without harming healthy cells (targeted therapy) is developing. ACPs can operate as carriers of cytotoxic drugs and radioisotopes by precisely targeting cancer cells, or they can be used directly as cytotoxic agents through a variety of ways (Cardoso & Lewis, 2019). In the current study, peptides from spider venom were investigated to determine their therapeutic potential against acute leukaemia. The complex cocktail of substances that make up spider venoms includes proteins, peptides, salts, and tiny chemical molecules (Chidambaram et al., 1970). They are a remarkable source of bioactive disulfide-rich peptides, which are frequently highly specific and affine for particular subtypes

of channels and receptor proteins (Davletov, Ferrari, & Ushkaryov, 2012), (Dubovskii et al., 2015). The majority of these peptides have an unusual configuration of disulfide links that gives them a high level of protease resistance (Glenn & Margaret, 2013). Spider-venom peptides are useful as pharmacological instruments and as drug development leads due to their distinct bioactivity and stability (He et al., 2013). These peptides display exceptional pharmacological characteristics.

Different spider venoms have produced a number of neurotoxic, cytotoxic, antimicrobial, anti-insecticidal, anti-arrhythmic, and antiparasitic peptides, which have been identified and described (Isbister & Gray, 2002) (King & Hardy, 2013) (Klint, Senff, & Rupasinghe, 2012) (Krissinel & Henrick, 2007) (Kuzmenkov et al., 2016) Some of the spider peptide toxins control the cell cycle, activate the caspase pathway, or deactivate mitochondria in tumor cells to cause deadly consequences (Mashkani et al., 2016) . Spider venom peptides have recently received substantial studies using proteome and transcriptome methods and have been widely applied for innovative medicinal purposes (Maupetit, Derreumaux, & Tuffery, 2009) (Nimmrich & Gross, 2012).

The current work emphasizes the *in silico* screening of spider venom peptides and their prospective therapeutic targets for Computer-Aided Drug Designing (CADD) for leukaemia in this context. The Flt3 protein and the Lck protein were the two target protein molecules used in the current investigation for molecular docking. Flt3 (Fms-like tyrosine kinase 3) is a type III receptor tyrosine kinase that is crucial for the survival, growth, and differentiation of hematopoietic cells. Similar to Flt3, Lck is a tyrosine kinase that catalysis the first phosphorylation of T-cell receptor components required for signal transmission and T-cell activation. However, unlike Flt3, Lck is a non-receptor tyrosine kinase. Interestingly, the Pax5-repressed target gene Lck works as an inducer of Stat5, and it may play a role in a variety of haematological malignancies.

MATERIALS AND METHODS

AntiCP and In silico screening of anticancer peptides: In order to identify effective antileukemic spider venom peptides, a thorough literature review, a systematic search in the SwissProt database, and the download of spider venom peptide amino acid sequences in FASTA format were all completed. Spider venom peptide in silico screening was done using the AntiCP web server. AntiCP is a web-based anticancer peptide prediction server. The core dataset, which includes experimentally confirmed anticancer peptides and random peptides generated from the SwissProt database, is based on amino acid composition and binary profile features as input features (Pineda et al., 2014).

PEP-FOLD3 and Prediction of Peptide Structure: Using the PEP-FOLD3 web service, the structure of the spider peptide was predicted. A de novo method called PEPFOLD3 is used to forecast peptide structures from amino acid sequences. In a matter of minutes, PEP-FOLD3 can produce models for new peptides with 5 to 50 amino acids (Rash & Hodgson, 2009)

Protein Data Bank (PDB) and Target Molecules: From the Protein Data Bank, the 3D structure of the identified medication (Flt3 and Lck protein) was retrieved (PDB). A database for the three-dimensional structural information of big biological molecules like proteins and nucleic acids is called the PDB. The information, which is frequently, collected using X-ray crystallography, NMR spectroscopy, or increasingly, cryo-electron microscopy.

HADDOCK, GalaxyPepDock, and Molecular Docking: Using HADDOCK and the GalaxyPepDock server, molecular docking was carried out following the prediction of peptide structure. The information-driven flexible docking server HADDOCK (High Ambiguity Driven Protein-Protein DOCKing) is used to model biomolecular complexes. It supports a number of different experimental data, such as NMR residual dipolar couplings, pseudo-contact shifts, and cryo-EM maps, and it enables the definition of precise, unambiguous distance

restrictions. Protein-protein, protein-nucleic acid, and protein-ligand complexes, including multi-bodies (N>2) assemblies, are among the many modelling issues that HADDOCK can handle. Similarly, the By selecting templates from a library of experimentally determined structures and creating models using energy-based optimization, the GalaxyPepDock web server, which is publicly available at http://galaxy.seoklab.org/pepdock, does similarity-based docking. This provides for structural flexibility. As a result, the server can successfully simulate the structural variations between the template and target protein-peptide complexes (Lee et al., 2015).

LIGPLOT, PDB ePISA and Analysis of Protein-Peptide Interaction: LIGPLOT and PDB ePISA (Protein interfaces, surfaces, and assemblies) Interface were used to investigate intermolecular interaction and strengths, including H-bonds, hydrophobic contacts, salt bridges, and atomic accessibilities between protein and venom peptide. For a given PDB file, LIGPLOT automatically creates schematic 2-D diagrams of protein-ligand interactions (Saez et al., 2010) (Saidijam et al., 2018).

RESULTS AND DISCUSSION

Eleven spider venom peptides were discovered to be related to anti-cancerous activities after a thorough literature review and AntiCP analysis. Table 1 displays specifics of the peptide in spider venom. Using the PEP-FOLD3 server and the peptide's primary amino acid sequence, the structures of eleven spider venom peptides were predicted. Flt3 (PDB ID: 1RJB) and Lck (PDB ID: 2PL0) protein three-dimensional structures were retrieved in PDB file format from the PDB database, and inhibitors were eliminated using PyMOL programme. Eleven peptides from spider venom were molecularly docked with the target proteins Flt3 and Lck. Tables 2 and 3 present the docking results for the two target molecules. Figs. 1 and 2 display the predicted Molecular Docking accuracy. Figures 3 and 4 display the docking outputs (Lycosin-I and Lck, Latarcin 2a, and FLT3). Examining protein-peptide interactions two spider venom peptides Latarcin 2a for FLT3 and Lycosin-I for Lck—were suggested to be the most effective inhibitors based on highly estimated docking accuracy.

The kinase inhibitory activity for both of the chosen target targets (Flt3 and Lck protein) was evaluated in an effort to find a possible anti-leukemic spider venom peptide. During the current work, 11 anti-cancerous spider venom peptide compounds were discovered using in silico screening. These peptide compounds were tested for protein-peptide interactions on Flt3 and Lck proteins. To assess the binding affinity and mechanism of binding with these molecules in the Flt3 and Lck, we used 11 spider venom peptides as ligands for molecular docking with proteins in the current work. Latarcin 2a (Ltc2a) peptide for Flt3 and Lycosin-I peptide for Lck were chosen from among 11 spider venom peptides based on their docking scores.

Ltc2a exhibits interaction with the binding pocket of Flt3 protein according to analysis of the binding site interaction with binding site amino acid residue. With the various amino acid residues, we discovered eight hydrogen bonds and three salt bridge interactions. The phosphorylation of Tyr-222, a highly conserved tyrosine residue, controls the function of Flt3. This Tyr-222 residue in Ltc2a interacted with Arg-22 in the current study. Between the Arg-22 of Ltc2a and Try-222 of Flt3, two hydrogen bonds were created.

All of the molecules taken into consideration in this study exhibit strong binding energies with the appropriate binding mode at the binding site, as demonstrated by the role of Flt3 in cell-singling binding site analysis of the protein-ligand complex. In 2011, Vorontsova et al. reported on the Ltc2a's activity and mode of action. A peptide antibacterial found in the venom of the spider *Lachesana tarabaevi* (Family: Zodariidae) is what it is. The primary mechanism underlying Ltc2a's cytotoxicity for erythroleukemia K562 cells (EC (50) = 3.3 M) has been identified as plasma membrane instability. K562 cells' membranes blebbed and swelled as a result of Ltc2a, and then the cells died. A key factor in the peptide's stimulation of cytotoxicity in K562 cells is the creation of tiny pores on the cell membrane during the growth of blebs. Small membrane pores are created by the

internalization of Ltc2a, which causes the mitochondria to become inactive and the externalization of phosphatidylserine (PS) (Shen et al., 2018) (Thundimadathil, 2012) (Ting et al., 2019). Small molecule kinase inhibitors' possible therapeutic target is the protein Flt3. Mashkani et al, revealed that homology modelling and molecular docking were used to determine the protein-ligand interactions between Flt3 and kinase inhibitors (CEP701, PKC412, sunitinib, imatinib, and dasatinib) (Tyagi et al., 2013). As FLT3 kinase antagonists, Bhujbal et al. presented docking experiments on a number of diaminopyrimidine derivatives (Tyagi et al., 2013). The docking analysis identified crucial active site residues involved in FLT3 kinase inhibition. There have been numerous investigations on the protein-ligand interactions between Flt3 and kinase inhibitors published by various researchers, but in the current study, Flt3 is inhibited by spider venom peptide. Lycosin-I exhibits good contact with the binding pocket of Lck protein according to an analysis of the binding site interaction with the binding site amino acid residue. With the various amino acid residues, we discovered four hydrogen bonds and three salt bridge interactions. *Acinetobacter baumannii* MDR infection may benefit from the development of an antibiotic called Lycosin-I, a peptide found in the venom of the spider *Lycosa singoriensis* (Vorontsova et al., 2011).

Table 1: *In silico* screening of Anticancer Peptides (Properties of Spider Venom Peptides)

Peptide ID	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	MW
Lycosin-I	0.82	Anticp	-0.24	-0.58	0.46	6.5	3002.1
Lycosin-II	0.52	Anticp	-0.04	0.26	-0.34	5	2531.49
Psalmotoxin	0.75	Anticp	-0.33	-0.91	0.52	3.5	4696.04
Gomesin	0.83	Anticp	-0.52	-0.87	0.33	6	2321.04
Brachyin	0.75	Anticp	-0.27	-0.97	0.04	1	4909.09
Raventoxin-I	0.75	Anticp	-0.22	-0.67	0.07	3.5	4849.15
Raventoxin-II	0.64	Anticp	-0.17	-0.15	-0.07	5	3293.46
Latrotoxin alpha	0.74	Anticp	-0.22	-0.72	0.48	-7.5	4091.97
Latarcin 2a	0.81	Anticp	-0.29	-0.62	0.46	9.5	2732.72
Lycotoxins-I	0.65	Anticp	-0.08	0.06	-0.25	6	2844.89
Lycotoxins-II	0.8	Anticp	-0.22	-0.66	0.39	6.5	3207.37

Table 2: Molecular Docking of FLT3 and Different Spider Venom Peptides

Protein -Peptide	Protein Template	Peptide Template	Protein Structure Similarity (TM-score)	Interaction Similarity Score	Estimated Accuracy
FLT3-Lycosin-I	2G2F_A	2G2F_C	0.869	85	0.687
FLT3-Lycosin-II	2G2F_A	2G2F_C	0.869	98	0.716
FLT3-Psalmotoxin	3BU3_A	3BU3_B	0.881	55	0.633
FLT3-Lycotoxin-I	2G2F_A	2G2F_C	0.869	98	0.716
FLT3-Lycotoxin-II	2G2F_A	2G2F_C	0.869	85	0.687
FLT3-Latarcin2a	3BU3_A	3BU3_B	0.881	102	0.738
FLT3-Gomesin	3BU3_A	3BU3_B	0.881	58	0.64
FLT3-Brachyin	3BU3_A	3BU3_B	0.881	72	0.671
FLT3-Latrotoxin alpha	2G2F_A	2G2F_C	0.869	93	0.705
FLT3-Raventoxin-I	3BU3_A	3BU3_B	0.881	92	0.715
FLT3-Raventoxin-II	3BU6_A	3BU6_B	0.883	80	0.691

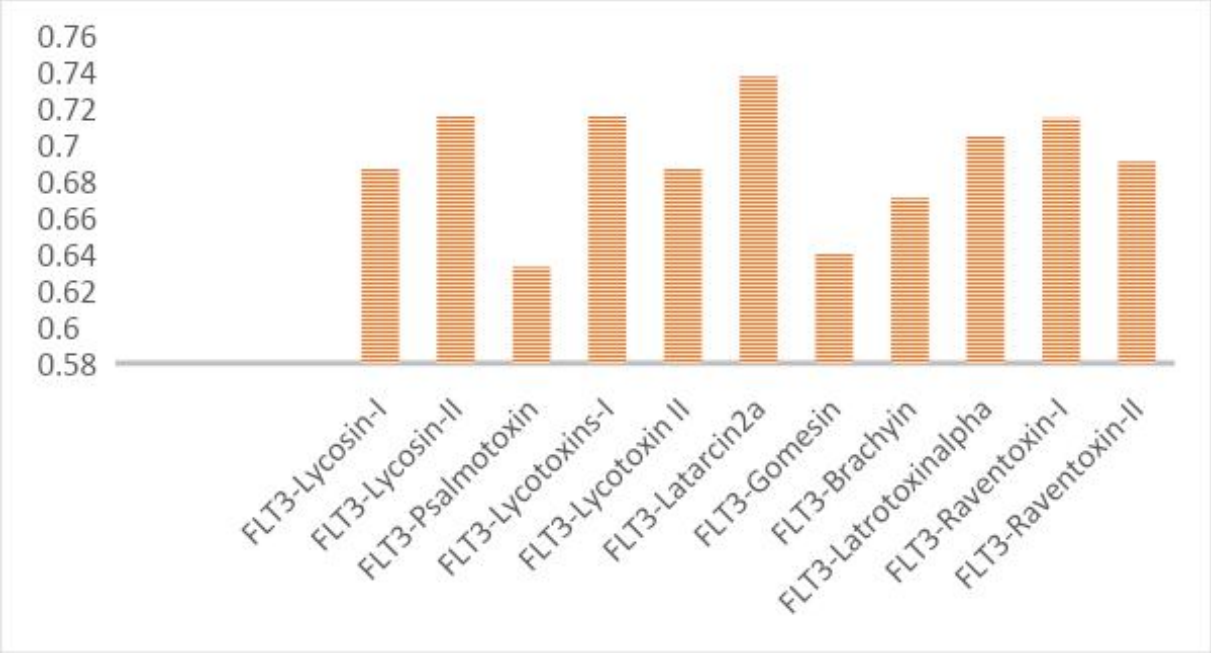

Figure 1: Estimated accuracy of Molecular Docking of FLT3 and Different Spider Venom Peptides

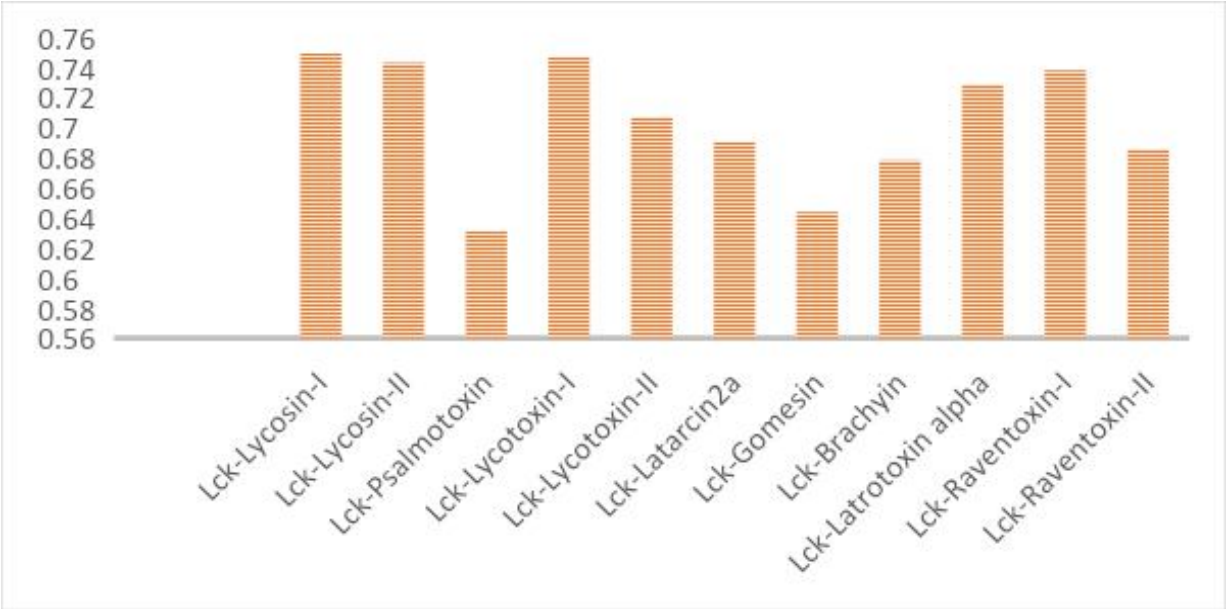

Figure 2: Estimated accuracy of Molecular Docking of Lck and Different Spider Venom Peptides

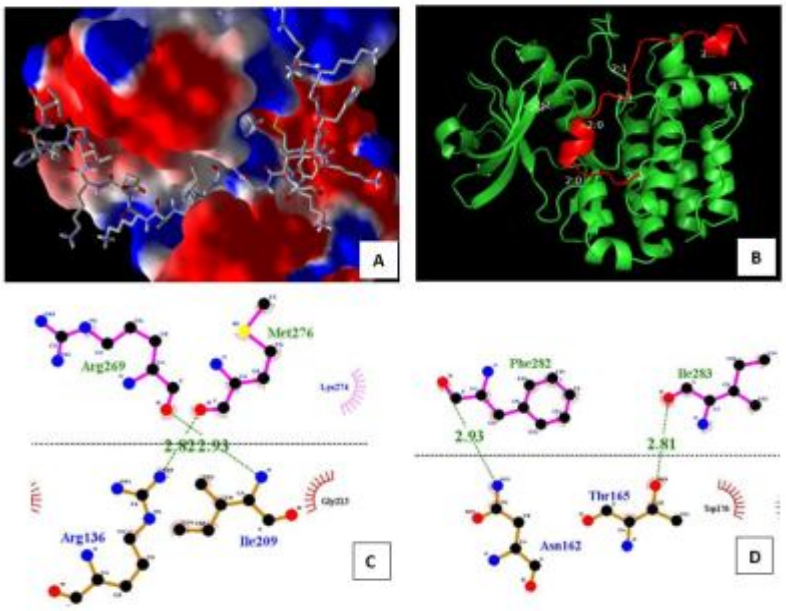

Figure 3: Docking outputs (Lycosin-I and Lck), A. Electrostatic Interaction B. Hydrogen Bonds, C and D: Important interaction of LCK-Lycosin-I Complex

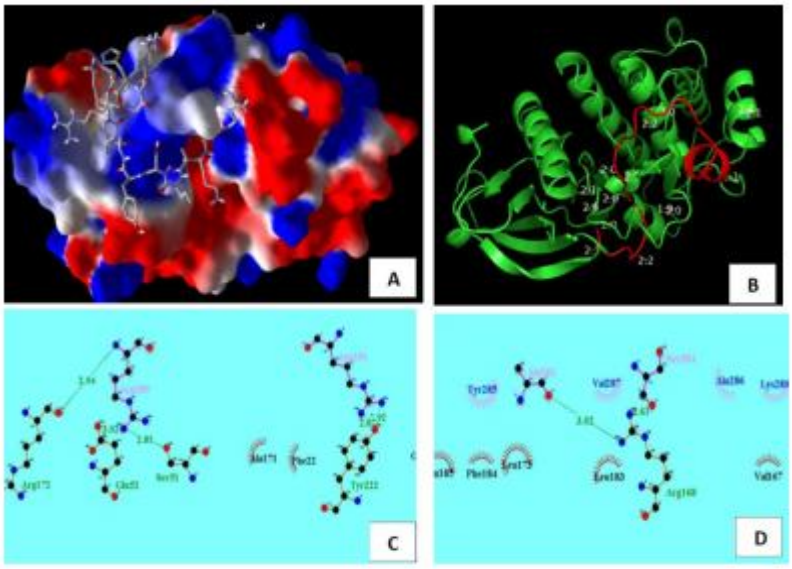

Figure 4: Docking outputs (Latarcin 2a and FLT3), A. Electrostatic Interaction B. Hydrogen Bonds, C and D: Important interaction of LCK-Lycosin-I Complex

According to Shen et al. Lycosin-I in high concentrations causes prostate cancer cells to undergo apoptosis whereas Lycosin-I at low concentrations prevents prostate cancer cells from migrating (Wang et al., 2014). This is because Lycosin-I inactivates the STAT3 pathway. Zhang et al. investigated the molecular modeling of the dihydropyridine pyrimidinones' method of binding as Lck inhibitors (Windley, Herzig, & Dziemborowicz, 2012).

A three-dimensional quantitative structure-activity relationship (3D-QSAR) model was created by Bharatham et al, to analyze the effectiveness of 2-amino-benzothiazole-6-anilide derivatives against lymphocyte-specific protein tyrosine kinase (P56 LCK) (Zhang et al., 2008).

REFERENCES

1. Bhujbal, S., Keretsu, S., & Cho, S. (2023). Design of New Therapeutic Agents Targeting FLT3 Receptor Tyrosine Kinase Using Molecular Docking and 3D-QSAR Approach. *Letters In Drug Design & Discovery,* 17(5), 585-596.
2. Cardoso, F., & Lewis, R. (2019). Structure–Function and Therapeutic Potential of Spider Venom-Derived Cysteine Knot Peptides Targeting Sodium Channels. Frontiers in Pharmacology, 10. doi: 10.3389 / fphar.2019.00366
3. Chidambaram, M., Manavalan, R., & Kathiresan, K. (2011). Nanotherapeutics to overcome conventional cancer chemotherapy limitations. *Journal of pharmacy & pharmaceutical sciences: a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques,* 14(1), 67–77.
4. Davletov, B., Ferrari, E., & Ushkaryov, Y. (2012). Presynaptic neurotoxins: An expanding array of natural and modified molecules. *Nature Reviews Neuroscience*, 13(7), 461-473. doi:10.1038 / nrn3314
5. Dubovskii, P. V., Vassilevski, A. A., Kozlov, S. A., Feofanov, A. V., Grishin, E. V., & Efremov, R. G. (2015). Latarcins: versatile spider venom peptides. *Cell & Molecular Life Sciences,* 72(23), 4501-4522.
6. Glenn, F., & Hamilton, M. C. H. (2013). Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. *Annual Review of Entomology*, 58(1), 475-496.
7. He, Q., Duan, Z., Yu, Y., Liu, Z., Liu, Z., & Liang, S. (2013). The Venom Gland Transcriptome of Latrodectus tredecimguttatus Revealed by Deep Sequencing and cDNA Library Analysis. *Plos ONE,* 8(11), e81357. doi: 10.1371 / journal.pone.0081357
8. Isbister, G. K., & Gray, M. R. (2002). A prospective study of 750 definite spider bites, with expert spider identification. *QJM,* 95(11), 723-731.
9. King, G. F., & Hardy, M. C. (2013). Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. *Annual Review of Entomology*, 58, 475–496
10. Klint, J. K., Senff, S., Rupasinghe, D. B., Er, S. Y., Herzig, V., Nicholson, G. M., & King, G. F. (2012). Spider-venom peptides that target voltage-gated sodium channels: pharmacological tools and potential therapeutic leads. *Toxicon: official journal of the International Society on Toxinology*, 60(4), 478–491.
11. Krissinel, E., & Henrick, K. (2007). Inference of macromolecular assemblies from crystalline state. *Journal of Molecular Biology,* 372, 774-797
12. Kuzmenkov, A. I., Sachkova, M. Y., Kovalchuk, S. I., Grishin, E. V., & Vassilevski, A. A. (2016). Lachesana tarabaevi, an expert in membrane-active toxins. *Biochemical Journal,* 473(16), 2495–2506.
13. Mashkani, B., Tanipour, M. H., Saadatmandzadeh, M., Ashman, L. K., & Griffith, R. (2016). FMS-like tyrosine kinase 3 (FLT3) inhibitors: Molecular docking and experimental studies. *European Journal of Pharmacology,* 776, 156–166. doi: 10.1016 / j.ejphar.2016.02.048.
14. Maupetit, J., Derreumaux, P., & Tuffery, P. (2009). PEP-FOLD: an online resource for de novo peptide structure prediction. *Nucleic Acids Research,* 37, 498-503. doi: 10.1093 / nar / gkp323.

15. Nimmrich, V., & Gross, G. (2012). P/Q-type calcium channel modulators. *British Journal of Pharmacology,* 167, 741–759

16. Pineda, S. S., Undheim, E. A., Rupasinghe, D. B., Ikonomopoulou, M. P., & King, G. F. (2014). Spider venomics: implications for drug discovery. *Future Medicinal Chemistry,* 6(15), 1699–1714. doi: 10.4155/fmc.14.103

17. Rash, L. D., & Hodgson, W. C. (2009). Pharmacology and biochemistry of spider venoms. *Toxicon,* 40, 225–254.

18. Saez, N. J., Senff, S., Jensen, J. E., Er, S. Y., Herzig, V., Rash, L. D., & King, G. F. (2010). Spider-Venom Peptides as Therapeutics. *Toxins,* 2(12), 2851-2871.

19. Saidijam, M., Yavari, B., Mahjub, R., Raigani, M., & Soleimani, M. (2018). The Potential Use of Peptides in Cancer Treatment. *Current Protein & Peptide Science,* 19(8), 759-770.

20. Shen, H., Xie, Y., Ye, S., He, K., Yi, L., & Cui, R. (2018). Spider peptide toxin lycosin-I induces apoptosis and inhibits migration of prostate cancer cells. *Experimental Biology and Medicine,* 243(8), 725–735.

21. Thundimadathil, J. (2012). Cancer treatment using peptides: current therapies and future prospects. *J. Amino Acids*, 1–13. doi: 10.1155/2012/967347

22. Ting, W., Meng, W., Wenfang, W., & Qianxuxan, L. (2019). Spider venom peptides as potential drug candidates due to their anticancer and antinociceptive activities. *J Venom Anim Toxins Incl Trop Dis*., 25, e146318.

23. Tyagi, A., Kapoor, P., Kumar, R., Chaudhary, K., Gautam, A., & Rghava, G. (2013). In Silico Models for Designing and Discovering Novel Anticancer Peptides. *Scientific Reports,* 3, 2984

24. Tyagi, A., Kapoor, P., Kumar, R., Chaudhary, K., Gautam, A., & Rghava, G. (2013). In Silico Models for Designing and Discovering Novel Anticancer Peptides. *Scientific Reports,* 3, 2984

25. Vorontsova, O. V., Egorova, N. S., Arseniev, A. S., & Feofanov, A. V. (2011). Haemolytic and cytotoxic action of latarcin Ltc2a. *Biochimie,* 93(2), 227-241.

26. Wallace, A., Laskowski, R., & Thornton, J. (1995). LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering, Design And Selection,* 8(2), 127-134.

27. Wang, L., Wang, Y. J., Liu, Y. Y., Li, H., Guo, L. X., Liu, Z. H., Shi, X. L., & Hu, M. (2014). In vitro potential of Lycosin-I as an alternative antimicrobial drug for treatment of multidrug-resistant Acinetobacter baumannii infections. *Antimicrobial Agents and Chemotherapy,* 58(11), 6999-7002. doi: 10.1128/AAC.03279-14.

28. Windley, M. J., Herzig, V., & Dziemborowicz, S. A. (2012). Spider-venom peptides as bioinsecticides. *Toxins,* 4, 191–227.

29. Zhang, G., Ren, P., Gray, N. S., Sim, T., Liu, Y., Wang, X., & He, Y. (2008). Discovery of pyrimidine benzimidazoles as Lck inhibitors: Part I. *Bioorganic & Medicinal Chemistry Letters,* 18(20), 5618–5621. doi: 10.1016/j.bmcl.2008.08.104.