Inhibiting Kholilah TN, Widodo, Nia Kurniawan

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isoaxazolines derivative. Although δ2-isoaxazolines derivative had been proved inhibitory activity in vitro and in vivo as known as IC50 in Group II svPLA2 [13], only a few studies in medicinal chemistry and structural biology evolved. Furthermore, since the characteristics of the interaction modes responsible for svPLA2 inhibition have not been investigated so far, we used the molecular docking approach to detect their interaction with VRV-PL-VIIIa toxin.

Material and Methods

The in silico study was done using the Window 10 platform of AMD A4-9125 RADEON processor with 4 GB RAM.

Protein target preparation

Protein target D. russelli’s PLA2 VRV-PL-VIIIa obtained from Protein Data Bank (PDB; https://www.rcsb.org/) with accession number 1SV3 [14]. Since there were several crystal structures, further multiple alignments between experiment published crystal structures 1SV3, 3HIx, 1FV0, 1KPM, 1OXL, 2QVD, and 2B17 protein target was executed using “blastp” (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The preferable sequence for the protein target based on the most preserved binding site sequences and mimics wild-type 3D crystal structure of D. russelli’s. The alignment displayed using ESPript v.3.0 [15]. The conserved site also validated using CASTp (sts.bioe.uic.edu/castp/) [16]. Before docking, the water and known ligands of the 3D structure removed using PyMol [17].

Ligand molecules preparation

The canonical SMILES of the small molecule of seven δ2-isoaxazoline derivatives and co-crystallized inhibitor [18] were mined from PubChem [19] and saved as a single SDF file using ChemMine [20]. To verify whether the in silico analysis simulated the previous experimental data, the co-crystal structure inhibitor must be re-docked. The ligands were minimized in energy and ready for docking using Open Babel on PyRx [21].

Molecular docking

Molecular docking executed using AutoDock Vina in PyRx version 0.9.8 [21] to identify the interaction mode between VRV-PL-VIIIa and small molecule inhibitors. The dimensions of the search space were defined 10 Å x 10 Å x 10 Å and confined to the active site of VRV-PL-VIIIa structure His48 and Asp49 [18]. The center coor-

dinate was X:52.7217, Y:36.7356, Z:2.4643. Semi-flexible docking refinement was chosen depending on their possible interaction to demonstrate the ligand capability. In semi-flexible docking, the residue act as the flexible macromolecule receptor, and the small molecules serve as the ligand with a rigid structure. The best binding pose was made based on the lowest binding affinity. Each molecular docking output was then saved as a.pdb file.

Determination of protein-ligand interaction

The interaction between the 3D structure of D. russelli’s PLA2 VRV-PL-VIIIa with each ligand visualized and unified as a single .pdb file using PyMol [17]. Further, the binding of the protein-ligand complex was determined by using PoseView [22] and LigPlot’ v.1.4 [23].

ADME analysis

The absorption, distribution, metabolism, excretion (ADME) properties was calculated using SwissADME (http://www.swissadme.ch) [24]. This website provides computer modeling to calculate physicochemical descriptors, predict ADME parameters, pharmacokinetic properties, drug-likeness (Lipinski rule of five), and chemical affability of all ligands considered in this study.

Results and Discussions

Two classes of secretory svPLA2 were classified, Group I of family Elapidae and Hydrophiinae and group II of family Crotalinae and Viperidae. Class II-A svPLA2 with a residue length of 120–125 amino acids divided into five subgroups at 49th residues: catalytically active Asp49, catalytically inactive or low activity Lys49, Ser49, Asn49, Arg49. The various subgroups identified a broad range of physiological and pathological impacts as their role in prey digestion [6].

Figure 1. The crystal structure of 1sv3 and predicted pockets on active site cleft
Figure 2. The δ2-isoxazoline derivatives and Anistic acid along with PubChem CID.

Figure 3. Interactions protein-ligand binding involving Anisic acid (a) and 3bI of δ2-isoxazoline derivate (b).
Two-thirds of Russell’s viper venom proteins composed by svPLA2s provide at least 7 isoforms among the subspecies of D. russelli [25]. For this first time, the VRV-PL-VIIIa snake venom structure used to learn the interaction and bonding actions with already identified svPLA2 inhibitors. VRV-PL-VIIIa’s multiple sequence alignment between 7 crystal structures showed that sequence identity shares 121 residues indeed with 100 percent query coverage. Since all 3D crystal structures preserved the same sequences, structure 1SV3 was preferred to be the macromolecule receptor, given the highest resolution (1.35 Å). Besides, CASTp confirmed the existence of highly conserved binding sites for Ca\(^{2+}\) in residues 27–32 and active site residues for His48 and Asp99, as stated in 1SV3 Uniprot (P59071). These conservative sequences correspond to the pocket, the concavities on the surface of a protein located at the active site cleft (Figure 1).

The crystal structure 1SV3 scaffolded by an \(\alpha\)-helix (2–13 residues), an external loop (17–22 residues), a calcium-binding loop (27–32 residues), a second \(\alpha\)-helix (39–52 residues), \(\beta\)-turn (66–65 residues) and a third \(\alpha\)-helix (80–98 residues) followed by several \(\eta\)-loop regions finally terminating with the C terminal (Figure 1). Anisic acid (ANN; 4-methoxy benzoic acid) was the co-crystallized inhibitor (Figure 2) [18].

The 82-isoxazoline derivatives were known to inhibit D. russelli PLA2 activity with \(IC_{50}\) ranged 2.5 – 208 \(\mu\)M. The ancestor, 82-isoxazoline (PubChem ID: 171639), also reported interfering D. russelli PLA2 with \(IC_{50}\) 86.2 \(\mu\)M [6]. The 82-isoxazoline derivative features an ideal filling at the enzyme hydrophobic active site with a butyl substituent. The expansion of ethyl groups increasing the inhibition potential. Based on in vivo study, only compounds 3bI and 3bIV appeared robust inhibition activity of svPLA2. These compounds did not cause edema [13]. However, we employed all seven derivatives in this study to double-check past findings. The structures of the 82-isoxazoline derivatives are shown in Figure 2.

**Molecular docking**

Molecular docking demonstrated putative binding modes and protein-ligand site interactions. According to the sequence source, the active site lies on the residue His48 and Asp99, while in the previous study, His48 and Asp49[11, 17]. Residue His48 and Asp49 were part of the svPLA2 domain that including the eliciting pro-inflammatory region. Besides, the Asp99 was part of the region that elicited anti-coagulants. Even so, the D. russelli PLA2 enzyme has classified the C-terminal enzyme as Group II Asp49 enzyme[13]. Thus, the molecular docking zones must be limited to the area of Asp49 and His48, so the procedure will be feasible and suitable for screening any other ligands.

In the previous experimental study [18], the carboxylate oxygen atoms of Anisic acid (ANN) bound the water molecule of PLA2 enzyme that interacts with the putative binding residues, Asp 49 and His 48 [18]. It also formed a hydrogen bond with residues from the calcium-binding loop Gly30. Several hydrophobic interactions have been found with residues of \(\alpha\)-helix H1 (Leu2, Phe5, Ile9), external loop residues (Ala18, Ile19, Tyr22), and Cys45.

Docking results indicated that all of the small molecules interact with the VRV-PL-VIIIa structure (Figure 3). Ligand ANN formed hydrogen bonds with Asp49 (2.96 Å) and hydrophobic interaction with Leu2, Phe5, Tyr22, Phe106. The contrast of the ANN docking result was the Gly30 and His48 residues interacted as non-hydrogen bonds (Figure 3). Additionally, there were hydrogen bonds with Cys45 (Table 1). Even though not accurately the same interaction, the denoted amino acid residues precisely the same as the previous finding, showing the results of this research mimic the binding mode of the experimental study.

All 82-isoxazoline derivatives interacted with the critical residues of Asp49 and His48, except for 3bI and 3bII ligands that did not interact with His48. Only small differences in binding affinity between ligand were observed. 3bI displayed the most favorable binding properties compared to other simulated derivatives, similar to the experimental result.

82-isoxazoline derivatives formed hydrogen bonds with Asp49 residues, except 3bII interacted as hydrophobic. Meanwhile, His48 active site residue has emerged in hydrophobic bonds. The ligand interaction with residues of Asp49 and His48 indicates binding in the pro-inflammatory eliciting area. Besides, several ligands formed hydrogen and hydrophobic bonds with Gly30 to stabilize protein-ligand interactions. Gly30, along with Tyr28 and Gly32, were part of the calcium-binding loop (27–32 residues) via carbonyl oxygen [26]. These discoveries affirmed the capacity of the ligand to cause inhibition activity of VRV-PL- VIIIa. Those small molecules might interfere with the Ca\(^{2+}\) catalytic activity, including with PLA2’s inflammatory functions [27]. Ca\(^{2+}\) plays...
as equilibration in the hemostasis process. Ca\(^{2+}\) binding by PLA2 was also related to the hemorrhagic occurrence. This study reinforced the study of VRV PL-VIIIa that targets the pituitary gland and injures the lung leading to hemorrhage [28].

Several hydrophobic interactions that also emerge were residue Leu2 and Phe5 of the \(\alpha\)-helix H1 and Tyr22 of the outer loop. It resembled numerous other 3D structures of the sPLA2, in which active sites are highly defined inside a hydrophobic invariant core [29]. Only ligand 3bI found hydrophobic interactions with the region eliciting an anti-coagulant response residue Gly53. The 3bIV ligand, which is also reported to have the best inhibition compared to other \(\delta\)-isoxazoline derivatives with the least IC50, showed binding affinity -4.9 or not equally with 3bI. The predicted binding affinity could not anticipate the IC50 of a ligand against a particular enzyme [26]. Molecular docking only gives a correlation of around 0.5 when compared to experimental data [30]. Hence, it is worth exploring advanced ligand-protein interaction through more exact strategies such as end-point free energy through molecular dynamics simulation.

**ADME properties**

Lead molecules were analyzed based on descriptors that were physically significant and
pharmacologically relevant using SwissADME (Table 2). The analysis also includes Lipinski’s five rules for understanding the drug-likeness. All descriptors that are biologically important and pharmacologically relevant are within the acceptable range for human use. Therefore, 3bI ligand was considered the most potent compound based on water solubility, pharmacokinetics absorption, and drug-likeness.

**δ2-isoxazoline derivatives as in silico PLA2 validator**

Flooding information on svPLA2 inhibitory activities results in a compromise on the study in silico [8]. To conducting in-silico research on D. russelli’s PLA2 while unavailable anti-venom serum chemical structure, δ2-isoxazoline derivatives seem suitable to be the validator. Besides, the δ2-isoxazoline also proven its inhibitory activity on a different subgroup of Group II svPLA2 D. russelli pulchella VRV-PL-V that presented His47, Gly48, and Phe5 as interacting residue [6]. These studies coincided that δ2-isoxazoline well conformed the receptor structure on the active site. The known putative binding site of D. russelli PLA2 will help to understand the structure and function interaction and in developing other novel and specific inhibitors.

**Conclusion**

The δ2-isoxazoline derivatives showed the VRV-PL-VIIa inhibition activity by interacting pro-inflammatory eliciting region on residue Asp49 via hydrogen bonding and His48 via hydrophobic interaction. Among the δ2-isoxazoline derivatives, 3bI possessed the most favorable binding as well as most ideal in the ADME properties. These finding sheds light on the experimental study of alternative serum anti-venom and developing other svPLA2 inhibitors. Further investigation utilizing molecular dynamics simulation is needed for better understanding.

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**References**

1. WHO (2016) Guidelines for the management of snake-bites, 2nd Edition. India.
2. Slagboom J, Kool J, Harrison RA, Casewell NR (2017) Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise. British Journal of Haematology 177(6): 947-959. doi: 10.1111/bjh.14591.
3. Gutiérrez JM, Calvete JJ, Habib AG et al. (2017) Snakebite envenoming. Nature reviews Disease Primes 3 (1): 1-21.
4. Roly ZY, Hakim MA, Zahan AS et al. (2015) ISOB: A Database of Indigenous Snake Species of Bangladesh with respective known venom composition. Bioinformation 11 (2): 107.
5. Berling I, Isbister GK (2015) Hematologic effects and complications of snake envenoming. Transfusie medicine Reviews 29 (2): 82 – 89. doi: 10.1016/j.tmrv.2014.09.005.
6. Sivaramakrishnan V, Illamathi M, Ghosh KS et al. (2016) Virtual analysis of structurally diverse synthetic analogs as inhibitors of snake venom secretory phospholipase A2. Journal of Molecular Recognition 29 (1): 22-32. doi: 10.1002/jmr.2492.
7. Marcussi S, Sant’Ana CD, Oliveira CZ et al. (2007) Snake venom phospholipase A2 inhibitors: medicinal chemistry and therapeutic potential. Current Topics in Medicinal Chemistry 7 (8): 743-756. doi: 10.2174/156802607780487614.
8. Ojeda PG, Ramírez D, Alzate-Morales J et al. (2018) Computational studies of snake venom toxins. Toxins 10 (1): 8. doi:10.3390/toxins10010008.
9. Tan KY, Tan NH, Tan CH (2018) Venom proteomics and antivenom neutralization for the Chinese eastern Russell’s vipers, Daboia siamensis from Guangxi and Taiwan. Scientific Reports 8 (1): 1-14. doi: 10.1038/s41598-018-25955-y.
10. Ghag-Sawant M, More TV, Samant LS, Chowdhary AS (2016) Study of neutralization of enzymatic activity of Daboia russelli venom by various plant extracts and their combinations using *in vitro* methods. International Journal of Pharmaceutical Sciences and Research 7(6): 2531. doi: 10.13040/IJPSR.0975-8232.7(6)2531-36.
11. Sakhivel G, Dey A, Nongalleima K et al. (2013) In vitro and in vivo evaluation of poly herbal formulation against Russell’s viper and cobra venom and screening of bioactive components by docking studies. Evidence-Based Complementary and Alternative Medicine 2013. doi: 10.1155/2013/781216.
12. Dhananjaya BL, Zameer F, Girish KSD, Souza CJ (2011) Anti-venom potential of aqueous extract of stem bark of Manjifera indica L. against Daboia russelli (Russell’s vipers) venom.
13. Basappa, Kumar MS, Swamy SN et al. (2004) Novel δ2-isoxazolines as group II phospholipase A2 inhibitors. Bioorganic Medicinal Chemistry Letters 14 (14): 3679-3681. doi: 10.1016/j.bmcl.2004.05.012.
14. Tsai IH, Lu PI, Su JC (1996) Two types of Russell’s viper revealed by variation in phospholipases A2 from venom of the subspecies. Toxicon 34(1): 99-109.
15. Robert X, Gouet P (2014) Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Research 42 (W1): W320-W324. doi: 10.1093/nar/gku316.
16. Tian W, Chen C, Lei X et al. (2018) CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Research 46 (W1): W363-W367. doi: 10.1093/nar/gky473.
17. DeLano WL (2002) Pymol: An open-source molecular graphics tool. CCP4 Newsletter on Protein Crystallography 40 (1): 82-92.
18. Singh N, Jabeen T, Pal A et al. (2006) Crystal structures
of the complexes of a group IIA phospholipase A2 with two natural anti-inflammatory agents, anisic acid, and atropine reveal a similar mode of binding. Proteins: Structure, Function, and Bioinformatics 64 (1): 89-100. doi: 10.1002/prot.

19. Kim S, Chen J, Cheng T, Gindulyte A et al. (2019) PubChem 2019 update: improved access to chemical data. Nucleic acids research 47(D1): D1102-D1109. doi: 10.1093/nar/gky1033.

20. Backman TW, Cao Y, Girke T (2011) ChemMine tools: an online service for analyzing and clustering small molecules. Nucleic acids research 39 (suppl 2): W486-W491. doi: 10.1093/nar/gkr320.

21. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry 31 (2): 455-461. doi: 10.1002/jcc.21334.

22. Stierand K, Rarey M (2010) Drawing the PDB: protein–ligand complexes in two dimensions. ACS Medicinal Chemistry Letters 1 (9): 540-545. doi: 10.1021/ml100164p.

23. Laskowski RA, Swindells MB (2011) LigPlot+: multiple ligand–protein interaction diagrams for drug discovery.

24. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports 7: 42717. doi: 10.1038/srep42717.

25. Deepa V, Sreekumar S, Biju C (2018) In silico validation of anti-russell’s viper venom activity in Phyllanthus emblica L. and Tamarindus indica L. International Journal of Pharmaceutical Sciences and Drug Research 10 (4): 217 – 226. doi: 10.25004/IJPSDR.2018.100403.

26. Apweiler R, Bairoch A, Wu CH et al. (2004) UniProt: the universal protein knowledgebase. Nucleic Acids Research 32 (suppl_1): D115 - D119. doi: 10.1093/nar/gkh131.

27. Mahmud S, Parves MR, Riza YM et al. (2020) Exploring the potent inhibitors and binding modes of phospholipase A2 through in silico investigation. Journal of Biomolecular Structure and Dynamics 38 (14): 4221-4231. doi: 10.1080/07391102.2019.1680440.

28. Kumar JR, Basavarajappa BS, Vishwanath BS, Gowda TV (2015) Biochemical and pharmacological characterization of three toxic phospholipase A2s from Daboia russelli snake venom. Comparative Biochemistry and Physiology Part C: Toxicology Pharmacology 168: 28-38. doi: 10.1016/j.cbpc.2014.11.005.

29. Dennis EA, Cao J, Hsu YH et al. (2011) Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chemical Reviews 111 (10): 6130-6185.

30. Cheng T, Li X, Li Y et al. (2009) Comparative assessment of scoring functions on a diverse test set. Journal of chemical information and modeling 49 (4): 1079-1093.
