Trop J Nat Prod Res, November 2023; 7(11):5211-5215
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Silico Study of Plumbagin as Potent Inhibitor of Pro-Inflammatory Molecules TNF-α, NF-$\kappa\beta$, and IL-17

Mitayani Purwoko[1]*, Trisnawati Mundijo[1], Yesi Astri[2,3], Siti Rohani[2], Dian P. Perkasa[4]

[1]*Department of Medical Biology, Faculty of Medicine, Universitas Muhammadiyah Palembang, Indonesia*
[2] *Department of Pharmacology, Faculty of Medicine, Universitas Muhammadiyah Palembang, Indonesia*
[3]*Department of Neurology, Faculty of Medicine, Universitas Muhammadiyah Palembang, Indonesia*
[4]*The National Research and Innovation Agency, Indonesia*

ARTICLE INFO

Article history:
Received 09 September 2023
Revised 10 October 2023
Accepted 19 October 2023
Published online 01 December 2023

ABSTRACT

Plumbagin is a flavonoid compound with an anti-inflammatory function, but its mechanism of action has yet to be explained in detail. The anti-inflammatory effect of Plumbagin may occur because Plumbagin interferes with signaling pathways in inflammatory pathways such as cytokines and transcription factors. This study aimed to examine the potential of Plumbagin as an inhibitor for pro-inflammatory molecules using a molecular docking approach. This study docked Plumbagin into TNF-α, NF-$\kappa\beta$, and IL-17A. The free binding energy between Plumbagin- IL-17A, - TNF-α, and -NF-$\kappa\beta$ were -6.04, -5.60, and -3.63 kcal/mol, respectively. The docking results suggest that Plumbagin has good binding affinities and interactions with IL-17A. These results can be used to conduct further *in vitro* and *in vivo* studies.

***Keywords*:** interleukin 17A, molecular docking, nuclear factor kappa beta, plumbagin, tumor necrosis factor

Introduction

Inflammation is the body's natural response to tissue damage. Inflammation is the body's attempt to activate or destroy invading organisms, remove irritants, and set the stage for tissue repair.[1] Herbal plants contain metabolites that can act as an antiinflammation agent, such as *Curcuma longa*, which contains Curcumin, and *Plumbago zeylanica* L., which contains Plumbagin.[2–4] Curcumin is known to have anti-inflammatory activity; however, this compound is difficult to dissolve, resulting in low bioavailability.[3] Therefore, this study explored the opportunity of Plumbagin as an anti-inflammatory agent. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone or 5-hydroxy-2-methylnaphthalene-1,4-dione) is reportedly abundant in the roots of the *Plumbago zeylanica* L. plants.[5–7] Many *in vitro* and *in vivo* studies have used extracts from *Plumbago zeylanica* L. roots, such as in the rat model of carrageenan-induced paw edema, a rat model of thermal stimuli, and *in vitro* study of A549 lung cancer cells.[8–10] However, few studies have directly used the active compound Plumbagin. Therefore, this study seeks to conduct *in silico* simulations of the target proteins of Plumbagin compounds in the inflammatory pathway.

Plumbagin is a member of the phenols group. Phenolic compounds can inhibit the production of pro-inflammatory mediators or their action, thus resulting in anti-inflammatory properties.[11,12] In the current study, we considered three key signaling molecules: tumor necrosis factor-alpha (TNF-α), interleukin-17A (IL-17A), and nuclear factor kappa beta (NF-$\kappa\beta$), which play essential roles in the inflammation process.

*Corresponding author. E mail: mitayani@um-palembang.ac.id
mitayani.dr@gmail.com
Tel: +62 81320074327

Citation: Purwoko M, Mundijo T, Astri Y, Rohani S, Perkasa DP. *In Silico* Study of Plumbagin as Potent Inhibitor of Pro-Inflammatory Molecules TNF-α, NF-$\kappa\beta$, and IL-17. Trop J Nat Prod Res. 2023; 7(11):5211-5215. http://www.doi.org/10.26538/tjnpr/v7i11.26

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Ligands stimulation will activate the I$\kappa\beta$ kinase (IKK) complex in macrophage cells. Activated IKK will cause phosphorylation of I$\kappa\beta\alpha$, which triggers the degradation of I$\kappa\beta\alpha$. The degradation of I$\kappa\beta\alpha$ induces NF-$\kappa\beta$ to become active in the cytoplasm, enters the nucleus, and triggers the induction of pro-inflammatory genes. Classically activated macrophages (M1) will produce various pro-inflammatory cytokines, including TNF-α. M1 macrophages also promote the differentiation of inflammatory T cells, including Th1 and Th17. Th17 produces the cytokine IL-17 and mediates the onset of inflammation.[13,14]

Interleukin 17A has two roles in the body: one side works with other mediators such as TNF-α, IL-1, and IL-6 to stimulate the summoning of neutrophils' task to destroy pathogens, and the other side also works with other pro-inflammatory cytokines to provoke an increased immune response.[15] As a cytokine, TNF-α is a significant regulator of inflammatory responses. It triggers a cascade of activation of various other inflammatory molecules. TNF-α has two receptors, TNF receptor-1 and -2.[16] Physiologically, TNF-α is a molecule that elicits a normal immune response from the body. Excess, however, it can be harmful and cause inflammation and diseases such as Psoriasis and Crohn's Disease.[17,18]

This study aimed to provide information on the potency of Plumbagin as an inhibitor for TNF-α, NF-$\kappa\beta$, and IL-17A using a molecular docking approach. Due to the small number of information regarding the involvement of Plumbagin in inflammatory pathways, it is necessary to conduct an *in silico* study utilizing Plumbagin as a candidate for inflammatory biomarkers inhibitor.

Materials and Methods

Data Retrieval

The Plumbagin's molecular structure was retrieved from the PubChem compound database CID 10205 (Figure 1a). Protein Data Bank (https://www.rcsb.org/) provides the 3-D structures of three chosen inflammation signaling targets, which are TNF-α (PDB ID: 2AZ5), human IL-17A (PDB ID: 4HR9), and NF-$\kappa\beta$ (PDB ID: 1SVC).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Drug likeliness

Analysis of drug-like properties used the SwissADME program by entering the Simplified Molecular Input Line Entry Specification (SMILES) of Plumbagin (CC1=CC(=O)C2=C(C1=O)C=CC=C2O). The analysis of drug-like properties yields a score of compound properties against Lipinski's rule of five, which includes the molecular weight of the compound ≤500, log P partition coefficient value ≤5, number of hydrogen bond donors ≤5, and number of hydrogen bond acceptors ≤10.[19]

Molecular Docking

PyMol version 2.4.0 facilitates the visualization and molecular docking analysis. AutoDockTools version 1.5.6 simulates the molecular ligand docking on the receptors' attachment side. Ligplot+ mediates the simulation process of molecular interaction between ligand and receptor. This research utilized a CPU equipped with Intel(R) Core(TM) i3-3110M CPU @ 2.40GHz, 2400 Mhz, 2 Core(s), 4 Logical Processor(s) and GPU NVIDIA GeForce GT 730M.

Results and Discussion

The selection of a compound that will become a human drug candidate is determined based on many factors. An easier way to assess drug likeliness is using SwissADME, an online application.[20] Plumbagin fulfills all those rules (Table 1); hence, it is worthy of being an anti-inflammatory drug candidate. This result also aligns with a previous ADMET study, which reported that Plumbagin had good drug likeliness.[21]

Molecular docking is a drug discovery method that uses computer modeling or simulation based on the structure of the compound under study. Docking allows the prediction of interactions between ligands and proteins at the molecular level to guide researchers to conduct adequate research at low cost.[22] The molecular basis for the activity of most pharmaceutical compounds lies in their binding to a pharmacological target. Therefore, predicting ligand binding is crucial to discovering new medicines.[23] After docking, the best binding pose between ligand and protein is selected based on the lowest estimated free binding energy.[24] Table 2 shows the binding affinity of Plumbagin and each pro-inflammatory molecule, ranging from -6.04 to -3.63 kcal/mol. Plumbagin-IL-17A exhibits the strongest binding affinity of -6.04 kcal/mol among them.

In addition to the free binding energy value and the inhibition constant value, consideration of the interaction between the residue, the ligand, and the hydrogen bonds that occur is important. The 3D complex of Plumbagin and TNF-α displayed the conformation of its binding (Figure 1b, 1c, and 1d). Hydrophobic interactions between Plumbagin and TNF-α appeared at Leu120, Tyr119, Ser60, and Tyr59, and hydrogen interactions at the residue of Gly121 and Tyr151 (Figure 2). The 3D complex of Plumbagin- NF-$\kappa\beta$1 displayed the conformation of its binding (Figure 3). With NF-$\kappa\beta$1, hydrophobic interactions were detected via Lys244(P) and Tyr60(P) and hydrogen interactions via Phe58(P), Glu63(P), and Arg57(P) (Figure 4). Previous studies have examined the interaction between Plumbagin and NF-$\kappa\beta$ *in silico*. However, previous studies used 3GUT ligand for NF-$\kappa\beta$, while this study used 1SVC. Therefore, the interaction residues between Plumbagin and NF-$\kappa\beta$ in this study differ from previous studies.[25]

Figure 5 displays the conformation binding of the Plumbagin-IL-17A 3D complex. Plumbagin-IL17A interaction are detected via hydrophobic bond with residue Thr21(A), Arg20(A), Phe110(A), Arg111(A), and Leu99(A) and also hydrogen bond with Leu112A and Val22(A) (Figure 6).

Based on the results in Table 3, the Plumbagin-IL-17A complex produces five residue interactions and two hydrogen bonds, which later the number of residue-ligand interactions will describe where the ligand binds to the protein. The number of hydrogen bonds that occur determines the ligand-receptor bond's stability because hydrogen bonds influence the structural stability of a protein.[26] Plumbagin-IL-17A complex produces low free binding energy and Ki values and produces the highest residue-ligand interactions. Free binding energy can predict the value of Ki and the number of interactions that occur; the lower the free binding energy value, the smaller the Ki value, and the more interactions occur.[27]

Table 1: Drug likeliness of Plumbagin

Chemical (ID Pubchem)	Molecule formula	Molecular weight (g/mol)	Log P	H-bond donor	H-bond acceptor
Plumbagin (10205)	$C_{11}H_8O_3$	188.18	2.3	1	3

Table 2: Docking analysis

Receptors	Center of Grid Box	Dimension of Grid Box	Free Binding Energy	Ki (Inhibition Constant)
TNF-α dimer-compound	*x*=-19.163 *y*=74.452 *z*=33.837	*x*=40 *y*=40 *z*=40	-5.60 kcal/mol	78.99 µM [at temp. = 298.15 K]
Nf-$\kappa\beta$1	*x*=29.025 *y*=29.093 *z*=33.837	*x*=40 *y*=40 *z*=40	-3.63 kcal/mol	2.17 mM [at temp. = 298.15 K]
IL-17A Chain A	*x*=30.068 *y*=28.476 *z*=33.938	*x*=40 *y*=40 *z*=40	-6.04 kcal/mol	37.18 µM [at temp. = 298.15 K]

Table 3: Residue-ligand interaction

Protein-Ligand Complex	Total of Hydrophobic interactions	Total of Hydrogen interactions
Plumbagin-TNF-α	4	2
Plumbagin- NF-$\kappa\beta$1	2	3
Plumbagin-IL-17A	5	2

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

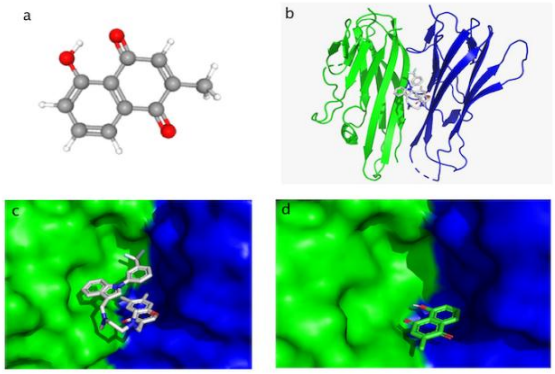

Figure 1: Docking result between Plumbagin and TNF-α. (a) 2D structure of Plumbagin. (b) Structure of TNF-α dimer-compound complex with native ligand. (c) The binding site of native ligand on the TNF-α dimer-compound complex. (d) Docking pose of Plumbagin in the binding site of TNF-α dimer-compound.

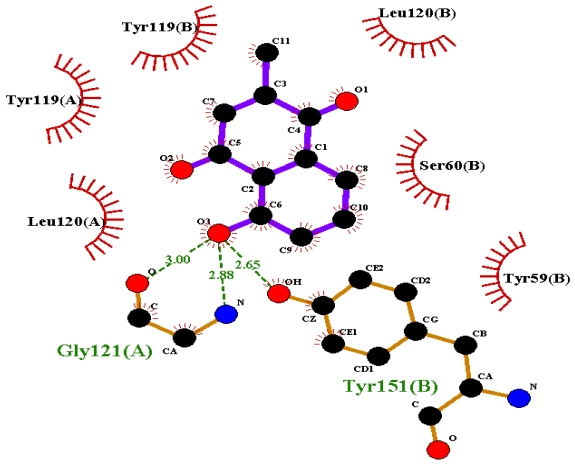

Figure 2: 2D ligand interaction diagram showing the hydrogen bond network (green dotted lines) and the hydrophobic interaction (red lines) of Plumbagin with the TNF-α dimer compound.

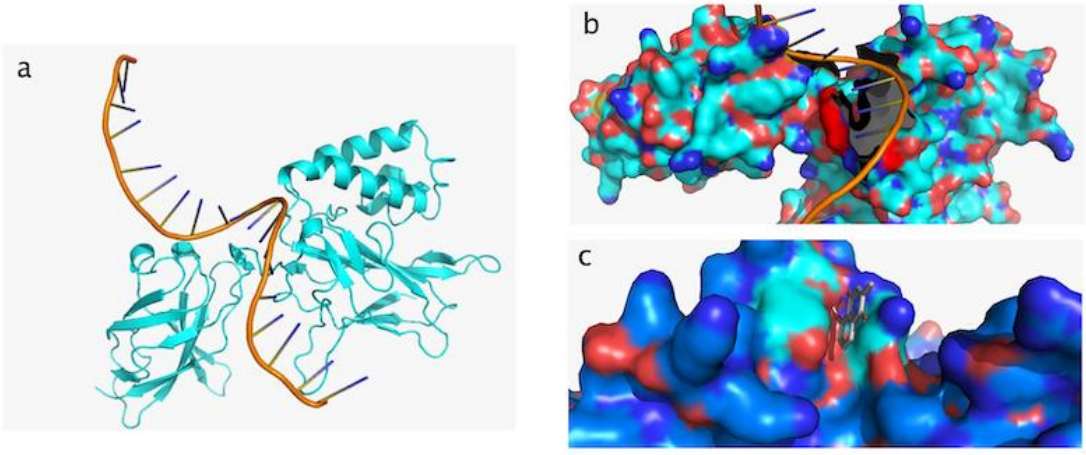

Figure 3: Docking result between Plumbagin and NF-$\kappa\beta$. (a) Structure of Nf-$\kappa\beta$1 with native ligand. (b) The binding site of native ligand on the NF-$\kappa\beta$1. (c) Docking pose of Plumbagin in the binding site of NF-$\kappa\beta$1.

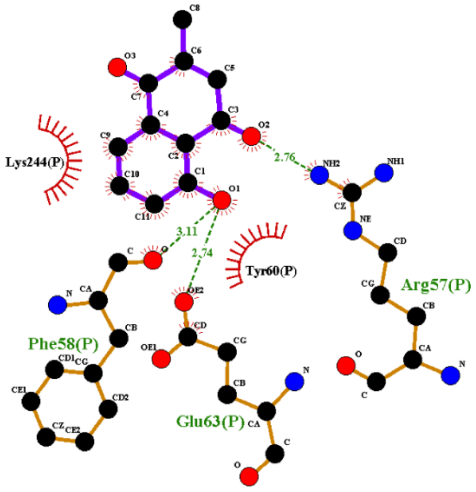

Figure 4: 2D ligand interaction diagram showing Plumbagin's hydrogen bond network (green dotted lines) and the hydrophobic interaction (red lines) with the NF-$\kappa\beta$1.

Conclusion

The results of the docking analysis showed various residues that act as the primary medium for inhibiting the inflammatory process by Plumbagin. In all three molecules targeted for signaling, the Dock score shows good binding affinity of Plumbagin into pro-inflammatory molecules. Among them, IL-17A is the most favorable pro-inflammatory molecule to be targeted by Plumbagin. However, this *in silico* study needed further validation using *in vitro* and *in vivo* studies to determine the pathways.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

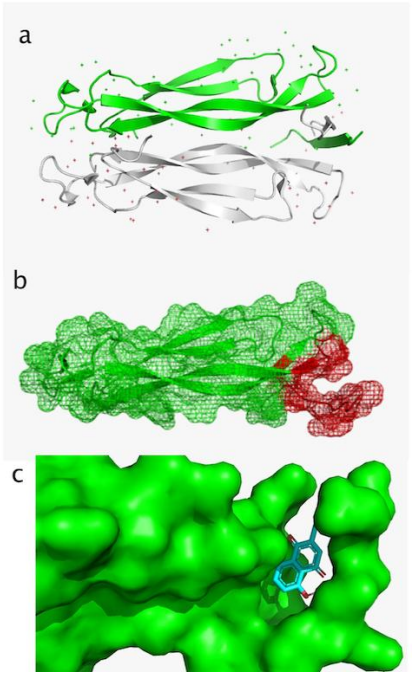

Figure 5: Docking result between Plumbagin and IL-17A. (a) 3D structure of homodimer interleukin-17A. Chain A is grey, and chain B is green. (b) The predicted druggable pocket of the target receptor (IL-17A). The residues are in red. (c) Docking pose of Plumbagin (blue) in a druggable pocket of IL-17A (green).

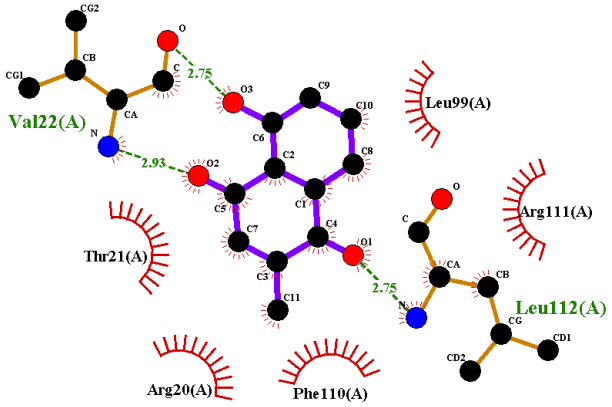

Figure 6: 2D ligand interaction diagram showing the hydrogen bond network (green dotted lines) and the hydrophobic interaction (red lines) of Plumbagin with the IL-17A chain A.

Acknowledgments

Authors are thankful to BIMA research grant 2023 from Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat the Ministry of Education and Culture Republic of Indonesia with contract number 178/E5/PG.02.00.PL/2023; 220/LL2/AL.04/2023; 154/H-5/LPPM.UMP/VII/2023.

References

1. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated disease in organs. Oncotarget. 2018;9(6):7204–18.
2. Yu Y, Shen Q, Lai Y, Park SY, Ou X, Lin D, Jin M, Zhang W. Anti-inflammatory Effects of Curcumin in Microglial Cells. Front Pharmacol. 2018;9(2018).
3. Kulkarni AS, Dias RJ, Ghorpade VS, Mali KK. Freeze-Dried Multicomponent Inclusion Complexes of Curcumin for Enhancement of Solubility and Anti-Inflammatory Activity. Trop J Nat Prod Res. 2020;4(11):887–94.
4. Purwoko M, Indarto D, Purwanto B, Soetrisno S, Kariosentono H. Identification of chemical compounds in *Plumbago zeylanica* Linn leaves from Indonesia. Trop J Nat Prod Res. 2022;6(9):1440–2.
5. Purwoko M, Sentono H, Purwanto B, Indarto D. Phytochemical evaluation of Plumbago zeylanica roots from Indonesia and assessment of its plumbagin concentration. Folia Med (Plovdiv). 2022;64(1):96–102.
6. Panicker S, Haridasan VK. *In vitro* Cytotoxicity Study on U87 Cells Using Root Extracts of Plumbago Species and GC-MS Study. Pharmacogn J. 2018;10(6s):s71–6.
7. Dohare B, Jain B, Khare S, Jain K. Comparative Estimation of Plumbagin in Aerial and Root Part of Plumbago zeylanica Using UV-Visible Spectrophotometric. UK J Pharm Biosci. 2015;3(3):9–14.
8. Subramaniyan V, Paramasivam V. Potential Anti-Inflammatory Activity of *Plumbago zeylanica*. Asian J Pharm Clin Res. 2017;10(10):372–5.
9. Thanigavelan V, Venkatachalam K, Venkatachalam L, Natarajan S, Murugan PK, Savarimuthu JA. Hydroalcoholic Extract of *Plumbago Zeylanica* Linn root bark exhibit Analgesic and Anti-Inflammatory activities in Experimental Rat models. Am J Pharm Heal Res. 2014;2(4):219–21.
10. Sundari B, Telapolu S, Dwarakanath B, Thyagarajan S. Cytotoxic and Antioxidant Effects in Various Tissue Extracts of *Plumbago zeylanica:* Implications for Anticancer Potential. Pharmacogn J. 2017;9(5):706–12.
11. Ambriz-Perez DL, Leyva-Lopez N, Gutierrez-Grijalva EP, Heredia JB. Phenolic compounds: Natural alternative in inflammation treatment. A Review. Cogent Food Agric. 2016;2(1):1131412.
12. Petrocelli G, Marrazzo P, Bonsi L, Facchin F, Alviano F, Canaider S. Plumbagin, a Natural Compound with Several Biological Effects and Anti-Inflammatory Properties. Life. 2023;13(6):1303.
13. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2(17023).
14. Mosca M, Hong J, Hadeler E, Hakimi M, Liao W, Bhutani T. The Role of IL-17 Cytokines in Psoriasis. Immunotargets and Therapy. Immunotargets Ther. 2021;10:409–18.
15. Ge Y, Huang M, Yao Y. Biology of Interleukin-17 and Its Pathophysiological Significance in Sepsis. Front Immunol. 2020;11(2020).
16. Jang D, Lee A-H, Shin H-Y, Song H-R, Park J-H, Kang T-B, Lee S-R, Yang S-H. The Role of Tumor Necrosis Factor Alpha (TNF-α) in Autoimmune Disease and Current TNF-α Inhibitors in Therapeutics. Int J Mol Sci. 2021;22(5):2719.
17. Bilgiç Ö, Sivrikaya A, Toker A, Ünlü A, Altınyazar C. Serum levels of TWEAK in patients with psoriasis vulgaris. Cytokine. 2016;77(2016):10–3.
18. Adegbola SO, Sahnan K, Warusavitarne J, Hart A, Tozer P. Anti-TNF Therapy in Crohn's Disease. Int J Mol Sci. 2018;19(8):2244.
19. Rowaiye AB, Oni SO, Uzochukwu IC, Akpa A, Esimone CO. The Structure-Based Virtual Screening for Natural Compounds that Bind with the Activating Receptors of Natural Killer Cells. Trop J Nat Prod Res. 2021;5(1):145–64.
20. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(42717).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

21. Khosla G, Shukla VK, Sharma V. Characterization of Plumbagin by implying various in silico studies. Int J Health Sci (Qassim). 2022;6(S1):7252-7265.
22. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. Int J Mol Sci. 2019;20(18):4331.
23. Cournia Z, Allen B, Sherman W. Relative Binding Free Energy Calculations in Drug Discovery: Recent Advances and Practical Considerations. J Chem Inf Model. 2017;57(12):2911–37.
24. Mohanty M, Mohanty PS. Molecular docking in organic, inorganic, and hybrid systems: a tutorial review. Monatshefte für Chemie - Chem Mon. 2023;154:683–707.
25. Jamal MS, Parveen S, Beg MA, Suhail M, Chaudhary AGA, Damanhouri GA, Abuzenadah AM, Rehan M. Anticancer compound Plumbagin and its molecular target structural insight into the inhibitory mechanisms using computational approaches. PLoS One. 9(2):e87309.
26. Fu Y, Zhao J, Chen Z. Insights into the Molecular Mechanisms of Protein-Ligand Interactions by Molecular Docking and Molecular Dynamics Simulation: A Case of Oligopeptide Binding Protein. Comput Math Methods Med. 2018;2018(3502514).
27. Iman M, Saadabadi A, Davood A. Molecular docking analysis and molecular dynamics simulation study of ameltolide analogous as a sodium channel blocker. Turkish J Chem. 2015;39:306–16.