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Original Article

IN SILICO STUDY OF ANTICANCER ACTIVITY OF PYRAZOLINE C AND M AS POTENTIAL SELECTIVE OF CYCLOOXYGENASE-2 (COX-2) INHIBITOR USING MOLECULAR DOCKING AND MD SIMULATIONS

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ABSTRACT

Objective: This study has been carried out with an in silico approach to predict interactions between drug candidates and receptor COX-2 (5IKT) and analysed the Molecular Dynamic (MD) simulation.

Methods: The docking procedure was executed with the MolDock algorithm, which was incorporated into Molegro Virtual Docker 5.0, employing the specific docking strategy. MD simulation was analysed with GROMACS 2019 for a duration of 50 nanoseconds. A graph is used to illustrate the interpretation of MD, depicting the Root mean Square Deviation (RMSD) on the backbone, the RMSF on C-alpha, and the Solvent-Accessible Surface Area (SASA) on the protein. This is accomplished via the qtGrace program.

Results: Pyrazoline C and M were used as ligands and celecoxib as a commercial drug. Pyrazoline M was the ligand with the highest affinity (-103.463 Kcal/mol) if compared with Pyrazoline C (-100.900 Kcal/mol), native ligand tolfenamic acid (-87.588 Kcal/mol) and celecoxib (-95.832 Kcal/mol). The molecular dynamics simulation for 50 ns was showed that RMSD, RMSF and SASA rigid and stable.

Conclusion: Pyrazoline C and M was the potential to develop as a breast cancer drug with COX-2 inhibitory activity.

Keywords: Pyrazolines C and M, COX-2, Anticancer drugs, Molecular docking, MD simulation

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INTRODUCTION

Breast cancer ranks as the second most prevalent form of cancer among women, behind cervical cancer. The primary factors that influence the occurrence of breast cancer are changes in lifestyle and dietary habits [1, 2]. Breast cancer often demonstrates an excessive expression of Cyclooxygenase-2 (COX-2). Multiple studies have shown that COX-2 is excessively produced in various types of cancerous tumors in humans. These studies indicate that metabolites originating from COX-2 may play a role in promoting tumor survival, stimulating the excessive growth of precancerous cells, facilitating the formation and advancement of tumors, inducing cell transformation, facilitating the invasion of neighboring tissues, and promoting the metastasis of cancer to other regions of the body. Pyrazoline derivatives are nitrogenous chemical compounds classified as heterocyclic compounds. These compounds display a variety of biological activity. Pyrazoline derivatives have demonstrated anti-cancer efficacy against breast cancer, hepatocellular carcinoma, lung cancer, and breast cancer cells [3-6]. Pyrazoline derivatives have been studied in many cancer cell lines and have demonstrated the ability to inhibit cell proliferation and induce programmed cell death. Prior studies have demonstrated that our synthetically produced N-phenyl pyrazolines possess anti-cancer characteristics that particularly target cells associated with breast cancer and colorectal cancer [2, 7-9]. The aim of this study was to investigate the effectiveness of N-pyrazoline derivative chemicals in inhibiting the production of COX-2, a key player in the metastasis of cancer.

MATERIALS AND METHODS

Ligand 3D preparation

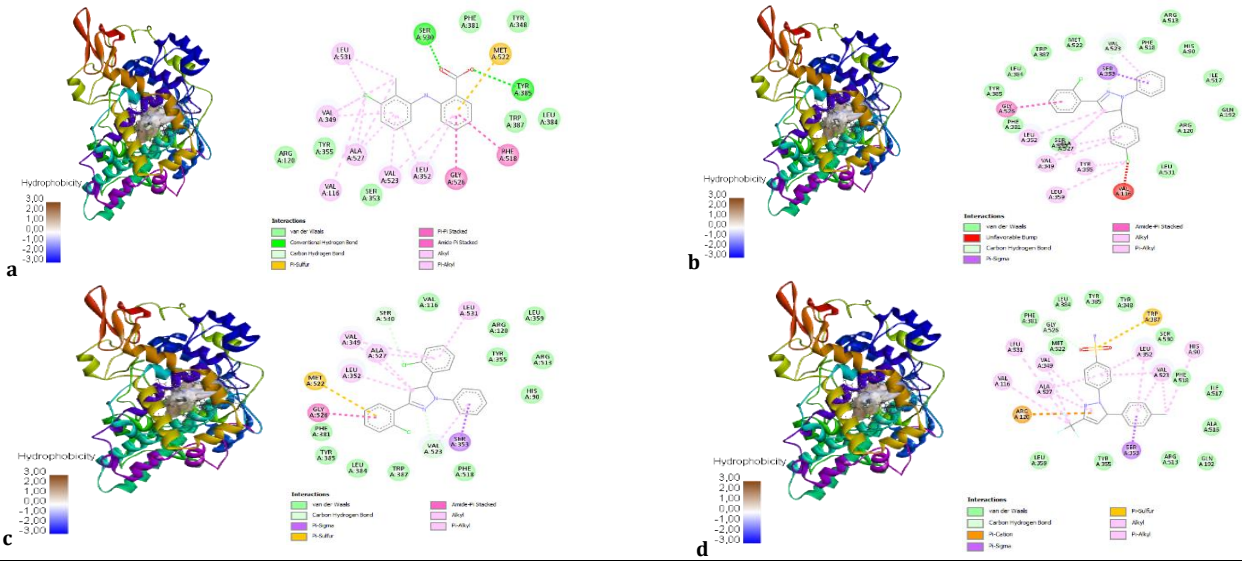
The molecular docking process was carried out using an Aspire Vivobook running on Windows 7 Home Basic. The laptop was equipped with an Intel® CoreTM i5 processor clocked at 3.4 GHz, 64-bit architecture, a 320 GB hard disc drive, and 4 GB DDR3 l RAM. The chemicals Pyrazoline C and Pyrazoline M were shown in their 2D structures using the ChemDraw 18.1 software. Then, the 2D structure is converted into a 3D structure using the chem3D 18.1 application by performing energy minimization (perform MMFF94 minimization) and saving in SDF format [10, 11].

Docking analysis

The 3D conformation of the chosen target proteins was obtained from the RSCB PDB database (https://www.rcsb.org/) for COX-2 (PDB ID: 5IKT), whilst the 3D arrangement of the reference ligand employed was taken from the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/). In addition, the re-docking process was performed via the MolDock algorithm, a particular docking approach that is incorporated into Molegro Virtual Docker 5.0. The grid box size corresponds to the control ligand (native ligand) that has been attached to the PDB protein [12, 13].

Molecular dynamics simulation

The protein and ligand were generated using GROMACS 2019, specifically through topological protein preparations utilizing the pdb2 gmx tool. The protein force field used is AMBER99SB. Acpype is utilized to ascertain the ligand's topology. Furthermore, the procedure encompassed the incorporation of protein and ligand structure, solvent inclusion, ion incorporation, stabilization, optimization, and the implementation of molecular dynamics simulations. The MD manufacturing process lasted for a duration of 50,000 picoseconds, which is comparable to 50 nanoseconds. The graph illustrates the MD interpretation by displaying the RMSD on the backbone, RMSF on C-alpha, and SASA on the protein. The utilization of the qtGrace software facilitates this process [14, 15].



Ligand	Interaction					
	Van der waals	Hydrogen conventional	Hydrogen carbon	Hydrophobic	Other	Unfavorable
Tolfenamic acid	A: TYR348 A: PHE381 A: TRP387 A: LEU384 A: GLY526 A: MET522 A: SER353 A: TYR355 A: ARG120	A: TYR385 A: SER530		A: ALA527 A: VAL349 A: LEU531 A: VAL116 A: VAL523 A: LEU352		
Pyrazoline C	A: TYR385 A: PHE381 A: SER530 A: LEU531 A: ARG120 A: GLN192 A: ILE517 A: HIS90 A: ARG513 A: PHE518 A: MET522 A: TRP387 A: LEU384		A: VAL523	A: GLY526 A: SER353 A: LEU352 A: VAL349 A: TYR355 A: LEU359 A: ALA527 A: VAL523		A: VAL116
Pyrazoline M	A: VAL116 A: ARG120 A: LEU359 A: TYR355 A: ARG513 A: HIS90 A: PHE518 A: TRP387 A: LEU384 A: TYR385 A: PHE381		A: VAL523 A: SER530	A: SER353 A: GLY526 A: VAL349 A: ALA527 A: LEU352 A: VAL349 A: LEU531 A: VAL523	A: MET522	
Celecoxib	A: MET522 A: PHE381 A: LEU384 A: TYR385 A: TYR348 A: SER530 A: PHE518 A: ILE517 A: ALA516 A: GLN192 A: ARG513 A: TYR355 A: LEU359		A: GLY526	A: LEU531 A: VAL349 A: VAL116 A: ALA527 A: LEU352 A: VAL523 A: HIS90 A: SER353	A: ARG120 A: TRP387	

Fig. 1: Visualization of docking results, a) COX2-control protein, b) COX2-pyrazoline C protein, c) COX2-pyrazoline M protein, d) COX2-celecoxib protein. The left part shows the 3D visualization and the right part shows the type of bond produced between the ligand-protein

RESULTS AND DISCUSSION

COX-2 protein docking analysis

Molecular docking is a computer approach to monitoring the formation of stable protein-ligand complexes in a protein's active region [16, 17]. The control RMSD results are in accordance with the standard, which is less than 2.0 Å [18]. The results of molecular docking of COX-2 protein (ID: 5IKT) with test compounds showed that two pyrazoline compounds (pyrazoline C and M) had a stronger binding affinity value than the control ligand Tolfenamic acid [12] and the commercial drug Celecoxib (table 2).

Furthermore, fig. 1 shows the interactions between each ligand and the COX-2 protein. The van der Waals and hydrophobic bonds are the most dominant. Residues with bold fonts are amino acid residues from the control retained by the sample. The analysis showed that Pyrazoline C and M respectively formed the same 9 and 11 amino acids as the control. In comparison, Celecoxib as a commercial drug formed the same 10 amino acids as the control.

Table 1: The grid box settings used are as follows

Protein	X	Y	Z	Radius	RMSD Re-docking	*Binding affinity (kcal/mol)*
5IKT	165.42	185.73	192.38	8	0.93	-87.5877

Table 2: Binding affinity between COX-2 protein and test compounds

Compounds	*Binding Affinity (kcal/mol)*	RMSD (Å)
Tolfenamic acid	-87.588	0.93
Pyrazoline C	-100.900	0.02
Pyrazoline M	-103.463	1.09
Celecoxib	-95.832	0.02

Pyrazoline M exhibits superior COX-2 inhibitory activity compared to Pyrazoline C, tolfenamic acid, and celecoxib, as indicated by the results. The connection between the ligand and protein with the lowest energy exhibits superior inhibitory action [19].

Molecular dynamics simulation

Root mean square deviation *(*RMSD)

A Molecular Dynamics Simulation was conducted for a duration of 50,000 picoseconds to assess the stability of COX-2 during its interaction with various test chemicals, including celecoxib, Pyrazoline C, Pyrazoline M, and tolfenamic acid. The results from MD simulations show that the native protein exhibits a RMSD of around 0.19 nm. Meanwhile, the COX-2 exhibited a slight drop in the RMSD value after its interaction with the test chemicals celecoxib, Pyrazoline C, Pyrazoline M, and tolfenamic acid, with scores of 0.17 nm, 0.15 nm, 0.17 nm, and 0.18 nm respectively (fig. 2). Although there was a drop in the RMSD value, the rise was not substantial, measuring less than 1 nm.

Root mean square fluctuation (RMSF)

We conducted for analysis on the flexibility of the COX-2 enzyme and its interactions with the test substances, as shown in fig. 3. The RMSF of the COX-2 value decreases while interacting with tolfenamic acid, celecoxib, Pyrazoline C, and Pyrazoline M compounds compared to COX-2 without a ligand. The RMSF score is in agreement with the RMSD score [20].

Solvent-Accessible Surface Area (SASA)

The COX-2 compounds lacking a ligand have a surface area of 245.22 nm2, as determined by the SASA measurement. During the interaction between the SASA COX-2 and the test chemicals celecoxib, Pyrazoline C, Pyrazoline M, and tolfenamic acid, there was a minor change in the surface area of 247.74 nm2, 249.84 nm2, 246.22 nm2, and 244.19 nm2, respectively. Nevertheless, this increment is really minimal. An elevation in the SASA value signifies a corresponding rise in the availability of the COX-2 surface (fig. 4) [21].

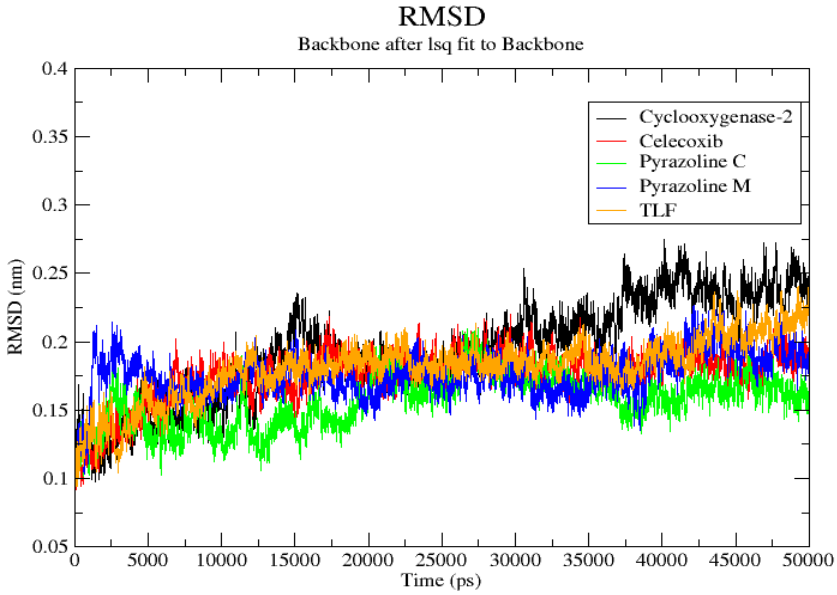


Fig. 2: Root mean square deviation kinase domain of COX-2 when interaction with celecoxib, Pyrazoline C, Pyrazoline M and tolfenamic acid

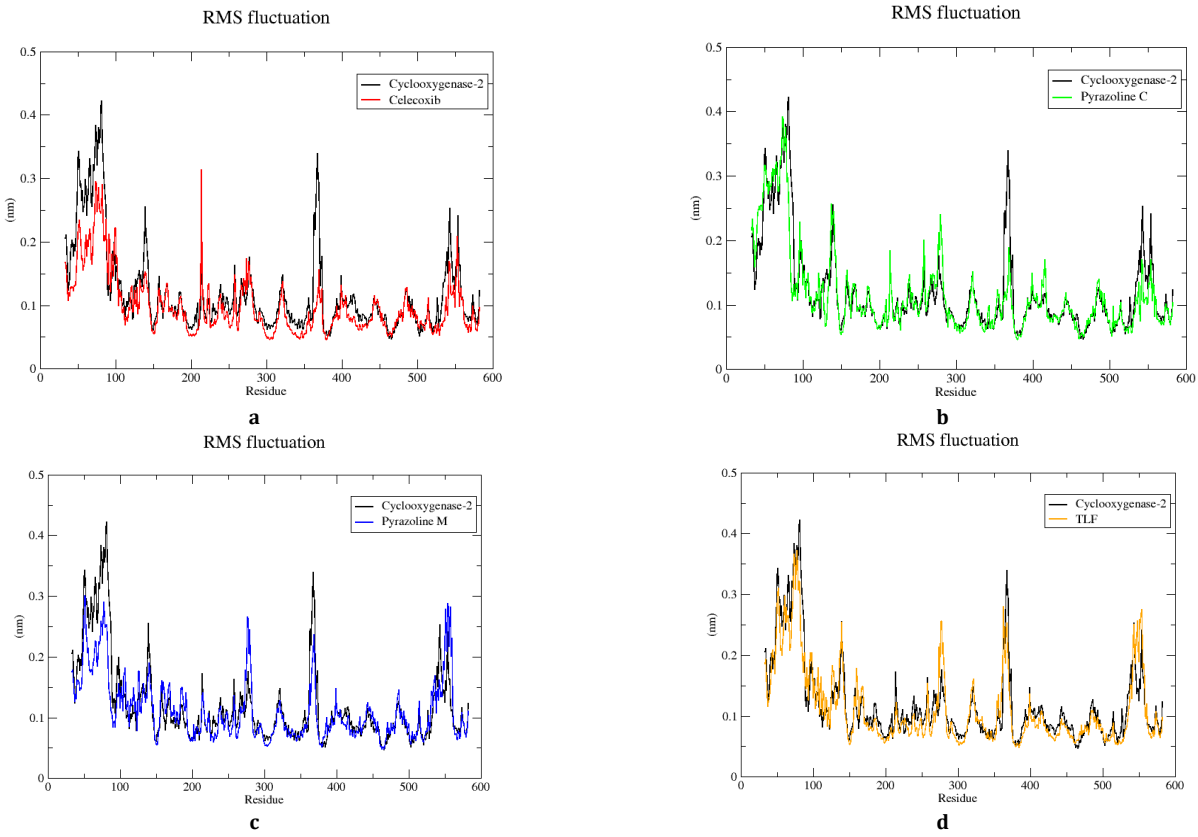


Fig. 3: *Root mean Square Fluctuation* kinase domain of COX-2 when interaction with celecoxib (a), Pyrazoline C (b), Pyrazoline M (c), tolfenamic acid (d)

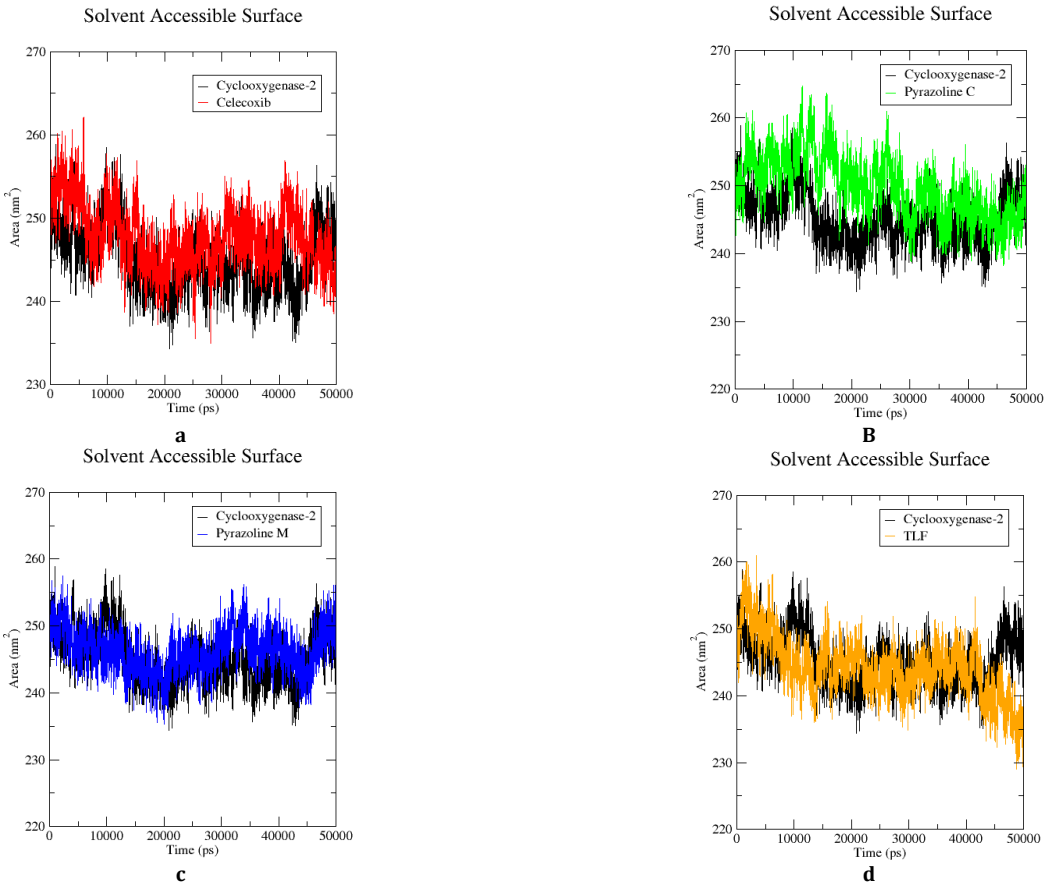


Fig. 4: Solvent-accessible surface area HER-2 when interaction with celecoxib (a), Pyrazoline C (b), Pyrazoline M (c), tolfenamic acid (d)

CONCLUSION

Based on these results, Pyrazoline M was the potential to develop as a breast cancer drug with COX-2 inhibitory activity.

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AUTHORS CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. Conceptualization (Denny Satria); methodology (Denny Satria and Eti Nurwening Sholikhah); software (Syukur Berkat Waruwu); validation (Mustofa); formal analysis (Denny Satria and Pamungkas Bagus Satriyo); investigation (Tutik Dwi Wahyuningsih and Syukur Berkat Waruwu); resources (Hesti l Wiraswati and Denny Satria) data curation (Ema Damayanti and Pamungkas Bagus Satriyo); writing—original draft preparation (Syukur Berkat Waruwu and Mustofa); review (Eti Nurwening Sholikhah and Hesti l Wiraswati); visualization (Syukur Berkat Waruwu); supervision (Ema Damayanti); project administration (Denny Satria); funding acquisition (Eti Nurwening Sholikhah).

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Satria D, Silalahi J, Haro G, Ilyas S, Zaitun Hasibuan PA. Chemical analysis and cytotoxic activity of nhexane fraction of Zanthoxylum acanthopodium DC. fruits. Rasayan J Chem. 2019;12(2):803-8. doi: 10.31788/RJC.2019.1225180.
2. Waskitha SS, Wahyuningsih TD, Sholikhah EN. Potent A EGFR inhibitor, N-phenyl pyrazoline derivative suppresses aggressiveness and cancer stem cell-like phenotype of cervical cancer cells. Drug Des Dev Ther. 2022;20(16):2325-39. doi: 10.2147/DDDT.S350913.
3. Chaudhary M, Kumar N, Baldi A, Chandra R, Babu MA, Madan J. 4-Bromo-4'-Chloro pyrazoline analog of curcumin augmented anticancer activity against human cervical cancer, HeLa cells: in silico-guided analysis, synthesis, and *in vitro* cytotoxicity. J Biomol Struct Dyn. 2020;38(5):1335-53. doi: 10.1080/07391102.2019.1604266, PMID 30957694.
4. Kumari P, Vishnu S, Mishra, Chintam N, Ashish K, Anindita C, Ram S. Design and efficient synthesis of pyrazoline and isoxazole bridged indole c-glycoside hybrids as potential anticancer agents. Scientific Reports. 2020;10(1). doi: 10.1038/s41598-02063377-x.
5. Wang H, Zheng J, Xu W, Chen C, Wei D, Ni W. A new series of cytotoxic pyrazoline derivatives as potential anticancer agents that induce cell cycle arrest and apoptosis. Molecules. 2017;22(10). doi: 10.3390/molecules22101635, PMID 28961210.
6. Yusuf M, Jain P. Synthetic and biological studies of pyrazolines and related heterocyclic compounds. Arab J Chem. 2014;7(5):553-96. doi: 10.1016/j.arabjc.2011.09.013.
7. Suma AA, Wahyuningsih TD, Pranowo D. Synthesis and antibacterial activities of n-phenyl pyrazolines from veratraldehyde. Mater Sci Forum. 2017;901:124-32. doi: 10.4028/www.scientific.net/MSF.901.124.
8. Wahyuningsih TD, Suma AA, Astuti E. Synthesis, anticancer activity, and docking study of N-acetyl pyrazolines from veratraldehyde. J App Pharm Sci. 2019;9(3):14-20. doi: 10.7324/JAPS.2019.90303.
9. Rana M, Arif R, Khan FI, Maurya V, Singh R, Faizan MI. Pyrazoline analogs as potential anticancer agents and their apoptosis, molecular docking, MD simulation, DNA binding and antioxidant studies. Bioorg Chem. 2021;108:104665. doi: 10.1016/j.bioorg.2021.104665, PMID 33571809.
10. Syahputra RA, Harahap U, Dalimunthe A, Nasution P, Haro G, Widodo. In-silico toxicity prediction of bioactive compounds of vernonia amygdalina delile and digoxin. Rasayan J Chem. 2020;13(2):1220-4. doi: 10.31788/RJC.2020.1325638.
11. Satria D, Waruwu SB, Sholikhah EN, Mustofa S, Satriyo PB, Wahyuningsih TD. In silico analysis of n-phenyl pyrazoline derivates as potential of human epidermal growth receptor-2 (her-2) inhibitor using molecular docking and md simulations. RJC. 2024;17(2):356-62. doi: 10.31788/RJC.2024.1728795.
12. Orlando BJ, Malkowski MG. Substrate-selective inhibition of cyclooxygeanse-2 by fenamic acid derivatives is dependent on peroxide tone. J Biol Chem. 2016;291(29):15069-81. doi: 10.1074/jbc.M116.725713. PMID 27226593.
13. Thomsen R, Christensen MH. MolDock: a new technique for high-accuracy molecular docking. J Med Chem. 2006;49(11):3315-21. doi: 10.1021/jm051197e, PMID 16722650.
14. Abraham MJ, Murtola T, Schulz R, Pall S, Smith JC, Hess B. Gromacs: high-performance molecular simulations through multi-level parallelism from laptops to supercomputers. Software X. 2015;1-2:19-25. doi: 10.1016/j.softx.2015.06.001.
15. Lindorff Larsen K, Piana S, Palmo K, Maragakis P, Klepeis JL, Dror RO. Improved side-chain torsion potentials for the amber Ff99SB protein force field. Proteins. 2010;78(8):1950-8. doi: 10.1002/prot.22711, PMID 20408171.
16. Mhatre S, Patravale V. Drug repurposing of triazoles against mucormycosis using molecular docking: a short communication. Comput Biol Med. 2021;136:104722. doi: 10.1016/j.compbiomed.2021.104722, PMID 34358995.
17. Warude BJ, Wagh SN, Chatpalliwar VA, Yildirim M, Celik I, Rudrapal M. Design, docking, MD simulation and in-silco ADMET prediction studies of novel indole-based benzamides targeting estrogen receptor alfa positive for effective breast cancer therapy. Pharmacia. 2023;70(2):307-16. doi: 10.3897/pharmacia.70.e100356.
18. Trott O, Olson AJ. Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. doi: 10.1002/jcc.21334, PMID 19499576.
19. Hasibuan PA, Harahap U, Sitorus P, Lubis MF, Satria D. In silico analysis of vernonioside d and vernonioside e from vernonia amygdalina delile. leaves as an inhibitor of epidermal growth factor receptor (egfr) and mammalian target of rapamycin (mTOR). Rasayan J Chem. 2021;14(3):1539-43. doi: 10.31788/RJC.2021.1436092.
20. Huang Y, Zhang X, Suo H. Interaction between β-lactoglobulin and EGCG under high-pressure by molecular dynamics simulation. PLOS ONE. 2021;16(12):e0255866. doi: 10.1371/journal.pone.0255866, PMID 34932559.
21. Krebs B. Baumgarten, and Joelma freire de Mesquita. "Amyotrophic lateral sclerosis type 20-in silico analysis and molecular dynamics simulation of HnRNPA1. Plos One. 2016;11(7):1-18. doi: 10.1371/journal.pone.0158939.