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Cinnamamide Derivatives as Anticancer Agent: Study of Molecular Docking, Molecular Dynamic Simulation, and ADMET Properties

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Cinnamamide has been known to have many prospect activities such as anti-inflammatory, antitubercular, anti-melanogenic, antitubulin, antidepressant, and anticancer. As an anticancer, there are 12 derivative compounds synthesized and tested in vitro against P388 cell. This study focuses the *in silico* analysis to know the interaction mechanism between cinnamamide derivatives and P-glycoprotein substrate through molecular docking, molecular dynamic simulation, and ADMET properties. Molecular docking revealed that compound **10** has a lower binding energy than the others about -5.57 kcal/mol and have a hydrogen bond interaction with Trp231 residue. Molecular dynamic simulation between compound **10** and the standard (ZQU) show a similar binding energy through MM-PBSA method. ADMET properties calculation show that some compounds satisfy the minimum standard parameters in ADMET properties.

Keywords: Cinnamamide; anticancer; molecular docking; molecular dynamic; ADMET

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Cancer is classified as a non-communicable disease and can occur due to the abnormal proliferation of various types of cells in the body [1]. Therefore, cancer being one of the leading causes of death in the worldwide [2]. According to estimates from the Global Cancer Burden, by 2040, there are 27.5 million new cancer cases and 16.3 million cancer deaths simply due to the growth and aging of the population. Chemotherapy is one of the most cancer treatments. However, a significant number of patients develop resistance to a broad spectrum of structurally anti-cancer drugs [3].

P-glycoprotein (P-gp) or ATP-binding cassette sub-family B (ABCB) plays a crucial role in determining response against medications, including cancer therapeutics. P-gp acts as an ATP dependent pump that pumps out small molecules from cells. In the upcoming years, several other proteins of the ABC transporter family were identified that shared a common mechanism of conferring chemotherapy resistance by drug efflux [4]. However, p-gp remains the best studied and most potent ABC transporter to induce chemoresistance [5]. None of P-gp inhibitor has passed clinical trials due to their high side-effects and approved by the US Food and Drug Administration (FDA) [6]. Hence, the scientists are searching for alternatives options, such as plant-based P-gp inhibitors because these products could be the future potential drug candidates for further research as P-gp inhibitors and more effective and less toxic [7].

Natural products are known for their low toxicity and higher specificity towards P-gp [8]. Several phytochemicals such as flavonoids and phenolic acids get much attention due to their antioxidant, anti-tumor, and anticancer activity. Cinnamamide is one example of cinnamic acid derivative compounds that showed a high potential to be developed as anti-inflammatory [9], antitubercular [10], anti-melanogenic [11], antitubulin [12], antidepressant [13], and anti-cancer [14-15].

As an anticancer Firdaus et al had been synthesized and tested cinnamamide twelve derivatives as anticancer for P388 cell lines since 2019 until 2022 [16-18], however some of synthesized compounds had not been studied the in silico analysis. Recently, computational methods are rapidly growing and play an important role in drug discoveries [19]. Duo to these findings, we investigated new potential cinnamamide derivative compounds with computer-based analyses by molecular docking study, molecular dynamic, and evaluated by ADMET properties [20]. By in silico and ADMET predictions, we could evaluate the pharmaco-kinetic and toxicity properties of compounds.

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MATERIALS AND METHODS

Materials

In this research, there are 12 cinnamamide derivatives used as ligands. All of ligands had been synthesized and tested as anticancer using P388 cell lines (Table 1). Protein structure of P-glycoprotein was downloaded from protein data bank website and entering code 6FN4.

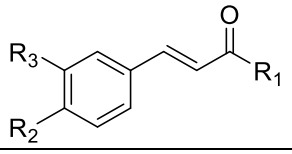
Ligand and Protein Preparation

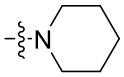
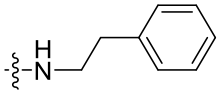
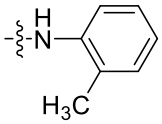
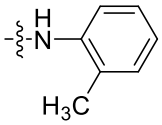
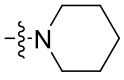
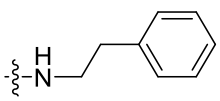
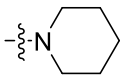
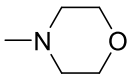
All of ligands were modelled and optimized by choosing GAFF method [21] in the Avogadro software [22]. Structure of P-glycoprotein were cleaning in Chimera software [23] by removing the water and standard ligand. Protein structure was added hydrogen and charges using Dock prep tools and then saved as pdb format file.

Molecular Docking

Molecular docking analysis of twelve ligands was done using AutoDock4 and AutoDockTools [24]. All ligands were set to be in the active site of the protein. The grid size used was 40 x 40 x 40 Å and spacing was 0.375 Å. Each complex was set to produce 10 conformations by choosing the Lamarckian Genetic Algorithm [25] and the best conformation was selected by the energy ranking which is the lowest energy belongs to the best conformation. Interaction between ligand and protein was visualized by using Discovery Studio Visualizer program [26].

Table 1. Structure of cinnamamide derivatives



Compounds	R_1	R_2	R_3	IC_{50}	References
1	$-NH_2$	-OH	-H	44	[16]
2	$-NHC_3H_7$	-OH	-H	53.56	[27]
3	$-N(C_2H_5)_2$	-OH	-H	23.50	[27]
4		-OH	-H	5.34	[18]
5		-OH	$-OCH_3$	29.14	[14]
6		-OH	-OH	0.91	[28]
7		-OH	-H	16.57	[28]
8	$-NH(C_4H_9)$	-OH	-H	0.609	[27]
9		-OH	-OH	0.86	[18]
10		-OAc	-OAc	0.5	[29]
11		-OH	$-OCH_3$	46.67	[17]
12		-OH	$-OCH_3$	57.10	[17]

ADMET Properties

There were 5 ligands (**4**, **6**, **7**, **10**, **11**) calculated for ADMET properties, those ligands were choosing due to the lower binding energy than the other ligands and compared to the native ligand, ZQU. All of ligand structure were converted into smiles format using Open Babel program [30]. The smiles format of each ligand was inserted to SwissADME to calculate drug likeness and pharmacokinetic properties [31], and also inserted to pkCSM web server to calculate toxicity properties [32].

Molecular Dynamic Simulation Protocol

Molecular dynamic simulation was performed for the complex between ligands **10** and standard ligand (ZQU) against P-glycoprotein and single protein structure without ligand. Complex of ligand **10** was chose due to the lower binding energy result in molecular docking analysis than the other cinnamamide derivatives. Totally there were 3 system in this simulation. All of simulation was conducting by using YASARA Dynamics program (YASARA Biosciences GmBH, Vienna, Austria) [33]. Simulation was performed by using Amber14 as the force field [34] and the system was set to have a physiological condition under pH 7.4 and temperature about 310 K. All of system run until 10 ns in a TIP3P solvent by adding counter ion NaCl to neutralize the charge in the system. [35] Trajectory data was used to analyze RMSD, RMSF, radius of gyration, and energy of each ligand.

RESULT AND DISCUSSION

Molecular Docking Analysis

Study of molecular docking for all compounds was started by redocking analysis using the native ligand (ZQU). Result of redocking analysis was depicted in Figure 1 and it was confirmed a successful redocking analysis due to the lower RMSD value about 0.609 Å. This low value is due to the good superimpose position of ligand. Successful redocking analysis achieved when the RMSD value is lower than 2 Å and indicated the method used had been validated [36].

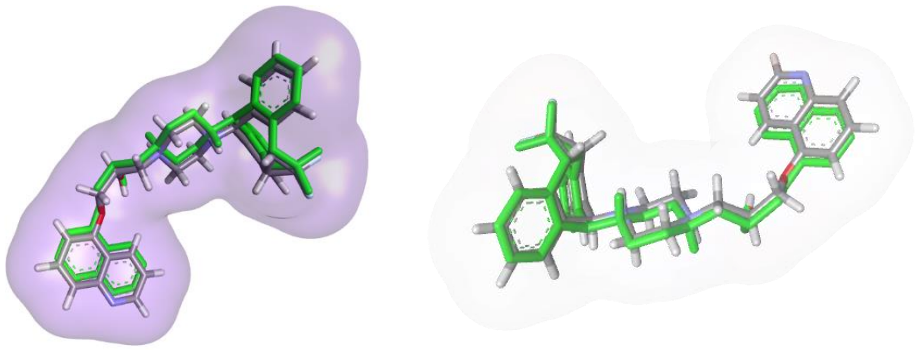


Figure 1. Superimpose of the native ligand (ZQU) in the P-glycoprotein structure (grey: conformation before docking, green: conformation after docking)

Table 2. Molecular docking result of cinnamamide derivatives in P-glycoprotein substrate

Compounds	Binding Energy (kcal/mol)	Inhibition Constant (μM)	Hydrogen bond interaction residue (Å)
1	-4.10	987.62	Ile399 (3.83); Gln346 (3.98); Tyr309 (5.44)
2	-3.90	139.00	Tyr309 (1.9)
3	-3.99	118.00	Tyr309 (1.9)
4	-5.13	173.81	Tyr309 (1.98)
5	-4.83	286.97	Trp231 (1.76)
6	-5.05	199.79	Tyr309 (1.8)
7	-5.40	110.62	Tyr309 (1.99)
8	-4.36	637.63	Ile339 (2.24)
9	-4.75	330.00	Glu874 (1.98; 1.85)
10	-5.57	83.04	Trp231 (2.07)
11	-5.07	191.58	Trp231 (1.85)
12	-4.46	539.27	Gln46 (1.82)
ZQU	-8.17	103.00	Trp231(2.96); Gln945(2.70)

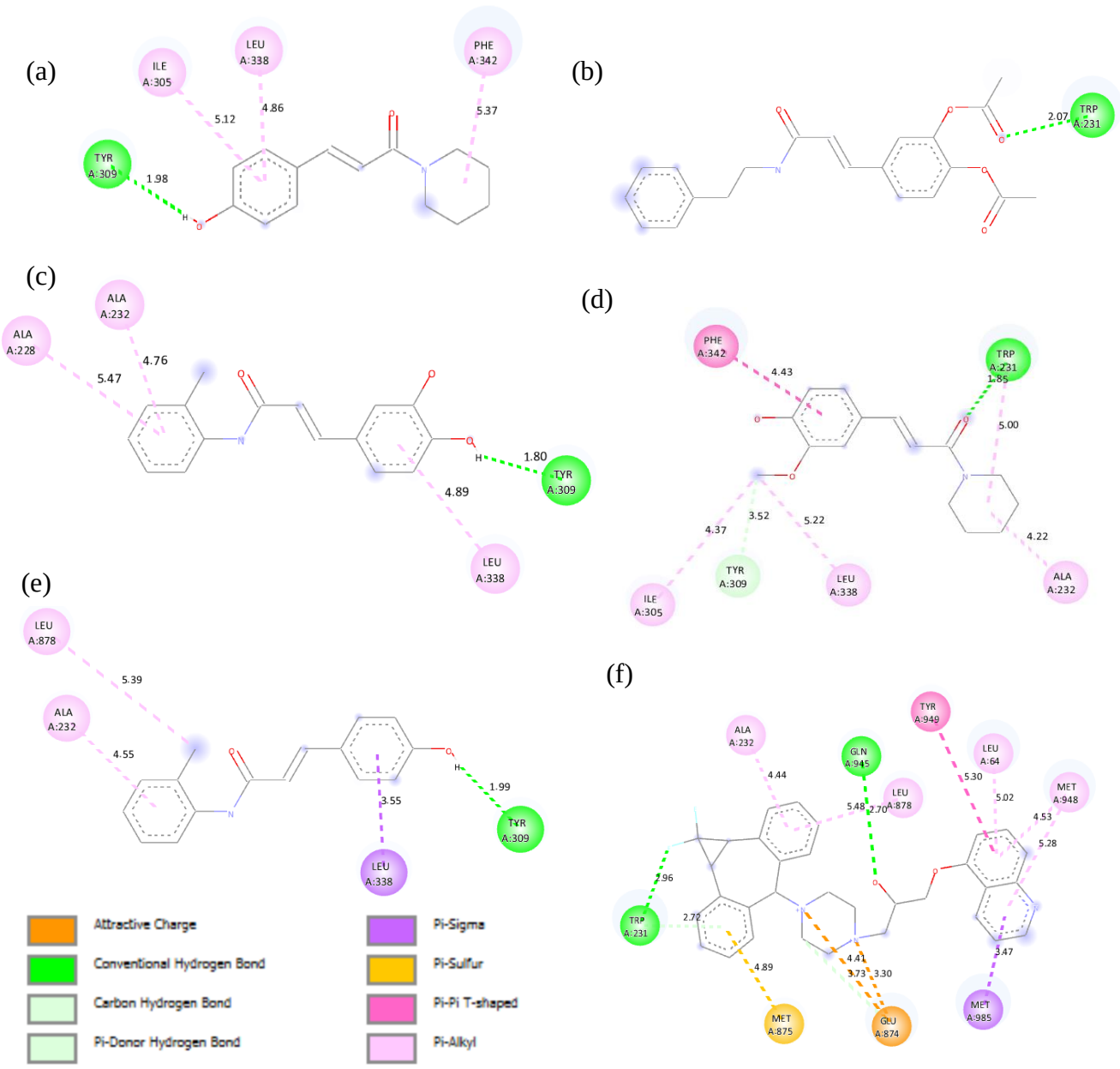


Figure 2. Interaction of coumaramide derivatives ligand (a-f) continued is 4**, 6, 7, 10, 11,** and **ZQU** compound on binding side of P-glycoprotein

After reaching a validated molecular docking method, twelve ligands of cinnamamide derivatives were docked into the active site of P-glycoprotein. Result of molecular docking was shown in Table 2 and all cinnamamide derivatives were compared to the native ligand (ZQU). Binding energy resulted in molecular docking showed that all cinnamamide derivatives had a higher energy value than ZQU, however there were 5 compounds (**4**, **6**, **7**, **10**, **11**) had a binding energy about -5 kcal/mol and indicating a potential property as an inhibitor [37]. The interaction found between ligands and protein are found as hydrogen bond and dominated by electron stacking interaction. Two-dimension interaction was shown in Figure 2 for some of ligands which is had a lower binding energy. All ligands had a hydrogen bond interaction with some of amino acid residue such as Tyr309, Trp231, Gln346, etc. However, most of ligands showed an interaction in the amino acid residue Trp231, the same interaction residue indicating the similar conformation of ligand in the active site of P-glycoprotein.

ADMET Properties

Calculation of ADMET properties had been conducted to the five compounds which had a lower binding energy in the molecular docking analysis. Result of ADMET calculation was shown in Table 3. There are four properties calculated to confirm the drug likeness property of each ligand based on the Lipinski rule [38].

Table 3. Result of ADMET calculation properties

Parameters	**4**	**9**	**10**	**13**	**14**	**ZQU**
			Drug likeness			
Molecular Weight (g/mol)	231.29	269.3	253.30	367.40	261.32	529.62
Log P	1.90	2.24	2.82	2.97	1.57	-3.71
Num. H-bond acceptors	2	3	2	5	3	5
Num. H-bond donors	1	3	2	1	1	3
Violation	0	0	0	0	0	1
			Pharmacokinetics			
GI absorption	High	High	High	High	High	High
BBB permeant	Yes	Yes	Yes	No	Yes	Yes
P-gp substrate	No	No	No	No	No	No
CYP1A2 inhibitor	No	Yes	Yes	Yes	No	No
CYP2C19 inhibitor	Yes	No	No	Yes	Yes	No
CYP2C9 inhibitor	No	Yes	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	Yes	Yes	No	No	No
Log Kp (skin permeation, cm/s)	-6.02	-5.93	-6.01	-6.46	-6.27	-6.04
			Toxicity			
AMES Toxicity	No	No	Yes	No	No	No
Max. Tolerated dose	0.551	0.269	0.637	0.332	0.454	-0.152
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	No	Yes	No	Yes	No	Yes
Oral Rat Acute	2.163	1.942	1.923	2.301	2.225	2.777
Oral Rat Chronic Toxicity	1.614	1.034	1.569	1.948	1.270	1.164
Hepatoxicity	No	No	No	Yes	No	Yes
Skin sensititation	No	No	No	No	No	No
T. Pyriformis toxicity	1.202	1.244	1.473	1.187	1.188	0.285
Minnow toxicity	1.649	1.314	0.774	1.075	1.798	1.128

All ligands fulfill all the minimum standard parameter, whereas **ZQU** presented one violation in the parameter of drug likeness due to the higher molecular weight. All ligands also tested their pharmacokinetics property and showed the high absorption in gastrointestinal. In the parameter of blood brain barrier, only compound **13** was not fulfil this parameter and all ligands were not acted as P-gp substrate. Prediction of toxicity property were confirmed by some parameters, compound **10** showed a toxicity property in AMES parameters but did not toxic for liver and skin.

Molecular Dynamic Simulation

Molecular dynamic simulation was performed to the complex between P-glycoprotein and ligand **10** as this ligand had a lower binding energy in the result of molecular docking. Complex of native ligand (ZQU) also evaluated and compared with single protein structure, P-glycoprotein. Binding energy of each ligand was calculated using MM-PBSA method and showed that binding energy of ligand **10** is quite similar with ZQU in the P-glycoprotein indicating that compound 10 is potential to be used as P-glycoprotein inhibitor (Table 4).

Table 4. Binding energy result using MM-PBSA method.

Energy	ZQU	**10**
E_{pot} Receptor	-40055.8	-40666.7
E_{solv} Receptor	-118406	-118139
E_{pot} Ligand	-40086.1	-40402.6
E_{solv} Ligand	-118602	-118139
E_{pot} Complex	-40055.8	-40666.7
E_{solv} Complex	-118406	-118139
Binding Energy [kJ/mol]	-158689	-158542

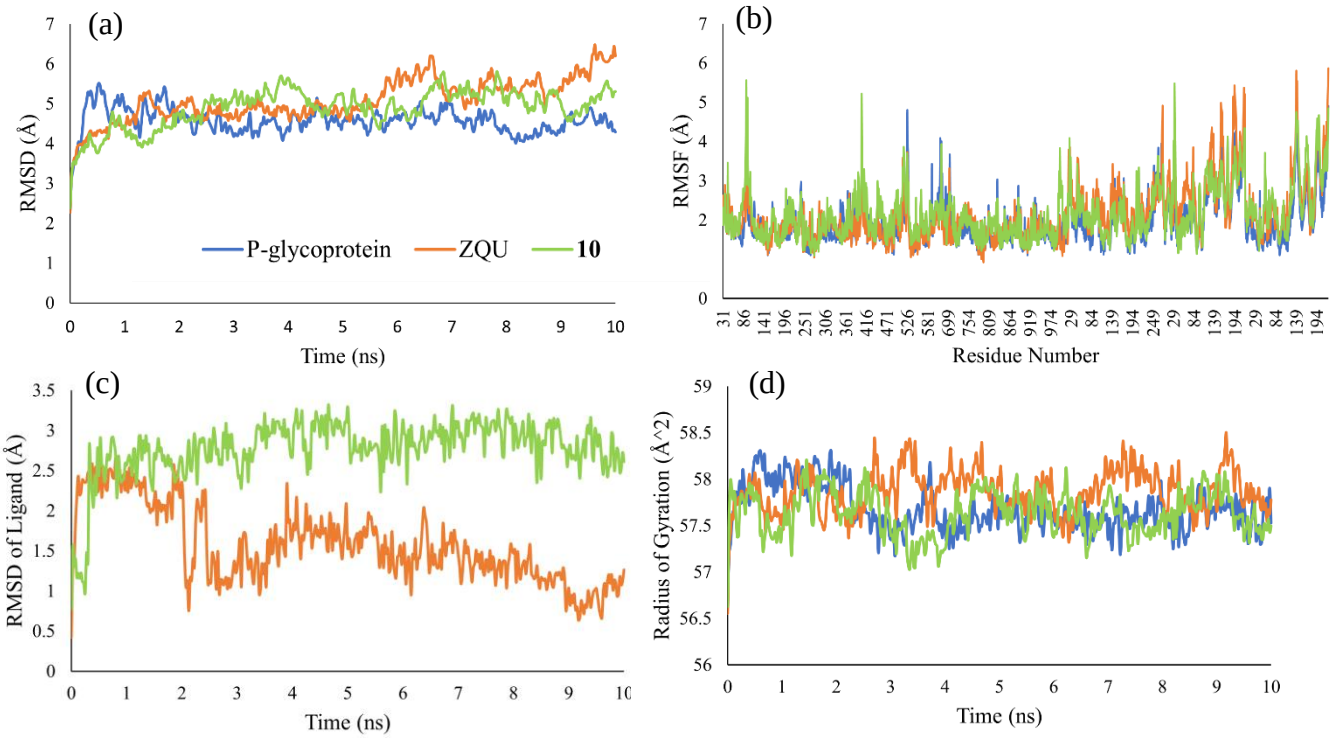


Figure 3. Molecular dynamic simulation result of single and complex P-glycoprotein (a) RMSD total, (b) RMSF (c) RMSD of ligand, and (d) radius of gyration graph.

Complex stability was evaluated using some of parameters such as RMSD, RMSF, RMSD of ligand, and radius of gyration (Figure 3). RMSD result of each complex showed a high deviation, it can be caused the structure of original protein. It was seen that in the beginning of simulation time, RMSD of P-glycoprotein was high about 5 until 6 Å (Fig.3a). Root mean square fluctuation (RMSF) is a score that provides a more detailed information about conformation change based on the fluctuation at the amino acid residue. Figure 3b showed that fluctuation of P-gp structure in both of single and complex structure showed a high value about 3 Å and it was predicted that there was a relaxation of bonds that can initiated an unfolding conformation of the protein target. It was in line with the result of radius of gyration (RG) (Fig. 3d), it was seen that addition of ZQU in the P-gp structure could make a significance change in the RG of P-gp structure, however addition of compound 10 did not change radius of gyration pattern of P-gp structure. Comparing the deviation of ligand conformation was seen in the Fig.3c which shown that RMSD value of ZAU is lower than compound 10, it can be caused due to the structure of ZQU is more planar and compact than compound 10, but both of compounds still give a low RMSD value about 2.5 Å.

CONCLUSIONS

In silico study of cinnamamide derivatives as anticancer agent had been done using molecular docking, ADMET properties, and molecular dynamic simulation. Binding energy resulted in molecular docking showed that all cinnamamide derivative had a higher energy than native ligand or ZQU, however there were 5 compounds presented an energy around -5 kcal/mol. These five compounds continued to test the ADMET properties and showed that all compounds fulfill minimum standard parameter in drug likeness property. Molecular dynamic simulation was attempted to single and complex P-glycoprotein with **10** and ZQU and showed that binding energy of compound **10** is quite similar with ZQU as standard ligand indicating that compound **10** is potential as anticancer agent.

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REFERENCES

1. Cooper, J. and Giancotti, F. G. (2019) Integrin Signaling in Cancer: Mechanotransduction, Stemness, Epithelial Plasticity, and Therapeutic Resistance. *Cancer Cell*, **35**, 3, 347–367. doi: https:// doi.org/10.1016/j.ccell.2019.01.007.

2. Zaorsky, N. G., *et al.* (2017) Causes of death among cancer patients. *Ann. Oncol.*, **28**, 2, 400–407. doi: https://doi.org/10.1093/annonc/mdw604.

3. Armesto, M., *et al.* (2021) Integrated mRNA and miRNA Transcriptomic Analyses Reveals

Divergent Mechanisms of Sunitinib Resistance in Clear Cell Renal Cell Carcinoma (ccRCC). *Cancers*, **13**, 17. doi: 10.3390/cancers13174401.

4. Sun, Y. L., Patel, A., Kumar, P. and Chen, Z. S. (2012) Role of ABC transporters in cancer chemotherapy. *Chin. J. Cancer*, **31**, 2, 51–57. doi: 10.5732/cjc.011.10466.

5. Leonard, G. D., Fojo, T. and Bates, S. E. (2003) The Role of ABC Transporters in Clinical Practice. *Oncologist*, **8**, 5, 411–424, Oct. 2003. doi: 10.1634/theoncologist.8-5-411.

6. Waghray, D. and Zhang, Q. (2017) Inhibit or Evade Multidrug Resistance P-glycoprotein in Cancer Treatment. *J Med Chem*, **176**, 3, 139–148. doi: 10.1021/acs.jmedchem.7b01457.Inhibit.

7. Yu, J., Zhou, P., Asenso, J., Yang, X. D., Wang, C. and Wei, W. (2016) Advances in plant-based inhibitors of P-glycoprotein. *J. Enzyme Inhib. Med. Chem.*, **31**, 6, 867–881. doi: 10.3109/14756366.2016.1149476.

8. Dewanjee, S., *et al.* (2017) Natural products as alternative choices for P-glycoprotein (P-gp) inhibition. *Molecules*, **22**, 6, 1–93. doi: 10.3390/molecules22060871.

9. Chen, G., *et al.* (2016) Discovery of a New Inhibitor of Myeloid Differentiation 2 from Cinnamamide Derivatives with Anti-Inflammatory Activity in Sepsis and Acute Lung Injury. *J. Med. Chem.*, **59**, 6, 2436–2451, Mar. 2016. doi: 10.1021/acs.jmedchem.5b01574.

10. Patel, K. N. and Telvekar, V. N. (2014) Design, synthesis and antitubercular evaluation of novel series of N-[4-(piperazin-1-yl)phenyl]cinnamamide derivatives. *Eur. J. Med. Chem.*, **75**, 43–56. doi: 10.1016/j.ejmech.2014.01.024.

11. Ullah, S., *et al.* (2018) Design, synthesis and anti-melanogenic effect of cinnamamide derivatives. *Bioorg. Med. Chem.*, **26**, 21, 5672–5681. doi: https://doi.org/10.1016/j.bmc.2018.10.014.

12. Wang, S. -F., *et al.* (2015) Synthesis, biological evaluation and 3D-QSAR studies of novel 5-phenyl-1H-pyrazol cinnamamide derivatives as novel antitubulin agents. *Eur. J. Med. Chem.*, **93**, 291–299. doi: https://doi.org/10.1016/j.ejmech.2015.02.018.

13. Han, M., *et al.* (2014) Synthesis and structure–activity relationship of novel cinnamamide derivatives as antidepressant agents. *Bioorg. Med. Chem. Lett.*, **24**, 22, 5284–5287. doi: https://doi.org/10.1016/j.bmcl.2014.09.050.

14. Firdaus, N. H., Soekamto, Seniwati, Islam, M. F. and Sultan (2018) Phenethyl ester and amide of Ferulic Acids: Synthesis and bioactivity against P388 Leukemia Murine Cells. *J. Phys. Conf. Ser.*, **979**, 1. doi: 10.1088/1742-6596/979/1/012016.

15. Ruwizhi, N. and Aderibigbe, B. A. (2020) Cinnamic acid derivatives and their biological efficacy. *Int. J. Mol. Sci.*, **21**, 16, 1–36. doi: 10.3390/ijms21165712.

16. Firdaus, Hariani Soekamto, N. and Karim, A. (2009) Sintesis Senyawa p-Hidroksisinamamida dari Asam p-Hidroksisinamat Melalui Reaksi Esterifikasi dan Amonolisis. *J. Chem. Acta*, **2**, 2.

17. Firdaus, *et al.* (2017) Synthesis of Piperidine and M orpholine Amides o f F erulic Acid and their Bioactivity a gainst P - 388 Leukemia Cells. *Int. J. Chem. Tech. Res.*, **10**, 27–33.

18. Firdaus, Soekamto N. H., Firdausiah, S. and Tahar, M. (2021) A Caffeic Acid Derivative Potential for Anticancer. *Indones. Chim. Acta*, **13**, 2, 38–42. doi: 10.20956/ica.v13i2.10962.

19 Yalcin, S. (2020) Molecular Docking, Drug Likeness, and ADMET Analyses of Passiflora Compounds as P-Glycoprotein (P-gp) Inhibitor for the Treatment of Cancer. *Curr. Pharmacol. Reports*, **6**, 6, 429–440. doi: 10.1007/s40495-020-00241-6.

20. Kamilah, T., Nazmi, T., Aminudin, N. I. and Hamzah, N. (2022) Molecular Docking and ADME Profiling of Xanthorrhizol Derivatives as Hyaluronidase Inhibitors. *Malaysian J. Chem.*, **24**, 2, 120–130.

21. Wang, J., Wolf, R. M., Caldwell, J. W., Kollman, P. A. and Case, D. A. (2004) Development and testing of a general AMBER force field. *J. Comput. Chem.*, **25**, 1157–1174.

22. Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeersch, T., Zurek, E. and Hutchison, G. R. (2012) Avogadro : an advanced semantic chemical editor , visualization, and analysis platform. *J. Cheminform.*, **4**, 17, 1–17.

23. Pettersen, E. F., *et al.* (2004) UCSF Chimera – A Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.*, **25**, 1605–1612.

24. Morris, G. M., *et al.* (2009) AutoDock4 and Auto DockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.*, **30**, 16, 2785–2791. doi: 10.1016/j.earlhumdev.2006.05.022.

25. Morris, G. M., *et al.* (1998) Automated docking using a Lamarckian genetic algorithm and an

empirical binding free energy function. *J. Comput. Chem.*, **19**, 14, 1639–1662. doi: 10.1002/(SICI)1096-987X(19981115)19:14<1639::AID-CC10>3.0.CO;2-B.

26. Dassault Systemes (2019) Biovia Discovery Studio Visualizer, San Diego.

27. Firdaus, *et al.* (2012) Sintesis senyawa turunan sekunder dan tersier p-Kumaramida dan uji aktivitasnya sebagai anti tumor sel leukimia P388. *Indones. Chim. Acta*, **5**, 2, 10–16.

28. Firdaus, Seniwati, Alamsyah, N. and Paramita, S. (2019) Synthesis and activity of N-(o-tolyl) caffeamide and N-(o-tolyl)-p-coumaramide against P388 leukemia murine cells. *J. Phys. Conf. Ser.*, **1341**, 3. doi: 10.1088/1742-6596/1341/3/032005.

29. Firdaus, Fattah, A. and Soekamto, N. H. (2020) Synthesis of a Cinnamic Acid Derivative and Bioactivity as an Anticancer Based on Result Quantitative Structure Activity Relationship (QSAR) Analysis. *Indones. Chim. Acta*, **13**, 1, 23–29.

30. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T. and Hutchison, G. R. (2011) Open Babel: An open chemical toolbox. *J. Cheminform.*, **3**, 33, 1–14. [Online]. Available: https://jcheminf.biomedcentral.com/track/pdf/10.1186/1758-2946-3-33.

31. Daina, A., Michielin, O. and Zoete, V. (2017) SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.*, **7**, 1–13, January. doi: 10.1038/srep42717.

32. Pires, D. E. V., Blundell, T. L. and Ascher, D. B. (2015) pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J. Med. Chem.*, **58**, 9, 4066–4072, May 2015. doi: 10.1021/acs.jmedchem.5b00104.

33. Land, H. and Humble, M. S. (2018) YASARA: A Tool to Obtain Structural Guidance in Biocatalytic Investigations BT - Protein Engineering: Methods and Protocols. *U. T. Bornscheuer and M. Höhne, Eds. New York, NY: Springer New York*, 43–67.

34. Case, D. A., *et al. (*2016) *AMBER 2016*. University of California, San Fransisco.

35. Mark, P. and Nilsson, L. (2001) Structure and dynamics of the TIP3P, SPC, and SPC/E water models at 298 K. *J. Phys. Chem. A*, **105**, 43, 9954–9960. doi: 10.1021/jp003020w.

36. Huey, R., Morris, G. M., Olson, A. J. and Goodsell, D. S. (2007) A Semiempirical Free Energy Force Field with Charge-Based Desolvation. *J. Comput. Chem.*, **28**, 6, 1145–1152. doi: 10.1002/jcc.

37. Yin, X. S., Zhang, X. Q., Yin, J. T., Kong, D. Z. and Li, D. Q. (2019) Screening and identification of potential tyrosinase inhibitors from Semen Oroxyli extract by ultrafiltration LC-MS and in silico molecular docking. *J. Chromatogr. Sci.*, **57**, 9, 838–846. doi: 10.1093/chromsci/bmz054.

38. Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (2001) Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Deliv. Rev.*, **46**, 3–26. doi: 10.1016/S0169-409X(00)00129-0.