Pharmacy Education (2024) 24(3) 178 - 184
https://doi.org/10.46542/pe.2024.243.178184

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Docking study of ferulic acid derivates on FGFR1, ADME prediction, and QSPR analysis

Darwin Riyan Ramadhan[1], Juni Ekowati[2,3], Denayu Pebrianti[1], Farrah Yulian Listyandi[1], Nuzul Wahyuning Diyah[2], Muhammad Faris Adrianto[2,4], Deepakkumar Mishra[4]

[1] Bachelor Programme Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3] Drug Development Research Group, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[4] School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom

Keywords
ADME
Good health
Ferulic acid
FGFR1
QSPR
Well-being

Correspondence
Juni Ekowati
Department of Pharmaceutical Sciences
Faculty of Pharmacy
Universitas Airlangga
Surabaya
Indonesia
juni-e@ff.unair.ac.id

Abstract

Background: FGFR-1 is an angiogenic receptor that plays a huge role in the cancer growth pathway. Angiogenesis inhibitory drugs released have significant side effects. Therefore, research into discovering anti-angiogenic agents to achieve good health and well-being is still necessary. **Objective:** To design the novel anti-angiogenic candidates from ferulic acid (FA) by docking study on FGFR1, to predict the ADME profile, and to find out the structural relationship of their pharmacokinetic properties as QSPR analysis. **Method:** Autodock Tools performed a docking study. ADME prediction was conducted using SwissADME. The MLR approach determined the QSPR model. **Result:** The docking results showed that FA-8 and FA-18 had the lowest free energy binding, inhibition constant, and GI absorption. The QSPR analysis obtained the equation model: Log HIA = 0,018 Log P^2 + 0,069 Log P + 0,020 CMR + 0,001 Etotal + 1,771 with n = 24, correlation coefficient (r)= 0.621, p-value= 0.046 and F-value= 2.975. **Conclusion:** Modifying FA on the phenolic moiety replaced by an ester increased the activity and ADME profile. The predicted bioavailability was supported by high Log P, molar refractivity (CMR), and electronic factor (Etotal). It is recommended that lipophilicity be increased to achieve better pharmacokinetic properties.

Introduction

Cancer is a major public health problem in the world and the second leading cause of death (Siegel *et al.*, 2022). Several mechanisms of cancer growth include self-sufficiency in growth signals, decreased sensitivity of growth inhibitory (antigrowth) factors, reduced apoptotic systems, continuous angiogenesis, and metastasis. Angiogenesis is one of the most important stages of cancer growth (Gotink & Verheul, 2010). Angiogenesis is the process of developing new blood vessels that will provide a pathway for tumour cell needs through the mechanism of endothelial cell branching from existing blood vessels (Wang *et al.*, 2012). Angiogenesis-inhibitory drugs have been used to reduce cancer growth, such as the monoclonal antibodies Avastin, celecoxib, sunitinib, and sorafenib (Gotink & Verheul, 2010). However, these drugs have shown major problems such as acquired resistance and bleeding (Bergers & Hanahan, 2008). Therefore, finding alternative compounds for cancer therapy through the angiogenesis pathway is important.

Ferulic acid (FA) is an alternative compound as an angiogenesis inhibitor with the mechanism of inhibiting the VEGF2 pathway (Ekowati *et al.*, 2020) and the FGF1 pathway (Yang *et al.*, 2015). FA inhibition in the VEGF2 pathway has been investigated using the silico method with molecular docking by Ekowati *et al.* (2020). This method utilised the VEGFR2 receptor or vascular endothelial growth factor receptor-2, and the results showed that the prediction of FA activity was still lower than that of standard drugs. Research conducted by Yang *et al.* (2015) showed that the FA inhibitory effect on the FGFR1 receptor was relatively high in vitro and in vivo, with an inhibition percentage of 92% at 1µM

concentration. Previous studies indicated that ferulic acid has the potential to act as an anticancer by inhibiting the angiogenesis pathway.

Apart from predicting anticancer activity, it is important to predict pharmacokinetic properties to determine bioavailability. In addition, QSPR is also important since it is one of the bases for developing drug compound derivatives (Hu *et al*., 2010). Therefore, this present study is aimed at developing new anti-angiogenic drug candidates from Ferulic acid (FA) derivates by docking study on FGFR1, evaluation of absorption, distribution, metabolism, and excretion (ADME), and finding out the structural relationship of their pharmacokinetic properties as quantitative structure-property relationships (QSPR) analysis.

Methods

Receptor

The receptor used is FGFR1. The molecular structure was obtained from the protein data bank (PDB) with PDB ID 4UWC, which contains 3,4-dimethoxy-N-ligand (5-phenyl-1H-pyrazol-3-yl) benzamide (ligand code: 4Y0) in chain A.

Ligand

The structure of the ligand (FA1-24) was drawn using the ChemDraw application version 17.1 (Chemoffice) and then copied into the Chem3D application version 17.1 (Chemoffice) to create the 3D structure and measure its minimum energy using MMFF94. This structure is then stored as mol2 {SYBYL2 (*.Mol2)}. The respective ligands used were by design; most have not been synthesised and are not yet available.

Validation of docking study

Molecular docking validation was performed by re-docking the native ligand into the receptor for three replications. If the root mean square deviation (RMSD) value is less than 2.0 Å, the docking procedure can be confirmed valid and used for docking FA derivative compounds.

Docking study

Molecular docking was performed on FA derivative compounds at the FGFR1 receptor (PDB ID: 4UWC) using the AutoDock Tools ver. 1.5.7. The computer used is an Asus X441MA laptop with an Intel Celeron N4000 operating system, CPU 1.10 GHz, 64-bit, and 4.00 GB RAM. The final result of molecular docking is the estimated constant prediction of free energy binding and inhibition in a file with the file.dlg format, which can be opened with the Notepad application. Free energy binding and inhibition constant data between FA derivatives were compared. The lowest value is predicted to indicate the greatest activity. The docking results contain receptor-ligand interaction and are visualised with the Discovery Studio 2021 Client application.

Prediction of ADME

ADME prediction was carried out using the SwissADME web programme. The initial procedure was to draw the molecule in 2D structure in the ChemDraw application, copy it to Chem3D, and save it in a file .sdf format. Then, it was converted into a SMILE structure with an online SMILE translator web programme. The SMILE was copied to the SwissADME website, and predictions were made simultaneously on the FA 1-24 derivative compounds. The structure of 24 FA derivates is shown in Figure 1.

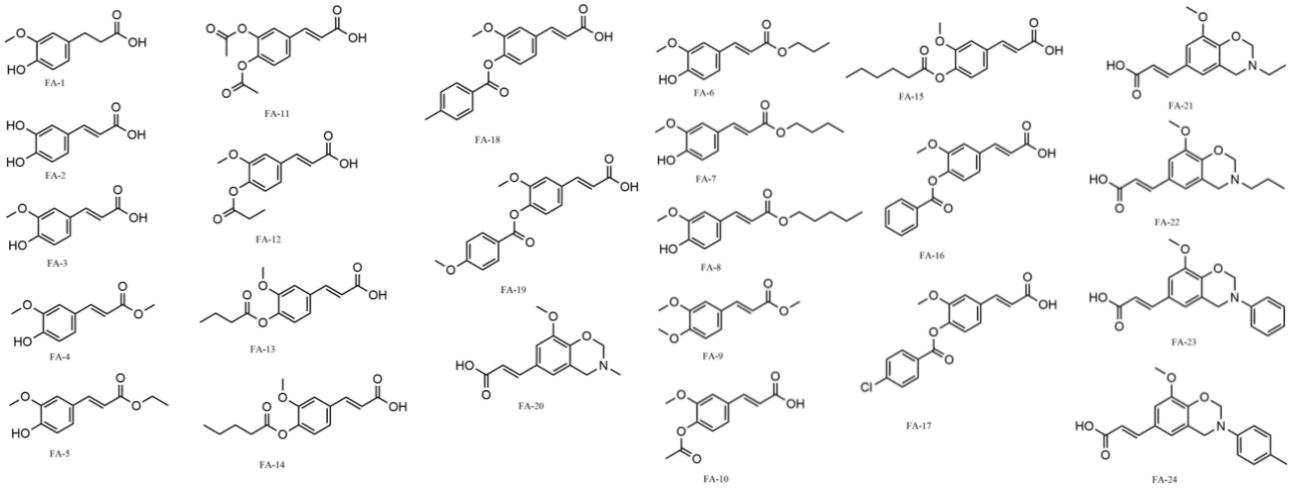

Figure 1: Structures of FA derivative compounds

QSPR analysis

Quantitative Structure-Property Relationship (QSPR) was conducted using IBM SPSS Statistics application version 26. Based on 24 FA-derived compounds, descriptors were determined that could represent the Hansch equation. Those descriptors were lipophilic (LogP), electronic (Etot), and steric (CMR) properties. LogP and CMR descriptors were obtained from predictions of physicochemical properties from ChemDraw, while Etot were obtained from minimal energy values using MMFF94 from Chem3D. The QSPR model was established using multiple linear regression (MLR) analysis with the SPSS software. The QSPR model relates the descriptor as the independent variable (X) and the HIA (Human Intestinal Absorption) prediction as the dependent variable obtained from the pkCSM online tool. The validity of the QSPR model was determined with statistical criteria, such as the correlation coefficient (r), F value, and p-value (Muhammad *et al.*, 2018). The best QSPR model is determined by establishing an equation with two, three, and four variables. Each requirement to accept the statistical parameter values is fulfilled based on the number of variables in the equation.

Results

Docking study

Docking study validation by re-docking the native ligand to the FGFR1 receptor was replicated three times and averaged. The RMSD value obtained is 1.022 Å. Docking validation showed that the mean RMSD value was less than 2.0 Å. This proves that the docking method is valid. Therefore, it can be used to dock FA derivative compounds. The free energy binding value and inhibition constant of the native ligand are -8.58 kcal/mol and 509 nM. This value can be used as a comparison, especially for the test ligand, since the native ligand is also an inhibitor of FGFR1.

The docking study results of 24 FA derivative compounds found that FA-8 and FA-18 had the lowest free energy binding (FA-8: -8.61 kcal/mol; FA-18: -8.30 kcal/mol) and the lowest inhibition constant (FA-8: 492 nM; FA-18: 826 nM). Figure 2 compares the values obtained for each FA derivative compound.

ADME prediction

Based on the structure of the FA-derived compounds used for molecular docking studies, it was followed by predictions of their pharmacokinetic properties with the SwissADME web programme. From the structure of FA 1-24 (Figure 2), ADME prediction was recapitulated in several properties such as GI-absorption, BBB permeability, P-glycoprotein substrate, CYP3A4 inhibitor, Log Kp, and bioavailability score. In addition, data obtained supported the Lipinsky rule of five, which includes donor and acceptor H-bonds, Log P, and molecular weight. There is also synthetic accessibility data to predict these compounds' potential to be synthesised. The prediction results of the 24 FA-derived compounds are shown in Table I.

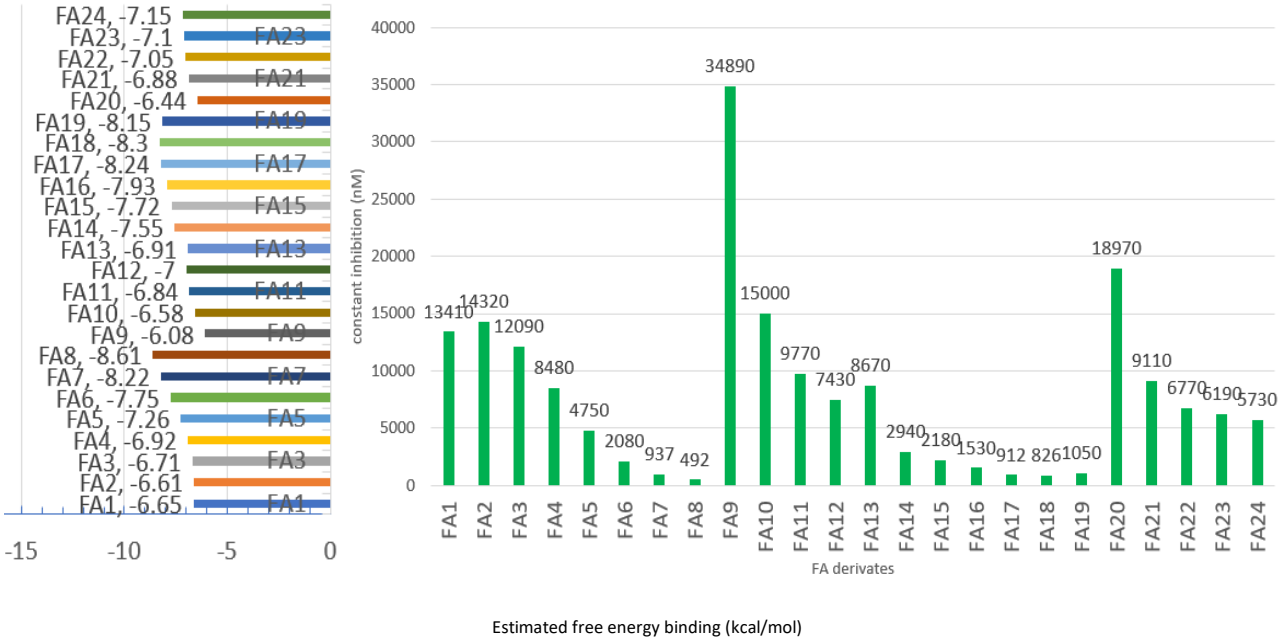

Figure 2: Chart of free energy binding (left) and inhibition constant (right) values of 24 FA-derivative compounds

Table I: Prediction of the pharmacokinetic properties of FA-derivative compounds by SwissADME web programme

Molecule	MW (g/mol)	H-bond acceptor	H-bond donor	LogP	GI Abs.	BBB permeab.	P-gp subst.	CYP3A4 inhib.	Log Kp (cm/sec)	Bioavail. score	Synthetic access.
FA1	196.20	4	2	1.31	High	Yes	No	No	-6.82	0.85	1.44
FA2	180.16	4	3	0.93	High	No	No	No	-6.58	0.56	1.81
FA3	194.18	4	2	1.36	High	Yes	No	No	-6.41	0.85	1.93
FA4	208.21	4	1	1.76	High	Yes	No	No	-6.26	0.55	2.13
FA5	222.24	4	1	2.11	High	Yes	No	No	-6.09	0.55	2.31
FA6	236.26	4	1	2.45	High	Yes	No	No	-5.80	0.55	2.43
FA7	250.29	4	1	2.80	High	Yes	No	No	-5.63	0.55	2.54
FA8	264.32	4	1	3.16	High	Yes	No	No	-5.34	0.55	2.66
FA9	222.24	4	0	2.22	High	Yes	No	No	-5.76	0.55	2.25
FA10	236.22	5	1	1.76	High	Yes	No	No	-6.24	0.85	2.25
FA11	264.23	6	1	1.73	High	No	No	No	-6.56	0.56	2.38
FA12	250.25	5	1	2.13	High	Yes	No	No	-5.99	0.85	2.33
FA13	264.27	5	1	2.46	High	Yes	No	No	-5.82	0.85	2.46
FA14	278.30	5	1	2.78	High	Yes	No	No	-5.52	0.85	2.57
FA15	292.33	5	1	3.18	High	Yes	No	No	-5.22	0.85	2.71
FA16	298.29	5	1	3.01	High	Yes	No	No	-5.44	0.85	2.53
FA17	332.74	5	1	3.54	High	Yes	No	No	-5.20	0.85	2.55
FA18	312.32	5	1	3.35	High	Yes	No	No	-5.27	0.85	2.67
FA19	328.32	6	1	3.01	High	No	No	No	-5.64	0.56	2.69
FA20	249.26	5	1	0.99	High	Yes	No	No	-8.36	0.55	2.79
FA21	263.29	5	1	1.32	High	Yes	Yes	No	-8.18	0.55	2.88
FA22	277.32	5	1	1.67	High	Yes	Yes	No	-7.89	0.55	2.96
FA23	311.33	4	1	2.62	High	Yes	No	No	-5.86	0.85	2.99
FA24	352.36	4	1	3.01	High	Yes	No	No	-5.86	0.85	3.10

MW: Molecular weight; GI: Gastrointestinal; Abs.: Absorption; BBB: Blood Brain Barrier; Permeab.: Permeability ; Subst.: substrate; Inhib.: Inhibitor; Bioavail.: Biovailability; Access.: Accessibility

QSPR result

The determination of the QSPR model is based on the best statistical parameters obtained. Multiple linear regression equations consisting of descriptors of lipophilicity, electronic and steric properties produce QSPR equations for various variables along with their statistical parameters as follows:

Log HIA = 0.018 Log^2 P + 0.069 Log P + 0.020 CMR + 0.001 Etot + 1.771

(n=24 ; r=0.621 ; F=2,975 ; p = 0.046) (1)

Equation (1) represents the equation that shows the highest significance between Log HIA (Human Intestinal Absorption) and the descriptor parameters Log P, CMR, and Etot.

Figure 3 visualises the interaction of the amino acid receptors with the test ligands (FA-8 and FA-18). It indicates that FA-8 heavily relies on hydrogen bond interactions while FA-18 relies on hydrophobic interactions (phi-alkyl interaction).

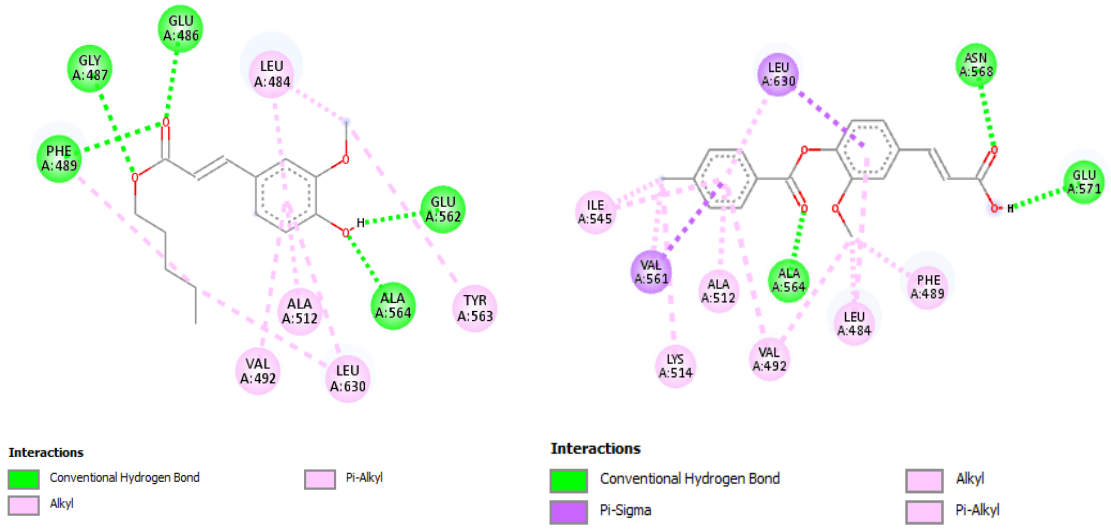

Figure 3: Visualisation of FA-8 (left) and FA-18 (right) ligand-receptor interactions

Discussion

This study consists of three main discussion points: activity prediction, pharmacokinetic properties prediction, and the study of structure-property relationships quantitatively. Each point is related to developing new drugs, especially in this study, which focuses on developing anti-angiogenesis drugs. Activity prediction with the Autodock tools application has an output of free energy binding values . This value is the sum of intermolecular free energy ($\Delta G_{interrmolecules}$) and free energy from torsion ($\Delta G_{torsion}$). The intermolecular energy consists of van der Waals interactions, hydrogen bonding and desolvation energy. Meanwhile, the torque energy is the multiplication of the weight factor ($W_{torsion}$) and the number of ligand torques ($N_{torsion}$) (Sriramulu *et al.*, 2019). Based on molecular docking data, ferulic acid (FA) derivatives that were predicted to have the greatest FGFR1 inhibitory activity are FA-8 and FA-18 with free energy binding values (FA-8: -8.61 kcal/mol; FA-18: - 8.30 kcal/mol) and the lowest inhibition constant (FA-8: 492 nM; FA-18: 826 nM). The results of these predictions are based on computational calculations and simulations of the interaction between ligands and receptors by software applications (Meng *et al.*, 2011).

Visualisation of the interaction of the amino acid receptors with the test ligands (FA-8 and FA-18) is shown in Figure 3. The visualisation showed that the interaction played a huge role in the hydrogen bond interactions in FA-8 and phi-alkyl interaction (hydrophobic interactions) in FA-18. The number of interactions with amino acid receptors on FA-18 was more (n=11) than FA-8 (n=10). This result showed an effect of the ester form on OH-carboxylate and ester on OH-phenolic. In addition, the type of substituent group also played a role in forming interactions with receptors where FA-18 was attached to an aromatic ring group. In contrast, FA-8 was attached to only an additional long-chain aliphatic alkyl group. Arthur and colleagues also reported Those types of interactions (2021).

Predictions of pharmacokinetic properties showed that each FA-derived compound had high gastrointestinal absorption and was not an inhibitor of CYP3A4 metabolic enzymes. Almost all compounds penetrate the blood-brain barrier (BBB) (except FA-2, FA-11, and FA-19) and not P-glycoprotein substrates (except FA-21 and FA-22). The value of the skin penetration coefficient (Log Kp) has a range of -5.20 to -8.36. While the Log P value range is 0.93 to 3.54 (<5). Among the compounds derived from FA, there is no violation of the Lipinsky rules of five. This rule states that a compound will have good oral absorption if the donor H-bond is not more than 5, the acceptor H-bond is not more than 10, the Log P is not more than 5, and the molecular weight is not more than 500 (Ekowati *et al.*, 2018).

The ADME predictions of the two compounds with the highest predicted activity (FA-8 and FA-18) differ. The prediction of GI absorption shows a high yield. However, the predicted bioavailability score of FA-18 (85%) showed a higher yield than FA-8 (55%). Meanwhile, most of the other pharmacokinetic parameters showed relatively similar results. This indicates that the compound FA-18 has the potential to be developed into pharmaceutical preparations with high bioavailability. On the other hand, to make FA-8 preparations, it may be necessary to modify the dosage form to increase its bioavailability (Gupta *et al.,* 2013).

The quantitative relationship of structure with HIA (Human Intestinal Absorption) pharmacokinetic parameters was analysed to determine the direction of drug development with better properties (Muhammad *et al*., 2018). Based on the QSPR analysis result, equation (1) is obtained, which fulfils the statistical parameters. The equation's F-value and correlation coefficient (r) meet the criteria for a linear regression equation with four variables and a total test sample (n) of 24 units. Besides that, the significance parameter also meets the statistical criteria ($p < 0.05$). Each physicochemical parameters show positive values , indicating a favourable relationship. This favourable relationship means that the greater the parameter value, the higher the value of the dependent variable, Log HIA, will increase. Nevertheless, the increase in physicochemical parameters also has a certain maximum limit to achieve a high Log HIA value.

Based on equation (1), information is obtained that the Log HIA value can be increased by increasing the lipophilicity of Log P, electronic properties of Etot, and steric properties of CMR. Based on the three parameters, the constant value of the Log P variable is the largest and has a positive value; therefore, it significantly affects the dependent variable Log HIA. This could be the basis that drug development can lead to increasing Log P to a certain maximum value. In addition, other parameters, such as CMR and Etot, also increase the Log HIA because the variable constants are positive. However, the increase in these parameters was not as significant as the lipophilic parameters. This QSPR model shows that the high prediction of GI absorption and bioavailability of FA-derived compounds is supported by high lipophilicity. Thus, drug development is recommended to modify the structure to increase lipophilic properties (Arnott & Planey, 2012).

Conclusion

The conclusions drawn from this study are several points. The lowest free energy binding and inhibition constant predicted by docking into FGFR1 from ferulic acid derivates were FA-8 and FA-18 derivative compounds. Compound FA-18 was predicted to have a higher bioavailability score (0.85) than FA-8 (0.55) by the Swiss ADME programme. Then, the QSPR model indicated that high GI absorption and bioavailability prediction was supported by high Log P, molar refractivity (CMR) and electronic factor (Etotal). Therefore, it is recommended that lipophilicity be increased to achieve better pharmacokinetic properties.

Acknowledgement

The author would like to thank LPPM Unair for their administrative and financial support.

Source of funding

Airlangga Research Funding (ARF) 2023 Scheme International Research Collaboration TOP#300. Ref. no 2564/UN3.LPPM/PT.01.03/2023.

References

Arnott, J. A., & Planey, S. L. (2012). The influence of lipophilicity in drug discovery and design. *Expert opinion on drug discovery*, **7**(10), 863–875. https://doi.org/10.1517/17460441.2012.714363

Arthur, D. E., Akoji, J. N., Sahnoun, R., Okafor, G. C., Abdullahi, K. L.., Abdullahi, S. A., & Mgbemena, C. (2021). A theoretical insight in interactions of some chemical compounds as mTOR inhibitors. *Bulletin of the National Research Centre,* **45**(67), 1–12. https://doi.org/10.1186/s42269-021-00525-x

Bergers, G., & Hanahan, D. (2008). Modes of resistance to anti-angiogenic therapy. *Nature Reviews Cancer*, **8**(8), 592–603. https://doi.org/10.1038/nrc2442

Ekowati, J., Diyah, N. W., Nofianti, K. A., Hamid, I. S., Siswandono. (2018). Molecular docking of ferulic acid derivatives on P2Y12 receptor and their ADMET prediction. *Journal of Mathematical and Fundamental Sciences*, **50**(2), 203–219. https://doi.org/10.5614/j.math.fund.sci.2018.50.2.8

Ekowati, J., Hamid, I. S., Diyah, N. W., & Siswandono, S. (2020). Ferulic acid prevents angiogenesis through cyclooxygenase-2 and vascular endothelial growth factor in the chick embryo chorioallantoic membrane model. *Turkish Journal of Pharmaceutical Sciences*, **17**(4), 424–431. https://doi.org/10.4274/tjps.galenos.2019.44712

Gotink, K. J., & Verheul, H. M. W. (2010). Anti-angiogenic tyrosine kinase inhibitors: What is their mechanism of action? *Angiogenesis*, **13**(1), 1–14. https://doi.org/10.1007/s10456-009-9160-6

Gupta, S., Kesarla, R., & Omri, A. (2013). Formulation strategies to improve the bioavailability of poorly absorbed drugs with special emphasis on self-emulsifying systems. *ISRN pharmaceutics*, **2013**, 1–16. https://doi.org/10.1155/2013/848043

Hu, J., Zhang, X., & Wang, Z. (2010). A review on progress in QSPR studies for surfactants. *International Journal of Molecular Sciences*, **11**(3), 1020–1047. https://doi.org/10.3390/ijms11031020

Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. *Current Computer Aided-Drug Design*,

7(2), 146–157. https://doi.org/10.2174/157340911795677602

Muhammad, U., Uzairu, A., & Ebuka Arthur, D. (2018). Review on: quantitative structure activity relationship (QSAR) modeling. *Journal of Analytical & Pharmaceutical Research*, **7**(2), 240–242. https://doi.org/10.15406/japlr.2018.07.00232

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. 2022. Cancer statistics, (2022). *CA: A Cancer Journal for Clinicians*, **72**(1), 7–33. https://doi.org/10.3322caac.21708/

Sriramulu, D. K., Wu, S., & Lee, S.-G. (2019). Effect of ligand torsion number on the AutoDock mediated prediction of protein-ligand binding affinity. *Journal of Industrial and Engineering Chemistry*., **83**, 359–365. https://doi.org/10.1016/j.jiec.2019.12.009

Wang, N., Wang, Z. Y., Mo, S. L., Loo, T. Y., Wang, D. M., Luo, H. bin, Yang, D. P., Chen, Y. L., Shen, J. G., & Chen, J. P. (2012). Ellagic acid, a phenolic compound, exerts anti-angiogenesis effects via VEGFR-2 signaling pathway in breast cancer. *Breast Cancer Research and Treatment*, **134** (3), 943–955. https://doi.org/10.1007/s10549-012-1977-9

Yang, G. W., Jiang, J. S., & Lu, W. Q., (2015). Ferulic acid exerts anti-angiogenic and antitumor activity by targeting fibroblast growth factor receptor 1-mediated angiogenesis. *International Journal of Molecular Sciences*, **16**(10), 24011–24031. https://doi.org/10.3390/ijms161024011