In Silico Molecular Docking Analysis of the Potential role of Reticuline and Coclaurine as Anti-colorectal Cancer Alkaloids

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Colorectal cancer (CRC) is a serious global epidemic, being the third most prevalent cancer worldwide, finding novel treatment alternatives for CRC is thus of the greatest importance. The atomic level interaction between a tiny molecule and a protein can be represented using molecular docking. Molecular docking is critical for visualizing ligand-protein interactions at the atomic level highlighting our knowledge of ligands behavior, which aids in the development of structure-based drugs. Methods: We used molecular docking to investigate the anticancer activity for two main ligands (reticuline and coclaurine) and four potential anticancer receptors (TNIK, VEGFR, EGFR and AKT2). Protein Data Bank provided the 3D structures of the receptor proteins, iGEMDOCK and AutoDock vina program were used for molecular docking. Results: Reticuline had the best docked postures and the highest interactive energy with CRC receptors: TNIK, VEGFR, EGFR and AKT2 with the following binding energy; -96.7, -117.8, -120.2, and -108.3 kcal/mol accordingly. Conclusion: According to this study, the investigated ligands were successfully docked onto reticuline and coclaurine ligands for drug interaction studies, the calculated binding energy demonstrate their importance as an anti-carcinogenic target. The current findings lay the groundwork for further research into reticuline and coclaurine as a potential CRC therapeutic option.

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1. INTRODUCTION

Colorectal cancer (CRC) is the third most frequent disease diagnosed and the second largest cause of cancer mortality globally, with over 1.9 million new cases and 935,000 fatalities expected in 2020 [1,2]. The prognosis of CRC is relatively dismal, with the patient's fate determined by the degree of local and metastatic tumor dispersion. Combined with advances in the detection and treatment of human CRC during the last decades, this disease remains one of the world's most difficult health issues [3]. Large comprehensive proteome and genomic studies of CRC have also been performed, resulting in the discovery of CRC subtypes, cancer antigens, therapeutic targets, and major signaling pathways linked to CRC development [4].

TNIK (Nck-interacting kinase) belongs to the germlinal center kinase family. TNIK was discovered to be a kinase that regulates cytoskeletal structure in several different kinds of cells, and it was lately suggested as a new curative candidate in many kinds of human malignancies [4]. While earlier research indicates that TNIK has an important function in tumor cell survival and predicting treatment, its intervention in hematological tumor cell survival has not been explored [5]. VEGFR-2 is tyrosine kinase receptor expressed in endothelial cells. VEGFR-2 is a main factor in anti-angiogenesis and a potent inhibitor of tumor cell growth and metastasis [6]. Angiogenesis inhibitors block the activities of vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2, and VEGFR-3) in downstream signaling pathways [7]. Preventing VEGFR-2 in tumor cells was discovered to start and expedite apoptosis, which simultaneously enhances the anticancer impact [8]. The Epidermal Growth Factor Receptor (EGFR) is a tyrosine kinase receptor that becomes active following the acquisition of different driver mutations within the kinase domain, causing aberrant cell replication. EGFR is a key indicator for treatment strategies since it is one of the most important goals for kinase inhibition in non-small cell lung tumors [9]. Akt, or protein kinase B, a serine–threonine kinase, has been elucidated in several types of human tumors, involving gastrointestinal, pulmonary, breast, ovarian, head and neck, prostate and thyroid tumors and may be involved in carcinogenesis [10].

Alkaloids have long been identified as vital secondary metabolites belongs to phytocannabinoids with a variety of biological characteristics [11]. The term “alkaloids,” is derived from the Arabic name al-qali, which is linked to the plant from which soda was originally extracted [12]. Due to their wide range of physiological and pharmacological properties such as antibiotics and anticancer, as well as their potential exploitation as narcotics, poisons, and stimulants, alkaloids have had a huge effect on human history, with about 12,000 alkaloids are isolated from different genera of the plant kingdom [11,13].

Reticuline (Fig. 1.A) and coclaurine (Fig. 1.B) are alkaloid’s chemical compounds that occur naturally and extracted in a wide range of plants [14–19].

An in silico experiment is one that is carried out on a computer or through computer simulation in biology and other experimental sciences [21]. The term refers to silicon in computer chips and is pseudo-Latin meaning ‘in silicon’ (in Latin, it would be in silico). In 1987, it must have been developed as a play on the Latin terms in vivo, in vitro, and in situ, which are usually utilized in biology (especially systems biology) [22]. In silico medical research has the ability to accelerate the rate of innovation, minimizing the requirements for costly laboratory efforts and new treatments. One method to accomplish this is to increase the efficiency with which drug candidates are produced and screened [23]. Researchers discovered possible inhibitors to an enzyme related with malignancy activity in silico and used the protein docking method EA Dock [24].

This approach varies from the use of delay and cost overrun screening (HTS) robotic labs that physically test hundreds of different compounds per day, with a predicted hit rate of 1% or fewer, and even lower projected to be genuine leads after additional examination (like drug discovery)
It has been attempted to develop computer models of cellular behavior. Researchers, for illustration, constructed an in silico model of tuberculosis to assist in medicinal development in 2007, with the major purpose of being better than meaningful predicted growth rates, providing phenomena of importance to be detected in minutes but instead of months [26].

The molecular docking methodology may be used to represent the atomic level interaction between a small molecule and a protein, allowing us to define novel molecular behavior in target protein binding sites as well as elucidate key biochemical pathways [27]. The lock-and-key theory proposed by Fischer [28], in which the ligand fits into the binding site like a lock and key, was the first explication of the ligand-receptor binding phenomenon. The initial docking approaches [29] were highlighted in this section, and the ligand and receptor were both considered as rigid entities. The “induced-fit” theory [30,31] proposed by Koshland extends the lock-and-key theory by claiming that as ligands engage with the protein, the active region of the protein is constantly altered by interactions with the ligands. Molecular docking has been utilized to identify potential inhibitors for numerous disease’s receptors and associated pathways, including but not limited to; inflammation, viral infection, Alzheimer’s disease, cardiovascular disease [32–37] and various cancer types [38–42].

In this research we used molecular docking approach to analyze and visualize ligand-protein interactions between (reticuline and coclaurine) and CRC receptors: TNIK, VEGFR, EGFR and AKT2.

2. MATERIALS AND METHODS

This study was carried out in the computer laboratories at King Abdulaziz University during 2020-2021.

2.1 Molecular Docking Analysis

The docking procedure consists of two main steps: predicting the ligand structure including its location and orientation within certain sites (known as pose) and determining the binding affinity. Knowing where the binding site would be before starting this same docking process improves docking efficiency dramatically. Throughout many cases, the target protein is identified already when ligands are docked into it. Also, by comparing the target protein to a group of proteins with comparable functions or proteins co-crystallized with other ligands, one can learn more about the locations.

Docking energy is utilized to pick the lowest-energy pose(s) among a huge variety of conformations created for each molecule. While binding energy is released when a drug molecule binds to a target, decreasing the total energy of the complex, the higher the energy released when a ligand binds to a protein, the greater the tendency of the ligand to associate with that protein [43].

2.2 Selection of Receptors

The receptors used in this investigation for performing In-silico studies, the receptors were...
chosen based on their physiological roles and pathways. The receptors for the current research were chosen based on the published target locations of ligands (Table 1). These receptors three-dimensional structures were obtained from the PDB (Protein Database) [44]. These receptors pathways were investigated utilizing KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways [45].

Four different types of receptors have been selected to perform this experiment, which mainly includes TNIK protein (Nck interacting kinase), VEGFR (Vascular endothelial growth factor), EGFR (Epidermal growth factor receptor), and AKT2 (Protein kinase B).

2.3 Selection of Ligands

For this study, ligands belong to alkaloid phytochemicals were screened with the specific anti-cancer receptor. Two alkaloids were selected for this study (reticuline and coclaurine) because of the highest binding energy exhibited by them. Chemspider database and PDB (Protein Database) [46] were used to obtain the structures of reticuline and coclaurine. The pKCSM (Prediction of small-molecule Pharmacokinetics and Toxicity) programme was used to screen the pharmacokinetic features of the various ligands. The absorption, distribution, metabolism, and excretion features of every substance are defined by its pharmacokinetics profile [47]. Many tools are accessible online for forecasting a compound’s pharmacokinetics and toxicological qualities depending on the chemical structure or composition, spanning from data-based approaches as QSAR (Quantitative Structure-activity Relationship), identical studies [48,49], and 3-dimensional QSAR [50-54].

2.4 Multi-Receptor Docking

The anticancer (colorectal tumour) capabilities of the selected reticuline and coclaurine were predicted using multi-receptor docking. iGEMDOCK and AutoDock vina software were used to conduct docking investigations [55]. The features of active sites, including as physical and chemical qualities, will allow the ligand to be recognized and bound. The parameters (population size 200, generations 70, and solutions 10) were used to produce different conformations of docked structures, and the best confirmation was chosen based on the lowest binding energy.

3. RESULTS

3.1 In silico Prediction of the Anti-Cancer Properties of Reticuline and Coclaurine

The inhibitory activity of the ligands Reticuline and Coclaurine with various cancer receptors that are thought to be potential therapeutic targets was investigated using multi-receptor docking. The optimum docked pose of structure was chosen based on the lowest binding energy, interacting residues, and hydrogen bond number. Among the tested alkaloids, reticuline had the best docked postures and the highest interactive energy. TNIK, VEGFR, EGFR and TNIK are the receptors that demonstrated the best binding relationships. -96.7, -117.8, -120.2, and -108.3 kcal/mol (Fig. 2 and Fig. 3). On the other hand with respect to Coclaurine ligand the receptors showed less binding energy which were -87.8, -102.3, -76.7, and -96.6 kcal/mol were discovered to represent the docked energies of receptor-ligand complex-1, accordingly (Fig. 4 and Fig. 5).

4. DISCUSSION

Colorectal cancer (CRC) is among the most lethal and diagnosed malignancies in the world. Targeted treatment is a novel optional strategy that has effectively prolonged overall survival in CRC patients. The current work provides comprehensive information on the binding ability of certain alkaloids, such as reticuline and coclaurine ligands, to prospective cancer treatment targets. In this study anti CRC activity was investigated with two main ligands and four receptors. The ability of reticuline and coclaurine to bind to specific targets was investigated using molecular docking and In-silico studies. TNIK, VEGFR, EGFR and AKT2 were found to have high affinity for reticuline and coclaurine as an anticancer agent according to this research, indicating a promising approach to medication discovery for CRC receptors. TNIK antagonists have recently been demonstrated to decrease cancer cell proliferation in vitro and in vivo, as well as to diminish CRC cell survival [56]. As a result, identifying TNIK inhibitors might be beneficial in understanding the process of TNIK-mediated cell cycle control and may have promising utility in cancer therapy. Breast cancer, colorectal cancer, and lung cancer all have TNIK protein [57]. Inhibiting VEGFR-2 in cancer cells was discovered to start and expedite apoptosis, which simultaneously enhances the anticancer
impact [8]. Another study found that the expression levels of TIE2 and VEGFR2 are
regulated in CRC angiogenesis [58]. In the treatment of metastatic CRC, EGFR inhibitors
are promising therapeutics target [58]. The EGFR signal transduction pathway is often
generally thought to have a significant role in tumor genesis and progression, and it is one of
the most key targets for a variety of malignancies [59]. The AKT family, which consists of three
highly associated isoforms, AKT1, AKT2, and
AKT3, has been linked to cell proliferation,
survival, and apoptosis [60]. Overexpressed
AKT2 promoted metastasis in CRC [61]. AKT2 is
the driving force behind a variety of cellular
activities such as DNA replication and DNA
repair, and its overexpression has been linked to
oncogenesis [62,63]. AKT2 knockdown was
reported to inhibit tumor cells proliferation
[64].

Table 1. Potential therapeutic targets from a variety of CRC receptors for structure-based drug
screening

| Gene name | Receptor name | PDB id |
|-----------|---------------|--------|
| TNIK      | Nck Interacting kinase | 5AX9   |
| VEGFR     | Vascular endothelial growth factor | 3VHK   |
| EGFR      | Epidermal growth factor receptor | 1XKK   |
| AKT2      | Protein kinase B | 1MRV   |

*Source: PDB (Protein Database)

Fig. 2. Molecular docking studies of reticulum ligand with CRC drug targets showing negative
binding energy

Fig. 3. Molecular docking studies of coclaurine ligand with CRC drug targets showing negative
binding energy
The inhibitory activity of the ligands reticuline and coclaurine with these anti-cancer receptors are thought to be potential therapeutic targets was investigated before using multi-receptor docking and for In-silico studies [65]. The optimum docked pose of structure was chosen based on the lowest binding energy interacting residues, and hydrogen bond number [66]. Among the alkaloids, reticuline had the best docked postures and the highest interactive energy [67]. Alkaloids have a wide range of inhibitory actions against a variety of cancer receptors making them ideal therapeutic medication for a number of cancers due to their high inhibitory efficacy and superior pharmacokinetic properties [68].

As a result of this research, it documented those alkaloids have an optimal binding feature with the selected cancer receptors.

To the best of our knowledge, this is one of the studies showing alkaloids’ therapeutic potential against cancer’s key targets. The present research improves our knowledge of how to select and test lead compounds as possible chemotherapeutic drugs in the future. Because of their effectiveness as an inhibitor, reticuline and coclaurine should be considered in in vitro and in vivo studies for a variety of cancer cell lines.

Fig. 4. Binding interaction of specific receptor target with reticuline (A): TNIK, (B): VEGFR, (C): EGFR), (D): AKT2

Fig. 5. Binding interaction of specific receptor target with coclaurine (A): TNIK, (B): VEGFR, (C): EGFR), (D): AKT2
5. CONCLUSION

Alkaloids are vital chemical substances that may be exploited to find new drugs. Various alkaloids isolated from medicinal plants and herbs were shown to have antiproliferative and anticancer effects on a broad range of malignancies in vitro and in vivo. By using computer-assisted virtual screening, the inhibitory effects of alkaloids against numerous cancer treatment targets were discovered.

According to this study, reticuline and coclaurine inhibited CRC pathogenic gene products of TNIK, VEGFR, EGFR and AKT2 better than their native ligands. These receptors were successfully docked onto reticuline and coclaurine ligands for drug interaction studies, with the best binding energy. Demonstrating its importance as an anti-carcinogenic target by alkaloids. The current findings lay the groundwork for further research into alkaloids as a potential CRC therapeutic option.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was carried out in the computer laboratories at king Abdulaziz University during 2020-2021. The study was approved by research ethics committee (29-CEGMR-Bioeth-2021) at the center of excellence in genomic medicine research (CEGMR). All of the data in this investigation was analyzed and presented in accordance with CEGMR ethical requirements.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in Globocan 2012. International Journal Cancer. 2015; 136(5):E359-86. DOI: 10.1002/ijc.29210 Epub 2014 Oct 9. PMID: 25220842.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2021;71:209–249.

3. Mattei J, Demissie S, Tucker KL, Ordovas JM. The APOA1/C3/A4/A5 cluster and markers of allostatic load in the Boston Puerto Rican Health Study. Nutrition, Metabolism and Cardiovascular Diseases, 2011;21(11):862-70. DOI: 10.1016/j.numecd.2010.02.024 Epub 2010 Jul 31. PMID: 20674306; PMCID: PMC3004022.

4. Li C, Sun YD, Yu GY, Cui JR, Lou Z, Zhang H, et al. Integrated Omics of Metastatic Colorectal Cancer. Cancer Cell. 2020;38:734-747.e9.

5. Taira K, Umikawa M, Takei K, Myagmar BE, Shinzato M, Machida N et al. The Traf2- and Nck-interacting Kinase as a Putative Effector of Rap2 to Regulate Actin Cytoskeleton. Journal of Biological Chemistry. 2004;279:49488–49496.

6. Goel HL, Mercurio AM. VEGF targets the tumour cell. Nature Reviews Cancer. 2013; 13:871–882.

7. Shen FQ, Shi L, Wang ZF, Wang CR, Chen JJ, Liu Y, Qiu HY, Zhu HL. Design, synthesis, biological evaluation of benzoyl amide derivatives containing nitrogen heterocyclic ring as potential VEGFR-2 inhibitors. Bioorg Med Chem. 2019;27(17): 3813-3824. DOI: 10.1016/j.bmc.2019.07.007 Epub 2019 Jul 4. PMID: 31327679.

8. Harame Y, Boucher-Hayes D. Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: Implications for anti-angiogenic therapy. BioEssays. 2002;24:280–283.

9. Wright NMA, Goss GD. Third-generation epidermal growth factor receptor tyrosine kinase inhibitors for the treatment of non-small cell lung cancer. Translational Lung Cancer Research. 2019;8:S247–S264.

10. Dummler B, Hemmings BA. Physiological roles of PKB/Akt isoforms in development and disease. Biochemical Society Transactions. 2007;35:231–235.

11. Kaur R, Arora S. Alkaloids-important therapeutic secondary metabolites of plant origin. Journal of critical reviews, 2015; 2:1–8.

12. Buchanan BB, Gruissem W, Jones RL. Biochemistry and molecular biology of plants. John Wiley & Sons; 2015.
13. Ziegler J, Facchini PJ. Alkaloid biosynthesis: metabolism and trafficking. Annual Review of Plant Biology. 2008;59: 735–769.

14. Morais LCSL, Barbosa-Filho JM, Almeida RN. Central depressant effects of reticuline extracted from Ocotea duckei in rats and mice. Journal of Ethnopharmacology. 1998;62:57–61.

15. Han Z, Zheng Y, Chen N, Luan L, Zhou C, Gan L, Wu Y. Simultaneous determination of four alkaloids in Lindera aggregata by ultra-high-pressure liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 2008;1212:76–81.

16. Leboeuf M, Legueut C, Cave A, Desconclos JF, Forgacs P, Jacquemin H. Alcaloïdes des Annonacées XXIX: Alcaloïdes de l’Annona muricata L. Planta Medica. 1981;42:37–44.

17. Da Silva IG, Barbosa-Filho JM, Da Silva MS, De Lacerda CDG, Da-Cunha EVL. Coclaurine from Ocotea duckei. Biochemical Systematics and Ecology. 2002;30:881–883.

18. Cacas JL, Buré C, Furt F, Maalouf JP, Badoc A, Cluzet S, Schmitter JM, Antajan E, Mongrand S. Biochemical survey of the polar head of plant glycosyl inositol phospho ceramides unravels broad diversity. Phytochemistry. 2013;96:191–200.

19. Sowemimo B, JL B, RW D, GH S. The Isolation of Stephiaradine And Coclaurine From Sarcopetalum Harveyanum. Isol. Stepharine Coclaurine From Sarcopetalum Harveyanum; 1972.

20. Bejugam PR, Kuppiil RR, Singh N, Gadewal N, Chaganti BK, Sastry GM, et al. Allosteric Regulation of Serine Protease HtrA2 through Novel Non-Canonical Substrate Binding Pocket. PLoS ONE. 2013;8(2):e55416. Available:https://doi.org/10.1371/journal.pone.0055416.

21. Ran T, Zhang Z, Liu K, Lu Y, Li H, Xu J, et al. Insight into the key interactions of bromodomain inhibitors based on molecular docking, interaction fingerprinting, molecular dynamics and binding free energy calculation. Molecular Biosystem. 2015;11:1295–1304.

22. Mashamba-Thompson T, Soliman MES. Insight into the binding theme of CA-074Me to cathepsin B: Molecular dynamics simulations and scaffold hopping to identify potential analogues as anti-neurodegenerative diseases. Medicinal Chemistry Research. 2014;24:701–713.

23. Poongavanam V, Kongsted J, Würtner D. Computational Analysis of Sterol Ligand Specificity of the Niemann Pick C2 Protein. Biochemistry. 2016;55: 5165–5179.

24. Decherchi S, Bertolotti A, Bottegoni G, Rocchia W, Cavalli A. The ligand binding mechanism to purine nucleoside phosphorylase elucidated via molecular dynamics and machine learning. Nature Communications. 2015;6: 6155.

25. Kacker P, Masetti M, Mangold M, Bottegoni G, Cavalli A. Combining Dyad Protonation and Active Site Plasticity in BACE-1 Structure-Based Drug Design. Journal of Chemical Information and Modeling. 2012;52:1079–1085.

26. McConkey BJ, Sobolev V, Edelman M. Quantification of protein surfaces, volumes and atom-atom contacts using a constrained Voronoi procedure. Bioinformatics. 2002;18:1365–1373.

27. Fischer E. Einfluss der Konfiguration auf die Wirkung der Enzyme. Berichte der deutschen chemischen Gesellschaft 27, Wiley; 1894.

28. Kuntz ID, Blaney JM, Oatley SJ, Langridge R, Ferrin TEA. geometric approach to macromolecule-ligand interactions. Journal of Molecular Biology. 1982;161: 269–288.

29. Hammes GG. Multiple Conformational Changes in Enzyme Catalysis. Biochemistry. 2002;41:8221–8228.

30. Koshland DE. Correlation of Structure and Function in Enzyme Action. Science (80-.) 1963;142:1533–1541.

31. Mahnashi MH, Alyami BA, Alqahtani YS, Jan MS, Rashid U, Sadiq A, Alqarni AO. Phytochemical profiling of bioactive compounds, anti-inflammatory and analgesic potentials of Habenaria digitata Lindl.: Molecular docking based synergistic effect of the identified compounds. Journal Ethnopharmacology. 2021;273:113976.

32. Khan FA, Nasim N, Wang Y, Alhazmi A, Sanam M, UI-Haq Z, et al. Amphiphilic desmuramyl peptides for the rational design of new vaccine adjuvants: Synthesis, in vitro modulation of inflammatory response and molecular docking studies. European Journal of Medicinal Chemistry. 2021;209:112863.

33. Al-Ostoot FH, Grisha S, Mohammed YHE, Vivek HK, Khanum SA. Molecular docking and synthesis of caffeic acid analogous and its anti-inflammatory, analgesic and...
ulcerogenic studies. Bioorganic & Medicinal Chemistry Letters., 2021;33: 127743.

34. Ahmad SS, Sinha M, Ahmad K, Khalid M, Choi I. Study of Caspase 8 inhibition for the management of Alzheimer’s disease: a molecular docking and dynamics simulation. Molecules. 2020;25:2071.

35. Pradeepkiran JA, Reddy PH. Structure based design and molecular docking studies for phosphorylated tau inhibitors in Alzheimer’s disease. Cells. 2019;8:26.

36. Hameed A, Zehra ST, Shah SJ, Khan KM, Alharthy RM, Furtmann N, et al. Syntheses, Cholinesterases Inhibition, and Molecular Docking Studies of Pyrido[2,3-b]pyrazine Derivatives. Chemical Biology & Drug Design. 2015; 86:1115–1120.

37. Ibrahim MAA, Badr EAA, Abdelrahman AHM, Almansour NM, Mekhemer GAH, Shawky AM, et al. In silico targeting human multidrug transporter ABCG2 in Breast Cancer: Database Screening, Molecular Docking, and Molecular Dynamics Study. Molecular Informatics. 2021;2060039.

38. Abdul-Rida NA, Farhan AM, Al-Masoudi NA, Saeed BA, Miller D, Lin MF. A novel pregnene analogs: Synthesis, cytotoxicity on prostate cancer of PC-3 and LNCPa-Ai cells and in silico molecular docking study. Molecular Diversity. 2021;25:661–671.

39. Vlasiou MC, Petrou CC, Sarigiannis Y, Pafiti KS. Density Functional Theory Studies and Molecular Docking on Xanthohumol, 8-Prenylnaringenin and Their Symmetric Substituted Diethanolamine Derivatives as Inhibitors for Colon Cancer-Related Proteins. Symmetry (Basel). 2021;13:948.

40. Govindarasu M, Ganeshan S, Ansari MA, Alomary MA, AlYahya S, et al. In silico modeling and molecular docking insights of kaempferitin for colon cancer-related molecular targets. Journal of Saudi Chemical Society. 2021;25:101319.

41. Zhang MM, Wang D, Lu F, Zhao R, Ye X, He L, Ai L, Wu CJ. Identification of the active substances and mechanisms of ginger for the treatment of colon cancer based on network pharmacology and molecular docking. BioData Mining. 2021;14: 1–16.

42. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank, 1999-. International Tables for Crystallography. 2006:675–684. DOI: 10.1107/97809553602060000722

43. Agarwal S, Mehrotra R. An overview of molecular docking. JSM Chemistry. 2016; 4:1024–1028.

44. Kanelhisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Research. 2000;28:27–30.

45. Pence HE, Williams A. ChemSpider: An Online Chemical Information Resource. Journal of Chemical Education. 2010:87: 1123–1124.

46. Pires DEV, Blundell TL, Ascher DB. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. Journal of Medicinal Chemistry. 2015;58:4066–4072.

47. Fröhlich H, Wegner JK, Sieker F, Zell A. Kernel Functions for Attributed Molecular Graphs – A New Similarity-Based Approach to ADME Prediction in Classification and Regression. QSAR and Combinatorial Science. 2006; 25:317–326.

48. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. ProTox: A web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Research. 2014: 42:W53–W58.

49. van de Waterbeemd H, Gifford E. ADMET in silico modelling: towards prediction paradise? Nature Reviews Drug Discovery. 2003;2:192–204.

50. KhanTHM. Predictions of the ADMET Properties of Candidate Drug Molecules Utilizing Different QSAR/QSAR Modelling Approaches. Current Drug Metabolism. 2010;11:285–295.

51. Obrezanova O, Csányi G, Gola JMR, Segall MD. Gaussian Processes: A Method for Automatic QSAR Modeling of ADME Properties. Journal of Chemical Information and Modeling. 47:1847–1857.

52. Lil MA. Multi-dimensional QSAR in drug discovery. Drug Discovery Today. 2007;12:1013–1017.

53. Hsu KC, Chen YF, Lin SR, Yang JM. iGEMDOCK: A graphical environment of enhancing GEMDOCK using pharmacological interactions and post-screening analysis. BMC Bioinformatics. 2011;12 Suppl 1:S33–S33.

54. Masuda M, Uno Y, Ohbayashi N, Ohata H, Mimata A, Kukimoto-Niio Met al. TNK inhibition abrogates colorectal cancer stemness. Nature Communications. 2016: 7:12586.
55. Lei H, Deng CX. Fibroblast Growth Factor Receptor 2 Signaling in Breast Cancer. International Journal of Biological Sciences. 2017;13:1163–1171.

56. Zhong M, Li N, Qiu X, Ye Y, Chen H, Hua J, Yin P, Zhuang G. Tlpe regulates VEGFR2 expression and promotes angiogenesis in colorectal cancer. International Journal of Biological Sciences. 2020;16:272–283.

57. Martinelli E, Ciardiello D, Martini G, Troiani T, Cardone C, Vitiello PP, Normanno N, Rachiglio AM, Maiello E, Latiano T, De Vita F, Ciardiello F. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: challenges and future perspectives. Annals of Oncology. 2020; 31:30–40.

58. Nam B, Rho JK, Shin DM, Son J. Gallic acid induces apoptosis in EGFR-mutant non-small cell lung cancers by accelerating EGFR turnover. Bioorganic and Medicinal Chemistry Letters 2016;26:4571–4575.

59. Zinda MJ, Johnson MA, Paul JD, Horn C, Konicek BW, Lu ZH, et al. Akt-1,-2, and-3 are expressed in both normal and tumor tissues of the lung, breast, prostate, and colon. Clinical Cancer Research. 2001;7: 2475–2479.

60. Rychahou PD, Kang J, Gulhati P, Doan HO, Chen LA, Xiao SY, Chung DH, B. Akt2 overexpression plays a critical role in the establishment of colorectal cancer metastasis. Proceedings of the National Academy of Sciences USA. 2008;105: 20315–20320.

61. Wang X, Hickey RJ, Malkas LH, Koch MO, Li L, Zhang S, et al. Elevated expression of cancer-associated proliferating cell nuclear antigen in high-grade prostatic intraepithelial neoplasia and prostate cancer. Prostate. 2011;71:748–754.

62. Stoimenov I, Helleday T. PCNA on the crossroad of cancer. Biochemical Society Transactions. 2009;37:605–613.

63. Wu L, Chen J, Ding C, Wei S, Zhu Y, Yang W, et al. MicroRNA-137 contributes to dampened tumorigenesis in human gastric cancer by targeting AKT2. PLoS One. 2015;10:e0130124.

64. Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, et al. Check your cultures! A list of cross-contaminated or misidentified cell lines. International Journal of Cancer. 2010; 127:1–8.

65. Rosdi M, Daud N, Zulkifli R, Yaakob H. Cytotoxic effect of Annona muricata Linn leaves extract on Capan-1 cells. Journal of Applied Pharmaceutical Science. 2015;45–48. DOI: 10.7324/japs.2015.50508.

66. Indrawati L, Ascobat P, Bela B, Abdullah M, Surono IS, Pramono S. Antiproliferative activity and caspase enhancement properties of Annona muricata leaves extract against colorectal cancer cells. 0. 25. Medical Journal of Indonesia. 2016;136–142.

67. Oviedo V, García M, Díaz C, Guerrero MF, Costa M, et al. Extracto y fracción alcaloídena de Annona muricata con actividad de tipo ansiolítica en ratones. Rev. Colomb. Ciencias Químico-Farmacéuticas. 2009;38.

68. Canny SA, Cruz Y, Southern MR, Griffin PR, PubChem promiscuity: A web resource for gathering compound promiscuity data from PubChem. Bioinformatics. 2012;28:140–141.