Molecular docking investigation of calotropone as a potential natural therapeutic agent against pancreatic cancer

Abstract

A natural bioactive compound named calotropone has been reported as a drug candidate for several cancers, including pancreatic cancers. Herein, we used molecular docking approach to test the possible mechanisms of action of calotropone in inhibiting the growth of pancreatic cell cancer with gemcitabine as the positive control. By employing AutoDock Vina, we studied the molecular interaction between calotropone and pancreatic cancer-associated proteins, namely Glucosaminyl (N-Acetyl) Transferase 3, Glutamic-Oxaloacetic Transaminase 1, Tyrosine-protein kinase Met (c-Met), peroxisome proliferator-activated receptor γ, Budding Uninhibited by Benzimidazole 1, A Disintegrin and Metalloproteinase 10, Sex-determining region Y and Nuclear Factor kappa Beta (Nf-κβ). Higher affinity energies of calotropone toward the aforementioned proteins (ranging from $-7.3$ to $-9.3$ kcal/mol) indicate that calotropone may work in the same manner as anticancer drug gemcitabine. Highest docking score was found at the interaction of calotropone and Nf-κβ ($-9.3$ kcal/mol).

Key words: Calotropis gigantea, calotropone, molecular docking, nuclear factor kappa beta, pancreatic cancer

INTRODUCTION

According to recent global epidemiological study on pancreatic cancer cases, numbers of incidence and mortality will keep increasing. In 2020, the global mortality rate for pancreatic cancer reached 90%, where difficult early diagnosis is the main cause. Nevertheless, administration of chemotherapy has been reported to give significant success on the treatment. Gemcitabine has been assigned as a standardized chemotherapeutic drug against pancreatic metastases. However, natural compounds have also become the focus of anticancer drug development due to their significant effective medicinal properties. Several plant-derived compounds are potential for pancreatic cancer treatment. Recently, Calotropis gigantea has been in the research spotlight due to its contents of multiple antiproliferative secondary metabolites. A study in vivo using pancreatic cancer cells (panc-1) revealed the superior anticancer properties of calotropone (one of secondary metabolites from C. gigantea), in comparison with gemcitabine. In the same research, the IC$_{50}$ of calotropone was observed to be as low as 18.7 µM.
Despite its high potential in treating pancreatic cancer, the mechanism of action of calotropone in inhibiting the cell growth and inducing apoptosis is still scarcely reported. *In silico* studies by means of molecular docking may aid the research in mapping the potential mechanism. Molecular docking has been implemented as a method of analyzing new drugs against their target proteins by predicting the affinity and activity of the compound. This method relies on the three-dimensional (3D) structure information of a protein target and the electronics of the ligand to the protein target.

Several pancreatic cancer-related proteins are the primary target of researchers in developing drugs. Glucosaminyl (N-Acetyl) Transferase 3 (GCNT3), Mucin Type GCNT3, Glutamate oxaloacetate transaminase 1 (GOT1), Tyrosine-protein kinase Met (c-Met), peroxisome proliferator-activated receptor (PPAR) γ, and Budding Uninhibited by Benzimidazole 1 (BUB1) are proteins that play a role in tumor cell development through the multiple schemes. A Disintegrin and Metalloproteinase 10 (ADAM10) and Sex-determining region Y (SOX2) play a role in immune regulation in pancreatic cancer cells. Nuclear factor kappa beta (Nf-Kβ) is an inhibitory protein in apoptosis. These aforementioned proteins have been proven to be regulated by gemcitabine. Therefore, by employing the molecular docking on those proteins and comparing the results with that of gemcitabine, we can obtain the information of possible main mechanism of calotropone. Study of calotropone interaction with the therapeutic molecular target of pancreatic cancer by means of molecular docking is the novelty of this work.

**METHODS**

**Hardware and software**

Docking simulation was performed on Intel Celeron N3350 Acer computer, 1.00 GB memory processor (RAM), 32-bit operating system, Windows 10 pro. Softwares used in this experiment were LigPlot + 1.5.4, PyMOL 2.4 (Delano Scientific LLC, Italy), and AutoDock Vina supported by AutoDock Tools 5.6.

**Docking study**

The docking study analyzed calotropone compounds which cytotoxic compounds obtained based on literature. Target proteins used in this present studies are similar to our previous research, where the preparation details had been presented. The 2D structure of calotropone (CID: 70680255) and gemcitabine (CID: 60750) (for comparison) was obtained from the website (www.pubchem.ncbi.nlm.nih.gov). The ligand structure was converted from SDF format into PDB format using Pymol 2.4 software. Ligand structures were also prepared using AutoDockTools 1.5.6.rc3 software La Jolla, California, USA. The preparation of proteins and ligands was docking with size validation and grid box separation. The parameter observed from this simulation represents the energy of the ligand affinity for the protein target. Hydrogen interactions, hydrophobic interactions, and bond distances were visualized using LigPLot + 1.5.4 (2D) and PyMOL 3.1 (3D).

**RESULTS AND DISCUSSION**

Calotropane is a derivative of a natural steroid compound known to be an agent for cancer treatment. This inhibitory activity led us to study the systematic mechanism of calotropane compounds against proteins of pancreatic cancer cells. The results of the molecular docking of gemcitabine and calotropane toward the focused proteins have been presented [Table 1].

From the docking results, each affinity value exceeds −5 kcal/mol confirming the role of the ligand in regulating the protein. The most efficient bonding is shown by calotropane with Nf-Kβ owing to its energy affinity approaching −10 kcal/mol. Nf-Kβ is a transcription protein factor that plays a role in tumorigenesis in several types of tumors. In pancreatic cancer cells, this protein has a role in the activation of oncogenic mutations of Kras (pancreatic cancer promoters). Gemcitabine, which is the standard drug for pancreatic cancer patients, has a smaller affinity value of −6.3 kcal/mol. Calotropane equally has a stable affinity for the GCNT3 (−9.0 kcal/mol). GCNT3 is a protein-coding gene that plays a role in mucin biosynthesis. Upregulation of mucin biosynthesis has an active role against Kras mutations and increases cell proliferation. Calotropane interactions with other proteins, GOT1, c-Met, PPARG, BUB1, SOX2, and ADAM10 possess good binding affinity with values ranging from −7.3 to −8.9 kcal/mol, where these values are higher than that of gemcitabine. The displays of ligand-protein interactions and their overlay in the active pocket for calotropane [Figure 1] and gemcitabine [Figure 2] have been presented.

The ligand and protein affinity occurs because of the hydrophobic and polar hydrogen interactions. In the case of Nf-Kβ, calotropane has polar hydrogen with amino acid Phe151 (3.5 Å) at N terminal and A Met208 (2.7 Å) at C terminal [Figure 1]. Compared with drug ligand, amino acids that interacted with gemcitabine are different. They are His67 (3.16 Å), dc15 (2.5 Å), dc13 (3.30 Å), dc13 (3.10 Å), dc14 (3.14 Å) and Arg-59 (3.12 Å) [Figure 2]. The interaction of Met208 and calotropane established the strongest bond with a bond length of 2.7 Å affecting the affinity energy. Previous analysis showed Met208 as one of the amino acids that play a role in the growth of B-cell activating factor (BAFF). The binding of inhibitor with Met208 causes the decrease of Nf-Kβ p65 activation via BAFF effect. Hydrophobic
Table 1: Comparative affinity energy and molecular interactions of calotropone dan gemcitabine with proteins

| Protein  | Ligand   | Affinity energy (kcal/mol) | Interaction        | Amino Acid                      |
|----------|----------|-----------------------------|-------------------|---------------------------------|
| GCNT3    | Gemcitabine | -7.2                      | Hydrophobic       | Lys246, Asn340, Ser345, Glu245, Leu344, Asn340, Asn348, Asn348 |
|          |          |                             |                   | Asp343, Asp343                  |
|          | Calotropone | -9.0                      | Hydrophobic       | Arg378, Nga1, Ala287, Glu320, Tyr288, Ser317, Ala188, Asp319, Val128, Cys217, Tyr187, Val185, His130 |
|          |          |                             |                   | AspH040, Asp155, Arg192, Lys401 |
| GOT1     | Gemcitabine | -7.0                      | Hydrophobic       | Thr43, Ser66, His47, Asp64, Trp49, Asn63, Asn65 |
|          |          |                             |                   | Edo1, Val50, Pro48               |
|          | Calotropone | -8.9                      | Hydrophobic       | Asn65, Lys55, Lys56, Gln59, Lys55, Lys56 |
|          |          |                             |                   | Asn65, Edo11, Trp49, Asn63       |
| c-Met    | Gemcitabine | -6.1                      | Hydrophobic       | 8Bz1402, Gly1085, Ala1226, Phe1223, Arg1227, Arg1208, Asp1164 |
|          |          |                             |                   | Arg1086                         |
|          | Calotropone | -8.6                      | Hydrophobic       | Pro1264, Gly1224, Glu1227, Asp1204, Lys1244, Tyr1235, Arg1227, Leu1225 |
|          |          |                             |                   | ArgH1086                        |
| PPARG    | Gemcitabine | -6.4                      | Hydrophobic       | Met169, Arg196, Asp186, Glu198, Val197, Asn200, Gly199, Leu201 |
|          |          |                             |                   | Met100, Ser99, Lys101, Gln100, Gln100                |
|          | Calotropone | -7.3                      | Hydrophobic       | Phe287, He262, Gly 284, He281, Met348, He341, Leu340, Asp333, Ser342, Arg288 |
|          |          |                             |                   | Cys285, Gly291                   |
| BUB1     | Gemcitabine | -5.8                      | Hydrophobic       | Gln816, Lys817, Glu867, Asn927 |
|          |          |                             |                   | Ser870, Asn927, Asn927, Leu868, Leu868 |
|          | Calotropone | -8.5                      | Hydrophobic       | Phe818, Lys817, Glu816, Leu868, Lys817, Asn927, Glu867 |
|          |          |                             |                   | Leu868, Tyr853, Asn927, Ser870 |
| Nf-Kβ    | Gemcitabine | -6.3                      | Hydrophobic       | Arg57                             |
|          |          |                             |                   | His67, dc15, Arg59, dc13, dc13, dc14                  |
|          | Calotropone | -9.3                      | Hydrophobic       | Lys147, Thr205, Lys148, Val150, Glu152, Lys206 |
|          |          |                             |                   | Met208, Phe151                     |
| SOX2     | Gemcitabine | -7.6                      | Hydrophobic       | Arg113, He108, da36               |
|          |          |                             |                   | Ser107, Ser107, dc35, dc35, dc16, Arg105                        |
|          | Calotropone | -8.6                      | Hydrophobic       | Arg113, Thr110, He108, Arg195, Ser107, da36, dt17, dc16, dg15, da18 |
|          |          |                             |                   | -                               |
| ADAM10   | Gemcitabine | -6.8                      | Hydrophobic       | Tyr415, Asp261, Leu434, He437, Gln39, Ser395, Pro392 |
|          |          |                             |                   | Asp651                           |
|          | Calotropone | -8.4                      | Hydrophobic       | Val333, Leu654, Leu654, Val333, Pro392, His393, Gln39, Ser395, Pro392 |

GCNT3: Glucosaminyl (N-Acetyl) Transferase 3, GOT1: Glutamic-Oxaloacetic Transaminase 1, BUB1: Budding Uninhibited by Benzimidazole 1, NF-Kβ: Nuclear Factor kappa Beta, ADAM10: A disintegrin and metalloproteinase 10, SOX2: Sex-determinin

interaction of calotropone also inhibits NF-Kβ through Lys147, Thr205, Lys148, Val150, Glu152, and Lys206 amino acids while gemcitabine maintains fewer bonds, namely Arg57 [Table 1]. The affinity value of calotropone in GCNT3 and c-Met is higher than that of gemcitabine because calotropone has more polar hydrogen and hydrophobic interactions due to the hydrogen interactions, except for GOT 1. BUB1 PPARG, SOX2, and ADAM10 which bind to calotropone are only superior in hydrophobic interactions. From the results of the dockings, calotropone binds to 7 amino acids in BUB1, 10 amino acids in PPARG, 10 amino acids in SOX2, and 9 amino acids in ADAM 10, while gemcitabine binds 4 amino acids in BUB1, 8 amino acids in PPARG, 3 amino acids in SOX2, and 8 amino acids in ADAM 10. SOX2 is a regulatory protein on ADAM10 and has a function in pancreatic cancer cell immunity. The suppression of SOX2 can suppress ADAM10 expression. This shows
the inhibition of ADAM 10 by calotrope can be carried out via SOX2 or directly targeting the protein (ADAM10).

In this study, the highest docking score was obtained from the interaction between calotrope and Nf-Kβ, suggesting the dominating mechanism of the anticancer activities. The increase level of Nf-Kβ during cancer development and progression is not only exclusive to pancreatic cancer.[27] It is the significance of our findings that calotrope may act as a nonspecific anticancer. Nf-Kβ has a role in the secretion of proinflammatory cytokines and chemokines such as interleukin (IL)-1 β, tumor necrosis factor, and IL-6.[28] The finding in our study can be substantiated by the fact that calotrope exhibited anti-inflammatory properties, which is even higher than ibuprofen.[29]

Molecular docking studies have some limitations attributed to various factors involved in drug interaction in the body. Of which, drug delivery may play a significant part in the treatment efficacy. Our research group have developed several biopolymers which could assist the delivery, such as chitosan,[30,31] cellulose,[32,33] and pectin.[34] Further studies in-vitro and in-vivo could also be conducted to confirm the drug interaction targeting the Nf-Kβ and other cancer growth-related proteins.

Figure 1: Interaction of calotrope with pancreas cancer proteins. (a) Glucosaminyl (N-Acetyl) Transferase 3. (b) Glutamic-Oxaloacetic Transaminase 1. (c) c-Met. (d) Peroxisome proliferator-activated receptor G. (e) Budding uninhibited by benzimidazole 1. (f) Nuclear factor kappa beta. (g) Sex-determining region Y. (h) A Disintegrin and Metalloproteinase 10; (i) Pose view of interaction of calotrope with proteins. (ii) Overlay of calotrope in active pockets of proteins.
CONCLUSIONS

Our study proved that calotropone has higher docking scores based on its interaction with pancreatic cancer-associated proteins (GCNT3, GOT1, c-Met, PPARγ, BUB1, ADAM10, SOX2, and Nf-Kβ), in comparison with that of gemcitabine. The highest score obtained from calotropone interaction with Nf-Kβ suggests its dominance in the mechanism of action. We further recommend to investigate the role of calotropone in the regulation of Nf-Kβ during the development and progression of cancer cells.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Huang J, Lok V, Ngai CH, Zhang L, Yuan J, Lao XQ, et al. Worldwide burden of, risk factors for, and trends in pancreatic cancer. Gastroenterology 2021;160:744-54.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.

3. Brada LJ, Walma MS, van Dam RM, de Vos-Geelen J, de Hingh IH, Creemers GJ, et al. The treatment and survival of elderly patients with locally advanced pancreatic cancer: A post-hoc analysis of a
multicenter registry. Pancreatology 2021;21:163-9.

4. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreatic cancer. A randomized trial. J Clin Oncol 1997;15:2403-13.

5. Pan F, Skaer C, Yu J, Zhao H, Ren H, Oshima K, et al. Berries and other natural products in the pancreatic cancer chemoprevention in human clinical trials. J Berry Res 2017;7:147-61.

6. Bimonte S, Barbieri A, Leongito M, Piccirillo M, Giudice A, Pivonello C, et al. Curcumin anticancer studies in pancreatic cancer. Nutrients 2016;8:433.

7. Hasballah K, Sarong M, Rusly R, Fitria H, Maïda DR, Iqbramullah M. Anti-proliferative activity of triterpenoid and steroid compounds from ethyl acetate extract of Calotropis gigantea root bark against P388 murine leukemia cell lines. Sci Pharm 2021;89:21.

8. Nguyen KD, Dang PH, Nguyen HX, Nguyen MT, Awale S, Nguyen NT. Phytochemical and cytotoxic studies on the leaves of Calotropis gigantea. Bioorg Med Chem Lett 2017;27:2902-6.

9. Ramirez D, Caballero J. Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? Molecules 2018;23:1038.

10. Pinzí L, Rastelli G. Molecular docking: Shifting paradigms in drug discovery. Int J Mol Sci 2019;20:4331.

11. Liu H, Zhou Q, Wei W, Qi B, Zeng F, Bao N, et al. The potential drug for treatment in pancreatic adenocarcinoma: A bioinformatical study based on distinct drug databases. Chin Med 2020;15:26.

12. Rao CV, Janakiram NB, Madka V, Kumar G, Scott EJ, Pathuri G, et al. Small-molecule inhibition of GCNT3 disrupts mucin biosynthesis and malignant cellular behaviors in pancreatic cancer. Cancer Res 2016;76:1965-74.

13. Holt MC, Assar Z, Beheshiti Zavareh R, Lin L, Anglin J, Mashadova O, et al. Biochemical characterization and structure-based mutational analysis provide insight into the binding and mechanism of action of novel aspartate aminotransferase inhibitors. Biochemistry 2018;57:6604-14.

14. Aier I, Semwal R, Sharma A, Varadwaj PK. In silico identification of therapeutic compounds against microRNA targets in drug-resistant pancreatic ductal adenocarcinoma. J Biomol Struct Dyn 2020;39(13):4893-4901.

15. Saeid G, Htoo HH, Hernandez JF, Iizasa H, Checner F, Knietzko U, et al. Sox2 functionally interacts with βAPP, the βAPP intracellular domain and ADAM10 at a transcriptional level in human cells. Neuroscience 2016;312:153-64.

16. Lin X, Huang M, Xie F, Zhou H, Yang J, Huang Q. Gemcitabine inhibits immune escape of pancreatic cancer by down regulating the soluble ULBP2 protein. Oncotarget 2016;7:70092-9.

17. Laskowski RA, Swindells MB. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51:2778-86.

18. Trotter O, Olsen AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31:455-61.

19. Purnama A, Mardina V, Puspita K, Qanita I, Rizki DR, Hasballah K, et al. Molecular docking of two cytotoxic compounds from Calotropis gigantea leaves against therapeutic molecular target of pancreatic cancer. Narra J 2021;1:637.

20. Hasballah K, Murniana M, Azhar A, Rahmi N. Cytotoxic potential of n-hexane extract of Calotropis gigantea L. leaves. Int J Trop Vet Biomed Res 2016;1:45-9.

21. Mishra A, Dey S. Molecular docking studies of a cyclic octapeptide-cyclosaplin from sandalwood. Biomolecules 2019;9:740.

22. Glorieux C, Huang P. Regulation of CD137 expression through K-Ras signaling in pancreatic cancer cells. Cancer Commun (Lond) 2019;39:41.

23. Rao CV, Janakiram NB, Mohammed A. Molecular pathways: Mucins and drug delivery in cancer. Clin Cancer Res 2017;23:1373-78.

24. Azam SS, Abbasi SW. Molecular docking studies for the identification of novel melatonergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. Theor Biol Med Model 2013;10:63.

25. Wang R, Wang R, Ma N, Guo Y, Xiao H, Chen G, et al. Identify the key amino acid of BAFF binding with TACI. Cell Immunol 2013;284:84-90.

26. Peron R, Vatanabe IP, Manzine PR, Camins A, Cominetti MR. Alpha-secretase ADAM10 regulation: Insights into alzheimer’s disease treatment. Pharmaceuticals (Basel) 2018;11:12.

27. Xia L, Tan S, Zhou Y, Lin J, Wang H, Oyang L, et al. Role of the NFκB-signaling pathway in cancer. Onco Targets Ther 2018;11:2063-73.

28. Smale ST. Hierarchies of NF-κB target-gene regulation. Nat Immunol 2011;12:689-94.

29. Kumar H, Sharma S, Vasudeva N. Pharmacological profile of Calotropis gigantea in various diseases: A profound look. Int J Great Res Thoughts 2021;9:2987-96.

30. Rahmi, Iqbramullah M, Audina U, Husin H, Fathana H. Adsorptive removal of Cd (II) using oil palm empty fruit bunch-based charcoal/chitosan-EDTA film composite. Sustain Chem Pharm 2021;21:100449.

31. Rahmi R, Libis S, Az-Zahra N, Puspita K, Iqbramullah M. Synergetic photocatalytic and adsorptive removals of metanil yellow using TiO2/grass-derived cellulose/chitosan (TiO2/GC/CH) film composite. Int J Environ Res 2021;5:2987-96.

32. Iqbramullah M, Suyanto H, Rahmi, Pardeed M, Karnadi I, Kurniawan KH, et al. Cellulose acetate-polyurethane film adsorbent with analyte enrichment for aqueous Pb using Laser-Induced Breakdown Spectroscopy (LIBS). Environ Nanotechnol Monit Manag 2021;16:100516.

33. Iqbramullah M, MARLINA M, Khalil HP, Kurniawan KH, Suyanto H, Hedwig R, et al. Characterization and performance evaluation of cellulose acetate-polyurethane film for lead II ion removal. Polymers (Basel) 2020;12:1317.

34. Sahil E, Humaira H, Murniana M, Nazaruddin N, Iqbramullah M, Md Sani ND, et al. Optical pH sensor based on immobilization anthocyanin from Dioscorea alata L. onto polyelectrolyte complex pectin-chitosan membrane for a determination method of salivary pH. Polymers (Basel) 2021;13:1276.