ORIGINAL ARTICLE

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

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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-Kβ). 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-Kβ (–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.[1] In 2020, the global mortality rate for pancreatic cancer reached 90%,[2] where difficult early diagnosis is the main cause. Nevertheless, administration of chemotherapy has been reported to give significant success on the treatment.[3] Gemcitabine has been assigned as a standardized chemotherapeutic drug against pancreatic metastases.[4] 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.[5,6] Recently, *Calotropis gigantea* has been in the research spotlight due to its contents of multiple antiproliferative secondary metabolites.[7] 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.[8] In the same research, the IC_{50} of calotropone was observed to be as low as 18.7 μM.

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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.[9] This method relies on the three-dimensional (3D) structure information of a protein target and the electronics of the ligand to the protein target.[10]

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.[11-14] A Disintegrin and Metalloproteinase 10 (ADAM10) and Sex-determining region Y (SOX2) play a role in immune regulation in pancreatic cancer cells.[15,16] 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,[17] PyMOL 2.4 (Delano Scientific LLC, Italy), and AutoDock Vina supported by AutoDock Tools 5.6.[18]

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.[19] 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

Calotropone is a derivative of a natural steroid compound known to be an agent for cancer treatment.[7,20] This inhibitory activity led us to study the systematic mechanism of calotropone compounds against proteins of pancreatic cancer cells. The results of the molecular docking of gemcitabine and calotropone 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.[21] The most efficient bonding is shown by calotropone 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).[22] Gemcitabine, which is the standard drug for pancreatic cancer patients, has a smaller affinity value of –6.3 kcal/mol. Calotropone 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.[23] Calotropone 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 calotropone [Figure 1] and gemcitabine [Figure 2] have been presented.

The ligand and protein affinity occurs because of the hydrophobic and polar hydrogen interactions.[24] In the case of Nf-Kβ, calotropone has polar hydrogen with amino acid Phe151 (3.5 Å) at N terminal and Å 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 (3.26 Å), dc13 (3.30 Å), dc13 (3.09 Å), dc14 (3.14 Å) and Arg-59 (3.12 Å) [Figure 2]. The interaction of Met208 and calotropone 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.[25] 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
			Polar H	Asp343, Asp343
	Calotropone	−9.0	Hydrophobic	Arg378, Nga1, Ala287, Glu320, Tyr288, Ser317, Ala188, Asp319, Val128, Cys217, Tyr187, Val185, His130
			Polar H	Lys401, Asp155, Arg192, Lys401
GOT1	Gemcitabine	−7.0	Hydrophobic	Thr43, Ser66, His47, Asp64, Trp49, Asn63, Asn65
			Polar H	Edo1, Val50, Pro48,
	Calotropone	−8.9	Hydrophobic	Asn65, Lys55, Lys56, Gln59, Lys55, Lys56
			Polar H	Asn65, Edo11, Trp49, Asn63
c-Met	Gemcitabine	−6.1	Hydrophobic	88z1402, Gly1085, Ala1226, Phe1223, Arg1227, Arg1208, Asp1164
			Polar H	Arg1086
	Calotropone	−8.6	Hydrophobic	Pro1264, Gly1224, Glu1127, Asp1204, Lys1244, Tyr1235, Arg1227, Leu1225
			Polar H	Gln1123, Gln1123, Arg1203
PPARG	Gemcitabine	−6.4	Hydrophobic	Met169, Arg196, Asp186, Glu198, Val197, Asn200, Gly199, Leu201
			Polar H	Gln100, Ser99, Lys101, Gln100, Gln100
	Calotropone	−7.3	Hydrophobic	Phe287, He262, Gly 284, He281, Met348, He341, Leu340, Leu333, Ser342, Arg288
			Polar H	Cys285, Glu291
BUB1	Gemcitabine	−5.8	Hydrophobic	Gln816, Lys817, Glu867, Asn927
			Polar H	Ser870, Asn927, Asn927, Leu868, Leu868
	Calotropone	−8.5	Hydrophobic	Phe818, Lys817, Gln816, Leu868, Lys817, Asn927, Glu867
			Polar H	Leu868, Tyr853, Asn927, Ser870
Nf-Kβ	Gemcitabine	−6.3	Hydrophobic	Arg57
			Polar H	His67, dc15, Arg59, dc13, dc13, dc14
	Calotropone	−9.3	Hydrophobic	Lys147, Thr205, Lys148, Val150, Glu152, Lys206
			Polar H	Met208, Phe151
SOX2	Gemcitabine	−7.6	Hydrophobic	Arg113, He108, da36,
			Polar H	Ser107, Ser107, dc35, dc35, dc16, Arg105
	Calotropone	−8.6	Hydrophobic	Arg113, Thr110, He108, Arg195, Ser107, da36, dt17, dc16, dg15, da18
			Polar H	-
ADAM10	Gemcitabine	−6.8	Hydrophobic	Tyr415, Asp261, Leu434, Leu434, He437, He437, Lys431, Ser433
			Polar H	Asp261, Phe 432, Phe432
	Calotropone	−8.4	Hydrophobic	Val333, Leu654, Leu654, Val333, Pro392, His393, Gln439, Ser395, Pro392
			Polar H	Asp651

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 g [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.[26] This shows

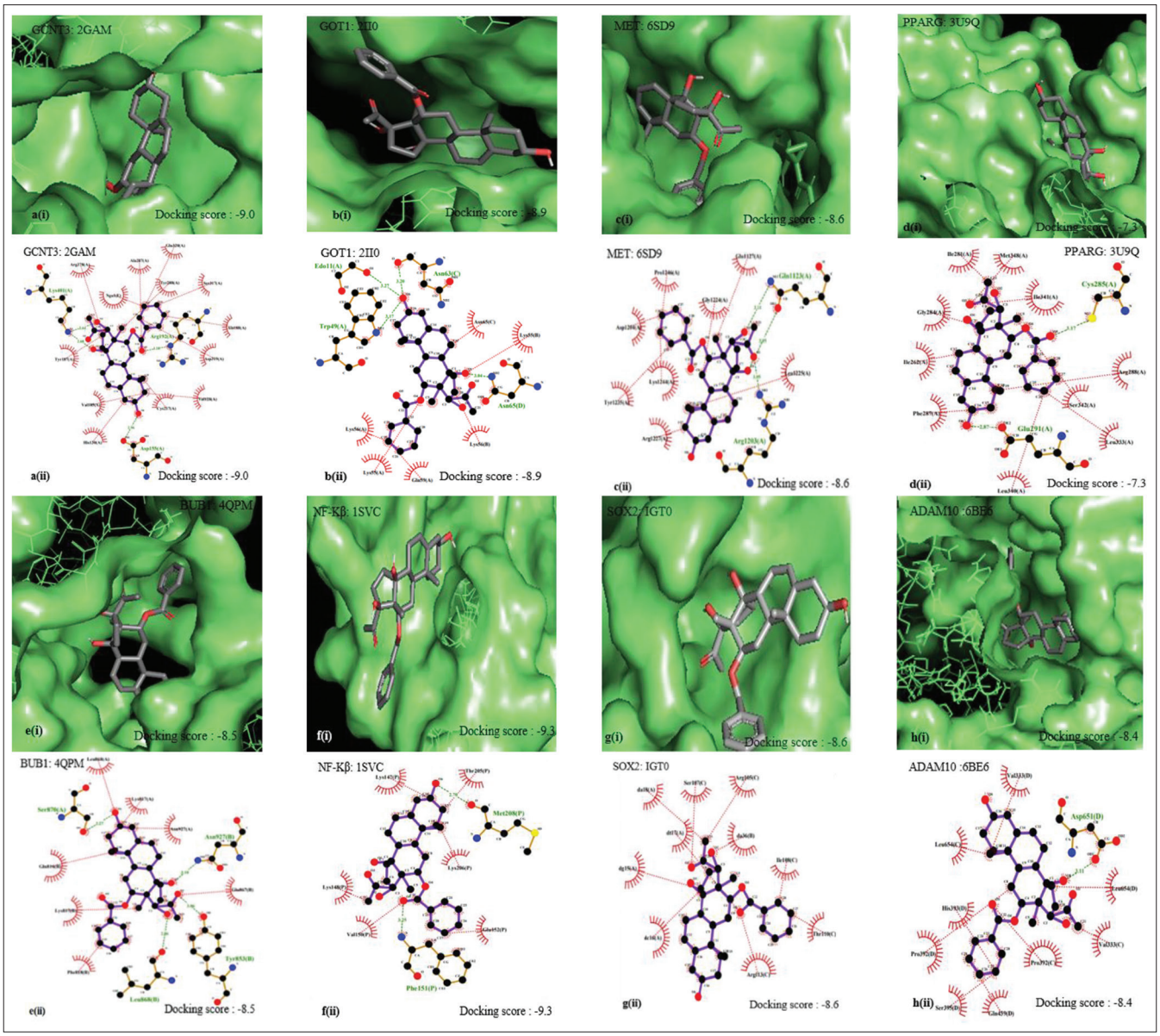

Figure 1: Interaction of calotropone with pancreas cancer proteins. (a) Glucosaminyl (N-Acetyl) Transferase 3. (b) Glutamic-Oxaloacetic Transaminase 1. (c) c-Met. (d) Peroxisome proliferator-activated receptor G. (e) Budding uninhibited by benzimidazole 1. (f) Nuclear factor kappa beta. (g) Sex-determining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

the inhibition of ADAM 10 by calotropone 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 calotropone 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 calotropone 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 calotropone 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.

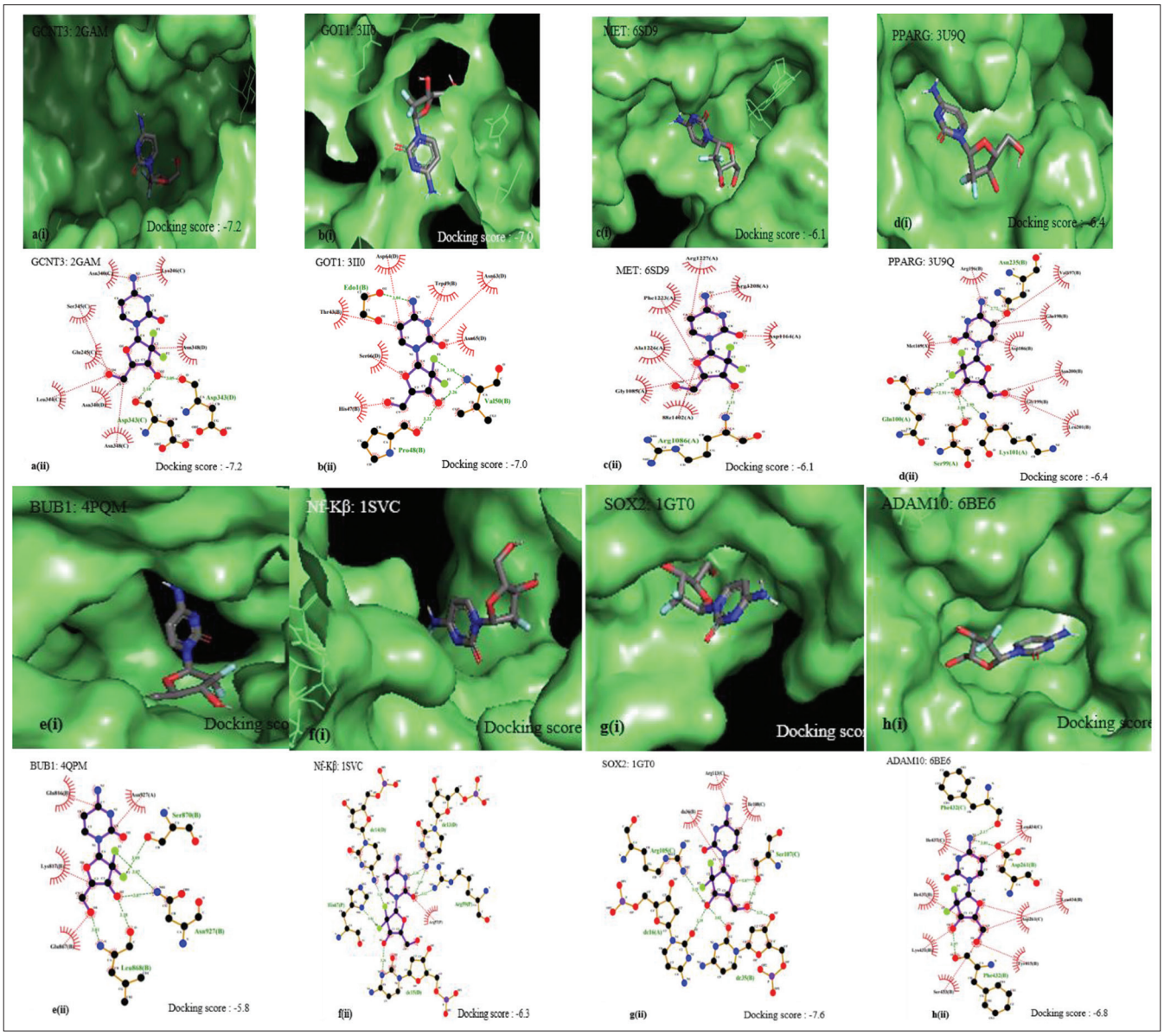

Figure 2: Interaction of gemcitabine with pancreas cancer proteins. (a) Glucosaminyl (N-Acetyl) Transferase 3. (b) Glutamic-Oxaloacetic Transaminase 1. (c) c-Met. (d) Peroxisome proliferator-activated receptor G. (e) Budding Uninhibited by Benzimidazole 1. (f) Nuclear factor kappa beta. (g) Sex-determining region Y. (h) A Disintegrin and Metalloproteinase 10; (i) Pose view of interaction of calotropone with proteins. (ii) Overlay of calotropone 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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Nil.

Conflicts of interest

There are no conflicts of interest.

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