Computational Study of Lactucine and its Derivatives with Apoptosis Inducing Proteins of Various Pathways

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Abstract

In the present computational study, we found that lactucin 15-oxalate a chemical component present in the *cichorium intybus* (chicory) has a potential apoptosis inducing effect in human leukemia cancer cell and may acts as an anticancer agent. Lactucine and its derivatives were used as ligand molecules to trace out its binding interactions with proteins involved in apoptosis and these ligands were docked with different apoptosis inducing protein such as caspases, cytochrome C, apaf-1, CDKs, etc involved in cell cycle regulation. Among lactucine derivatives, the lactucin 15-oxalate showed virtuous affinity for the apoptosis inducing protein. In addition to this, the number of the hydrogen bonding was higher with lactucin 15-oxalate as compare to other derivatives which indicates its suitability as an anticancer agent. An apoptosis inducing agent if implemented for the treatment of leukemia cancer, then it can reduce the use of multidrug dose therapy and chemotherapeutic drugs. However, different in vitro as well as clinical trials are needed for further validation of the lactucin 15-oxalate.

1. Introduction

Leukemia is cancer of blood forming tissues; including bone marrow and it lead to formation of high number of abnormal white blood cells. These white blood cells are also known as blasts or leukemia cells due to its incomplete formation. There are four types of leukemia as lymphoblastic, chronic lymphocytic, chronic myeloid, acute myeloid leukemia **(Vardiman et al., 2009).** Leukemia is treated by the individual therapeutic options like radiotherapy, chemotherapy, or their combination. Lactucine is having anti-tumor and apoptotic effect in the HL-60 human leukemia carcinoma cells. In these cells, lactucine induces significant cytotoxic effects in a dose and time dependent manner **(**Zhang et al., 2016). With the increase in the incubation time, the cytotoxicity against leukemia cells also increases. Phase contrast microscopy studies have indicated that lactucine-treated HL-60 cells show the appearances of a large number of cytoplasmic vacuoles which indicates changes in cellular morphology. With the increase in doses of lactucine, there is increase in size and the number of vacuoles.

The lactucine-treated cells show yellow and reddish orange fluorescence, while control cells (untreated) only show green fluorescence which indicates that control cells are active **(**Zhang et al., 2016). At higher doses of lactucine, the late apoptotic features appears. Transmission electron microscopy (TEM) also shows the formation of vacuoles in the cytoplasm in lactucine treated cells and increase in lactucine dose also increases vacuoles number. TEM results also show that lactucine treatment led to swelling of the mitochondria and endoplasmic reticulum (ER). Flow cytometry studies have proven that lactucine has a notable effect on cell cycle phase distribution of HL-60 human leukemia cells. Sub-G1 cell cycle arrests in these cells are induced by lactucine in a dose-dependent manner **(**Zhang et al., 2016). Lactucine is related to the sesquiterpene lactone group of naturally occurring compounds, and have a variety of pharmacological effects including anticancer properties. Sesquiterpene lactones are a wide and diverse group of biologically active plant compounds. Sesquiterpene lactones are found in Chicory, Wormwood, hrysanthemum, Laurus nobilis, Pyrethrum, Chamomile etc. (Kreuger et al., 2012; Chadwick et al., 2013; **Dall' Acqua** et al., **2006).**

Apoptosis is also called as programmed cell death, which is a systematic process of death of any cell under certain abnormal or necessary conditions. It is an element of natural homeostatic mechanism to keep the number of the cells constant in an organism and helps the tissue to get rid of increasing number of redundant cells that are injured or no longer manageable throughout development, growth or aging **(Elmore et al. 2013).** In apoptosis regulatory pathways, a number of gene families regulate the morphological and biochemical changes in the cells throughout the process. Morphological event cascade as well as nuclear condensation, cytoplasmic filament aggregation, cellular fragmentation, and plasma membrane blebbing finally results in the formation of apoptotic bodies. Every morphological hallmark of apoptosis can be represented in three points; (i) the changes arise in nucleus; (ii) those occur in mitochondria; (iii) cell membrane and cytosolic changes (**Elmore** et al., **2013; Savill** et al., **2000**).

Here, in this study, different *in silico* approaches have been used to screen and explore the role of lactucine and its derivatives in the induction of apoptosis process. This study has been proposed to find the anticancer mechanism of lactucine and its derivatives, and also to find the molecular targets that are being induced as a result of binding interactions with lactucine and its derivatives.

2. Materials And Methods

2.1 Protein and ligand file

Three dimensional structure of lactucine and its derivatives were retrieved from Pubchem database in the SDF format (https://pubchem.ncbi.nlm.nih.gov/). Sequence information of apoptosis inducing proteins were retrieved from Uniprot database (https://www.uniprot.org/) in FASTA format for 3D structure modeling where as PDB file of known 3D structures of apoptosis inducing proteins were retrieved from the protein databank (https://www.rcsb.org/). Proteins whose structures were not available in the PDB database were modeled through Swiss-Modeller by searching and selecting suitable templates of highest sequence identity (https://swissmodel.expasy.org/).

2.2 Active site prediction

During docking process, information about active site is required to proceed for the docking. Active sites of proteins structures were determined by the in-build module of Mastero (Scrodinger) i.e. Sitemap (https://www.schrodinger.com/products/sitemap), which predicts the active site on the basis of the binding affinity using default parameters.

2.3 Molecular docking

2.3.1 Ligand Preparation

Ligand preparation is the preliminary step for molecular docking studies. LigPrep module (version 2.4, 2019) was used for geometrical refining of chemical structures (drawn in Maestro module) of lactucine and its derivatives and LigPrep was concise to set up refine 3D structures with precise chiralities. Ionizations original states were maintained, conformations and tautomers were generated by the Monte

Carlo method as executed in MacroModel version 9.8, 2010 using OPLS-2003 force field. Generated conformers were eventually minimized by means of the truncated Newton conjugate gradient (TNCG) minimization method up to 500 iterations. The conformational searches were carried out for aqueous solution using the generalized born/solvent accessible surface (GB/SA) continum solvation model (Still et al., 1990; Kaushik et al., 2012).

2.3.2 Protein Preparation

Protein preparation wizard of Maestro (Scrodinger) was used for protein preparation. Protein*s* involved in induction of apoptosis basically from caspase pathway were chosen for the docking with lactucine and its derivatives. Total of 46 protein structures were selected and prepared for missing hydrogens atoms and proper bond orders. Optimizations of hydrogen bonds were performed using sample orientation and then, all the polar hydrogens were displayed. Finally, the minimization of protein structures was done to a default root mean square deviation (RMSD) value of 0.30 Å.

2.3.3 Receptor Grid Generation

From the receptor-complex structure, the cocrystallised ligands were removed from its active site position of the receptor chain. The size of atom was equal to Van der Waals radii of (greater than) > 1.0 while 0.25 was the partial atomic charge by default. All ligands were docked using default Glide settings for a grid centered on the ligand and structure.

2.3.4 Molecular Docking Analysis

Flexible docking was performed using the extra precision (XP) feature of Glide module (version 12.1, 2019) **(Glide, Schrodinger, LLC, New York, NY, 2021).** The interaction of receptor and ligand was visualized by the ligand interaction viewer. Docking studies of lactucine and its derivatives were performed against the chosen proteins that are related to induction of apoptosis in the human leukemia carcinoma cell.

2.3.5 Selection of the best scored pose

Best docking poses for the lactucines and its derivatives were selected based on the docking scores. Values of different energies, number of hydrogen bonds, and visual inspection of all docking poses in Mastero (schrodinger, USA) were also taken into account. Protein and ligand interaction energy can be related to the binding affinities. Selection of best docked protein for each ligands was based on lower energy criteria. The rank was given by using glide D score.

2.3.6. ADMET properties prediction

The ADMET properties of lactucin 15-oxalate has been predicted from software admetSAR. ADMET is chemical adsorption, distribution, metabolism, excretion, and toxicity and it plays vital roles in every stage of drug discovery and development (Guan et al., 2019). Consequently, it is essential to find effective molecules with preferable ADMET properties. It is important to perform ADMET due to lack of efficacy and safety which is the two major causes leading to drug failure (Siramshetty et al., 2016). ADMET prediction software admetSAR is most compendious, curated resource for distinctive chemicals

interrelated with known absorption, distribution, metabolism, excretion, and toxicity profiles (Cheng et al., 2012).

3. Results And Discussion

3.1 Protein modeling

Proteins and ligands three dimensional structures were retrieved from PDB and Pubchem databases respectively. 3D structure of nine proteins BLK, Caspase 5, Caspase 10, Cyclin A1, Cyclin B2, Cyclin B3, Cyclin E2, Endonuclease G and NOXA 1 are not available in protein data bank, and structures of these proteins were generated using Swiss-Model tool. Template information for modeling different proteins is given in the Table 1. In all cases, template with highest identity percentage was used for model building.

Table 1
Target proteins and template information (identity, length and sequence coverage) used for modeling

Target protein	Template PDB ID	Identity	Sequence similarity	Coverage	Length of modeled protein
BLK	3VS6	69.98	0.52	0.88	494
Caspase 5	6NS7	61.15	0.48	0.64	309
Caspase 10	1M72	35.74	0.38	0.45	313
Cyclin A1	1V1N	67.69	0.50	0.56	185
Cyclin B2	6GU2	64.50	0.49	0.66	309
Cyclin B3	4BCQ	39.35	0.39	0.15	281
Cyclin E2	1W98	61.62	0.49	0.67	315
Endonuclease G	6NJU	93.33	0.60	0.81	292
NOXA 1	1WM5	37.31	0.38	0.42	243

3.2 Active site/pockets analysis

The active sites of the selected proteins were determined by the Sitemap which is present itself in Schrodinger. Sitemap identifies the active site of the protein which can be around four to five residue position inside the protein structure. Criteria to select the active site are based on three parameters (i) Dscore that is the drug ability score which should be more than 1, and if less than 1 then the active site position is not suitable to dock (ii) Sitescore which also gives information on the possibility of binding of a ligand to the target protein and this should be more than 1 and (iii) volume, this parameter is crucial as active site space required for the binding of ligand, and it should be above 500 Å^3. Active sites were

selected based on these criteria and these sites have shown remarkable binding affinity for the ligand molecules (Table 2**)**. Active site information of some other proteins was obtained from the Uniprot database (Table 3**)**. 3D Structure of some proteins such as AIF, Caspase-1, CDK-2, and CDK-1 were available in complex with their respective ligand in PDB database, and in that case the active site information of protein was depicted by removing the pre-existing ligand molecule (Table 4**)**.

Table 2
Active site of protein predicted by the Sitemap

S. No.	Protein	D score	Site score	Volume	Amino acid
1.	APO-3	0.9372	0.916	280.23	LYS335
2.	BLK	0.998	0.941	276.115	ASP355
3.	FADD	0.437	0.483	235.91	ARG142
4.	TRAF2	1.074	1.05	384.15	ASN418
5.	Cyclin B3	0.959	1.127	221.57	ARG1161
6.	Cyclin E2	0.955	0.915	235.641	GLU220
7.	Endonuclease G	1.152	1.11	386.218	HIS141
8.	CDK-4	0.965	1.064	414.0	ALA157
9.	Cyclin A2	1.13	1.13	153.664	VAL18
10.	Cyclin B2	1.189	1.17	131.71	LYS266
11.	Cyclin D2	1.09	1.045	319.33	ALA203
12.	FLIP	0.793	0.79	156.40	GLN346
13.	TNFR-1	1.042	1.028	302.86	GLN142
14.	TRAIL	0.952	0.971	447.95	SER238
15.	FASLG	0.7263	0.8227	2921.55	ALA254
16.	Cyclin E1	0.97	0.93	241.2	ASP383
17.	HtrA	1.043	1.0495	649.98	SER306
18.	Noxa	0.914	0.943	409.61	PHE186
19.	CDK-6	1.074	1.0464	600.25	PHE164
20.	Cyclin A1	1.19	1.172	138.57	CYS372
21.	Cyclin B1	1.06	1.142	220.20	ARG299
22.	Cyclin D1	1.09	1.02	323.7920	THR191
23.	Cyclin D3	1.077	1.189	117.64	PHE195

Table 3
Active site of protein without bound ligand (obtained from Uniprot)

S. No.	Protein	Amino acid
1.	BAD	ASP160
2.	BAX	SER184
3.	BID	GLY61
4.	Caspase-4	HIS210
5.	Caspase-5	HIS267
6.	TNF-Alpha	GLY24
7.	Caspase-2	HIS277
8.	Caspase-8	HIS317
9.	Caspase-10	HIS358
10.	P53	HIS178
11.	TRADD	GLN74
12.	PUMA	TYR71
13.	TNF-alpha	GLY24
14.	APAF-1	ARG265
15.	FAS	ARG52
16.	BAK	ASP160
17.	CASPASE 6	HIS121
18.	Caspase-7	CYS186
19.	Caspase-9	HIS237

Table 4
Active site of protein with ligand (determined by the PDB)

S. No.	Protein	Ligand	Amino acid
1.	AIF	FAD	ARG285
2.	Caspase-1	3-(2-mercapto-acetylamino-4-oxo-pentanoic acid)	CYS285
3.	CDK-2	ATP	VAL180
4.	CDK-1	(2,6-difluorophenyl)carbonyl]amino}-N-(4-fluorophenyl)-1H-pyrazole-3-carboxamide	ASP146

3.3 Molecular docking

In this study, lactucine and its derivatives were evaluated for their binding interaction for various targets through docking studies (Tabassum et al. 2014; Zahra et al. 2013). Literature survey indicates that lactucine have potential for treatment of human leukemia cancer by inducing apoptosis in the cell (Zhang et al., 2016). For a single drug, it is not possible to inhibit the continuous cell growth; therefore induction of apoptosis in cancer cells by some chemical compound can reduce the risk of cancer.

3.4 Docking Analysis

Molecular docking studies revealed that all the apoptosis inducing protein showed a good interaction with lactucine. Lactucin 15-oxalate interacts with all proteins which are responsible for apoptosis with maximum six H-bonds. Other types of interactions are also involved like Pi-cation, Pi-Pi stacking, salt bridges and halogen bonds. Protein CDK-4 has shown the highest number of H-bond (LYS142 (salt brigdes), ALA16, VAL14, ASP99, LYS35, TYR17, and ASN145) with the Lactucin 15-oxalate. All lactucine derivatives suitably docked on the apoptosis inducing proteins with ample Glide scores.

Lactucine and its derivatives have shown different Glide score and Dock score which is assumed due to the structural difference between these chemical compounds. This can be illustrated from the fact that the value of the Glide score and Dock score was lowest for the docking of cyclin A2 and cyclin B1 with lactucin 15-oxalate. Lower Dock score and Glide score value indicate the low binding affinity of a compound for protein target.

Molecular docking also determines the type of interaction exits between a protein and the ligand. Pi stacking is frequently seen in protein crystal structures, and provides interactions between small-molecules and proteins. The large polarizabilities of aromatic ring are due to its shape and electronic properties and substantial quardruple moment resulted in favored interaction geometry (Bissantz et al., 2010). Several studies recommended that the stacking interaction favors the binding energy of receptor-ligand (Boehr et al., 2002). Other compounds have shown cation –Pi bonding with the active site of

protein. Cation-pi bond interaction is a crucial contributor of stability and architecture of protein (Wintjens et al., 2000). The aromatic ring of the small molecule and the protein cation (Arg 142) provides the pi system. Energy wise, the cation-pi interaction is analogous to or robust than a hydrogen bond **(Gallivan et al,. 1999).** Many studies suggested that the cation-pi interaction is a strong force between proteins and ligands, and is a worthwhile predictor of drug-receptor interactions **(Supplementary table 1) (Zacharias et al., 2002).**

Consistent Glide score and Dock score suggests that lactucine would be a suitable lead for designing therapeutic against human leukemia. All docked proteins are responsible for inducing apoptosis in the normal cell whenever cell find any kind of damage or abnormality. Compound with high number of the H-bond and good binding affinity with apoptosis inducing proteins can be chosen for the in vitro studies. Different bonding interactions are shown in **Supplementary table 1**, and can be easily anticipated that lactucin 15-oxalate (L6) is best docked ligand with the apoptosis inducing proteins. In elucidating fundaments of biochemical process and rational drug designing, the binding behaviors play a crucial role. Based on these points, it can be asserted that lactucin 15-oxalate may provide novel anticancer agent. The amino acid involved in the interaction of CDK-1 with lactucin 15-oxalate is shown in the Fig. 2b. Cyclin A1, and Cyclin D3 have not shown docking interactions with lactucin 15-oxalate.

Many chosen targets have shown adequate contact with the ligand molecules which indicates that there endured an appropriate interaction. But, some target molecules such as BAD, BAK, Cyclin A1, Cyclin A2, Cyclin D3, and Caspase 7 do not show any kind of interaction with the ligand molecules. Dock score and Glide score of lactucin 15-oxalate for Caspase-5 was found the − 11.16 and − 11.13 (Kcal/mol) respectively which indicates reliable docking interactions. The interacting amino acids of Caspase-5 are ARG209, ARG371, and HIS320 which formed hydrogen bonding (-3.26 Kcal/mol) with the lactucin 15-oxalate (Table 5). Caspase 5 is an enzyme involved in apoptosis which activates Caspase cascade and degrades those cell which functions abnormally. Lactucin 15-oxalate may initiate apoptosis after binding with Caspase 5. Lactucine and its derivatives docked with the apoptosis inducing proteins and found adequate glide score for all targets except cyclin D3 and cyclin A1.

Table 5
Proteins with Glide score, DOCK Score, Hydrogen-bond information for compound lactucin 15- oxalate

S. No.	Protein	Glide score (Kcal/mol)	DOCK Score (Kcal/mol)	Hydrogen-bond (Kcal/mol)
1.	AIF	-8.52	-8.49	-1.89
2.	APAF-1	-8.57	-8.54	-2.14
3.	APO-3	-7.62	-7.59	-0.83
4.	BAD	-3.78	-3.76	-2.74
5.	BAK	-5.65	-5.63	-0.39
6.	BAX	-2.65	-2.63	-0.96
7.	BID	-6.19	-4.29	-0.33
8.	BLK	-4.57	-2.67	-1.1
9.	Caspase-1	-8.68	-8.66	-2.42
10.	Caspase-2	-4.28	-4.25	-1.07
11.	Caspase-4	-5	-4.9	-1.6
12.	Caspase-5	-11.16	-11.13	-3.26
13.	Caspase-6	-3.1	-1.19	-1.33
14.	Caspase-7	-4.89	-4.87	-2.24
15.	Caspase-8	-4.37	-4.34	-1.2
16.	Caspase-9	-1.35	0.56	-1.8
17.	Caspase-10	-4.85	-4.82	-1.11
18.	CDK-4	-5.91	-5.88	-1.71
19.	CDK-2	-6.87	-6.85	-1.78
20.	CDK-1	-6.6	-6.58	-1.01
21.	CDK-6	-4.9	-3	-1.95
22.	Cyclin A1	-	-	-
23.	Cyclin A2	-0.48	1.42	0
24.	Cyclin B1	0.33	0.35	-0.54
25.	Cyclin B2	3.2	5.11	0

S. No.	Protein	Glide score (Kcal/mol)	DOCK Score (Kcal/mol)	Hydrogen-bond (Kcal/mol)
26.	Cyclin B3	-0.71	-0.69	-1.59
27.	Cyclin D1	-3.11	-1.2	-1.22
28.	Cyclin D2	-2.83	-0.93	-0.35
29.	Cyclin D3	-	-	-
30.	Cyclin E1	-5.27	-3.36	-0.8
31.	Cyclin E2	-3.75	-3.73	-0.68
32.	Cytochrome C	-4.88	-2.97	-1.18
33.	Endonuclease G	-3.84	-3.82	-0.88
34.	FADD	-3.52	-3.5	-1.05
35.	FAS	-3.64	-3.23	-0.91
36.	FASLG	-4.7	-2.8	-1.9
37.	FLIP	-6.6	-6.57	-2
38.	Noxa	-2.46	-2.44	-2.16
39.	P53	-2.63	-0.72	-1.5
40.	PUMA	-2.02	-0.12	-2.35
41.	TNF-alpha	-2.58	-2.55	-1.26
42.	TNFR-1	-4.96	-3.06	-1.27
43.	TRADD	-3.46	-1.55	-1.6
44.	TRAF2	-6.42	-6.4	-1.23
45.	TRAIL	-5.61	-3.71	-1.4
46.	HtrA2	-4.1	-2.2	-1.1

3.5 ADMET properties prediction of Lactucin 15-Oxalate

The ADMET properties of the lactucin 15-oxalate have been generated by the software with various parameters (Table 6). Molecular weight of the selected molecule was 348.31Da and range for the 95% drugs is 130–725 Da. For the number of H-acceptor it was 7 and the range for the 95% drugs is 2–20 and similarly for the H-donor it was 2. Rotatable bonds of the drug were 2 in numbers which are indicating that drug have molecular flexibility and oral bioavailability (**Khanna et al., 2009**). A log p which is the

logarithm of the octan-1-ol/water partition coefficient and its value is -0.08, which is a good score for drug candidate **(Ntie-Kang et al., 2013).** These parameters signify that lactucin 15-oxalate has requisite ADMET properties and it is eligible to be a novel drug for the treatment of human leukemia cancer.

Table 6
ADMET profile of Lactucin 15-Oxalate

S.No.	ADMET prediction profile	Value
1.	Water solubility	-2.012 logS
2.	Plasma protein binding	0.519 (100%)
3.	Acute Oral Toxicity	2.404 kg/mol
4.	Tetrahymena pyriformis	0.573 pIGC50 (ug/L)
5.	Molecular Weight	348.31
6.	A logP	-0.08
7.	H-Bond Acceptor	7
8.	H-Bond Donor	2
9.	Rotatable Bonds	2

3.6 Validation of ligand protein interaction

Interaction of a reference drug with the apoptosis inducing proteins has also been determined through docking for the purpose of a comparative study. The reference drug is Dasatinib which is best known drug for the treatment of leukemia cancer. Dasatinib (anhydrous) is an aminopyrimidine that is 2-methylpyrimidine which is substituted at position 4 by the primary amino group of 2-amino-1,3-thiazole-5-carboxylic acid and at position 6 by a 4-(2-hydroxyethyl)piperazin-1-yl group, and in which the carboxylic acid group has been formally condensed with 2-chloro-6-methylaniline to afford the corresponding amide **(**Sankar et al., 2021).

Table 7
Docking scores and interacting amino acids of apoptosis inducing proteins with Dasatinib

S. No.	Protein	Dock score	Glide score	H-bond	Interacting amino acids
1.	AIF	-5.3	-4,8	-0.7	GLU164, ASP438(2), ARG285 (pi-cation), HIE455 (halogen)
2.	APAF-1	-8.2	-7.8	-1.3	GLN121, HIE438 (Pi-Pi stacking), ARG129 (halogen)
3.	APO-3	-7.4	-7.0	-1.3	LYS43, GLU45, GLU154, PHE157, LYS16, TRP63, ASP66, ARG67
4.	BAD	-4.5	-4.1	-0.4	TYR108 (Pi-Pi stacking), GLN153, GLU25
5.	BAK	-4.5	-4.1	-0.7	TYR108 (Pi-Pi stacking), GLN153 (halogen), GLU25
6.	BAX	-3.3	-2.9	-1.0	TYR108 (Pi-cation), PHE157 (halogen), ARG156(2)
7.	BID	-3.1	-2.7	-1.4	ASP57(3), TRP53 (Pi-Pi stacking, Hbond)
8.	BLK	-5.2	-4.8	-0.3	ASP378
9.	Caspase-1	-4.8	-4.4	-2.9	GLN283, THR180, ASP185 (2, salt bridge)
10.	Caspase-2	-5.6	-5.2	-1.2	GLN129, GLU287, CYS289, THR291, LYS225 (halogen), ARG156 (Pi-cation), ARG156 (Pi-cation), LYS225 (Pi-cation), GLU115
11.	Caspase-4	-4.2	-3.8	-1.6	GLY260, ARG256(2), HIE210, HIE309,
12.	Caspase-5	-3.5	-3.0	-1.5	HIE267 (Pi-Pi stacking), GLU318 (2), ALA208
13.	Caspase-6	-3.4	-3.0	-1.1	PHE55 (halogen), GLU191
14.	Caspase-7	-4.3	-3.9	-1.3	TRP232 (Pi-Pi stacking), PHE282, TYR230, ARG233
15.	Caspase-8	-5.2	-4.8	-2.0	TYR412, ARG258 (Pi-cation), ASP259, ARG413
16.	Caspase-9	-5.1	-4.7	-1.2	LYS409, THR337, GLY269, LYS409
17.	Caspase-10	-6.3	-5.9	-1.1	ARG360, GLY362
18.	CDK-4	-10.2	-9.7	-1.3	GLU8, LEU83(2)
19.	CDK-2	-7.7	-6.6	-0.7	LYS129, THR165
20.	CDK-1	-4.5	-4.0	-1.0	VAL176, LYS142, GLU144, LYS35 (Pi-cation)
21.	CDK-6	-4.3	-3.9	-1.6	LYS43, ASP145, ARG186
22.	Cyclin A1	-	-	-	-
23.	Cyclin A2	-4.7	-4.3	-0.7	ASN237 (halogen), LYS194, SER386,
24.	Cyclin B1	-9.4	-8.9	-1.7	SER227 (halogen), VAL336, GLU182, GLU181

S. No.	Protein	Dock score	Glide score	H-bond	Interacting amino acids
25.	Cyclin B2	-3.9	-3.5	-1.7	HIE302 (Pi-Pi stacking), ARG196, LEU148, ILE151
26.	Cyclin B3	-3.2	-2.8	-0.9	LYS1187, GLU1298, LYS1299 (Pi-cation, H-bond), LYS1302
27.	Cyclin D1	-2.5	-2.1	-0.3	GLU36(2), ILE237
28.	Cyclin D2	-6.2	-5.8	-1.3	GLU73, PHE77
29.	Cyclin D3	-	-	-	-
30.	Cyclin E1	-8.3	-7.8	-0.9	GLU188 (2)
31.	Cyclin E2	-6.9	-6.5	-0.7	GLU200 (2), TRP107
32.	Cytochrome C	-4.7	-4.3	-1.3	LYS13, LYS73 (Pi-cation), GLY84, ASN70,
33.	Endonuclease G	-3.9	-3.5	-0.7	LEU75
34.	FADD	-4.5	-4.0	-1.7	GLU94, HIS91, ARG142 (Pi-cation, H-bond), CYS105
35.	FAS	-2.6	-2.2	-2.2	ARG52 (Pi-cation), GLU51, ARG105, PRO49, PRO68
36.	FLIP	-4.7	-4.3	-0.9	TR407, TRP466 (Pi-Pi stacking), ASN359,
37.	Noxa	-3.2	-2.8	-1.6	HIE190, ARG39, HIE187 (Pi-Pi stacking), ARG188 (halogen, H-bond), THR111, ARG116 (Pi-cation)
38.	P53	-7.2	-6.8	-1.8	HIE115, PHE113 (halogen), ASP238, GLY226
39.	PUMA	-1.8	-1.4	-1.2	GLU74, TRP71 (Pi-Pi stacking), GLN70, GLU69
40.	TNF-alpha	-2.2	-1.8	-0.7	LYS90
41.	TNFR-1	-7.3	-6.9	-2.1	SER86
42.	TRADD	-3.8	-3.4	-0.8	HIE65 (Pi-cation), TYR16 (Pi-cation), ARG148, GLN74, GLN143
43.	TRAF2	-6.5	-6.1	-1.1	GLU346, LYS357(2)
44.	TRAIL	-5.6	-5.2	-2.6	ASN134, HIS265 (Pi-Pi stacking), ASN143, ASN134 (halogen), LEU239
45.	FASLG	-2.2	-1.8	-2.1	GLU42 (2), ASP40, ARG60 (halogen), GLN59
46.	Htra2	-6.1	-5.7	-2.7	ARG226, VAL90, ARG299 (Pi-cation), ASP91, GLU292(2)

Docking studies of Dasatinib drug with the target proteins showed reliable binding interaction (Table 7). Glide and dock score are significant parameters from binding point of view, and it is considerable for the comparative binding study of the drug and ligands. Top Dock score of the Dasatinib with APAF-1, APO-3,

CDK-4, cyclin-B1, cyclin E1 targets with value – 8.2, -7.4, -10.2, -9.4 -8.3 Kcal/mol showing prominent binding. In case of lactucin 15-oxalate, proteins with top dock score were caspase-5, AIF, caspase-1, APAF-1, and APO-3 with value of -11.13, -8.49, -8.66, -8.59, -7.59 Kcal/mol convicts preferable scores for better interactions. On comparison, best interaction shown by protein was APAF-1, APO-3 for both the Dasatinib and lactucin 15-oxalate, which specify that lactucin 15-oxalate have exquisite binding affinity for the apoptosis inducing proteins. Dock score of both the Dasatinib drug and the lactucin 15-oxalate with the apoptosis inducing protein stipulates that selected ligand have equitable interaction with the target proteins.

As it reveals that ligand have acceptable score for further research. In the case of Dock score and Glide score, the most significant protein from binding interaction point of view was Caspase 5 with lactucin 15-oxalate and Dasatinib. On the other hand, H-bond energy of the Dasatinib with CDK-4 has shown adequate H-bond energy. In comparison of both the Dastanib and Lactucin 15-oxalate, their highest H-bonding with CDK-4 are nearby equivalent and shows efficient interaction As CDK-4 has shown highest H bonding with lactucin 15-oxalate and for the drug the numbers of bonds are also higher in comparison to ligand **(**Fig. 3**).**

Docking of Caspase 2 with Dasatinib have shown nine amino acids GLN129, GLU287, CYS289, THR291, LYS225 (halogen), ARG156 (Pi-cation), ARG156 (Pi-cation), LYS225 (Pi-cation), and GLU115 in interaction. For the lactucin 15-oxalate, there are four amino acids GLU115, LYS225 (2), TYR221, and GLN129 in interaction with Caspase 2. But for CDK-4, the interacting amino acids for the Dasatinib are VAL176, LYS142, GLU144, and LYS35 (Pi-cation) where as lactucin 15-oxalate interacts with LYS142 (salt brigdes), ALA16, VAL14, ASP99, LYS35, TYR1, and ASN145 of CDK-4, which indicates a better interaction with the CDK-4.

Three dimensional interactions of protein CDK-4 and TNFR-1 with Dasatinib and lactucine 15-oxalate has been depicted in Fig. 3 and also showing hydrogen bond interactions. The hydrogen bond can be measured and which is found to be near about 2.59 Å for the Dasatinib and 2.56 Å with lactucin 15-oxalate on interaction with CDK-4 implies appropriate distance for binding affinity. In the case of TNFR-1, hydrogen bond was found to be near about 2.16 Å with Dasatinib and 3.20 Å with the lactucin 15-oxalate. On the basis of these analysis, it can be emphasized that lactucin 15-oxalate having reasonable interaction with CDK-4 which can be interest for the further research on Leukemia drug discovery.

Lactucin 15-oxalate docked with the apoptosis inducing proteins and found adequate Glide score for all targets except Cyclin D3 and Cyclin A1. Lactucin 15-oxalate can be considered as the most reliable ligand as it exhibit high affinity with the apoptotic inducing protein and can be used as anticancer agent. Lactucin 15-oxalate bears the best glide score for each macromolecule except Cyclin D3 and Cyclin A1. Cyclin D3 and Cyclin A1 is involved in cell cycle's G1 and S phase respectively and there function is to activate cyclin dependent kinase (CDK). Molecular docking results indicates that lactucin 15-oxalate having best Glide score, Dock score and highest number of H bonding with the CDK-4 may serve as a potential lead molecule for development of novel anticancer drugs. On the other hand, these results are

based on preliminary computational analysis and surely need experimental confirmation. Lactucin 15-oxalate may possibly be a first choice as a lactucine based lead which could be used to design anticancer agents of future.

4. Conclusion

Human leukemia cancer is a life threatening disease with a mortality rate of 9.4 % and requires treatment to save the life of people. Lactucine have anticancer effect which may induce apoptosis in cancerous cell and protect other cell from getting infected. In this study, lactucine derivatives are docked with the apoptosis inducing proteins for the prediction of its anticancer effect. Lactucin 15-oxalate has shown highest binding affinity for the CDK-4 target and can be used as a lead compound for the cancer treatment. Glide and Dock score for docking of lactucin 15-oxalate with CDK-4, well as the number of hydrogen bonding is in agreement to use this ligand for study. These *in silico* results are valuable to proceed for the in vitro and in vivo studies related to anti-cancer role of lactucin 15-oxalate.

Declarations

Conflict of interest

The authors declare that there are no conflicts of interests.

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Figures

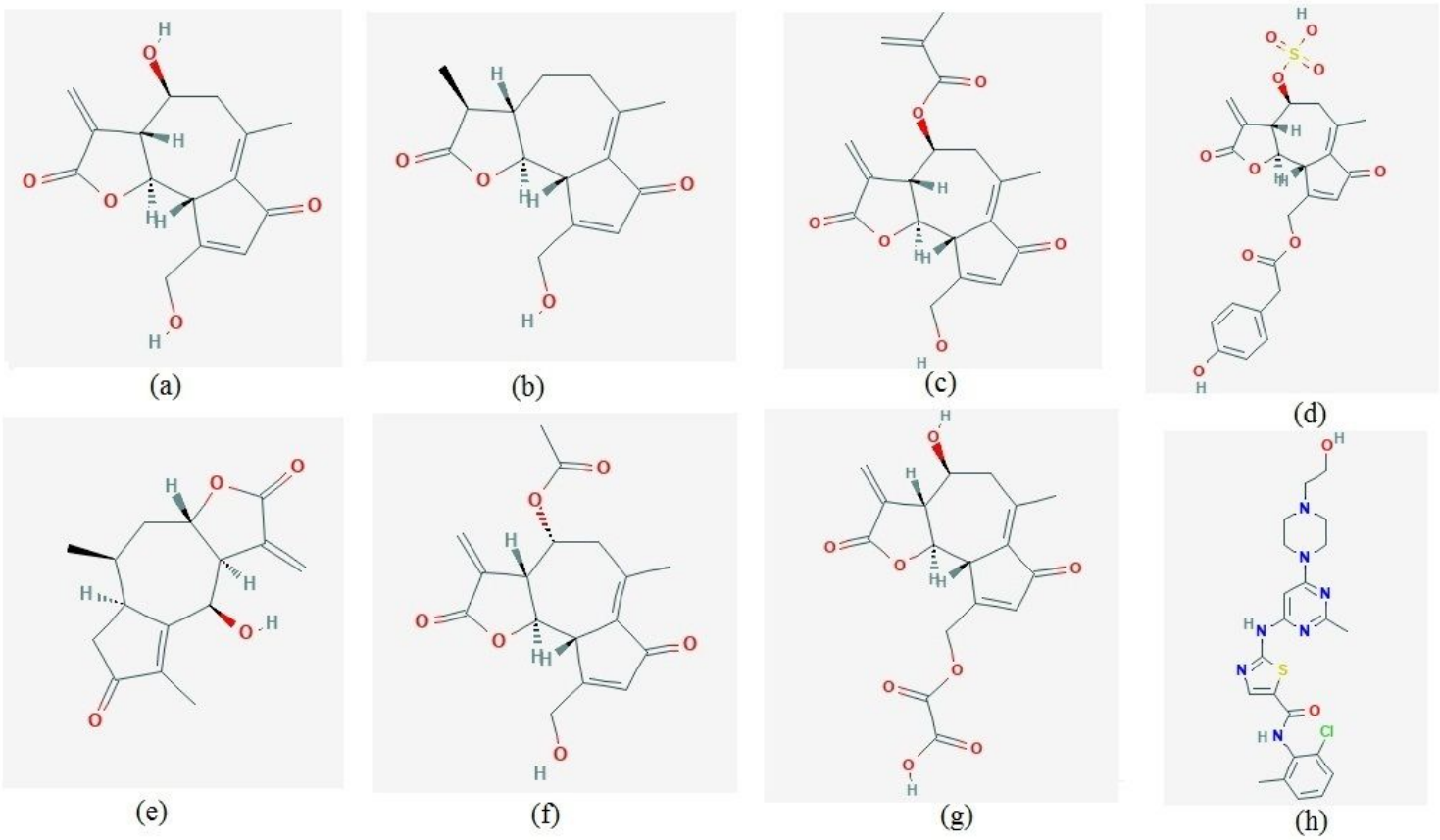

Figure 1

Two dimensional structures of lactucine and its derivative, and drug Dasatinib (a) Lactucine (b) 11beta,13-Dihydro-8-deoxylactucine (c) Lactucine-8-O-methacrylate (d) 15-(p-hydroxypphenylacetyl) lactucine-8-sulphate (e) Mikanokryptin (f) Lactucine-8-acetate (g) Lactucin 15-oxalate (h) Dasatinib

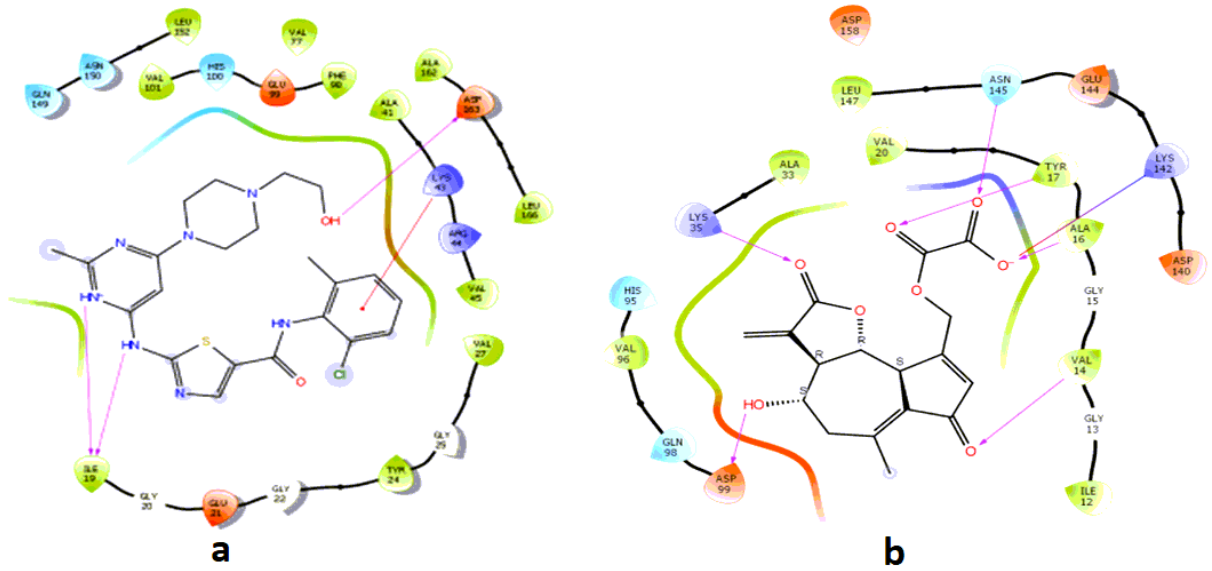

Figure 2

Interaction of CDK-1 (a) Dasatinib (b) Lactucin 15-oxalate. Different types of bonding have been represented in different colours. Purple: hydrogen bond; green: Pi-Pi stacking; red: Pi-cation; brown: halogen; bluish red: salt bridges

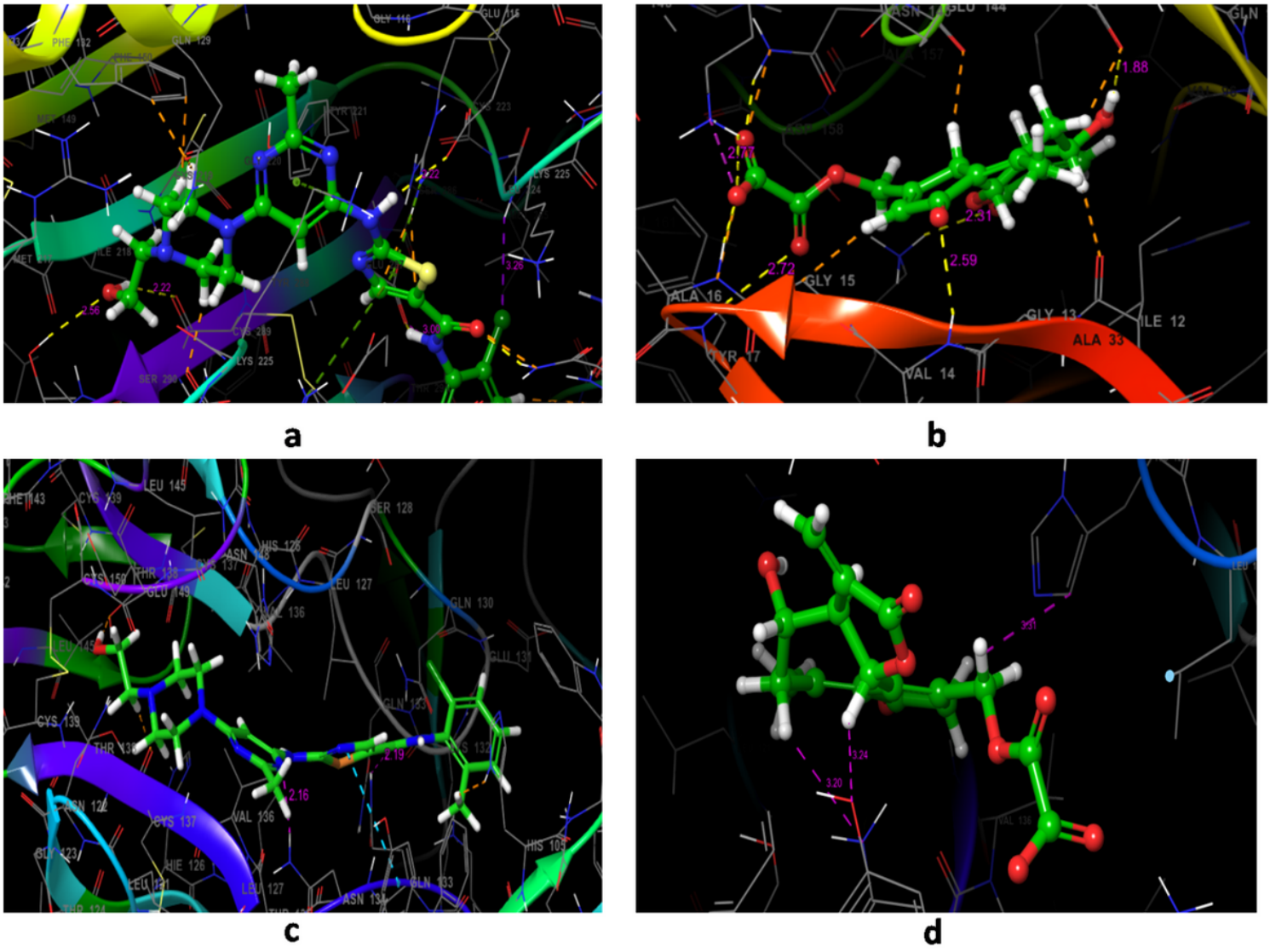

Figure 3

Three Dimensional docking images of interaction between protein with Dasatinib and lactucine 15-oxalate. (a) CDK-4 with Dasatinib (b) CDK-4 with lactucin 15-oxalate (c) TNFR-1 with Dasatinib (d) TNFR-1 with lactucin 15-oxalate

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