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DESIGN DOCKING STUDIES ON ANTIAPOPTOTIC PROTEIN INHIBITORS AS A NOVEL TARGET ON BREAST CANCER

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ABSTRACT

Cancer mainly Breast cancer being one of the most common type of cancer effecting large number of population in the present day life, various drug molecules were developed against breast cancer where the cancer cells are being resistant to the drug molecules. Inhibition of apoptosis pathway is one of the leading causes of cancer, where there is uncontrolled growth of cells leading to the formation of tumors. C-FLIP is one of such antiapoptotic protein which inhibits the apoptosis process, which can be considered as drug target by inhibiting the c-FLIP activity there will be increase in the apoptosis process which would be of potential use. In our present study we modeled c-FLIP protein containing death effector Domains (DED's) and have taken some of natural and synthetic inhibitors that inhibit c-FLIP protein and studied the interaction studies of these ligand molecules with the protein. Among the taken 37 ligands 12 ligands were interacting with the c-FLIP protein. Among the synthetic compounds droxinostat and in natural compounds chirac in are showing highest dock scores of 44.169 and 19.758. These studies could of potential use in generating new drug molecules by creating analogues with the highest interacting molecules.

Keywords: c-FLIP, Modeling, Natural compounds, synthetic compounds, Death Effect or Domains (DED)

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INTRODUCTION

Breast cancer is one of the leading disease that is affecting large number of population in the world. Damage in the Apoptotic pathway may leads to the continuous growth of the cells which in turn leads to cause of cancer. Now-a-days various studies have been done on the apoptotic signaling pathway which acts a novel drug target for breast cancer. Apoptosis of the cells is mainly caused in two different pathways, death receptor- induced pathway and mitochondria-mediated pathway [1]. In the Death receptor induced pathway death ligand binds to the death receptor, this helps in the formation of death inducing signaling complex followed by cleavage of Caspase-8 activation. Tumor necrosis factor related apoptosis inducing ligand (TRAIL) is attaining a high attention due to its activity in apoptosis pathway, Present mutated TRAIL's are being used as the anti-apoptotic agents which are in their phase trails [2,3]. Cellular FLICE-like inhibitory protein(c-FLIP) is a catalytically inactive Caspase-8 homologue, Death receptor –mediated apoptosis is mainly inhibited by c-FLIP by preventing the Caspase-8 binding with death inducing signaling complex [4,5.]c-FLIP contains various variants ,among all the variants c-$FLIP_L$ and c-$FLIP_S$ which are well characterized. These 2 variants contain two death effectors domains (DED) [6-9.]

Due to the increase in resistance to apoptosis which is mediated by TRAIL and FAS leads to the over expression of c-FLIP [10]. In c-FLIP two proteins short form and long form ($FLIP_L$ and c-$FLIP_S$) plays a key role in the death receptor mediated apoptosis by binding with the DISC and inhibiting the Caspase-8, Caspase-10 activation [11]. Several studies have proved that TRAIL and FAS mediated apoptosis can be sensitized by down-regulating the c-FLIP activity [12-15].Various studies have been showed that down-regulation of c-FLIP can be done by various chemical and natural compounds which can inhibit or regulate the activity of the protein molecule [16-18].

It has been studied that various synthetic and natural compounds are showing activity against the c-FLIP protein which is one of the most studied drug target in the death receptor mediated apoptosis pathway. It has also been studied that some of the natural available plant extracts not only inhibit the c-FLIP function but they in turn can inhibit the growth of certain type of cancer cells [19-24].

The main purpose of this study is identifying the best synthetic and natural inhibitor molecules for the c-FLIP protein using the receptor-ligand interaction studies. In the present study we have considered a list of synthetic and natural ligand molecules and have done docking studies to identify the best active synthetic and natural compound.

MATERIALS AND METHODOLOGY

Selection of protein molecule:

Protein molecule selection is done using swissprot database. In the swissprot database availability of 3D structure is verified and the functional domains of the protein molecules were studied using the Swissprot database.

Template selection and Sequence alignment:

Structure similar to the protein is selected using the NCBI Blast algorithm. In which highest similarity structure is selected. The 3D structure of the protein and the FASTA format were collected and then using. Template sequence and the protein sequence were aligned using the sequence alignment algorithm in Discovery Studio Software.

Homology modeling and model verification of protein:

Using the template selected and the alignment file structure of the protein molecule is modeled in the Discovery studio software using Build Homology model protocol in the parameters file. Once the structure is modeled the structure of the protein is verified using the various model verification servers like Pro check, pro sa, RMSD.

Protein preparation and energy minimization:

Modeled protein molecule is then prepared by cleaning and applying the charm force fields to the protein molecule. The energy of the prepared protein molecule is minimized using various algorithms like steepest descent and conjugate gradient methods in which the potential energy of the protein molecule is decreased. [25-26].

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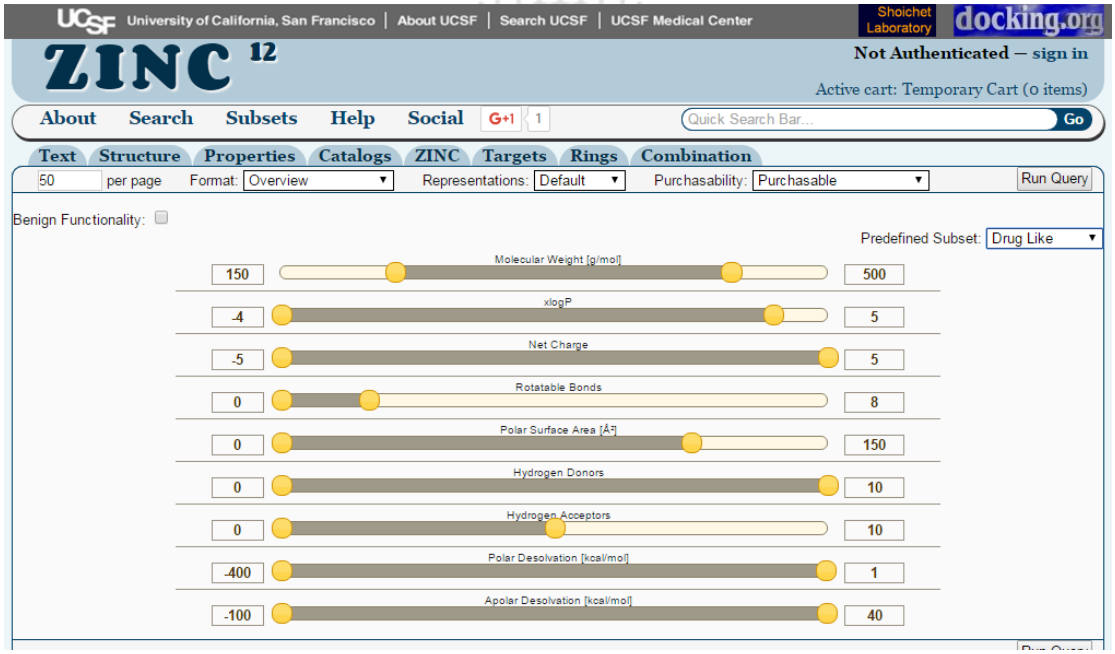
Virtual Screening in Zinc Database:

Zinc an acronym for zinc is not commercial, a free database for virtual screening' contains over 21 million compounds in ready-to-dock, 3D formats, available at the URL http://zinc.org. Molecules in zinc are annotated by molecular property that include molecular weight, number of rotatable bonds, calculated log P, number of hydrogen-bond donors, hydrogen-bond acceptors, chiral centers, chiral double bonds (E/Z isomerism), polar and a polar desoluation energy (in kcal/mol), net charge and rigid fragments.

1] We filter-out molecules with molecular weight 150 to 350, calculated Log P greater than 6 and less than 4, number of hydrogen-bond donors greater than 6, number of hydrogen bond acceptors greater than 2, and number of rotatable bonds greater than 15. We also remove all molecules containing an atom other than H, C, N, O, F, S, P, Cl, Br, or I. We do make exceptions, for example, to include a number of actual drugs that violate these constraints; these rules are guidelines toward making the database loosely conform to current opinion in the field.



2] After selecting the search option on Zinc Database home page we performed the search according to properties specifically.

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3] For the same we selected predefined subset-Drug Like and then submitted for virtual screening.

RESULT AND DISCUSSION

Selection of protein molecule:

Protein molecule is selected from Swissprot database with Accession number: O15519.The FASTA format of the protein sequence is taken from 1-376 amino acids which contain DED1 and DED2 functional domains and the FASTA format is submitted for protein blast to obtain the structure which is similar to the protein sequence.

Selection of template:

Selection of template is done using PBLAST search 3H11 is obtained as the template sequence with an identity of 99%.3H11 is a zymogene caspase-8: c-Flip protease domain complex. The structure of the template is downloaded from the PDB database and loaded into Discovery studio.

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A, Zymogen Caspase-8:c-Flipl Protease Domain Complex >pdb|3H13|A Chain A, C-Flipl Prc	353	353	44%	3e-120	99%	3H11_A
Chain A, Crystal Structure Of Mc159 Reveals Molecular Mechanism Of Disc Assembly And Vflip Inh	85.9	85.9	61%	4e-19	30%	2BBZ_A
Chain A, Crystal Structure Of A Viral Flip Mc159	82.4	82.4	45%	3e-18	32%	2F1S_A
Chain A, Crystal Structure Of Mc159 Reveals Molecular Mechanism Of Disc Assembly And Vflip Inh	82.4	82.4	48%	4e-18	32%	2BBR_A
Chain A, Crystal Structure Of A Vflip-Ikkgamma Complex: Insights Into Viral Activation Of The Ikk Sig	75.1	75.1	44%	1e-15	34%	3CL3_A
Chain B, Crystal Structure Of The Caspase-8/p35 Complex >pdb|2FUN|B Chain B, Alternative P35-	75.1	75.1	35%	3e-15	33%	1I4E_B
Chain A, Solution Structure Of The Catalytic Domain Of Procaspase-8	75.1	75.1	35%	3e-15	33%	2K7Z_A
Chain A, Caspase-3 Specific Unnatural Amino Acid-based Peptides	75.1	75.1	35%	4e-15	33%	4JJ7_A

Fig 1: Showing the BLAST results in NCBI server where 3H11 protein molecules 'A' chain is showing the highest identity with the modeled protein structure.

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Sequence Alignment:

The protein sequence and the template sequences were aligned in the Discovery Studio software and the alignment is done with a sequence identity of 33.9%.

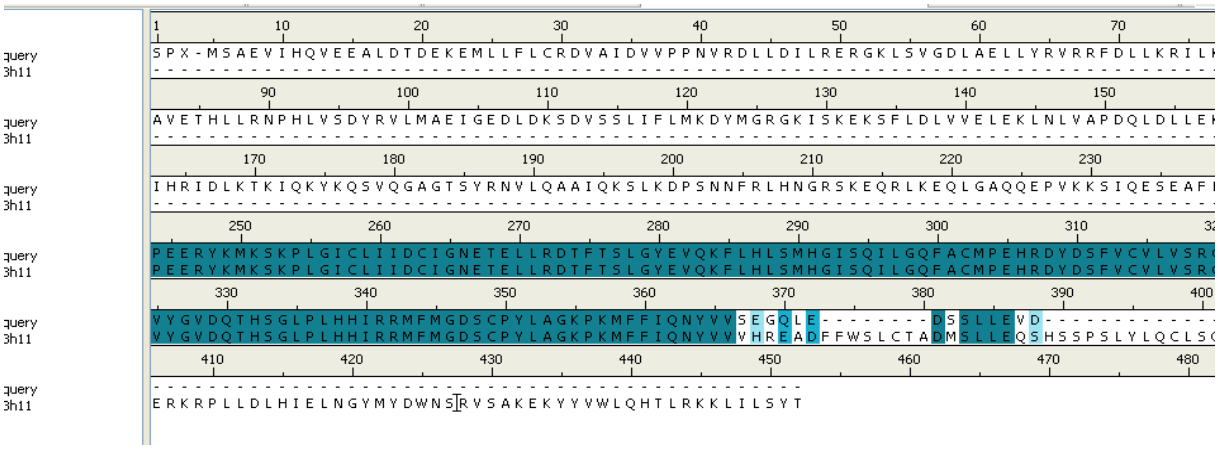

Fig 2: Showing the sequence alignment of C-FLIP and 3H11 in Discovery studio software where the shaded regions in figure represent the similar amino acids in the two sequences.

Modeling:

Homology modeling of the protein molecule is done using Discovery studio software using build homology models in the protocols

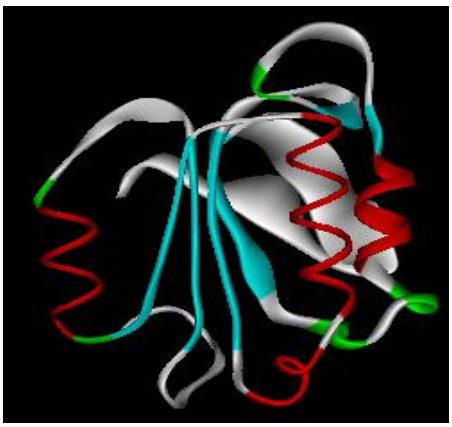

Fig 3: Showing the modeled structure of the protein molecule in discovery studio in solid ribbon format.

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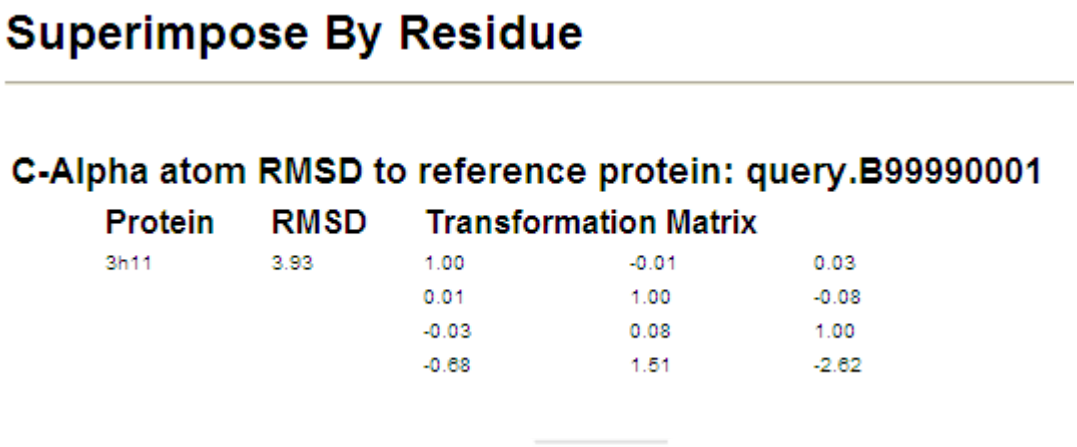
Model Verification:

Model verification of the protein molecule is done using the various servers to check the quality of the modeled protein molecule.

RMSD:

RMSD of the modeled structure is calculated in the Discovery studio software, first the two structures query and the template were superimposed then the RMSD is calculated as 3.93



Superimpose By Residue

C-Alpha atom RMSD to reference protein: query.B99990001

Protein	RMSD	Transformation Matrix		
3h11	3.93	1.00	-0.01	0.03
		0.01	1.00	-0.08
		-0.03	0.08	1.00
		-0.68	1.51	-2.62

Fig 4: Showing the calculated RMSD value of the modeled protein molecule when it is superimposed with 3H11 protein molecule.

Pro check:

Pro check is used for the assessment of the stereochemical properties of modeled protein structure. Pro check is done using online server SAVES-Pro check

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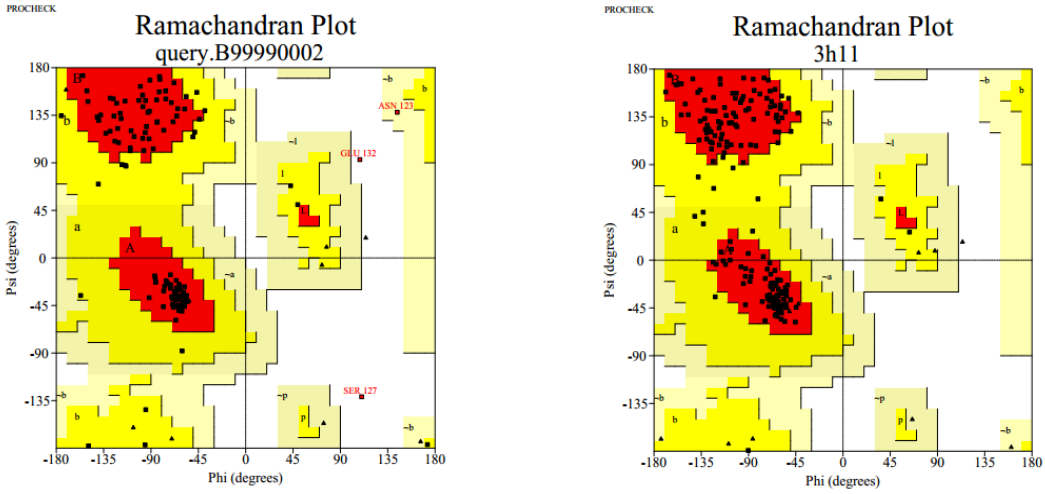

(A) **(B)**

Fig 5: Showing results of Ramchandran plot analysis for the modeled protein molecule (A) and the template 3H11 protein (B). The graphs in figure are showing the amino acids in the allowed and disallowed regions.

Table 1: showing the results of pro check for modeled sequence and template 3H11 where the modeled protein have a 83.3 % of amino acids present in the core region and 14.4 % in the allowed , 1.7 % in the disallowed regions.

Structure	Core	Allowed	Generous	Disallowed
B9999002	83.3	14.2	0.8	1.7
3H11	91.5	8.5	0.0	0.0

Virtual Screening in Zinc Database:

Docking (Ligand Fit):

Docking results of synthetic ligand molecules

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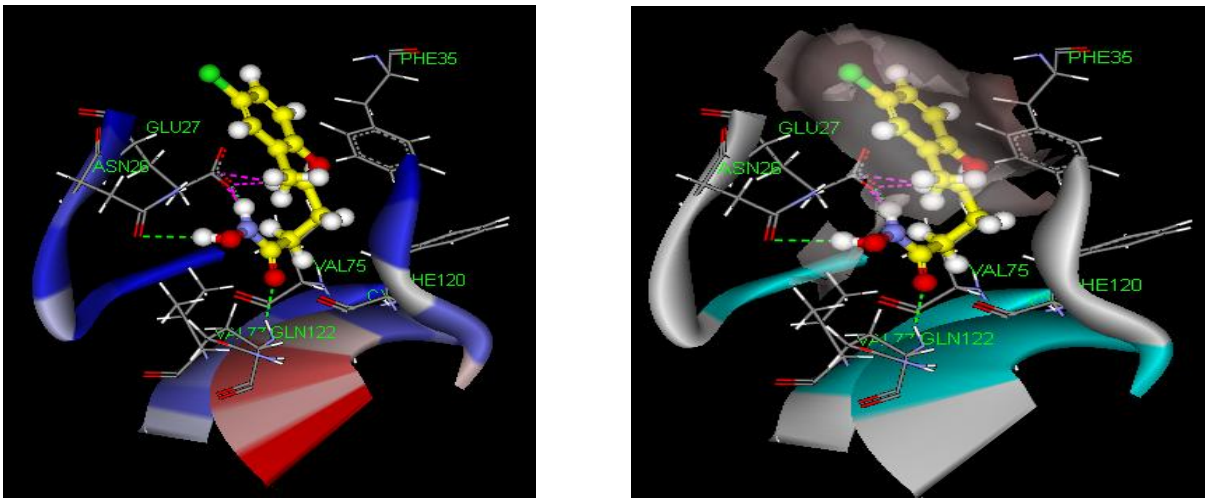
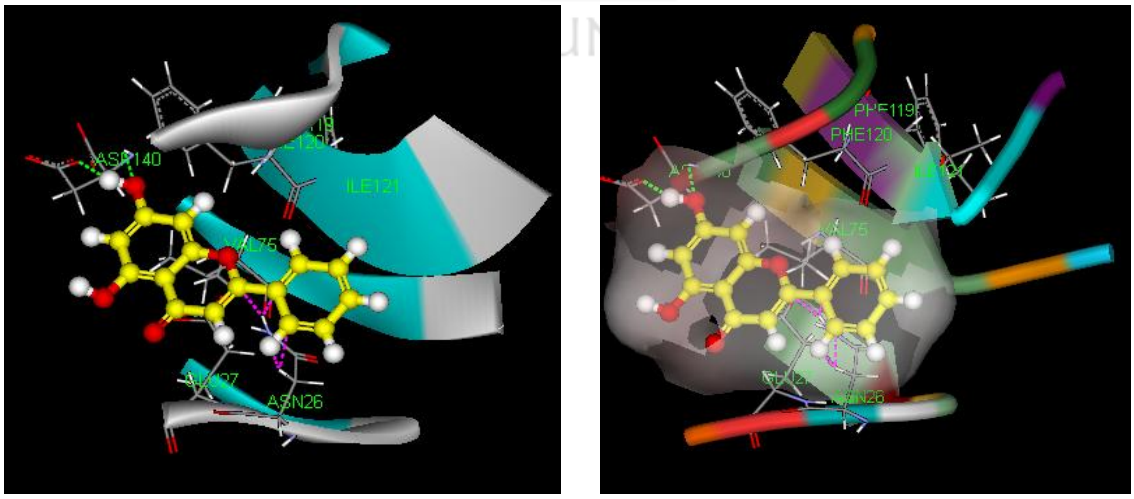


(A) **(B)**

Fig 7: Fig 7 (A) Droxinostat is interacting with ASN26, GLU27, GLN122 amino acids of modeled protein molecule. Fig 7(B) is showing the interaction of the Droxinostat with modeled protein molecule where the ligand molecule Droxinostat is covered with electrostatic surface area.

Docking results of natural ligand molecules



(A) **(B)**

Fig 8: Fig 8 (A) Chyrisin is interacting with ASP140, GLU27, ASN26 amino acids of modeled protein molecule. Fig 8(B) is showing the interaction of the Chyrisin with modeled protein molecule where the ligand molecule Chyrisin is covered with electrostatic surface area.

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Table 2: showing the docking results of synthetic and natural compounds with modeled protein molecule

Ligand	Ligscore1	Ligscore2	-PLP1	-PLP2	Jain	-PMF	Dock_score
Anisomycin	1	2.22	26.77	27.94	1.37	24.12	3.659
Cystamine	0.13	3.27	20.28	16.42	-0.99	5.82	26.32
Droxinostat	3.13	2.67	38.93	42.62	2.24	22.34	44.169
Pyrido[2,3-d]pyridine	1.55	2.99	30.81	27.67	1	30.42	20.346
Toxol	1.16	2.1	29.97	34.11	0.08	4.39	2.934
Valproicacid	1.36	1.24	20.73	30.89	-0.02	13.97	32.583
Vorinostat	1.93	1.4	43.49	46.38	1.9	24.63	12.422
Apigenin	2.01	3.38	37.99	37.44	-0.46	23.22	18.608
Chyrisin	1.45	3.07	34.19	32.15	0.17	23.88	19.758
Genistein	1.64	2.71	24.83	30.85	0	19.93	14.763
Honokiol	1.1	2.79	31.7	39.52	0.98	18.67	8.38
Wagonin	1.3	1.9	10.86	20.25	0.42	20.22	14.249

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In the fig 8 and fig 9 are showing the hydrogen bond interactions of the highest docked ligand molecules with the modeled protein molecule. In the figures yellow color molecules are ligand molecules which are represented in the form of ball and stick model and the carbon atoms are colored in yellow color. Green and red color dotted lines represent the H-Bond interactions and bumps of Ligand molecule with the receptor. Modeled protein molecule is represented in the form of solid ribbon.

CONCLUSION

c-FLIP is one of the important drug target in case of TRAIL and Drug/chemotherapy resistant cell lines. C-FLIP has attained a much importance in cancer treatment; inhibition of c-FLIP could help in increasing the apoptosis of cancer cells. In our present study, we studied the interaction of the C-FLIP with the natural and synthetic inhibitors that stop the activity of c-

FLIP. C-FLIP contains two death effector regions (DED1, DED2) which have their activity in inactivating c-FLIP, here we have taken the C-FLIP protein containing the two death receptor and modeled the protein molecule by taking 3H11 as the template structure in Discovery studio. Modeled protein structure is the validated to predict the quality of the structure using Ramachandran plot analysis, Prosa and RMSD which proved that modeled protein is of good quality. Modeled structure is prepared and energy of the protein minimized using various algorithms, later structure is selected as receptor and active site were identified for the protein molecule. All the ligand molecules were docked against the protein molecules active sites and the inhibitors with highest binding were identified as Droxinostat and chyrisin which are showing highest dock scores of 44.169 and 19.758. In this study Droxinostat is interacting with ASN26, GLU27, GLN122 amino acids of c-FLIP and chyrisin is interacting with ASP140, GLU27, ASN26 amino acids of c-FLIP protein. Among all the 37 ligands taken only 12 ligands were showing interactions with c-FLIP protein, these studies also revealed that synthetic compounds are showing highest interactions when compared to the natural compounds. These studies could be helpful in studying the highest interacting ligand and could be helpful in creating analogues for highest binding molecule to create novel drugs for inhibiting C-FLIP protein.

REFERENCES

1. Hengartner MO. (2000) The biochemistry of apoptosis. Nature , 407,770–6.
2. Kelley SK, Ashkenazi A. (2004) Targeting death receptors in cancer with Apo2L/TRAIL. Curr Opin Pharmacol ,4,333–9.
3. Rowinsky EK. (2005) Targeted induction of apoptosis in cancer management: the emerging role of tumor necrosis factor-related apoptosis inducing ligand receptor activating agents. J Clin Oncol ,23, 9394 –407.
4. Krueger A, Baumann S, Krammer PH, Kirchhoff S. (2001) FLICE-inhibitory proteins: regulators of deathreceptor-mediate d apoptosis. Mol Cell Biol 21, 8247–54.
5. Budd RC, YehWC , Tschopp J. (2006) FLIP regulation of lymphocyte activation and development. Nat Rev Immunol, 6,196–204.
6. Golks, A.; Brenner, D.; Fritsch, C. (2005) Krammer, P. H.; Lavrik, I. N. c-FLIPR, a new regulator of death receptor-induced apoptosis. *J. Biol. Chem. 280*, 14507-14513.
7. Tschopp, J.; Irmler, M. (1998) Thome, M. Inhibition of Fas death signals by FLIPs. *Curr. Opin. Immunol.* , *10*, 552-558
8. Wajant H. (2003)Targeting the FLICE inhibitory protein (FLIP) in cancer therapy. Mol Interv 3,124–7.
9. Kataoka T.(2005) The caspase-8 modulator c-FLIP. Crit Rev Immunol ,25,31–58
10. M. Djerbi, V. Screpanti, A.I. Catrina, B. Bogen, P. Biberfeld, A. Grandien, (1999) The inhibitor of death receptor signaling, FLICE-inhibitory protein defines a new class of tumor progression factors. J Exp Med 190 , 1025-1032.

11. Scaffidi C, Schmitz I, Krammer PH, Peter ME.(1999) The role of c-FLIP in modulation of CD95-induced apoptosis. J Biol Chem , 274,1541–8.
12. Galligan L, Longley DB, McEwan M, Wilson TR, McLaughlin K, Johnston PG. (2005) Chemotherapy and TRAIL-mediated colon cancer cell death: the roles of p53, TRAIL receptors, and c-FLIP. Mol Cancer Ther 4,2026–36.
13. Fulda S, Meyer E, Debatin K-M. (2000) Metabolic inhibitors sensitize for CD95 (APO-1/Fas)-induced apoptosis by down-regulating Fas-associated death domain-like interleukin 1-converting enzyme inhibitory protein expression. Cancer Res 60, 3947–56.
14. Kim Y, Suh N, Sporn M, Reed JC. (20020 An inducible pathway for degradation of FLIP protein sensitizes tumor cells to TRAIL-induced apoptosis. J Biol Chem ,277,22320–9.
15. Longley DB, Wilson TR, McEwan M, et al. (2006) c-FLIP inhibits chemotherapy-induced colorectal cancer cell death. Oncogene 25, 838–48.
16. Wajant H. (2003) Targeting the FLICE inhibitory protein (FLIP) in cancer therapy. Mol Interv 3,124–7.
17. Abedini MR, Qiu Q, Yan X, Tsang BK.(2004) Possible role of FLICE-like inhibitory protein (FLIP) in chemoresistant ovarian cancer cells in vitro. Oncogene 23, 6997–7004
18. Rogers KM, Thomas M, Galligan L, et al. (2007) Cellular FLICE-inhibitory protein regulates chemotherapy-induced apoptosis in breast cancer cells. Mol Cancer Ther 6, 1544–51.
19. Hibasami H, Achiwa Y, Katsuzaki H, et al. (1998) Honokiol induces apoptosis in human lymphoid leukemia Molt 4B cells. Int J Mol Med 2, 671–3.
20. Yang SE, HsiehMT, Tsai TH, Hsu SL. (2002) Down-modulation of Bcl-XL, release of cytochrome c and sequential activation of caspases during honokiol-induced apoptosis in human squamous lung cancer CH27 cells. Biochem Pharmacol ,63,1641–51.
21. Battle TE, Arbiser J, Frank DA. (2005) The natural product honokiol induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (BCLL) cells. Blood 106, 690–7.
22. Shitsuka K, Hideshima T, Hamasaki M, et al. (2005)Honokiol overcomes conventional drug resistance in human multiple myeloma by induction of caspase-dependent and -independent apoptosis. Blood ,106, 1794–800.
23. Wolf I, O'Kelly J, Wakimoto N, et al.(2007) Honokiol, a natural biphenyl, inhibits in vitro and in vivo growthof breast cancer through induction of apoptosis and cell cycle arrest. Int J Oncol ,30,1529–37.
24. Ahn KS, Sethi G, Shishodia S, Sung B, Arbiser JL, Aggarwal BB. Honokiol potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through modulation of nuclear factor-nB activation pathway. Mol Cancer Res 2006;4:621–33.
25. Michael Levitt; Protein Folding by Restrained Energy Minimization and Molecular Dynamics, J. Mol. Hiol. (1983) 170, 723-764 .
26. Kini RM, Evans HJ. Molecular modeling of proteins: a strategy for energy minimization by molecular mechanics in the AMBER force field. J Biomol Struct Dyn. 1991 Dec;9(3):475-88.