ejpmr, 2017,4(9), 452-458

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DOCKING STUDIES ON ANTI APOPTOSIS PROTEIN INHIBITORS AS A NOVEL TARGET ON BREAST CANCER.

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Article Received on 27/06/2017 **Article Revised on 17/07/2017** **Article Accepted on 06/08/2017**

ABSTRACT

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 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 containg 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 Chyrasin 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, Modleing, Natural compounds, synthetic compounds, Death Effector Domains (DED).

INTRODUCTION

Breast cancer is one of the leading diseases 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 Procheck, prosa, RMSD.

Protein preparation and energy minimization

Modeled protein molecule is then prepared by cleaning and applying the CHARMm forcefields 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]

Ligand sketching and preparation

All the ligand molecules were sketched using the chemsketch software and then the preparation of ligand molecules is done by prepare ligands protocol in discovery studio.

RESULTS 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.

Sequence Alignment

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

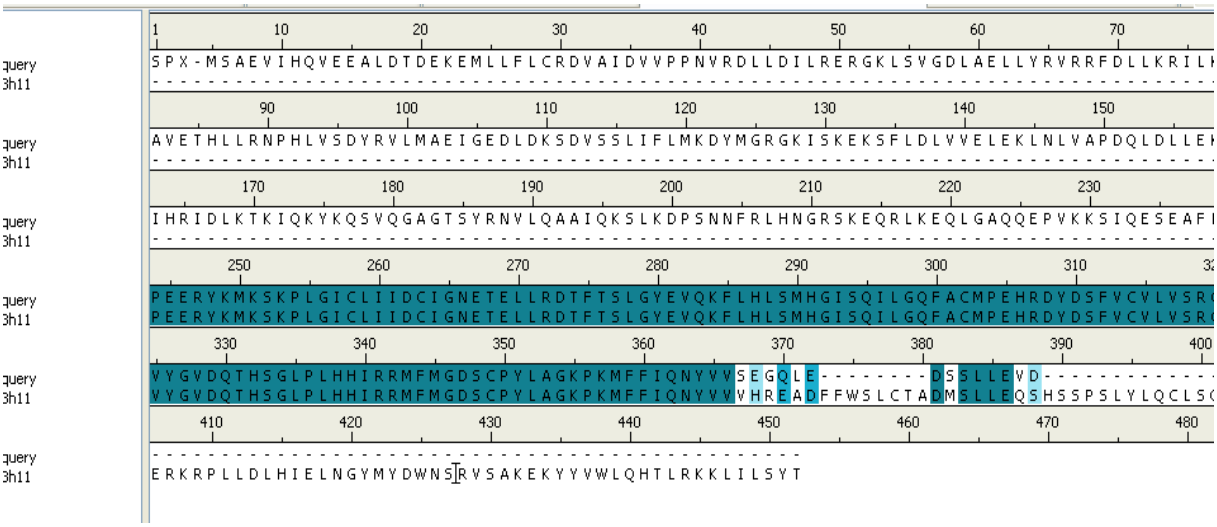


Fig 2: Showing the sequence alignment of C-FLIP and 3H11 in Discovery studio software where the shaded regions in figure represent the similar amino acids in the two sequences.

Modeling

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

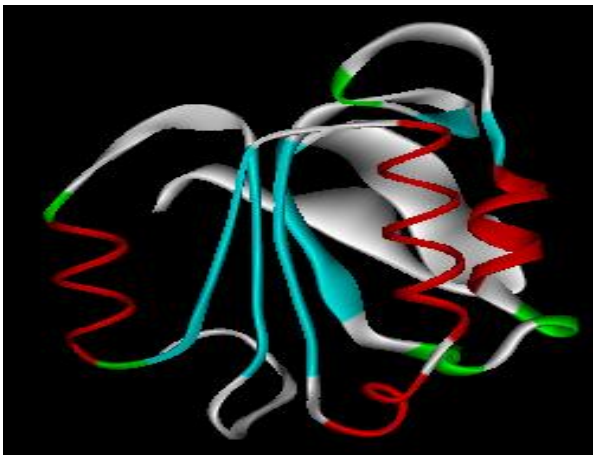


Fig 3: Showing the modeled structure of the protein molecule in discovery studio in solid ribbon format.

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.

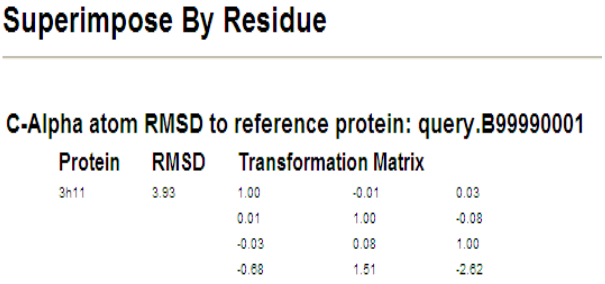
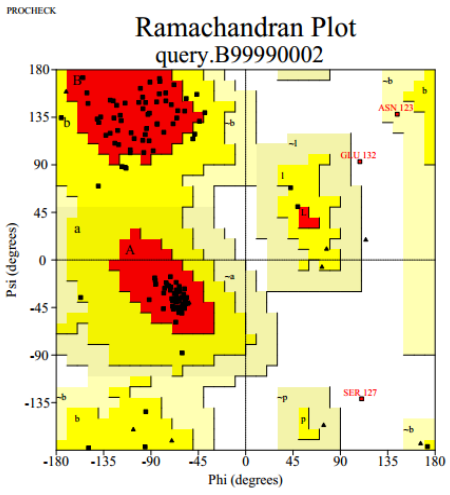


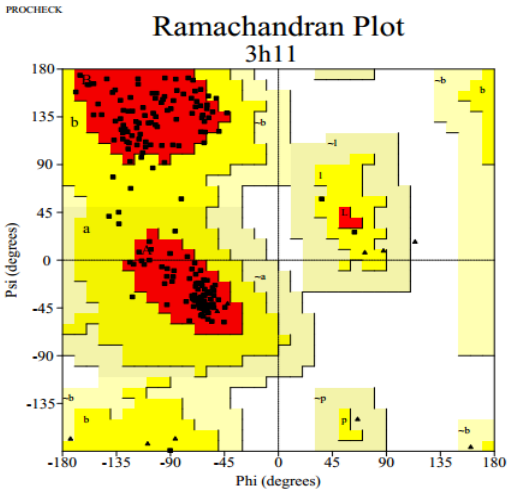
Fig 4: Showing the calculated RMSD value of the modeled protein molecule when it is superimposed with 3H11 protein molecule.

Procheck

Procheck is used for the assessment of the sterochemical properties of modeled protein structure. Procheck is done using online server SAVES-Procheck.



(A)



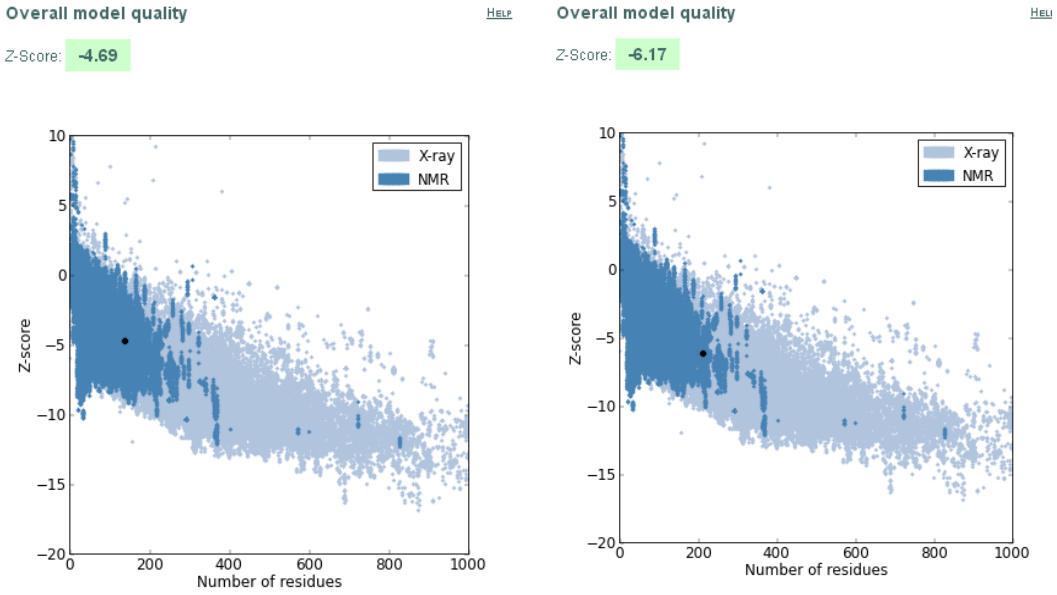
(B)

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.

Tabel 1: showing the results of procheck for modled 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

Prosa



(A) **(B)**

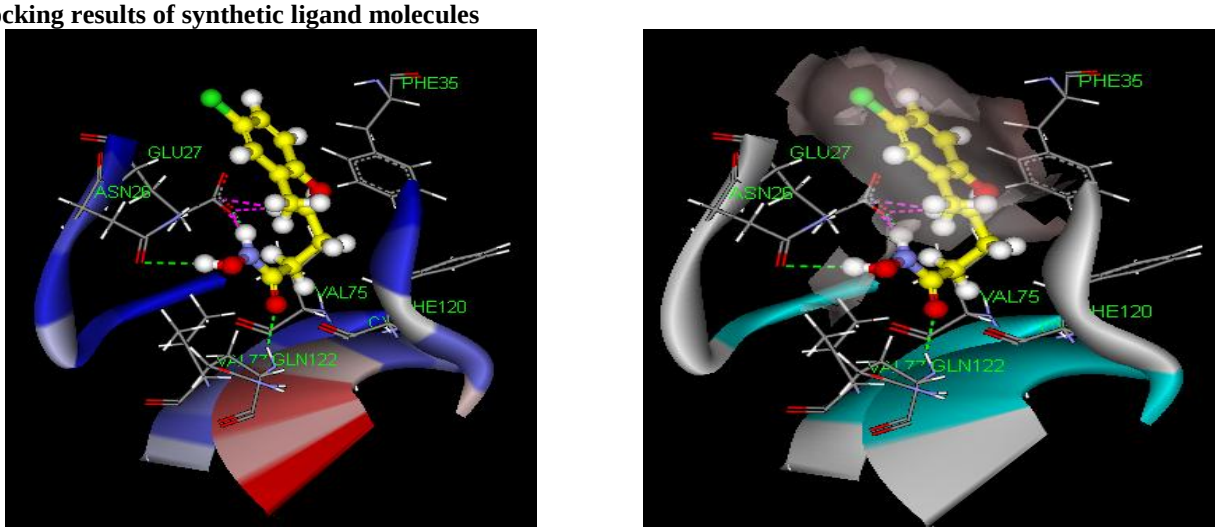
Fig 6: graphs showing the Prosa results of the modled protein molecule (A) and the template 3H11 (B) which are showing the Z-scores as -4.69 and -6.17.

Sketching of ligand molecules

All the ligand molecules synthetic and natural ligand molecules were sketched using the Chemsketch software and were prepared using prepare ligand protocol in Discovery Studio. List of ligand molecules taken (Wagonin, apigenin, chyrisin, honokiol, genistin, kahweol, β-elemene, camptothecin, 9-nitocamptothecin, irinotecan, lupeol, celastrol, zerumbone, withaferin-A, quinacrine, rocaglamide, silibinin, genestin, eupatolide, Pyrido[2,3d] pyrimidine, celecoxib, oxaliplatin, cisplatin, doxorubicin, vorinostat, trichostatin, droxinostat, valproicacid, romidepsin, salirasib, rapamycin, toxol, cycloheximide, anisomycin, sorafenib, cystamine).

Docking (Ligand Fit)

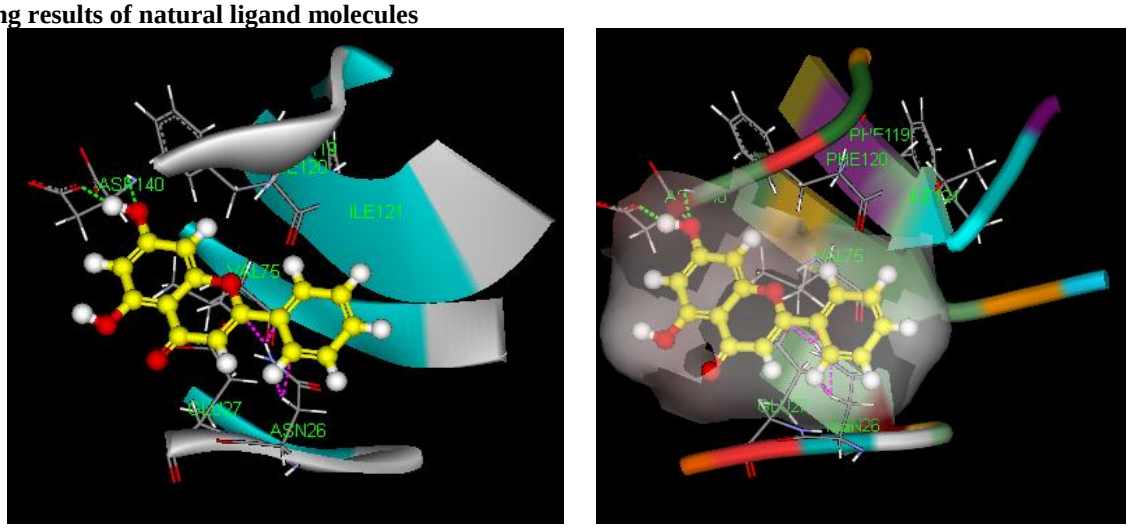
Docking results of synthetic ligand molecules



(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.

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

In the fig8 and fig9 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

Anti apoptotic protein 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 amio 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.

Acknowledgement: The author sincerely thanks to Principal Dr. V. K. Deshmukh M.E.S. College of Pharmacy, Prashant Patil Gadakh Secretary, Mula Education Society, Sonai, Maharashtra, India for encouragement and availing of the laboratory facilities during the course of investigation.

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