

Molecular Docking studies of Synthetic and Natural compounds against cFLIP protein in cancer

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ABSTRACT

Cellular FLICE – inhibitory proteins (c-FLIP) plays a major role in apoptosis. There are a few diseases reported which are associated with the apoptosis mechanism such as autoimmunity, AIDS and cancer. In majority of the cancers the c-FLIP over expression is seen. The aim of the present investigation is to evaluate a ligand / drug molecule which could resist the over expression of the c-FLIP and hence establishing itself as the major therapeutic drug target. In this quest, the docking of the c-FLIP with 28 ligands on GOLD software was performed. The docking results were found to be very encouraging with doxorubicin elevating itself as the ace ligand with the highest fitness score of 55.81.

INTRODUCTION

Caspase-8 activation is essential and plays a pivotal role in the apoptotic cell death (Wang *et al.*, 2008). Caspases are cysteine dependent aspartate specific proteases act by splitting the substrates in a very systematic, highly specific and in a much regulated manner (Fuentes-Prior and Salvesen, 2004). It was noticed that apoptosis occurs in intrinsic and extrinsic pathways (Micheau, 2003). Activation of intrinsic pathway occurs by the formation and release of proapoptotic molecules which in turn activates the Apaf- 1, referring to Apoptotic Protease – Activating Factor -1 (Zou *et al.*, 1999). In contrast extrinsic pathway activates by stimulation of the cell membrane associated with death receptor (DR) (Olivier, 2003). So far 8 death receptors have been identified amongst which TRAIL- R1/R2 and Fas

(Inazawa *et al.*, 1992 ; Oehm *et al.*, 1992; Macfarlane *et al.*,1997; Screaton *et al.*, 1997; Sheridan *et al.*, 1997; Wu *et al.*, 1997), when are bound to their cognate ligands, activates the adaptor molecule FADD (MORT1) (Chinnaiyan *et al.*, 1996) forms the Death- Inducing Signalling Complex (DISC) during which the caspase-8 is activated (Kischkel *et al.*, 1995). Initiator caspase -8 is composed of FLICE, MACH, and MCH5 (Muzio *et al.*, 1996).

C-FLIP are also known by different names like FLAME-1, I – FLICE, CLARP, CASH, MRIT, usurpin and Casper (Goltsev *et al.*,1997; Inohara *et al.*,1997; Rasper *et al.*, 1998; Han *et al.*,1997; Hu *et al.*, 1997) are present on2q33-34 chromosome along with caspase-8 and caspase-10 (Rasper *et al.*, 1998). On the protein level c FLIPs and c FLIP_L were absorbed (Scaffidi *et al.*, 1999). Structurally it was noted that the c FLIP lacks the amino acid residue which are crucial for caspase activity (Andreas *et al.*, 2001). Known as a major anti-apoptotic regulator, c FLICE works by inhibiting and regulating the cell death by the death receptor Fas, DR 4, DR 5 and TNFR1 (Sarah *et al.*, 2013).

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There are reports of c FLIP over- expression associated with several cancers like colon rectal cancer, cervical cancer, Burkitt's lymphoma and non-Hodgkinis lymphoma (Djerbi *et al.*, 1999; Wang *et al.*, 2007; Ullenhag *et al.*, 2007; Valnet-Rabier *et al.*, 2005). C FLIP that inhibits TRAIL induced cell death is seen as a tumor- progression factor. Not only in cancers, c FLIP also has an impressive role to be portrayed in cardiovascular diseases (Valente *et al.*, 2006), multiple sclerosis (Antel, 1999), leading to myelin damage , Rheumatoid arthritis (Janossy *et al.*, 1981), Alzheimer's diseases (De Felice and Ferreira, 2002) and diabetes mellitus (Hilliard *et al.*, 2001). These observations emphasize on c FLIP being a potential drug target for cancers as many types of cancers associated with the over expression of c FLIP. The aim of the present article is to identify a drug molecule which could resist the over expression of the c FLIP and hence regulating the cancers.

MATERIALS AND METHODS

Protein selection and preparation

The crystal structure of human c- FLIP protein (PDB ID: 3H13) was retrieved from protein data bank with a resolution of 2.2 Å. Preparation of the retrieved protein was performed by using prepare protein protocol of Discovery Studio (DS). Initially all the water molecules were removed and hydrogens were added to satisfy the valances. Energy minimization of the protein structure was done by employing CHARMM forcefields and smart minimization algorithm followed by conjugant gradient algorithm in DS until the convergence gradient is satisfied.

Ligand selection and preparation

28 ligands were selected from various literature sources (Safa and Pollok, 2011; Noh *et al.*, 2012 ; McCourt *et al.*, 2012 ; Zhang *et al.*, 2008 ; Yodkeeree *et al.*, 2009). Using Chems sketch (ACDLABS 12.0), the selected compounds are drawn and saved in mol file format. The saved ligand compounds were later imported in DS and further ligand preparation with constraint parameters such as ionization change, tautomer and isomer generation and all the duplicate structures are removed followed by geometry optimization and energy minimization of the lowest energy structure using the CHARMM forcefield. The individual compounds were finally converted to 3D structures using DS catalyst algorithm.

Docking

Genetic Optimization Ligand Docking-GOLD, one of the finest molecular docking mechanisms, used to dock the selected ligands into the active site of the protein c-FLIP. GOLD uses Genetic Algorithm which renders ligand full flexibility and partial flexibility to proteins. This algorithm is an optimizing method, allowing the direct random searches (Shahper and Asad, 2008) utilizing the electronic, lipophilicity and shaped related properties. To simplify the docking calculations the protein's active site is defined within 10 Å radius and the amino acids present within this radius are used as an input to run GOLD docking calculations with

default parameters. The default evolutionary parameters used are population size: ¼100; selection pressure: ¼ 1.1; operations: ¼ 100,000; islands: ¼ 5; niche size: ¼ 2; migration: ¼ 10; mutation: ¼ 95; crossover: ¼ 95. Using the default GOLD fitness function, ten docking runs were performed per ligand compound unless three of the 10 poses were within 1.5 Å RMSD of each other with an early termination of Generic Optimization calculation is enabled. The ability of ligand compound interaction with the protein ability of a compound depends on the fitness score, greater the GOLD fitness score better the binding affinity. Ligand compounds which showed the expected interactions with the amino acids present in the active site of the protein, and with high binding scores were selected as potent inhibitors of c-FLIP. The best pose of each ligand was chosen based on the GOLD fitness score and visualized using silver.

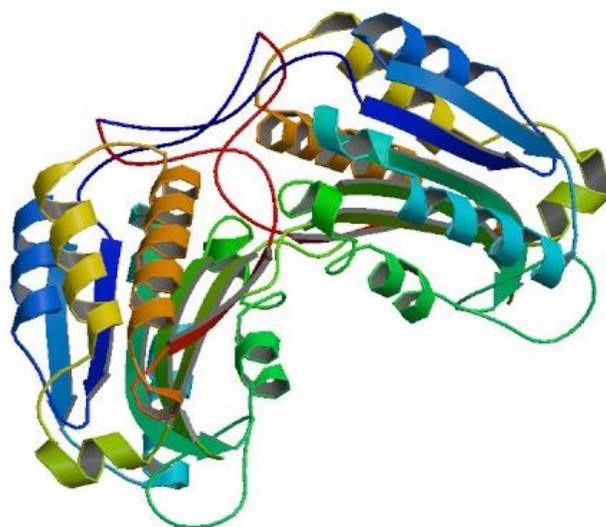


Fig. 1: Secondary Structure of human c- FLIP (PDB ID: 3H13).

RESULTS AND DISCUSSION

Docking studies was performed using GOLD to find the interactions between the active site of c-FLIP and ligands. The prepared protein and the ligands were imported on to the GOLD software facilitating them to undergo flexible docking process commenced with default parameters. For each ligand, 10 different poses with their corresponding GOLD fitness scores were saved. The selection of best ligand pose was called on their affinity towards the amino acids which renders them the highest fitness score. Higher the GOLD fitness score of the ligand pose was better because it was calculated based on the negative of the sum of the component energy terms. The optimized fitness function was used for the prediction of well fitted ligand binding position that has the least energy with average Gold fitness score. From this analyzes it was elucidated that the Doxorubicin and Sorafenib as the active molecules against c-FLIP which shows the good fitness score and is selected as potent inhibitors which are further validated by using the visualization method to find the suitable binding mode of the inhibitor based on the critical interactions with the active site

residues. Silver was used to visualize the protein ligand complex interactions. From the table 1, it is therefore noted that the ligand Doxorubicin and Sorafenib has the highest fitness scores of 55.81 and 52.28. From the figure 2, it can be elucidated that the interacting amino acids are Val 324, Asp 325, Arg 315 and Trp 322. The ligand pose was further analyzed and studied based on their hydrogen bond interaction as the hydrogen bonding plays a pivotal role in the proteins structural and biological functions. Close observation of the interactions revealed that the Try322 forms a Hydrogen bond with the OH, Asp 325 with O, Val 324 with O and Arg 315 forms the hydrogen bond with NH1 respectively.

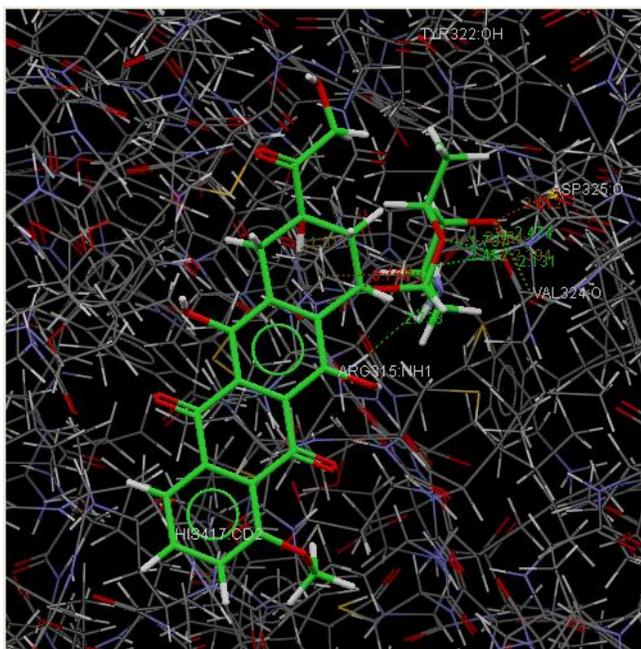


Fig. 2: Shows H-bonding interactions of Doxorubicin with active site residues of human c- FLIP (PDB ID: 3H13).

Table 1: Protein-Ligand fitness scores

Ligand name	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
5-Fluorouracil	15.96	0.25	11.43	0.00	0.00
9-NC	32.55	0.00	26.91	0.00	-4.45
Camptothecin	38.18	0.81	28.14	0.00	-6.63
Celastrol	34.75	6.00	25.29	0.00	-30.12
Celecoxib	43.55	6.00	28.88	0.00	-10.81
Chrysin	31.47	1.32	22.97	0.00	-7.13
Anisomycin	41.79	6.00	29.10	0.00	-21.10
Cisplatin	-501.36	0.00	79.83	0.00	-3055.64
Cycloheximide	36.96	6.00	23.99	0.00	-10.11
Etoposide	46.17	6.00	34.00	0.00	-32.87
Flavopiridol	43.63	7.75	29.22	0.00	-21.50
Nocodazole	40.21	6.00	26.15	0.00	-8.72
Paclitaxel	-1196.97	5.29	76.69	0.00	-6538.54
Sorafenib	52.28	7.82	34.84	0.00	-17.25
Doxorubicin	55.81	1.73	43.69	0.00	-29.96
Droxinostat	40.10	6.83	26.04	0.00	-12.73
Genistein	33.48	2.00	24.23	0.00	-9.22
Irinotecan	47.38	0.00	38.29	0.00	-26.31

Lupeol	21.92	0.00	22.74	0.00	-46.75
Oxaliplatin	-18.69	6.00	29.36	0.00	-325.28
Quinacrine	27.58	2038	38.73	0.00	-140.31
Silybin	44.70	7.32	31.27	0.00	-28.04
Taxol	-1203.03	5.86	67.57	0.00	-6508.96
Trichostatin A	43.76	5.72	31.85	0.00	-28.83
valproic acid	29.43	6.00	19.94	0.00	-19.95
Vorinostat	46.66	6.00	32.64	0.00	-21.13
Withaferin	35.15	0.00	29.67	0.00	-28.21
Zerumbone	26.52	0.00	19.80	0.00	-3.49

Figure 3 shows the protein docking to the ligand Sorafenib. The interacting amino acids are Val 324, Trp 322 and His 417 and the Hydrogen bonds were observed between Val 324 and O, Trp 322 binding with OH and subsequently, His 417 binding to C/OD2.

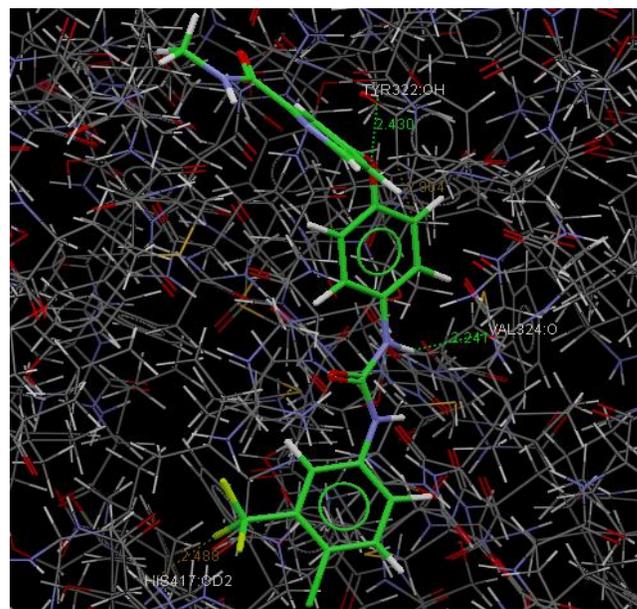


Fig. 3: Shows H-bonding interactions of Sorafenib with active site residues of human c- FLIP (PDB ID: 3H13).

CONCLUSION

Cancer is one of the major causes of death in the present day. Researchers are involved in finding a solution for this disease. It is known from the reports that the c FLIP is a cellular protein whose levels are elevated during certain types of cancers. Not only in cancers, it's over expression has also been noticed in other health associated diseases as reported in the article making c-FLIP a prime drug target. Hence, therefore, we tried to evaluate a ligand molecule which could inhibit its over expression by targeting the c-FLIP with 28 ligands. The GOLD fitness scores were very promising with the highest fitness score of 55.81 and 52.28 for Doxorubicin and Sorafenib respectively. Henceforth, monitoring the changes in the levels of c-FLIP could be of great diagnostic value.

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Conflict of Interests: There are no conflicts of interest.

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