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Insilico Identification of Novel Natural Compound Inhibitors Targeting the XIAP Protein for Cancer Management

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Apoptosis dysregulation is a significant factor in cancer pathogenesis and may result from the overexpression of anti-apoptotic proteins such as inhibitor of apoptosis proteins (IAPs). Among these IAPs, the most potent and well-characterized anti-apoptotic member is X-linked inhibitor of apoptosis protein (XIAP), which directly inhibits caspase-9 via its BIR3 domain and caspase-3 and -7 via its BIR2 domain. Current chemotherapeutic agents that target XIAP are frequently associated with toxicity and unfavorable side effects. Here, a virtual screening approach was utilized to screen a total of 2,777 phytochemicals from the ZINC database in order to find putative XIAP inhibitors. The top hit compounds, ZINC1725698, ZINC4104879, ZINC257053067, and ZINC6585367 were chosen for their high binding affinity and specificity for the XIAP binding pocket. Notably, as compared to the control drug (Hydroxythio Acetildenafil), these hit compounds exhibited a greater affinity for the XIAP binding site. Furthermore, these compounds were found to possess the drug-like properties, making them viable candidates for future experimental validation as possible anticancer agents.

Keywords: Cancer, apoptosis dysregulation, natural compounds, virtual screening

INTRODUCTION

Cancer is one of the world's leading causes of death. Cancer treatments are generally designed to trigger programmed cell death in highly proliferating cancer cells. However, if tumor cells develop the ability to evade drug-stimulated cell death, this can result in the failure of chemotherapy. This resistance can occur as a result of defects in pro-apoptotic death regulators, such as pro-survival protein overexpression. These events can result in the selection of chemotherapy or radiation-resistant tumor cell subclones, which can eventually lead to therapeutic relapse (Obexer and Ausserlechner 2014).

Resistance to apoptosis is a defining characteristic of cancer and is controlled in part by a crucial family of proteins known as inhibitors of apoptosis proteins (Lewis et al. 2004; Gyrd-Hansen and Meier 2010; Hanahan and Weinberg 2000). XIAP is regarded as the most potent apoptosis suppressor due to its ability to inhibit caspase 3, 7, and 9 activation. This leads to the suppression of both extrinsic and intrinsic pathways of cell death (Tu and Costa 2020). Increasing evidence strongly suggests that XIAP exhibit vital role in conferring therapeutic resistance to multiple tumors by suppressing apoptosis induced by cancer therapies (Abbas and Larisch 2020). Hence, targeting XIAP could be a promising treatment strategy for various cancer types.

It is evident that XIAP bind to CASP3 and CASP7 active site pocket and obstructs substrate entry (Twiddy et al. 2006; Denault et al. 2006). Inactivates CASP9 by keeping it in a monomeric, inactive state (Shiozaki et al. 2003). The interaction analysis by the canSAR web server (Mitsopoulos et al. 2021) explored that XAIP binds (via BIR3 domain) with DIABLO/SMAC; the interaction prevents apoptotic suppressor activity (Bornstein et al. 2011; Liu et al. 2000). Several other genes directly or indirectly interacting with XIAP are CASP (6,7,8), UBC, UBB, MDM2, TBK1, GSK3B, AKT1, 2 and others (Figure 1).

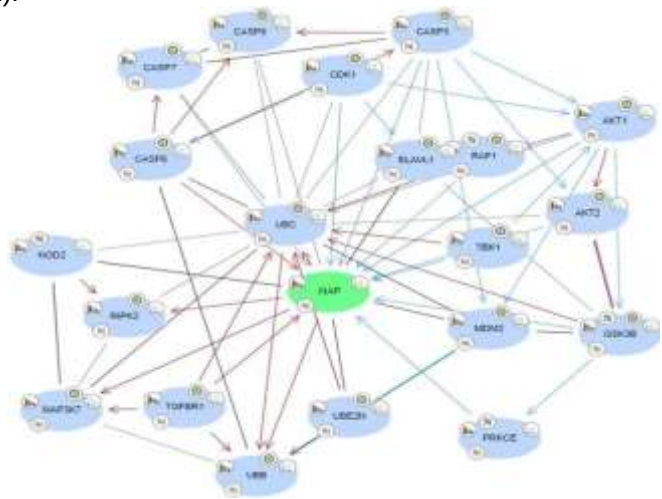

Figure 1: Interactome network of XIAP.

In-silico drug design, which includes both computational and theoretical methods, can be used to identify new candidate compounds that target specific proteins or receptors. Currently, computer-aided drug design (CADD) is widely used in the identification, development, and analysis of drugs and other biologically active molecules (Yu and MacKerell, 2017).

The pharmacokinetics, drug-like properties, and even toxicity of a compound can be predicted using CADD process (Kalyaanamoorthy and Chen, 2011). The goal of this study was to use computational methods like structure-based virtual screening (VS) and ADMET analysis to find potential natural XIAP antagonists to combat the cancer.

MATERIALS AND METHODS

Protein preparation

The 3D structure of XIAP (PDB ID: 5OQW) was obtained from the protein data bank. The protein was then cleaned by removing bound ligand and water molecules present. Subsequently, the protein structure was subjected to a minimization process and saved in the .pdb format.

Phytocompound library preparation

A database of 2,777 phytochemicals from the ZINC database under the catalog 'Biopurify Phytochemicals' in SDF format was accessed. These compounds were minimized and prepared for docking analysis by converting them to PDBQT format.

Virtual screening

The process of VS has a crucial role in the development of novel drug candidates (Lionta et al., 2014). One of the widely used approaches in VS is molecular docking, which involves the analysis of the interactions between small molecules and target proteins. For this study, AutoDock Vina and AutoDock were employed for VS and detailed molecular docking investigations. The top hits were selected based on their binding affinity towards XIAP.

Physicochemical and druglikeness estimation

The SwissADME web tool was used in the research study to gain insight into the physicochemical and druglikeness characteristics of the top ten phytochemicals. This powerful tool enabled a thorough evaluation and estimation of various properties associated with these compounds, providing essential details about its chemistry and potential as drug candidates (Daina et al. 2017).

RESULTS AND DISCUSSION

Apoptosis dysregulation is a significant factor in cancer pathogenesis. Different malignancies are caused by XIAP overexpression (Gao et al. 2017). Chemicals developed to target XIAP are typically toxic and have side effects. Therefore, the development of natural inhibitor targeting XIAP is critical in order to avoid the negative consequences associated with its inhibition. Here, a database of 2777 phytochemicals from the ZINC database under the catalog 'Biopurify phytochemicals' were screened targeting XIAP. Hydroxythio Acetildenafil was selected as control for screening as it has been reported to be a potent antagonist of XIAP (Opo et al. 2021). Structure-based VS resulted in the identification of multiple compounds exhibiting better binding efficacy towards the active site residues of XIAP when compared to the control compound. However, the top 10 phytochemicals were found to be more potent after 2D and 3D visualization of their docking poses (Table 1, Figure 2, and Figure 3).

Table 1: Top 10 screened phytochemicals and their binding affinity with XIAP.

Natural compound	Binding affinity (kcal/mol)
ZINC1725698	-8.4
ZINC4104879	-8.2
ZINC257053067	-7.9
ZINC6585367	-7.7
ZINC100018343	-7.5
ZINC33832503	-7.1
ZINC49872462	-7.1
ZINC6585367	-7
ZINC2169830	-6.9
ZINC15146195	-6.9
Hydroxythio Acetildenafil*	-6.7

*Control compound

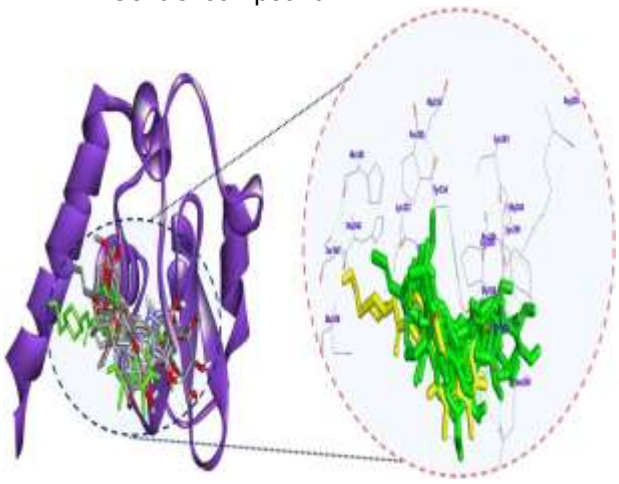

Figure 2: Binding interaction of selected phytochemicals and control in the XIA active pocket.

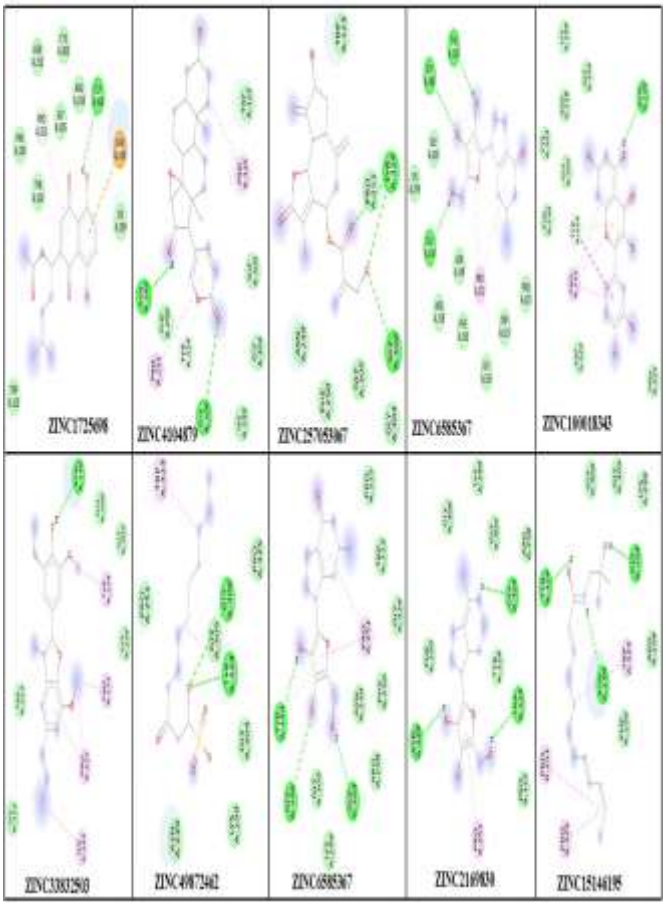

Figure 3: Interacting residues of XIAP with top 10 screened phytochemicals.

A comprehensive analysis of the interaction details was performed for the top four (ZINC1725698, ZINC4104879, ZINC257053067, and ZINC6585367) compounds. ZINC1725698 interacted with Tyr324, Phe250, Pro251, Asn252, Gly305, Cys303, Arg258, Gly304, Asn249, Lys299, and Trp323 residues of XIAP. Gly304 residue was H-bonded with ZINC1725698. ZINC4104879 interacted with Pro325, Gly305, Gly304, Lys299, Gly306, Tyr324, Phe250, Pro251, Asn249, and Trp323 residues of XIAP. Gly306 and Asn249 residues were H-bonded with ZINC4104879. ZINC257053067 bind with Trp323, Tyr324, Pro251, Gly306, Gly304, Gly305, Phe250, and Asn249 residues of XIAP. Gly306 and Tyr324 residues were H-bonded with ZINC257053067. Further, ZINC6585367 interacted with Pro325, Trp323, Gly326, Pro251, Phe250, Asn249, Arg258, Gly304, Lys299, Gly305, Gly306, and Tyr324 residues of XIAP. Gly306, Gly304, Tyr324 residues were H-bonded with ZINC6585367. The XIAP residues Lys297, Leu292, Gly304, Gly305, Tyr324, Gly306, Leu307, Gln319, Lys311, Asp309, Thr308, Glu314, Trp310, Lys299, and Trp323 have been identified as critical for inhibitor binding (Opo et al. 2021). Notably, the findings of this investigation demonstrate that the hit compound exhibits binding affinity towards these XIAP residues (Figure 3).

The control compound (Hydroxythio Acetildenafil) was found to interacted with Pro251, Phe250, Pro325, Glu350, His343, Ser347, His346, Trp323, Lys322, Tyr324, Gly304, Gly305, Gly306, and Asn249 residues of XIAP (Figure 4). Interestingly, Pro251, Phe250, Trp323, Tyr324, Gly304, Gly305, and Asn249 were the common binding residues of XIAP with hit compounds (ZINC1725698, ZINC4104879, ZINC257053067, and ZINC6585367) as well as the Hydroxythio Acetildenafil (Figure 3 and Figure 4).

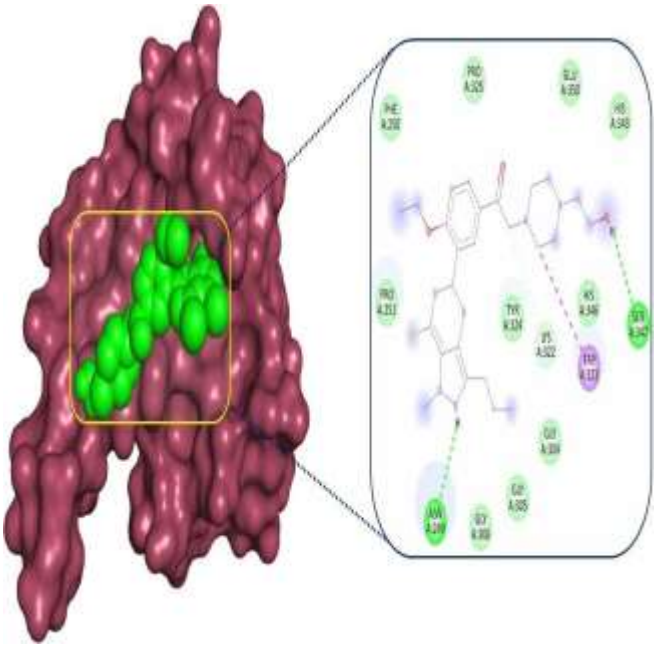

Figure 4: Interacting residues of XIAP with Hydroxythio Acetildenafil.

The application of molecular docking has become widely used in the prediction and evaluation of interactions between enzymes/proteins and their inhibitors (Sait et al. 2019). In this context, a high negative binding affinity value observed in docking studies indicates a strong interaction between the ligand and the protein within the ligand-protein complex (Sait et al. 2020). Remarkably, the hit compounds (ZINC1725698, ZINC4104879, ZINC257053067, and ZINC6585367) exhibit considerably lower binding affinities in comparison to the Hydroxythio Acetildenafil (control). This observation signifies a strong interaction between these hit compounds and the XIAP.

To evaluate the physicochemical property and drug like efficacy of top 10 selected phytochemicals, a computational estimation was performed by the SwissADME web tool. This powerful tool assessed and estimated various properties of these compounds, providing crucial information about their chemistry and drug candidate potential (Daina et al. 2017). The resulted values indicate that these phytochemicals have tendency to be a drug molecule (Table 2 and Table 3).

Table 2: Physicochemical properties of top 10 screened phytochemicals.

Phytochemicals	MW	RB	HBA	HBD	MR	TPSA	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
ZINC1725698	330	5	6	2	87.56	100.9	2.64	3.54	2.69	0.82	2.99	2.54
ZINC4104879	401	1	5	2	108.83	83.2	2.97	2.77	3.23	2.75	3.43	3.03
ZINC257053067	346	4	6	2	90.1	93.06	2.42	0.64	1.06	1.49	2.06	1.54
ZINC6585367	283	2	7	5	65.5	159.51	0.64	-1.89	-3	-2.36	-2.22	-1.77
ZINC100018343	304	1	7	5	74.76	127.45	0.71	0.95	0.86	-0.64	0.66	0.51
ZINC33832503	356	5	5	1	101.48	57.15	3.91	4.35	4.26	2.39	4.43	3.87
ZINC49872462	280	11	5	2	71.7	100.05	1.69	2.32	3.37	1.2	1.81	2.08
ZINC6585367	283	2	7	5	65.5	159.51	0.64	-1.89	-3	-2.36	-2.22	-1.77
ZINC2169830	267	2	7	4	62.67	139.54	0.61	-1.05	-2.3	-1.91	-2.37	-1.4
ZINC15146195	263	8	3	2	83.59	52.82	0.61	-1.05	3.74	-1.91	-2.37	-1.4

RB=Rotatable bonds HBA= H-bond acceptors; HBD=H-bond donors

Table 3: Drug likeness properties of top 10 phytochemicals

Phytochemicals	Violation of druglikeness rules						BS	PAINS	SA
	Lipinski	Ghose	Veber	Egan	Muegge	Lead likeness			
ZINC1725698	0	0	0	0	0		0.55	2	3.85
ZINC4104879	0	0	0	0	0	1	0.55	0	6.25
ZINC257053067	0	0	0	0	0	1	0.55	0	4.7
ZINC6585367	0	1	1	1	1	0	0.55	0	3.86
ZINC100018343	0	0	0	0	0	0	0.55	1	3.51
ZINC33832503	0	0	0	0	0	0	0.55	0	4.12
ZINC49872462	0	0	1	0	0	2	0.56	0	3.92
ZINC6585367	0	1	1	1	1	1	0.55	0	3.86
ZINC2169830	0	1	0	1	0	0	0.55	0	3.86
ZINC15146195	0	1	0	1	0	0	0.55	0	3.86

(BS=Bioavailability Score; SA=Synthetic Accessibility)

In spite of the notable progress in the development of chemically synthesized pharmaceutical agents, natural sources remain the primary reservoir for bioactive molecules. The investigation of natural products is a valuable strategy in the search for new pharmacologically active molecules with distinct structures and action mechanisms (Dehelean et al. 2021). Extensive efforts have been made in recent decades to explore and extract novel natural products from a variety of sources, including microorganisms, plants, and other organisms. Thorough investigations have focused on the examination of natural compounds for anticancer properties and the elucidation of the underlying mechanisms of action, resulting in the development of a variety of anti-cancer therapeutics. Surprisingly, it has been estimated that the use of natural ingredients was responsible for approximately 25% of newly approved anticancer drugs between 1981 and 2019 (Newman and Cragg 2020; Huang et al. 2018).

CONCLUSION

XIAP is a potent and well-studied anti-apoptotic member protein. The present study found that ZINC1725698, ZINC4104879, ZINC257053067, and ZINC6585367 have high binding affinity for XIAP, interact with its essential residues, and have good druglike properties. These compounds have potential as XIAP inhibitors, potentially opening up new avenues for cancer therapy. However, further experimental validation is required.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

HMB designed the study, conducted the methods, analyzed the data, wrote the manuscript, and approved the final version.

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