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Interactions of Chloroquine With the Xiap Induced Oral Cancer, and Analysis of Molecular Mechanism - Using in Silico Validation Tools

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ABSTRACT

*Aim***:** The present study employed a computational Insilco approach to confirm the mechanism of interaction for antibacterial activity against the XIAP gene, which is comparable to chloroquine, explicating characteristics and ADMET- drug likeness of cinnamaldehyde along with apigenin.

Materials and methods: Using an online database called ZINC15, which enables virtual screening by downloading the database subset for the two natural compounds that were chosen, the features of the selected compounds were examined. The molecular docking studies were performed with XIAP gene using swissdock database, employing a ligand docking approach and their binding energies are determined. The SwissADME tool was used to estimate the pharmacokinetic and other molecular properties of the compounds, utilizing their canonical SMILE structures.

Results and discussion: The docking study reveals the potential of the selected compounds as an inhibitor of XIAP gene, thereby predicting anti-carcinogenic activity. The SwissADME prediction results showed that novel compounds ZINC00128902 and ZINC79496741 satisfy Lipinski's rule of five with zero violations.

Conclusion: The results predict a development of an inhibitor molecule acting against the XIAP gene causing oral cancer that could bring about the development of new drugs for oral cancer.

KEY POINTS

- Novel compounds derived by In Silico analysis, showed high potential for inhibition of XIAP gene.

This holds high potential for targeted therapy against oral cancer, which currently requires development of such treatments to improve quality of life for the patient.

I. INTRODUCTION

A growing number of potential therapeutic targets for drug discovery have emerged due to the progress made in the study of the human genome. The development of nuclear magnetic resonance spectroscopy, crystallography, and protein purification methods has also led to the understanding of several structural features of proteins and protein-ligand complexes.

Early drug concepts were frequently inspired by active components of conventional treatments or by chance encounters. Identifying and verifying a target, creating assays to locate lead compounds, and tweaking lead compounds to maximize affinity and efficacy and to minimize potential side effects are more modern laboratorial development steps in drug discovery. The time and money needed to create a medicine from an idea through laboratory bench work, to clinical trials, and finally to a product have increased as the process has become more complex. The majority of the research's sources stated that the typical development time is 12 years or longer. In addition, the types of researchers involved in the drug discovery and development process have changed from chemists, physiologists, and statisticians to biochemists who research the chemistry of biological processes, molecular biologists who research the molecules that make up living things, toxicologists who investigate the potential harm from chemicals, and pharmacologists who study how these drugs act. The role of computer scientists in accessing, matching, and evaluating data from, for instance, chemical libraries or gene sequencing studies, increases as computing power becomes more widely available [1].

To overcome these hurdles in drug discovery, computer-aided drug design became popular, with a crucial technique in this toolkit being molecular docking. In order to reduce expenses and accelerate drug discovery, it was first developed in the middle of the 1980s and early 1990s for the purpose of digitally screening sizable digital chemical libraries and predicting the binding mode of known active compounds. It belongs to the category of "structure-based drug design" approaches [2]. Another method, the hit-to-lead optimization technique also utilizes docking technological advances. This method presents the greatest challenge since the worst flaw in docking software has been the inability to predict relative binding affinities for a set of related molecules since the program's inception [3]. However, by informing the user of whether

the generated analogues of a hit molecule have superior chemical interactions with the target molecule, docking can still be used in hit-to-lead optimization. Due to these developments, computational methods can now be used in all stages of the drug discovery process.

The term "in-silico," which describes computer-based research, is related to the more well-known biological terms "in vivo" and "in vitro." The term's origins are uncertain; however, many researchers claim to have contributed to its invention. However, Sieburg (1990) and Danchin et al. are two of the authors who used the term in some of the early published cases [4]. Danchin (2002) offers a passage that provides a succinct and persuasive description of the possibilities of these computational tools in all realms of pharmacobiology [5,6]. Pharmacology, commonly referred to as computational therapeutics, is a fast-growing area that focuses on developing techniques for applying software to gather, process, and combine biological and medical data from multiple sources [7]. Additionally, it has been recommended that to capitalize on the advancements made by the human genome project, findings must be integrated to develop in silico pharmacology, which links all other forms of available data [8]. Despite such intensive research, our understanding is limited, which may significantly oppose our ability to advance the pharmaceutical industry's ability to discover new medications using simulations and data [9].

Another application of in silico is docking of the drug for specific genes and their types [10]. As far as genes are concerned, inhibitory regulators of apoptosis are known to be crucial for the way cancer cells react to cytotoxic substances [11]. A unique family of inhibitor of apoptosis (IAP) proteins includes the X-linked inhibitor of apoptosis protein (XIAP). The goal is to demonstrate that non-small cell lung cancer is more radiation resistant due to translational upregulation of XIAP following acute low dose ionizing radiation. The 'X linked inhibitor of apoptosis (XIAP)' is a member of the inhibitor of apoptosis and one of the indicators of oral cancer, making it a potential target in cancer treatment [12,13]. Chloroquine possesses lysosomotropic action and is crucial for gene-targeted cancer treatment because it sensitizes TRAIL-mediated apoptosis.

Hence, the principal aim of the research was to establish a new pharmacophore with emphasis towards XIAP inhibition for the potential curative agent for oral cancer [14,15].

II. MATERIALS AND METHODS

2.1 Ligands identification

Chloroquine, Embellin, Cyanidin, Irisoquin-A and Ubiquinone were chosen as the five previously described XIAP targeting compounds. Using an online database called ZINC15, which enables virtual screening by downloading the database subset (SMILE) for the five natural chemicals that were chosen, the features of the selected compounds were examined. Following the ligand discovery, open Babel software was used to convert the downloaded molecules' format to mol2 format. The selected structures were sent to the pharmacist program, a ligand-based method for pharmacophore detection, to reveal 3D pharmacophores from the collection of selected ligands. The log P value, bioavailability, and drug likeness rules were all tested and tabulated for the five selected herbal ligands [16].

2.2 Target Protein structure

The target of interest, the protein XIAP, has its 3-dimensional structural data obtained from the online protein data bank (PDB) database as shown in Figure 1.

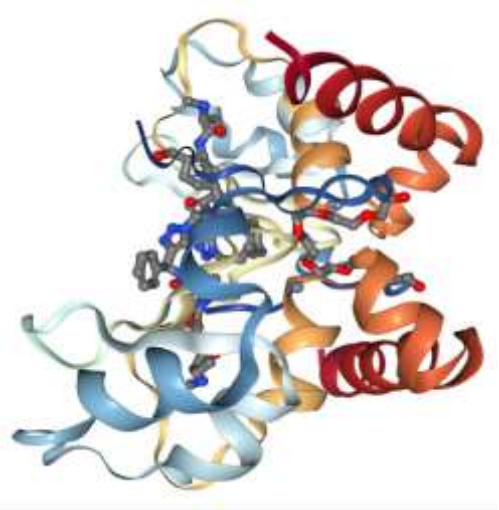

Figure 1 : Protein structure of XIAP

The structure of ligands for pharmacore detection is described in Figures 2-6.

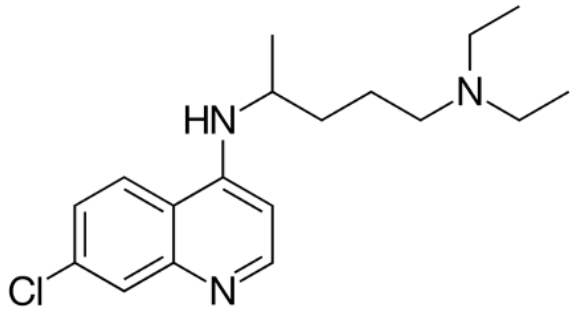

Figure 2 : Structure of Chloroquine

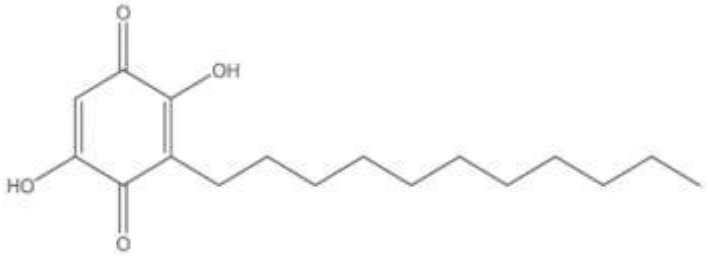

Figure 3 : Structure of Embellin

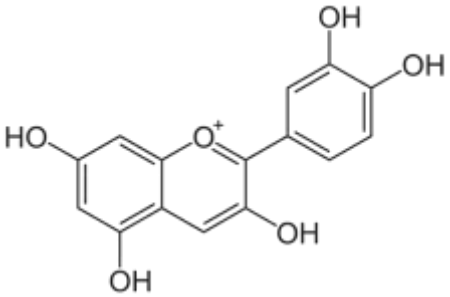

Figure 4 : Structure of Cyanidin

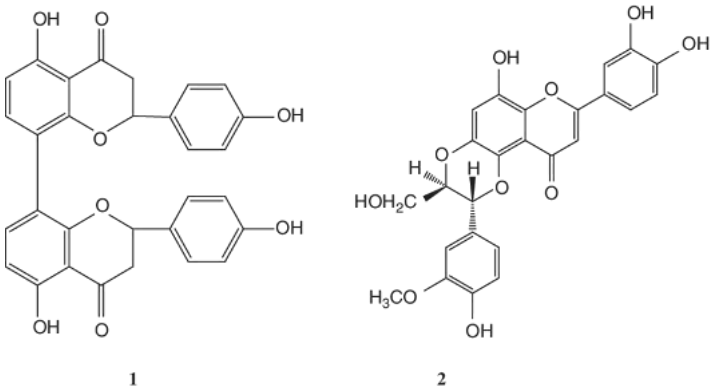

Figure 5 : Structure of Irisquin-A

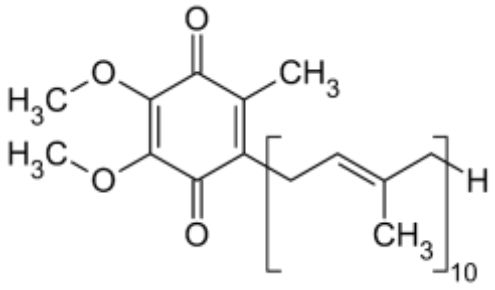

Figure 6 : Structure of Ubiquinone

2.3 *Pharmacophore detection*

The spatial configuration of properties needed for a chemical to interact with a particular target receptor is known as a pharmacophore. Using the Pharmagist programme, the pharmacophore detection is carried out by inputting the five herbal compounds' downloaded inputs in mol2 format. We obtained many pharmacophore hits for the uploaded data. Only 20 outputs were chosen by Pharmacophore virtual screening from thousands produced, and their traits were examined using SWISS ADME software and their parameters were tabulated [17].

2.4 *Docking*

The Swissdock programme was used to individually dock each of the 25 pharmacophores selected from the hits found on the pharmacy website with the target protein in order to determine the complex molecular transfers between the ligands and the protein [18,19]. Each pharmacophore's binding energy was noted from the results and values were tabulated.

III. RESULTS

ZINC79496741 has the greatest binding energy of the 20 newly derived candidates chosen from the hits, with a value of -9.68, followed by ZINC00128902, which has a binding energy of -7.14. Also, it complied with all five guidelines for drug-likeness. Consequently, it could be employed as a possible medication that targets the beta-catenin protein. The final structure obtained after docking shown in Figure 7.

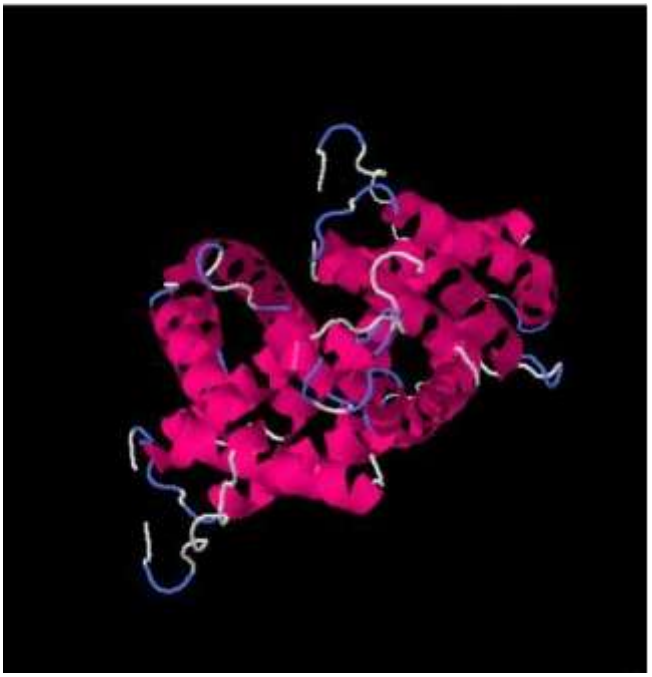

Figure 7 : XIAP protein structures with binding sites (Structure obtained after docking)

Pharmacophore modeling shown for various novel molecules shown in Figure 8.

Molecule ID	rule 1	rule 2	rule 3	rule 4	rule 5	TPSA	Log P
ZINC04024879	Y	Y	Y	Y	Y	32.59	3.33
ZINC93356082	Y	N	Y	Y	Y	110.56	1.74
ZINC05908995	Y	Y	Y	Y	Y	43.09	1.61
ZINC16123974	Y	N	Y	Y	N	40.46	1.74
ZINC34303159	Y	N	Y	Y	N	40.46	1.95
ZINC19793770	Y	Y	Y	Y	Y	90.82	2.51
ZINC04024879	Y	Y	Y	Y	Y	32.59	3.33
ZINC00128902	Y	Y	Y	Y	Y	66.4	1.9
ZINC80348128	Y	N	Y	Y	N	104.27	3.92
ZINC93408648	Y	Y	Y	Y	Y	67.84	2.03
ZINC01753297	Y	N	Y	Y	N	55.12	1.33
ZINC34303160	Y	N	Y	Y	N	40.46	1.87
ZINC93409142	Y	Y	Y	Y	Y	60.64	2.86
ZINC00128899	Y	Y	Y	Y	Y	66.4	2.03
ZINC85695240	Y	Y	Y	Y	Y	49.25	2.17
ZINC86866106	Y	Y	Y	Y	N	56.05	2.12
ZINC63233052	Y	Y	Y	Y	N	72.26	2.44
ZINC18141053	Y	N	Y	Y	N	110.24	0.24
ZINC00128902	Y	Y	Y	Y	Y	66.4	1.9
ZINC79496741	Y	Y	Y	Y	Y	49.91	3.66

Figure 8 : Pharmacophore modelling seen with the binding energy and values

Molecules with favorable properties showing highest binding to XIAP gene represented in Table 1.

Molecule ID	Lipinski rule	Ghose rule	Veber rule	Egan rule	Muegge rule	TPSA	Log P	Bioavailability	GI absorption	Binding energy

ZINC00128902	Yes	Yes	Yes	No	Yes	66.4	1.9	0.55	High	-7.14
ZINC79496741	Yes	Yes	Yes	Yes	Yes	49.91	3.66	0.55	High	-9.68

Table 1 : Two compounds showing the highest binding energy to XIAP gene

IV. DISCUSSION

One of the leading causes of disease-related mortality is still cancer. In 2015, the World Health Organization (WHO) recorded 8.8 million deaths from cancer and predicts a roughly 70% increase in the number of new cases. The disorderly, diverse, and highly differentiated architecture of tumor cells are a determining factor in the deficiency of potent anticarcinogenic medications [20].

Predictive models in the early stages of developing clinical drugs and therapies, which incorporate data from in vitro, in vivo, and in-silico research, essential to comprehending the intricacy of tumor biology and physiological processes premediating it [21]. It is frequently difficult to explain in full the causal links because of the complexity. Model-based methods can explain these physio biological systems and aid in the interpretation of the complex interactions related to the pathophysiology of various chronic diseases [22]. Preclinical research currently mostly uses in vivo models that are derived from animal testing. Combining computational modelling and analysis with biological investigations could help to cut down on the number of experiments needed and enhance the quality of the data obtained from them. [23,24]

Clinical trials, in vivo screens, and validation studies, as well as functional studies employing a variety of in vitro experimental techniques, including cell-based models, spheroid systems, and screening systems for cytotoxicity, mutagenicity, and carcinogenesis, provide a wealth of data that are used in cancer research.

Organ-on-a-chip technology will progress, but so will the in-silico area of systems biology, which aims to construct a virtual physiological human [25].

XIAP was employed in this investigation as an indication of oral cancer. The main way that XIAP stops cell apoptosis is by preventing caspase-3/-7/-9, which are important initiators and effectors of apoptosis, from becoming activated and maturing. Although preventing apoptosis is XIAP's primary role, it has been found to play other roles as well. Diablo homolog (DIABLO), also known as SMAC, is a mitochondrial intermembrane space protein. SMAC–XIAP interaction prevents the XIAP binding to caspases and promotes cell apoptosis [26].

In patient tissue samples and cell lines from human breast cancer, XIAP was shown to be substantially expressed. Foster et al. demonstrated that, as opposed to non-cancerous tissues, the XIAP levels in breast cancer samples were elevated. XIAP encourages the development, growth, and progression of tumors by suppressing cell death and boosting pro-survival pathways. The development of anti-XIAP medications has received attention due to the harmful effects of XIAP and accumulating evidence associating XIAP to various malignancies [27].

Therefore, this study identified 5 ligands which inhibit XIAP. When lysosomal inhibitor Chloroquine was treated in lung adenocarcinoma cells, XIAP was significantly reduced after the treatment indicating that XIAP can be degraded. Embelin treatment in human leukemia cells led to apoptosis, which is mostly responsible for the loss of cell viability and reduction of proliferation in a dose- and time-dependent manner. The potential of the mitochondrial membrane was depolarized by embelin. Embelin inhibited the expression of the anti-apoptotic protein X-linked inhibitor of apoptosis (XIAP), according to a Western blot investigation.

Similarly, Cyanidin when treated in human hepatoma cells, cyanidin-3-*O*-glucoside (C3G) was more effective in apoptosis, and this protected the cytotoxicity caused by quinoline. C3G had a more bonding affinity to XIAP than other proteins.

Furthermore, Irisoquin A had cytotoxic properties, but inhibited XIAP mechanism which induced apoptosis. Also, there is coenzyme Q10 or ubiquinone which significantly inhibits glioma growth by inducing apoptosis of XIAP.

But the limitations of the current mode of study is that all these ligands were studied in an insilico manner to detect the most affiliated one but lacks the in vitro or in vivo aspect. Apart from this, the amount and number of ligands that were detected to inhibit XIAP were less in numbers. 5 ligands were identified that were seen to inhibit XIAP gene.

V. CONCLUSION

Among the 25 compounds, the current study found that ZINC00128902 and ZINC79496741 had better binding energy and had features that were superior to those of the five selected inhibitors. Studies assessing pharmacodynamics and pharmacokinetics are needed for the newly discovered phytocompound's pharmaceutical development. It is necessary to conduct both in vitro and clinical research.

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