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Research Article

IN SILICO EVALUATION OF SELECTED TRITERPENE GLYCOSIDES AS A HUMAN DNA TOPOISOMERASE II ALPHA (α) INHIBITOR

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ABSTRACT

Triterpenoids are natural glycosides which possess anticancer activities. DNA topoisomerase II α plays a key role in DNA replication and is target for multiple chemotherapeutic agent. This study in silico demonstrates potential anticancer effect of selected triterpenoids bivittoside A, holothurin A, holotoxin A, holothurinoside A and cucumarioside A. Homology modeling of human DNA topo II α was done. Possible binding site of DNA binding domain of Topo II α was identified .Selected triterpenoids are screened for QSAR and in silico ADME/TOX analysis and treated as ligands. Ligands are docked with topo II α DNA binding domain using Autodock Vina. All ligands show potent anticancer activity in silico. Out of all cucumarioside a showed best result. The current work has potential for application in design of novel structure based DNA topo II α inhibitors discovery.

Keywords: Triterpenoids, DNA topo II α, Homology modeling, QSAR, Autodock Vina

INTRODUCTION

DNA topoisomerases Type II α (topo II α) and II Beta catalyze the ATP-dependent transport of one intact DNA double helix through another[1]. Structural and biochemical studies showed topo II selectively negatively supercoil or decatenate DNA[2].Topo II α plays a key role in DNA replication with main functions are chromosome segregation, chromosome condensation, arrest in meiosis I and recombination suppression[3].

It is recognized that topo II α high level expression correlate with drug sensitivity and topo II-α low levels correlate with drug resistance[4].Increased topo II α expression is associated with an aggressive form of breast cancer predicts disease-related death, lymph node metastasis, and advanced tumor stage as prognostic markers [5].

Topo II α is a well-known anticancer target. For the treatment of human cancer, one of the most effective anticancer drug doxorubicin is inhibitor of topo II α used in the therapy of breast cancer. Based on insertion into DNA double strands Top2 inhibitors are DNA intercalative and non-intercalative agents[6].

Two groups are identified as topo II inhibitors. The first group contains those that directly bind to the ATP pocket of the enzyme and competitively inhibit the ATPase activity of topo II α, represented by novobiocin[7], cyclothialidine[8] and salvicine[9].Second group bind DNA binding domain and prevent interaction of DNA with protein.

Novel and diverse chemical structures of natural products are motivating the search for new types of anticancer agents. Natural marine compounds with effective chemo-preventive and chemotherapeutic activities indicate source of unique leads[10]. Naturally derived anticancer drugs such as taxol, adriamycin, etoposide and vincristine are the backbone of clinical cancer chemotherapy. In quest of discovery of drug candidates such as ecteinascidin, squalamine and psammaplin A, with unique structures and reaction mechanism, the attention of search has been moving to marine organisms from terrestrial lives. These drugs and several in clinical trials exhibit excellent therapeutic effectiveness in treating chronic or obstinate cancers[11]. Marine organisms derived natural compounds exhibit excellent-tumor, anti -inflammatory, anti-viral, immunomodulatory and analgesic properties[12].

For present work marine based triterpeneglycosides ,bivittoside A, holothurin A, holotoxin A, holothurinoside A and cucumarioside A isolated from the sea cucumber are selected[13]. Here we report in silico potent anticancer activities of selected triterpene glycosides. These results show prospective activity of selected triterpenoids with the saponin skeleton as anticancer drug and for application in design of novel structure based DNA topo II inhibitors discovery.

MATERIALS AND METHODS

Homology modeling of protein sequence

Primary sequence of human DNA topoisomerase II α was retrieved in FASTA format from Uniprot public domain protein database (accession no P11388). Retrieved sequence was submitted to SWISS-MODEL homology modeling program for modeling of three dimensional structure of protein[14]. The SWISS- MODEL depended on the quality of the sequence alignment by BLAST and template structure .Tertiary structure was predicted using homology modeling by taking template PDB 1zxm and modeled protein energy was minimized. Validation of tertiary structure was done by Procheck. Tertiary structure of topo II α showed presence of ATP binding domain and DNA binding domain. For docking simulation DNA binding domain was considered.

Protein structure preparation

DNA binding domain preparation was done using AUTODOCK 4.2 tools. The Autodock Tools package version 1.4.6 was employed to generate the docking input files. All the nonpolar hydrogens were merged and the water molecules were removed. For the docking, a grid spacing of 0.375 Å and 60×60×60 number of points was used. Before docking all water molecules were removed from the protein structure followed by addition of Hydrogen atoms to receptor and merging non-polar hydrogens. Receptor protein was assigned by Kollman united atom charges and solvation parameters while saponin ligands were assigned by Gasteiger charge. Rigid roots were also assigned to the ligand and five bonds were made rotatable. Modeled three dimensional structure of topo II α, and the structure of each ligand were converted to PDBQT format.

Ligand structure preparation

A dataset of 5 triterpene glycosides isolated from sea cucumber were used as ligand molecules. The Saponin structures were retrieved from Pubchem. ChemAxon, freeware developed by Advanced Chemistry Development, Inc. was used for generating chemical structure of triterpene glycosides followed by 2D structure cleaning, 3D optimization and viewing, InChl generation and conversion, drawing of polymers, organometalics and Markush structures.

QSAR property study of saponins

In silico prediction of biological activity of a compound is widely used tool in drug discovery process[15].The molecular descriptors such as Molecular weight, hydrogen donor, acceptors, LogP, Total Polar Surface Area (TSPA), were obtained using ACD/I-Lab web-based service.

Binding site prediction

Q site finder server was used for the identification of the most potential active site where the ligand can bind and interact with the target protein topo II α[16, 17].

Molecular docking study of saponins against DNA binding domain

Docking simulation was done using AutoDockVina suite as molecular-docking tool[18]. In Windows operating system, Cygwin interface was used to launch AutoDockVina. The default optimization parameters were used for with the Lamarckian Genetic Algorithm was used with a population size of 150 dockings. Autodock 4.2 tools generated 10 possible binding conformations, i.e. 10 runs for each docking by using Genetic Algorithm (GA-LS) searches. A default protocol constituting a maximum number of 2.5 x 10^5 energy evaluations, a maximum number of 2.7 x 10^4 generations and an initial population of 150 randomly placed individuals was applied. A mutation rate of 0.02 and a crossover rate of 0.8 were used. The grid box used for specifying the search space was set at 60 × 60 × 60 centered on of topo II α with a default grid point spacing of 0.375 Å. Autogrid was used to obtain pre-calculated grid maps.After completion of docking most suitable conformation was chosen based on lowest docked energy. Selected conformations were analysed using Pymol software.

RESULTS

Homology modeling of protein sequence

Pymol was used for structural analysis, figure illustration and creation of modeled topo II α protein. Validation of the tertiary structure by PROCHECK revealed that the structure modeled through SWISS-MODEL was of high quality with 90% of residues in the most favored region (Fig.1)

The predicted structure conformed well to the stereochemistry indicating reasonably good quality. The topo II α homology modeled structure was shown in (Fig.2).

Ligand structure preparation

The structures of ligands used for docking were constructed using Marvin sketch tool of ChemAxon software were shown in Table 1. From the assessment of ligand molecules, it was observed that the ligand molecule cucumarioside A showed better molecular properties than the other molecules. Thus it could lead to the development of drug discovery process.

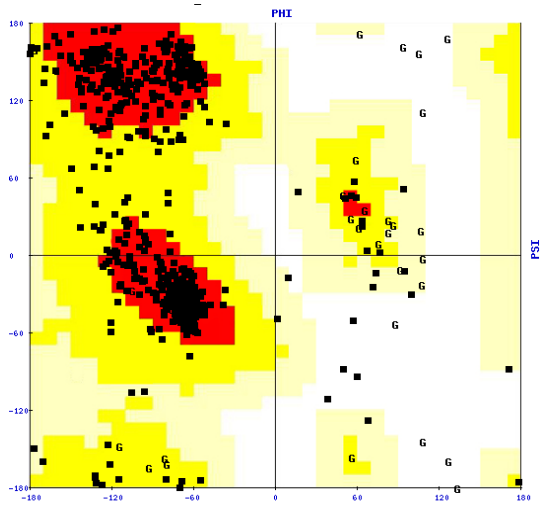


Fig. 1: Procheck analysis of human DNA topo II α

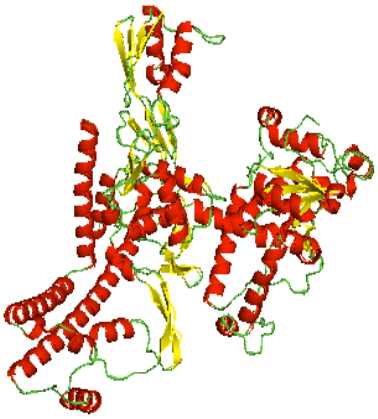
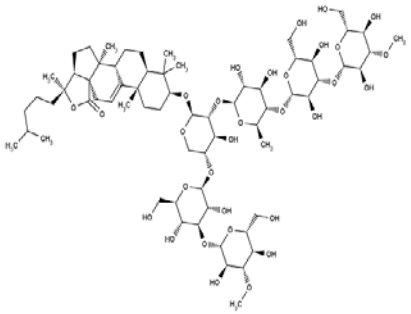
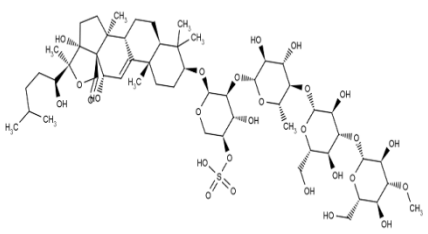


Fig. 2: Homology modeled structure of human DNA topo II α

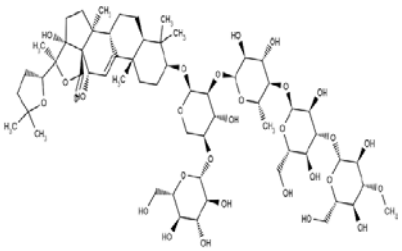
Table 1: Bioactive triterpene glycosides from marine source



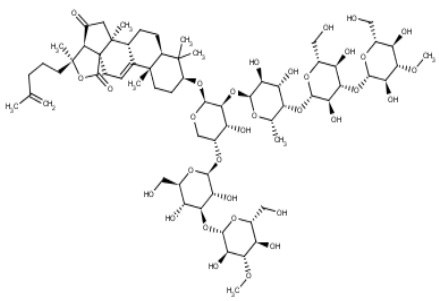
Bivittoside A



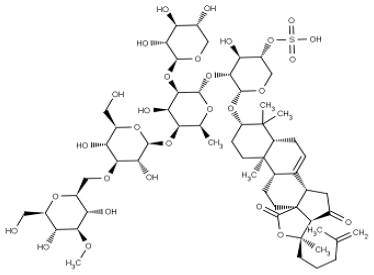
Holothurin A



Holothurinoside A



Holotoxin A



Cucumarioside A

Binding site prediction

Most potential active site of target protein topo II α where the ligand can bind and interact was identified with the Q site finder server**.** Residues PRO 716, ASP 720, GLY 721, LEU 722, LYS 723, GLN 726, ASN 770, LEU 771 , GLN 773, PHE 775, GLY 777, SER778, ASN 779, LEU 781, LEU 783, GLY 796, LYS 798, MET 847, VAL 848, LEU 849, ILE 850, ASN 851, GLY 852, ALA 853, GLU 854, LYS 863, ILE 864, PRO 865, ASN 866, TYR 892 and ARG 929were predicted as active site in the target protein topo II α .

QSAR property study of saponins

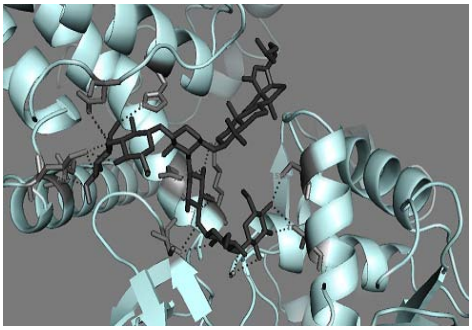
QSAR and toxicity studies were performed to find the molecular properties of selected triterpene glycosides is shown in Table 2. All triterpene glycosides showed less than 30% bioavailability, log RBA value less than -3 and negative PAT probability. QSAR studies of the ligand reveal that all selected ligands were passed, and acted as hydrophilic neutral drug molecule by their adherence to the properties such as Absorption, Distribution, Metabolism, and Excretion (ADME) as per the ACD/I-Lab web-based service.

Table 2: QSAR/ADME TOX study of triterpene glycosides

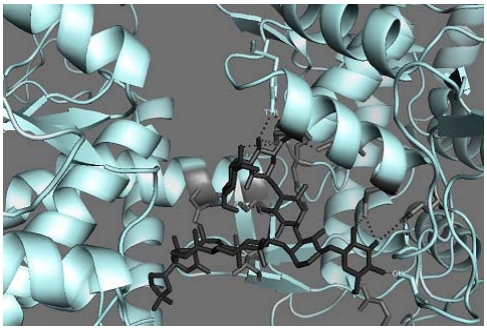
Compound	Energy value (kcal/mol)	Molecular wt	LogP	HD	HA	LogPS	TPSA	Vd (L/kg)
Bivittoside A	-10.0	1411.57	0.354	15	31	-8.7	458.97	0.63
Holothurin A	-10.5	1201.32	3.215	13	27	-10.8	424.11	0.29
Holothurinoside A	-10.5	1281.38	-3.43	15	29	-9.4	440.51	0.51
Holotoxin A	-9.7	1423.54	-2.104	15	32	-9.2	476.04	0.49
Cucumarioside A	-11.1	1297.44	-3.24	12	29	-10.5	439.41	0.29

Molecular docking study of saponins against DNA binding domain

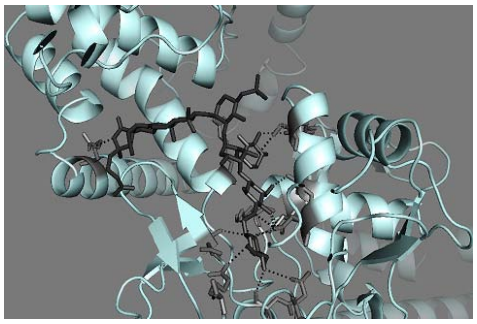
The docking scores were obtained from the analogues with topo II α as the receptor. The output of all the ligands were given by energy values in kcal/mol is shown in Table 2. The docking score was highest for cucumarioside A with docking score -11.1kcal/mol followed by holothurinoside A and holothurin A with -10.5 kcal/mol, bivittoside A with -10 kcal/mol, holotoxin A with -9.7 kcal/mol. Crucial interaction between the ligands and target topo II α DNA binding domain were shown in figure 3. Based on the results of molecular docking, all bioactive compounds showed significant binding energy with topo II α receptor, and is therefore considered as the active compounds. Known anticancer agent etoposide showed binding energy of -9.5 kcal/mol demonstrating efficacy of the bioactive compounds in the treatment of cancer with little or no cytotoxicity.



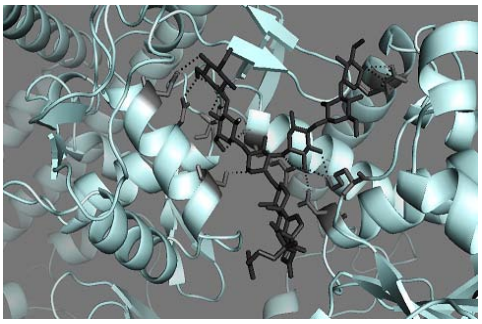
Bivittoside A docking interaction



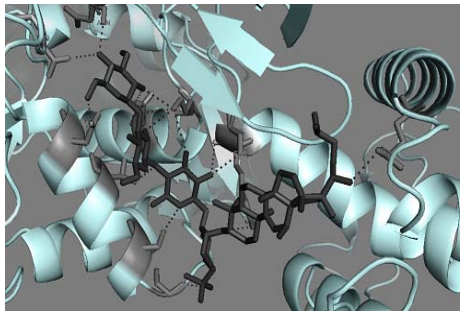
Holothurinoside A docking interaction



Holotoxin A docking interaction



Holothurin A docking interaction



Cucumarioside A docking interaction

Fig. 3: **Docked triterpene glycosides with human DNA topo II α**

DISCUSSION

In the present study ADME/TOX study and docking simulation was performed between topo II α protein with five marine derived bioactive compounds using Autodock vina to find out the binding orientation and binding affinities of the ligands. Q site finder was used to predict the active site of the target protein topo II α with a higher average precision.

According to this study, the triterpene glycoside cucumarioside A was identified as a possible better inhibitor against topo II A and follow most of the ADME properties, leading to a hydrophilic neutral drug candidate for anti cancer activity. Thus with the least binding energy, least TPSA, with a reasonable hydrogen bond interaction and with no toxicity risk at all ensures this ligands is a better source for inhibiting the topo II α oncoprotein and can be useful for further drug development studies.

The docking study revealed the binding orientation of the phenolic principles in the topo II α binding pocket. The phenolic principles of triterpene glycosides formed hydrogen bonds with the active basic and acidic amino acid residues in the conserved zinc binding motif and could chelate the Zn^{2+} atom of the topo II α which may result in inhibition of the enzymatic activity.

It will be important to study whether it interferes with the balance between the topo II α-mediated DNA cleavage and relegation by enhancing the cleavage and suppressing the religation, with more notable alterations in the prestrand passage equilibrium than in the poststrand passage event.

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