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# Studies on structural basis of epidermal growth factor receptor target using Tunicamycin on human cancer cell line

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Cancer is the leading cause of death in both economically developed and developing countries. It is a fatal disease caused by an uncontrolled division of abnormal cells in the body. These abnormal cells called malignant cells which can invade nearby tissues can spread through bloodstream and lymphatic system to other parts of the body. Tunicamycin is an antibiotic isolated from *Streptomyces lysosuperificus* that inhibits the synthesis dolichyl-N-acetylglucosamine diphosphatase essential for the assembly of oligosaccharide chains and their subsequent transfer to aspargine residues in proteins. The epidermal growth factor receptor (EGFR) is the cell-surface receptor, it's over expression or over activity has been associated with a number of cancers, including breast, lung, ovarian, and anal cancers. EGFR, an N-glycosylated transmembrane protein used to study whether inhibition of N-glycosylation and stimulation of endoplasmic reticulum (ER) stress by Tunicamycin enhances growth inhibition in cancer cell line. The protein-ligand (EGFR and Tunicamycin) and the inhibitor is Aldose reductase. The structure prediction was done using 3D pymol and maestro analysis. The structure of the ligand is elucidated and it was docked with EGFR active pocket site. The docking score was –9.87 kcal/mol and gliding energy was –69.17 kcal/mol. In conclusion the structural details and docking interaction predicts that this model can be used for drug target delivery in cancer. It also anticipated that the findings may provide useful information or clue for designing effective drugs for the therapeutic treatment of EGFR-related cancer.

Keywords: EGFR, tunicamycin, aldose reductase, docking, drug target.

## Introduction

Cancer is the leading cause of death in both economically developed and developing countries. The burden of cancer is increasing annually especially in economically developing countries, as a result of population growth as well as increased adoption of cancer-associated lifestyle including smoking, physical inactivity and western diets. In 2008, the International Agency for Research on Cancer (IARC), estimated that around worldwide 12.7 million new cancer cases, in which 5.6 million occurred in economically developed countries and 7.1 million in economically developing countries. The cancer deaths in 2008 were 7.6 million including 2.8 million in economically developed countries and 4.8 million in economically developing countries. The World Health Organization (WHO) estimates that 84 million people will die due to cancer between the year 2005 and 2018 without intervention. In 2030, the global burden is expected to grow with 21.4 million new cases and 13.2 million cancer deaths in developing countries[1].

Epidermal growth factor receptor (EGFR) is a key factor in epithelial malignancies and its activity enhances tumor growth, invasion and metastasis[2]. EGFR is a member of the ErbB family of tyrosine kinase receptors that transmit a growth-inducing signal to cells that have been stimulated by an EGFR ligand (e.g. TGF$\alpha$ and EGF)[3,4]. In normal tissues, the availability of EGFR ligands is tightly regulated to ensure that the kinetics of cell proliferation precisely match the tissues requirements for homeostasis. In cancer, however, EGFR is often perpetually stimulated because of the sustained production of EGFR ligands in the tumor microenvironment[5,6] or as a result of a mutation in EGFR itself that locks the receptor in a state of continual activation[7]. Aberrant expression of TGF$\alpha$ or EGFR by tumors typically confers a more aggressive phenotype and is thus often predictive of poor prognosis[8–11]. Not surprisingly, EGFR has emerged as a principal target for therapeutic intervention.

Tunicamycin is a nucleoside antibiotic produced by *Streptomyces lysosuperficus* that blocks N-linked glycosylation and its forms N-glycosidic protein-carbohydrate linkages in eukaryotic cells[12]. In specific tunciamycin blocks

the transfer of GluNac-1-p form UDP-GluNAc to dolichol phosphate, the first step in the synthesis of N-linked oligosaccharide chains on maanoproteins. The cell surface glycoproteins are involved in the progression of cell through the cell cycle[13,14]. Tunicamycin alters the composition of cell surface glycoproteins composing the glycocalyx[15–18]. Tunicamycin inhibits the replication of Gram-positive bacteria, fungi, yeast and viruses[19,20]. It also inhibits many biochemical functions such as glycoprotein biosynthesis in yeast[21], peptidoglycans[22], procollagen[22] and polymer cell walls[23]. Cell division in *Tetrahymena pyreformis*[24] and immunoglobulin M and G secretion by plasma cells[25]. It inhibits cell wall polymer synthesis[26,27].

Molecular docking is the process by which two molecules fit together in 3-dimensional space to predict the binding modes of a ligand with a protein of known sequences. Docking problem is concerned with generation and evaluation of possible structures of protein-ligand complexes. The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures. The study is to dock a database of potential metabolites into a protein binding site and then rank them based on their calculated binding affinities[28]. The objective of the study was to find out the affinity of the Tunicamycin have been performed using EGFR ligand. Docking analysis was carried out using Auto dock 4.0 and Maestro (10.2).

*Materials and methods:*

*Protein preparation:*

Protein EGFR was downloaded from the Protein Databank (PDB)[29]. The downloaded proteins energy was minimized by GROMOS 96 in SPDB viewer software after removed hetero atoms and water molecules from the complex structure. Active site of protein EGFR was found by the Catalytic Site Atlas serer.

**Table 1.** Docking results of compound Tunicamycin and drug EGFR calculated with AutoDock 4.0

| Drug targets | Compound | Docking score (kcal/mol) | Glide energy (kcal/mol) |
|---|---|---|---|
| EGFR-1M17 | Tunicamycin C (PubChem CID::56927832) | –9.87 | –69.17 |

*Ligand preparation:*

The chemical formula of Tunicamycin (6433557) has been retrieved from PubChem[30]. After optimization, the compound was converted to PDB format for further docking studies. The structure of drug molecule Tunicamycin was downloaded from the Drug Bank database[31]. The structures of the compounds were downloaded from the pubchem database. These compounds format was converted from .sdf to .pdb format by the software Open babel.

*Docking studies:*

The docking studies were carried out by AutoDock 4.0. Hydrogen atoms, charges were added to protein structure and grid co-ordinates were calculated based upon the active site and high volume surface area of the protein[32]. Grid co-ordinates were set to generate the grid box. The docked structure was analysed and visualised by the software Molegro viewer

*ADME properties:*

Absorption, Distribution, Metabolism and Excretion (ADME), the four criteria influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug. The properties of Rule of 5 (log p < 5, molecular weight < 500, H-bond donors < 5, H-bond acceptors < 10) was calculated by the online server mol inspiration.

## Results and discussion

AutoDock 4.0 was recently reported to be the most popular docking program. Its high accuracy and versatility had expanded its usage. To qualify the docking result in terms of accuracy of the predicted binding conformation in comparison with experimental structure[33].

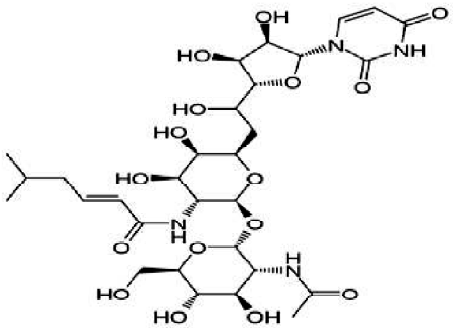


**Fig. 1.** Structure of Tunicamycin (Source - en.wikipedia.org).

The compound was obtained from *Streptomyces lysosuperficus* and it is used to cause Endoplasmic reticulum stress both *in vitro* and *in vivo* models. The severe ER stress leads to cell death of the cells or organism termed as apoptosis. The compound was sketched using Maestro software analysis. Tunicamycin was used as a testing molecule. The docking result indicated that the binding conformation of Tunicamycin derived by the AutoDock operation superposed well with the crystallographical structure. The ligand structures with the most favorable free binding energies were selected as the optimal docked conformations. Similar results was obtained in Lapatinib. They used this as a testing molecule. The docking result indicated that the binding conformation of Lapatinib derived by the AutoDock operation superposed well with the crystallographical structure, with the RMSD of 0.91 Å for the heavy atoms[34].

The ribbon shaped epidermal growth factor receptor was downloaded from protein data bank. The crystal structure was shown in Fig. 2. The docking results of the table shows that, the compound Tunicamycin binds with the drug target with the docking score as –9.87 kcal/mol which clearly shows that the compound is tightly bound to the EGFR pocket site and it exhibits the gliding energy of –69.17 kcal/mol. Molecular docking of active compounds into the active site of the protein is one of the methods used for obtaining the active conformation. These molecules were docked into the active site of EGFR to obtain the receptor based conformations. The compound superpose very well with each other, suggesting that the inhibitors have a similar binding orientation when docked into the ATP binding site of EGFR. The binding pocket[35] of EGFR structure reveals the presence of the tube with a single opening for the definition of "binding pocket". The docked structure of Valacyclovir with Thymidine kinase had the binding energy of –7.42 kJ/mol.

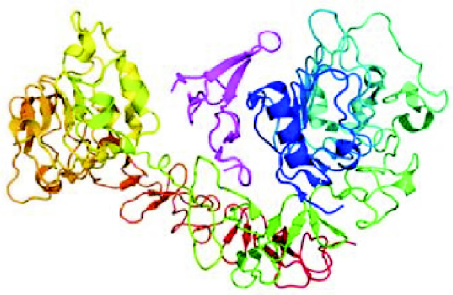

**Fig. 2.** Epidermal growth factor receptor (Source - PDBe RCSB).

*Aldose reductase:*

Aldose reductase is an enzyme and act as an inhibitor. Inhibition of AR prevented the epidermal growth factor in the adhesion of the cancer cells to endothelial cells. To study the dynamic interaction of AR with the docked phytochemicals, molecular dynamics (MD) simulations of the AR structure (PDB ID: 4GCA) complexed with gingerenones A and B were performed using Desmond. 100 ns simulations of these structures were performed to observe how the binding site adapts to the docked ligand. RMSD of the protein Cα atoms with respect to the initial structure in these simulations stabilized to under 2 Å indicating a stable conformation of the protein[36].

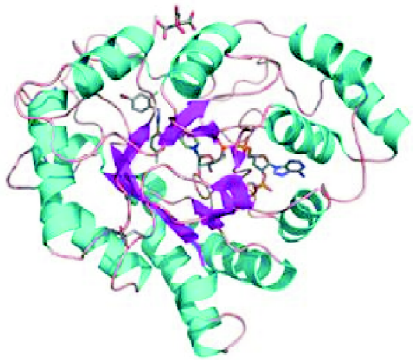

**Fig. 3.** Aldose reductase (Source - PDB: 1US0).

Molecular docking analysis was performed using Auto dock 4.0 and Maestro version 10.2, Schrodinger suite. The Fig. 4A and B shows that the structure of Tunicamycin (Ligand) and the epidermal growth factor with its amino acid sequences such as ASN-818, ASP-831, THR-766, LYS-72 and its interaction with 2D and 3D view using maestro analysis. The complex of Valacyclovir with Thymidine Kinase showed 8 hydrogen bond interactions with MET-60, GLY-61, LYS-62, THR-63, THR-64 and GLU-83 with the bond length of 2.116 Å, 2.586 Å, 2.557 Å, 2.276 Å, 1.864 Å, 2.310 Å, 2.869 Å and 2.032 Å respectively[37].

The Tunicamycin compound is bind to the EGFR receptor site and exhibits high energy level. The surface presentation of the compound is showed in the Fig. 5. The

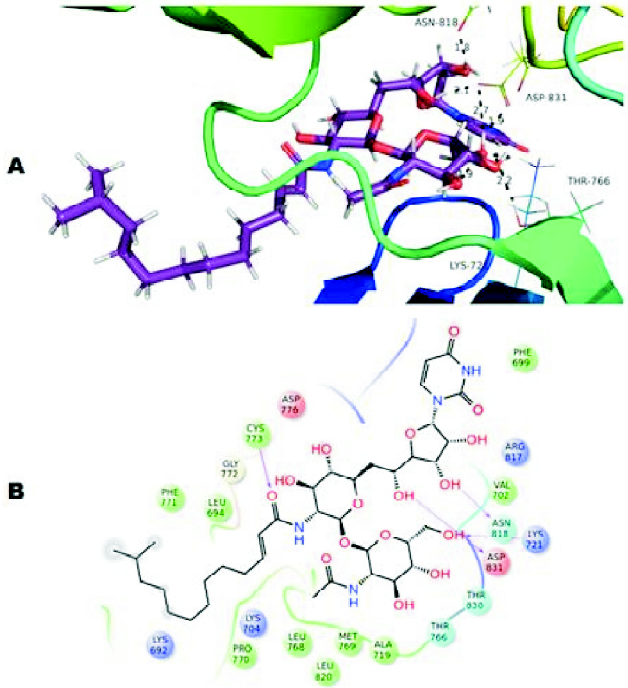


**Fig. 4.** Natural compound docked with cancer drug target for epidermal growth factor receptor (PDB ID:1M17) ligand interaction: (A) 3D Pymol view and (B) 2D Maestro view.

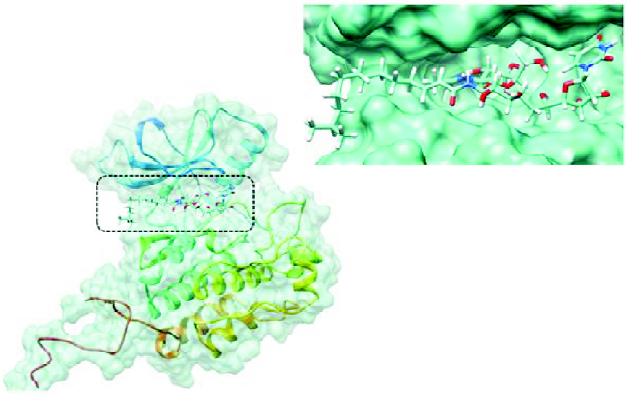

**Fig. 5.** Cartoon and surface representation of compound docked with egfr active site pocket.

binding of ligand-EGFR interaction with Aldose reductase as an inhibitor is shown by the cartoonistic representation. This clearly shows the inhibitory capacity of the Tunicamycin at the active pocket site of EGFR. Simliar results was obtained for the binding mode of the compound Phe-856 is used as a paradigm to probe the hydrogen bonding and hydrophobic interactions between inhibitors and EGFR. This compound is chosen because of its potent biologic activity. The residues involved in hydrophobic interactions between compound Phe-856 and EGFR, obtained by the Chimera program[33].

The docking studies performed give better structural insights and understanding on how the various ligands interact with the kinase domain of EGFR in acting as competitive inhibitors. The natural compound is docked with cancer target (EGFR) with AR as inhibitor is represented with pymol and maestro view analysis with THR-113 and TRP-20 amino acid sequence in Fig. 6A and B. The structure-activity relationships of the ligands may shed light on the important structure and conformation which could be applied in the design of new compounds. Many marvelous biological functions in proteins and DNA and their profound dynamic mechanisms, cooperative effects[38], allosteric transition[39], intercalation of drugs into DNA[40], and assembly of microtubules[41], can be revealed by studying their internal motions[47]. Likewise, to really understand the action mechanism of EGFR with its ligands, we should consider not only the static structures but also the dynamical information obtained by simulating their internal motions or dynamic process.

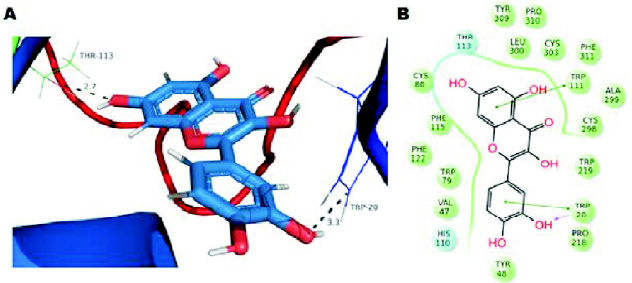


**Fig. 6.** Natural compound docked with cancer drug target for Aldose reductase AR2 (PDB ID: 1US0) ligand interaction: (A) 3D Pymol view and (B) 2D Maestro view.

## Conclusion

In this study, the automated molecular docking was used to find the binding mechanisms for a series of Tunicamycin based EGFR with Aldose reductase as a inhibitor. The docking results have been further validated as robust by the molecular dynamics simulation. The inhibitor occupy the ATP binding pocket of EGFR with various binding orientations. The findings may provide useful clues or stimulate new strategies for designing more effective drugs against EGFR related cancers. The results of the current study concluded

that Tunicamycin with high binding energies as well as a good ADME profile against target be taken into consideration, suggesting them as potential hits for drug development against cancer after testing through *in vitro* experiments. *In silico* approaches have paved the way to solve many biological problems, which have led to the identification of novel inhibitors against numerous diseases.

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