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Full Length Research Paper

Evaluating the *in silico* activity of bioactive compound iressa, tarceva and capsaicin against epidermal growth factor receptor tyrosine kinase

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Epidermal growth factor receptor (EGFR) protein tyrosine kinases (PTKs) are known for their role in cancer. Lapatinib drug have been reported to be the molecules of interest, with potent anticancer activity and they act by binding to adenosine triphosphate (ATP) site of protein kinases. ATP binding site of protein kinases provides an extensive opportunity to design newer analogs. Here we aimed to do the molecular docking studies for the potent anti-cancer drugs iressa, tarceva and capsaicin against the breast cancer treatment. The estimated free energy of binding and inhibition constant are highly differed with each drugs compared to the current market available drugs and bioactive compounds. Our results strongly suggest that the bioactive compound capsaicin activity would be comparable with the commercially available cancer drug. Further study indicates that *in silico* method would be an important tool for the drug design and development against cancer.

Key words: Epidermal growth factor receptor (EGFR), inhibitors, docking studies, protein tyrosine kinases (PTKs), breast cancer.

INTRODUCTION

The development of tyrosine kinase inhibitors has become an active area of research in pharmaceutical science. Epidermal growth factor receptor (EGFR) that plays a vital role as a regulator of cell growth is one of the intensely studied tyrosine kinase targets of inhibitors (Carpenter and Cohen, 1990; Yarden and Sliwkowski, 2001; Cohen et al., 1982; Yarden and Schlessinger, 1982, 1987). EGFR is over expressed in numerous tumors, including those derived from brain, bladder, lungs, breast, colon, neck and head. EGFR hyper activation has also been involved in other diseases including, psoriasis polycystic kidney disease and asthma (Albuschat et al., 2004; Bridges et al., 1996; Ma et al., 2005). Since the hyper activation of EGFR has been associated with these diseases, inhibitor of EGFR has potential therapeutic value and it has been extensively studied in the pharmaceutical industry. One could not, however, confirm that the compounds designed would always possess good inhibitory activity to EGFR, while experimental assessments of inhibitory activity of these compounds are

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compounds are time-consuming and expensive. Consequently, it is of interest to develop a prediction method for biological activities before the synthesis. Many of the tyrosine kinase enzymes are involved in cellular signaling pathways and regulate key cell functions such as proliferation, differentiation, anti-apoptotic signaling and neurite outgrowth. Unregulated activation of these enzymes, through mechanisms such as point mutations or over expression, can lead to a large percentage of clinical cancers (Yarden and Sliwkowski., 2001; Thompson et al., 2011). The importance of tyrosine kinase enzymes in health and disease is further under-scored by the existence of aberrations in tyrosine kinase enzymes signal occurring in inflammatory diseases and diabetes. Inhibitors of tyrosine kinase as a new kind of effective anticancer drug are important mediators of cellular signal transduction that affects growth factors and oncogenes on cell proliferation (Saloman et al., 2000; Harris et al., 1989). In the present investigation, we have taken two commercially available compounds as well as select a bioactive compound for docking analysis.

MATERIALS AND METHODS

EGFR crystal structure selection

Various different X-ray crystal structures were available in the Protein Data Bank server. Using model evaluation method that involved analysis of stereochemistry and overall quality factor distribution identified the best crystallographic structure of EGFR kinase protein. The stereochemistry of protein structure quality and verification in both side and main chain was checked by employing WHAT_CHECK, PROCHECK, ERRAT, VERYFY_3D and PROVE. Following this server helps to check the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry and checking of many sterochemical parameters of the residues in the protein model. It also analyzes the statistics of non-bonded interactions between different atom types and plots calculated by a comparison with statistics from highly refined structures, determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc), calculates the volumes of atoms in macromolecules using an algorithm which treats the atoms like hard spheres and calculates a statistical Z-score deviation for the model from highly resolved (2.0 Å).

Ligand structure and pharmacophore preparation

There are eleven commercial and bioactive compounds available as drugs for breast cancers. Information about them were downloaded from pubchem (http://pubchem.ncbi.nlm.nih.gov/). All the compounds were prepared by using LigX module in Molecular Operation Environment (MOE) (2009) version. LigX is a strong collection of conducting interactive ligands modification and energy minimization in the active site of a flexible receptor. The verification of drug ligand step will isolate all such molecules. The chosen drug ligand were identified to be treated as though they were part of the receptor. The LigX/protonate application was used to add hydrogen atoms according to the ionization state of the current atom. The heavy atoms are then fixed and a brief energy minimization is conducted to refine the positions of the added hydrogen atoms. The protonate 3D application is used to add hydrogen atoms. The ligands preparation was based on geometry optimization method and charge calculation method, a pH of 7.6 was used. Pharmacophore function group predictions were predicted from LigandScout, this also analyzed the collection of chemical features that characterize a specific mode of interaction of a drug in the active site of the macromolecule in 3D space. Examples of chemical features include charge interactions, hydrogen bonds and hydrophobic areas. It also includes unfavorable steric interactions which can be the optimization of a potential drug, which also analyzes pharmacophoric elements. Also we analysed hydrogen bond donor, hydrogen bond acceptor, positive ionizable area, negative ionizable area, hydrophobic interactions, aromatic ring, metal binding feature and excluded the volume.

Protein structure preparation

One of the best x-ray crystal structure was selected for the docking simulations and prepared using ‘protein preparation module’ (Berman et al., 2000; Berman, 2008; Hooft et al., 1996). The hydrogen atoms were added and unwanted water molecules were removed from the target protein; EGFR partial charges and atom types were assigned by using MOE. Automatically, type of information was connected and assigned using element and coordinate information. Hydrogen and refine/relax structures was added using AMBER '89, '94, MMFF94 or Engh-Huber parameters augmented with an implicit solvent model Energy minimization which was done until the average Root-Mean-Square Deviation (RMSD)of non-hydrogen atoms was reached

Binding site prediction

The active sites were predicted by using MOE. An alpha shape algorithm is used to determine potential active sites in 3D protein structures (Westbrook et al., 2005). We analysed active site of a receptor, protein surface calculations and of course molecular docking used to search for favorable binding configurations between one or more small, flexible ligands and a protein target. These are most likely to contribute to tight protein-ligand binding. Typically, scoring functions emphasize favorable hydrophobic, ionic and hydrogen bond contacts. By using this information, we detected the candidate of protein-ligand binding sites using a fast geometric algorithm, based on Edelsbrunner's alpha shapes. EGFR binding site on a macromolecular structure is ranked according to its accessible hydrophobic contact surface and active sites analysis to identify polar, hydrophobic, acidic and basic residues. Visualize solvent exposed ligand atoms and residues in close contact with ligand atoms as well as side chain and backbone acceptor.

Molecular docking method

The bioactive compound and the other known drugs were docked into the active site of the receptor (PDB ID: 1XKK) protein docking server (http://www.dockingserver.com/web). This server is based on AutoDock version 4 (Schames et al., 2004). This application was employed for accurate ligand geometry optimization, energy minimization, charge calculation, docking calculation and protein-ligand complex representation. It is shown that more accurate partial charge calculation and as a consequence, more accurate docking can be achieved by using quantum chemical methods. For docking calculations, quantum chemical partial charge calculation as a routine was only used for ligands and also, the use of application MOPAC2009 allowed fast partial charge calculation of

Table 1. Docking results in between EGFR (epidermal growth factor receptor kinase) and commercially available.

Compound	Est. Free Enery of Binding (Kcal/mol)	Est. Inhibition constant. Ki (µM)	Vdw+Hbond+desolv energy (Kcal/mol)	Total Interamolecular energy (Kcal/mol)	Interact surface
Iressa (drug)	-8.79	358.75	-11.33	-11.24	981.316
Lapatinib (drug)	-8.33	779.60	-11.78	-11.78	1201.519
Capsaicin	-6.84	9.62	-8.85	-8.95	796.202

proteins by quantum mechanical semi-empirical methods.

RESULTS AND DISCUSSION

First we validated the protein structure generated from our modeling study. For that purpose for the first step, the model protein x-ray crystal structures from Brookhaven Protein Data Bank was extracted (Wolber and Langer, 2005). Further, we used the following programme such as ERRAT, VERYFY_3D, PROCHECK, PROVE and WHAT_CHECK to validate and find the best model. Specifically, the following software and online tools such as SMART Server (EMBL) were used for domain identification, FISH Server for domain analysis and SAVES Server for procheck, to verify 3D and error checking.

Our results suggest that the protein 1XKK crystal structure was good enough to proceed for further docking analysis based on stereochemistry and overall quality factor among six EGFR crystal structures. ERRAT is a best protein structure verification algorithm that was especially well suited for evaluating the progress of crystallographic model building and refinement. This program was worked by analyzing the statistics of non-bonded interactions between different types of atoms. A single output plot was produced that gives the value of the error function versus position of a 9-residue sliding window. By comparison with statistics from highly refined structures, the error values have been calibrated to give confidence limits. This is extremely useful in making decisions about the reliability. After confirming the quality of the modeled structured, we carried out the molecular docking analysis with different bioactive compounds with our model. Our model structures of EGFR do not have any substrate or co-crystallized ligand, so the binding site was predicted by using MOE Site-finder. The site-finder generates information on the character of binding sites using novel search and analysis facilities, and provides site points for ligand binding.

Based on the docking result, it was found that phytochemical of the bioactive compound of capsaicin possess a high docking score of estimated free energy of binding (-6.84 Kcal/mol) and good estimated inhibition constant, Ki (9.62 µM), other bioactive compound, such as lapatinib and iressa drug showed similar docking score of binding free energy (-8.033 and -8.79 Kcal/mol, respectively) with good interactions, respectively (Table 1). The phytochemical of bioactive compounds lapatinib, capsaicin and iressa were predicted by pharmacophore (Figure 1).

Based on the docking score, Hydrogen Bond Acceptor (HBA) and Hydrogen Bond Donor (HBD) bonding and energy, our results suggest that the natural derivatives of capsaicin showed better scoring function than the commercially available drugs like lapatinib and iressa (Figure 2). Despite the intensive efforts and substantial advances that have occurred through focusing on improving treatments, cancer is still a leading cause of death worldwide. Our data shows that phytochemicals with precisely defined best target identification can be linked to successful drug discovery. The MOE molecular modeling software was used to search for three-dimensional shape complementarity and to examine the common elements of ligands with a reference molecule. Similar chemicals are identified and then 'docked' into the target protein by computer, enabling detailed protein-ligand interactions to be obtained. All these three approaches require the computational processes of docking and scoring using known and hypothetical drug targets on a protein, coupled with databases of virtual chemical compounds. The molecular docking will determine the binding interactions between two molecules either protein to protein or protein to ligand.

Once a compound is docked, it is then scored using mathematical models. Scoring estimates the chemical interactions, such as binding strength and energy state, between the ligand and protein to assist in the in ranking the efficacy of the compound being scored (Nagarajan et al., 2009; Perez-Sanchez and Wenzel, 2011; Oi et al., 2010). From these approaches, several potential candidate phytochemicals that directly interact with target proteins can be identified in future for the development of effective drug against cancer. Bioinformatics approach would help and provide an enhanced approach to personalized cancer drug development.

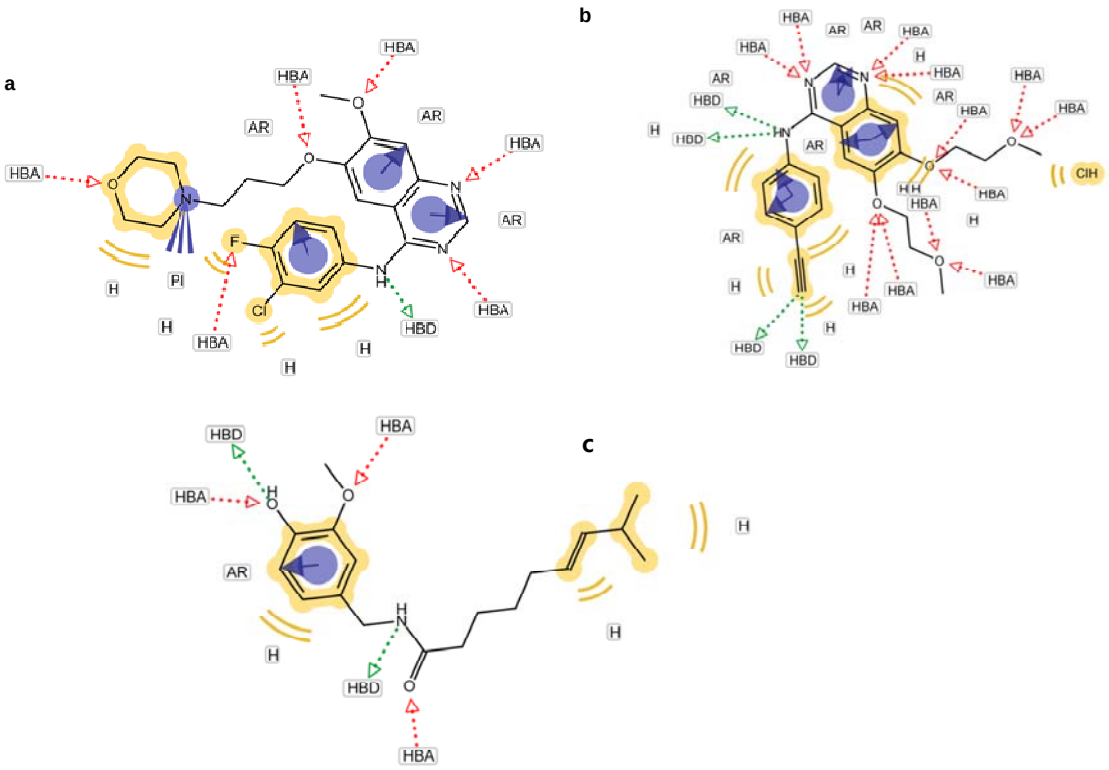

Figure 1. Pharmacophore structure prediction of drugs and bioactive compounds. A) Iressa, B) Tarceva, C) Capsaicin.

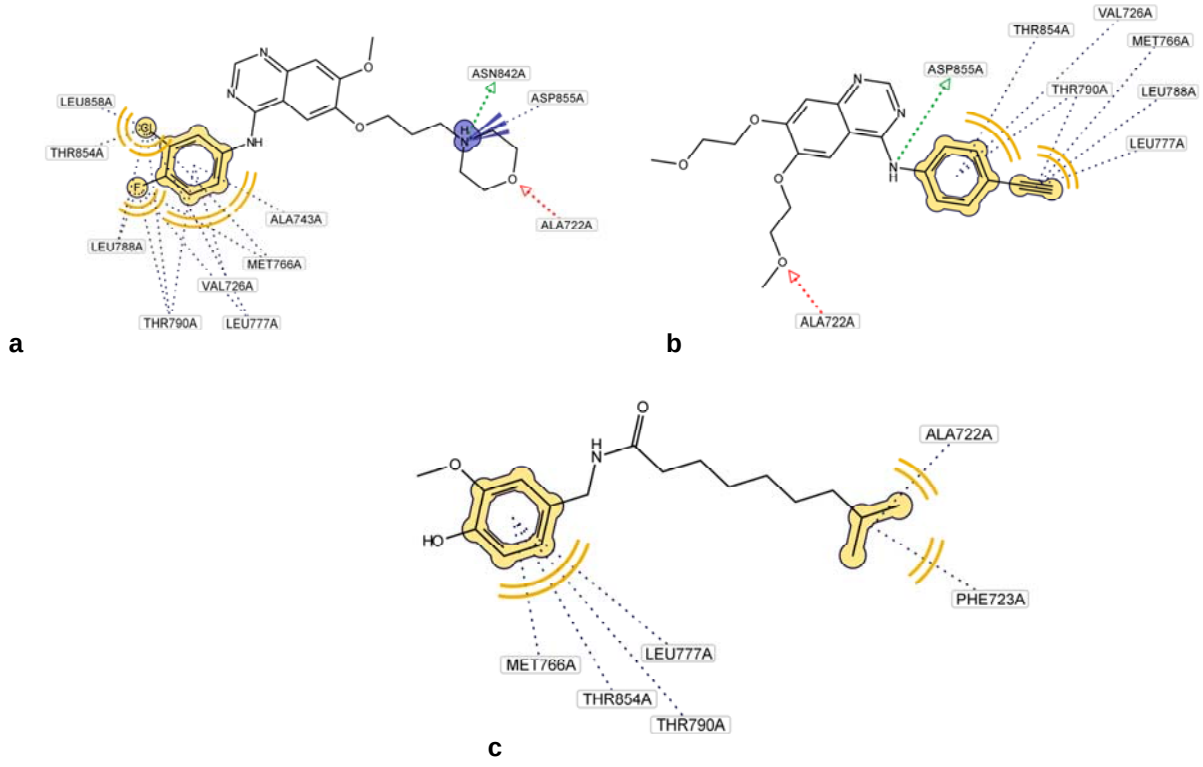

Figure 2. Active binding site and interacts residues in both drugs and bioactive compounds. (A) Iressa, (B) tarceva and (C) capsaicin.

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