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Original Article

Inhibiting EGF Receptor of Colon Cancer by Bioactive Compounds from *Momordica charantia* through Molecular Binding Approach

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ABSTRACT

Epidermal Growth Factor Receptor (EGFR) regulates cell growth and differentiation and are implicated in human cancers. EGF activates its receptor extracellular region. It is well-understood that signaling is initiated by EGF to EGF receptor. Overexpression of EGFR is associated with several different human cancers, including breast cancer and colon cancer. Thus EGFR becomes potential targets of therapeutic approach in developing EGFR inhibitors. The aim of this research is to investigate potential inhibitors for EGFR isolated from *Momordica charantia*. In order to achieve this goal, chemical structures of all compounds were designed using ChemDraw program and energies are minimized. Molecular binding was performed by AutoDock Vina program and The resulting binding modes were analyzed with AutoDock Tools and Pymol programs. Among the all compounds, triterpene-a showed the best binding modes compared to other two inhibitors based on the lowest binding energies (LBE = -5.5 kcal/mol). This indicated favorable interaction with the key amino acid residues at active site of EGFR. These in silico results can thus serve as a compound model for further studies in vitro and in vivo.

Keywords: EFG receptor, *Momordica charantia*, inhibitor, molecular binding

1. INTRODUCTION

Colorectal cancer is the third most diagnosed cancer type in males and the second in females worldwide, and its incidence is increasing even in traditionally low-risk countries. Moreover, mortality rates caused by colorectal cancer remain high, being the fourth and third cause of cancer-related mortality in males and females, respectively[1]. CRC presents with a broad spectrum of neoplasms, ranging from benign growths to invasive cancer. CRC starts in the inner lining of the colon and/or rectum as a growth of tissue called a polyp slowly growing through some or all of its

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layers. A particular type of polyp called the adenomatous polyp or adenoma is a benign tumor that may undergo malignant transformation to cancer. This malignant transformation is the result of mutation or deletion of major regulator genes, resulting first in hyperplasia moving toward adenoma to carcinoma and then metastasis[2].

Rectal bleeding, diarrhea, weight loss, abdominal pain, and anemia are considered to be the common symptoms of this malignancy[3]. In developing countries, the incidence of colorectal cancer increases sharply after the age of 50 years; whereas only 3% are found among those patients less than 40 year of age and complied with the Amsterdam / Bethesda modification criteria for HNPCC (hereditary non-polyposis colon cancer) with the following characteristics: 1) right-sided location; 2) a lower pathologic stage; 3) less tendency of metastasis; and 4) a better prognosis.6 In Asia and Africa, colorectal cancer cases at young age are also reported with a higher incidence,7,8 which may reach 4-5 times that of developed countries, but neither of those reports explain about biologic characteristics of colorectal cancer.

Epidermal growth factor receptor (EGFR) is a key factor in epithelial malignancies, and its activity enhances tumor growth, invasion, and metastasis [1]. 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., TGFα and EGF) [2, 3]. 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 [4, 5] or as a result of a mutation in EGFR itself that locks the receptor in a state of continual activation [6]. Aberrant expression of TGFα or EGFR by tumors typically confers a more aggressive phenotype and is thus often predictive of poor prognosis [7–10]. Therefore, EGFR has emerged as a principal target for therapeutic intervention [1].

Epidermal growth factor receptor (EGFR) is a therapeutic target in colorectal cancer (CRC). The benefit from EGFR inhibitors appears to be limited to a subset of patients with CRC. it is important to find appropriate therapeutic methods to prevent the development of CRC or to prolong survival after its occurrence. A number of studies have shown that diet can play a significant role in the development of colon cancer as a higher risk is associated with consumption of high-fat, low-fiber diet and red meat [2]. Bitter melon (*Momordica charantia*) is a tropical and subtropical vine, widely grown in Asia, Africa, and the Caribbean for its edible fruit. Studies have shown that bitter melon extracts are well tolerated in both acute and chronic doses in animals [3]. Recent studies have demonstrated that aqueous extracts of bitter melon can inhibit the growth of breast and prostate cancers [4-7]. Though the mechanisms behind the antitumor activity are poorly known, the previous studies hint towards induction of apoptosis as one potential mechanism [8]. Previous study showed that methanolic extract of bitter melon is potent in inhibiting the growth of colon cancer cells. Since, cancer is a disease involving uncontrolled proliferation of cells, if a drug product is able to deter this cell division, it can potentially possess anticancer activity. Bitter melon extracts has shown having potent inhibition of proliferation of HT-29 and SW480 colon cancer cells. In bitter melon, the active ingredient most probably resides in the flesh as it inhibited cell proliferation with a much higher efficiency than the extracts from the skin[8,11].

The aim of this study is to delineate the inhibition mechanism by bioactive compounds of *Momordica charantia* interact with EGFR. To study the binding interactions of bioactive compounds with EGFR through molecular docking methods. Computational methodologies have become a crucial component in drug discovery program, which involves identification to lead optimization. Molecular modeling is one of the methodologies primarily used as hit identification tool when only structure of target and its active or binding site are available [9,10]. Docking method is an energy-based scoring function which identifies the energetically most favorable ligand conformation that binds to the target.

2. MATERIALS AND METHODS

1. Protein structure preparation

The amino acid sequence of hP-gp (Entry code : 1nql) was retrieved from RSCB Protein Databank [11]. The protein is commonly found to has four subunits with sequence within 1-618, which domains I and III have the β helix or solenoid topology. These domain structures have a sequence-related extracellular region of the insulin-like growth factor-1. Domains I and III line from amino acids 1-165 and 310-481, respectively. Domains II and IV with amino acids sequence 166–309 and 482–618, respectively, contain a succession of small disulfide-bonded modules that comprise an extended rod-like structure. The H subunit is the tyrosinase domain with a molecular mass of 43 kDa. All chains EGF molecules of crystallization were removed from the complex using Pymol. All missing hydrogens were added and nonpolar hydrogens were united to their corresponding carbons using Autodock tools. The final protein structure is saved in pdbqt file format.

2. Ligand structures preparation

Ligands that we use consisting of three 3D structures of natural bioactive compounds originally belong to *Momordica charantia* and one anticancer drug for molecular docking experiments and their conformational energy were minimized by using MMFF94 force field. 3 molecule structures of *Momordica charantia* bioactive compounds were selected from leaf, fruit and seed parts. Gefitinib is used as commercial structure compared in this study. The structures were scored based on their physicochemical properties under Chemicalize (ChemAxon).

3. Drug likeness analysis of *Momordica charantia* bioactive compounds

3D structures of *Momordica charantia* bioactive compounds were analyzed using a program based on the physicochemical properties, Molsoft Drug - Likeness. Determination of physicochemical properties is important in the development of drug candidates in all stages ranging from study design to analysis studies.

4. Molecular docking of EGFR and *Momordica charantia* bioactive compounds

The preparative protein and ligand coordinates were saved as pdb files. Molecular docking experiment is performed using Autodock Vina program (Vina, The Scripps Institute). The AutodockTools is used to add partial charges using Gasteiger method and to arrange the polar hydrogens in the protein. The ligands are set to have flexible torsion angles at all rotatable bonds, while the protein is prepared as a rigid structure. Both protein and ligand are saved as output pdbqt files. For specific docking of ligands of *Momordica charantia* bioactive compounds onto human EGFR protein, the grid box volume was adjusted to 30x30×30 Å in the x, y and z axes, respectively, with grid-sizes have a space up to 1 Å. The binding energy values were calculated based on the total intermolecular energies (kcal/mol) including hydrogen bond energy, Van Der Walls energy, desolvation energy and electrostatic energy. On the other hand, the appropriate torsion angles of ligand are also induced as internal ligand energy. The docking program will evaluate this energy to obtain the best binding mode. The Root-Mean-Square Deviation (RMSD) which less than 2.0 Å was scored during running docking program. At the end of molecular docking, the binding modes of protein-ligand complexes were analyzed in AutoDockTools and Pymol programs.

3. RESULTS AND DICUSSIONS

Bioactive compounds isolated from *Momordica charantia*, including α-eleostearic acid, momordicin and triterpene-a. and commercial drug compound, gefitinib (Figure 1) were docked into binding pocket of EGFR (Figure 2).

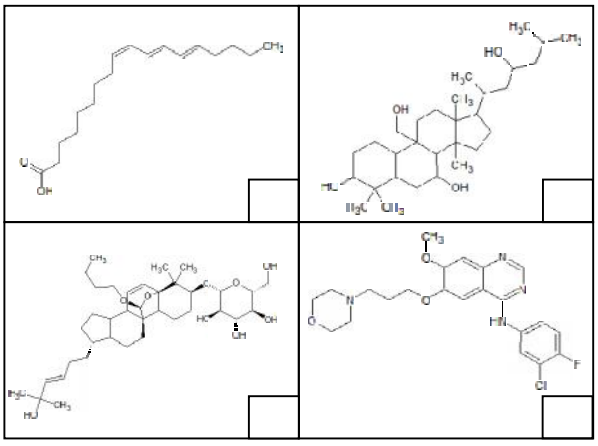

Fig 1: *Momordica charantia* compounds: a. α-Eleostearic acid; b. momordicin; c. triterpene-a. Commercial drug compound: d. gefitinib

The lowest binding energy (LBE) of the best bound conformation is triterpene-a (-5.5 kcal/mol). The molecular interaction of triterpene-a with EFGR is illustrated in Figure 3. Triterpene-a interacts with EGFR is stabilized by hydrogen bond between nitrogen atom of Carboxamide group of Asparagine-91 side chain with hydrogen from hydroxyl group of triterpene-a (Figure 3).

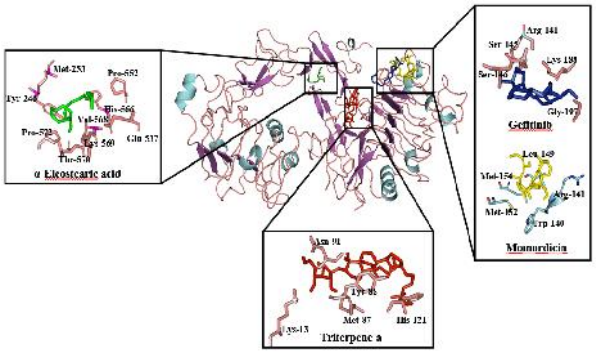

Fig 2: Molecular binding between EGFR with its potential inhibitor

Table 1: Binding Interactions between Momordica charantia bioactive compounds with of EGFR binding pocket residues.

Compound	LBE (kcal/mol)	H-bonding	Hydrophobic Interaction with EGFR residues
α-Eleostearic acid	-4.8	-	Tyr-246, Met-253, Glu-537, Pro-552, His-566, Val-568, Lys-569, Pro-572, Thr-570
Momordicin	-4.9	-	Trp-140, Arg-141, Leu-149, Met-152, Met-154
Triterpene-A	-5.5	-NH of Asn-91 with –OH of carboxamide group	Lys-13, Met-87, Tyr-88, Asn-91, His-121
Gefitinib	-5.7		Arg-141, Ser-145, Ser-146, Lys-188, Gly-197

This compound interacts hydrophobically with Lys-13, Met-87, Tyr-88, Asn-91, His-121 in the biding pocket of EGFR as shown on Table 1. Docking analysis revealed that all active compounds, occupied the same space as triterpene with a similar binding mode. The estimated lowest binding energy (LBE) value of the docked positions are listed in Table 1. LBE is the sum of the intermolecular energy and the torsional free energy which indicates favorable interactions and tight binding with key amino acid residues at the active site. On the other hand, LBE also performs the intermolecular energy which is calculated based on the sum of van der Waals, hydrogen bonding, desolvation and electrostatic energies. The favorable interactions with the key amino acid residues at the binding pocket of the receptor are presented in Table 1. Based on the structural diversity of the compounds, they are divided into three different groups. Interactions of the most active compounds in each group are provided in related sections.

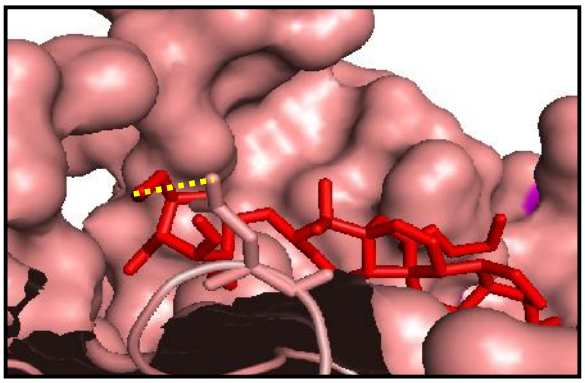

Fig 3: Hydrogen bond interaction of nitrogen atom of carboxamide group of Asparagine-91 side chain with hydrogen from hydroxyl group of triterpene-A (red)

Table 2: Drug likeness properties of *Momordica charantia* bioactive molecules and commercial anticancer drug molecule

Compound	Drug Likeness	Log P	Molecular weight (g/mol)	TPSA (A^2)	Stereocenter number**	Violation of Lipinski`s Rule**
α-Eleostearic acid	-0,08	5.65	278.20	28.29	0	1
Momordicin	0.04	5.7	478.40	65.52	0	1
Triterpene-A	-0,99	4.81	648.42	112.24	0	1
Gefitinib	-0.24	4,33	446.15	56.07	0	0

In order to find the best candidate as anticancer from *Momordica charantia* bioactive compound, we evaluated drug likeness properties of 3 compounds compared them with 1 commercial anticancer drug compound. It is found that all bioactive compounds have 1 violation of Lipinski`s rule of five, based on molecular weight. All molecular weight is above 500 g/mol. However, according to docking result, triterpene-A is the best candidate as anticancer and it could be used as a model for further analysis both in-vitro and in-vivo.

4. CONCLUSION

In agreement with the lowest binding energy, α-eleostearic acid, triterpene-A, momordicin, and gefitinib are 4.8, 5.5, 4.9 and 5.7 kcal/mol, respectively. Triterpene-A showed the closest LBE value with gefitinib. Thus triterpene-A is selected to be the best candidate as anticancer in-silico. These in-silico results can thus serve as a model for further studies in-vitro and in-vivo.

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