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Virtual screening for identification of novel potent EGFR inhibitors through Autodock Vina molecular modeling software

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ABSTRACT

*Cancer is a malignant disease and causing the high rate of mortality and morbidity. Current strategies for the treatment include chemotherapy, radiation therapy and surgery. There are various validated targets are explored and among them EGFR (Epidermal growth factor receptor) is most recently known to control the cancer and also implicated in more than 30% case of epidermal cancers. EGFR is a cell surface protein which involves in the cell proliferation and plays a crucial role in progression of cancer. Mutation in EGFR leads to the development of cancer. Only few drugs are available targeting the EGFR such as gefitinib, erlotinib, afatinib, brigatinib, icotinib and cetuximab. So herein we tried to explore the EGFR for identification of novel ligands through virtual screening by using Autodock Vina as molecular docking software. Among tested ligand indole ligand (**5**) showed better binding affinity. The novel identified ligands can be helpful in drug discovery and development and also can serve a better therapeutic substitute in case of drug resistant.*

Key words: EGFR, Autodock Vina, molecular docking, cancer,

INTRODUCTION

Cancer is a most common disease involve in great amount of morbidity and mortality in the world. It engross an abnormal growth of cells that tends to proliferate and in some case it spread i.e. metastasize [1]. It can be characterized by signs and symptoms such as fatigue, fever, pain, skin changes (jaundice, redness, darkening), trouble in swallowing, enlargement, and difficulty with bowel or bladder function, constant cough and excessive blood loss [1]. Cancers are classified as either liquid i.e. blood cancer; or solid i.e. prostate, lungs or breast cancer. It can also be classified according to the tissue such as (i) carcinomas i.e. prostate, breast, skin, lung, colon; (ii) leukaemias i.e. blood cancers; (iii) myelomas i.e. bone marrow (iv) sarcomas i.e. fat, bone, muscles, cartilage and blood vessels; (v) lymphomas i.e. cancer of immune system cells [2].

Treatment of cancer includes chemotherapy, radiation or immunosuppressive drugs etc. [1,2]. Chemotherapy includes the targeted therapy which utilizes the molecular targets involves in the growth, progression, and spread of cancer. There are various validated targets which are explored and among them EGFR (Epidermal growth factor receptor) is most recently known to control the cancer and also implicated in more than 30% case of epidermal cancers. The epidermal growth factor receptor (EGFR) is a growth factor receptor that causes cell differentiation and proliferation by activating the binding of its ligand [3]. EGFR situated at the cell surface and activated by a tyrosine kinase upon binding with ligand of four transmembrane receptors i.e. EGFR (HER1/erbB-1), HER2 (erbB-2/neu), HER3 (erbB-3) and HER4 (erbB-4). The EGFR (HER1/erbB) is a cytoplasmic tyrosine kinase domain which is

hydrophobic in nature [4,5]. The conformational changes in EGFR takes place after binding of a ligand, which leads to phosphorylation of intracellular substrates responsible for cell growth, DNA synthesis as well as expression of oncogenes [6-8]. The cellular substrates such as phospholipase C-y, (PLC-y), mitogen-activated protein kinase (MAPK) and the *ras* GTPase-activating protein (GAP) are activated [3,7]. EGFs and their transmembrane receptor kinases play a vital role in cell differentiation, proliferation, migration, survival and adhesion [6,7].

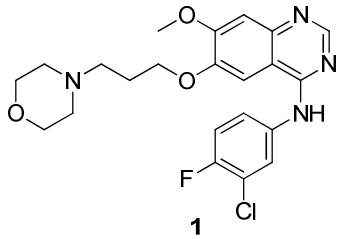
Over-expression or dysregulation of EGFR results in malignancies and stimulate tumor growth, progression as well as invasion [4]. Various epithelium cancers such as head and neck cancer along with breast, uterine, bladder, brain, cervical, esophageal, colon, ovarian, renal cell, pancreatic, glioma and non-small-cell lung cancer (NSCLC) are due to dysregulation of EGFR [9-11].

Monoclonal antibodies (MAbs) such as cetuximab [4,10], panitumumab [4,8], ABX-EGF (Abgenix) [4], EMD 72000 and MDX-447 (Medarex) [4] act by binding extracellularly which will cause internalization of receptor–antibody complexes that inhibit the EGFR signal pathway and potentiate stimulation of an immunological response [4,9]. Various EGFR mAbs are in clinical trial and considered as potential alternate for the treatment of cancers (Table 1).

Table 1. EGFR mAbs and TKIs inhibitors in clinical trials

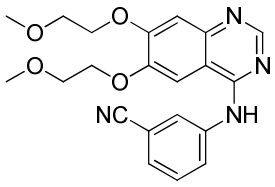
mAb	Developer	TKIs	Developer
IMC-C225	ImClone/BMS/Merck KGaA	ZD1839	AstraZeneca
ABX-EGF	Abgenix/Amgen	OSI-774	OSI/Genentech/Roche
EMD 72000	EMD Pharms/Merck KGaA	CI-1033	Pfizer
MDX-447	Medarex/Merck KGaA	EKB-569	Wyeth Ayerst
H-R3	YM Biosciences/CIM	GW572016	GlaxoSmithKline
Mab 806	Ludwig Institute	PKI-166	Novartis

Due to complexity and cost of mAbs other important class of EGFR inhibitors have gained considerable attention known as tyrosine kinase inhibitor (TKIs) such as gefitinib (ZD1839) [4], erlotinib hydrochloride (Tarceva) [4] (Figure 1), PKI-166, GW572016, CI-1033 and EKB-569 which act by binding intracellularly, and prevent activation of tyrosine kinase leads to inhibition of EGFR signal pathway which involves in phosphorylation [11-14]. Development of TKIs generated the new era of cancer treatments and interest for the scientist (Table 1). Our current research in heterocyclic scaffolds and their potential application in drug development [15] paved the way to study the newer ligands based on erlotinib.



1

Gefitinib



2

Erlotinib

Figure 1. TKIs drugs as EGFR inhibitors

EXPERIMENTAL SECTION

Multi drug resistance (MDR) is a common problem in the treatment of cancer and hence new drug development is an essential need to overcome this problem. TKIs are simpler and cost effective than the mAbs EGFR inhibitors, so we have chosen the erlotinib as the basic pharmacophore to design the novel EGFR inhibitors. Various structural analogue are designed on the basis of erlotinib EGFR inhibitor (**3-8**) by the modification in heterocycle (ring B) as well as varying the side chain at ring A (Figure 2).

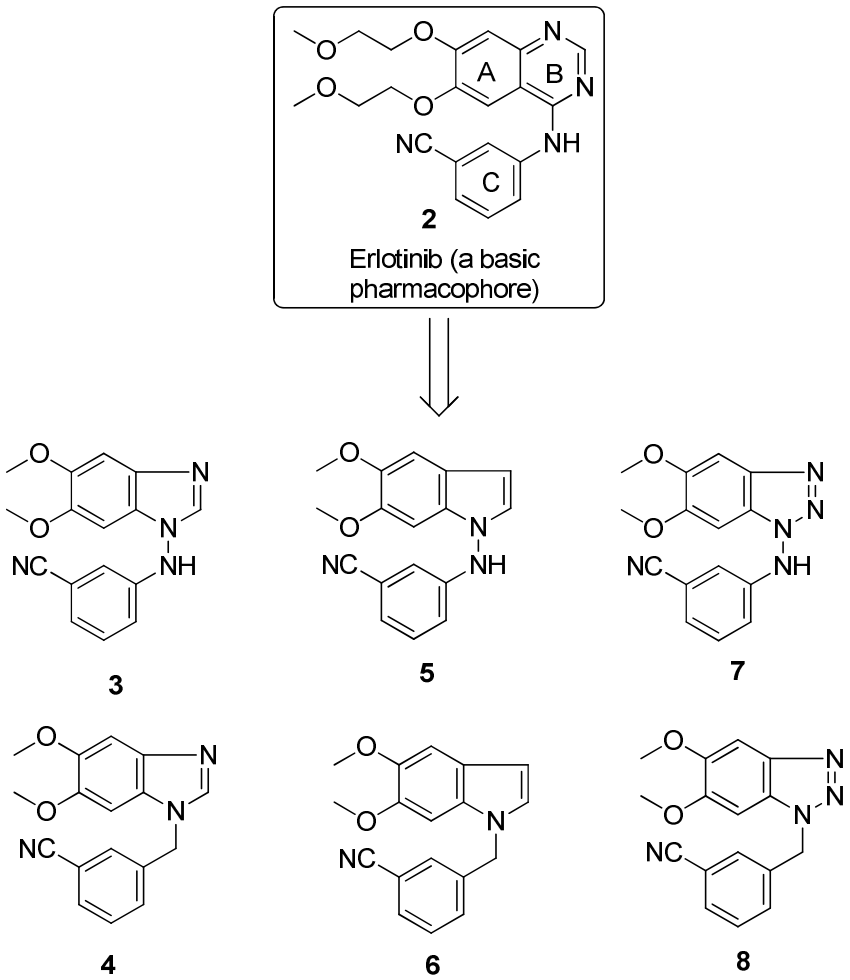


Figure 2. Designing of new ligand on the basis of erlotinib

The designed molecules can be analysed through molecular docking (figure 2) using Autodock Vina 1.5.6 software [16], which is useful molecular docking software to identify the potentially active ligands. The desired protein 1m17 is downloaded from protein data bank [17] and loaded into Autodock Vina for extraction and preparation of ligand by adding polar hydrogen to the ligand (AQ4999; erlotinib) and saved as ligand.pdbqt (Figure 3).

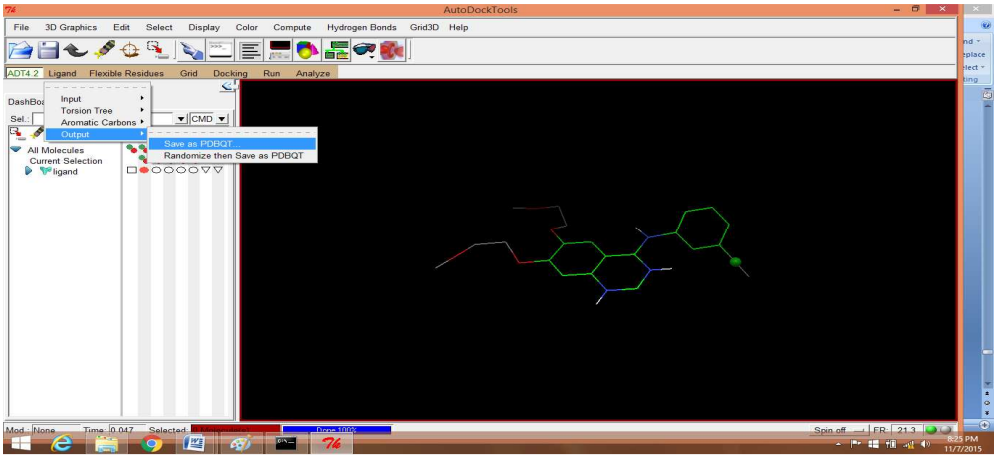


Figure 3. Preparation of ligand (AQ4999, erlotinib)

Validation of selected protein 1m17 is done by extraction of ligand and docking it in a same manner as actual ligand (AQ4999; erlotinib). For preparation of protein 1m17 protein is reloaded and various problems were fixed such as missing bonds or atoms, and remove extraneous structures such as water molecules. Before beginning to molecular docking the PDB file is inspected for such structures and cofactors as may be bound to protein naturally and also affect the binding of ligand. X-ray crystallography usually does not locate hydrogens; hence most PDB files do not include them. But hydrogens, particularly those that can involve in hydrogen bond, are important in binding of ligands; hence polar hydrogens are added along with the Kollaman charges. Later on macromolecule saved as 1m17.pdbqt file (Figure 4). Afterward ligand.pdbqt is loaded and set it as map type by choosing ligand and grid box generated by selecting "Center on ligand" and saved it by close saving current as precautionary measures like search space volume should be more than 27000 $A^{3.}$

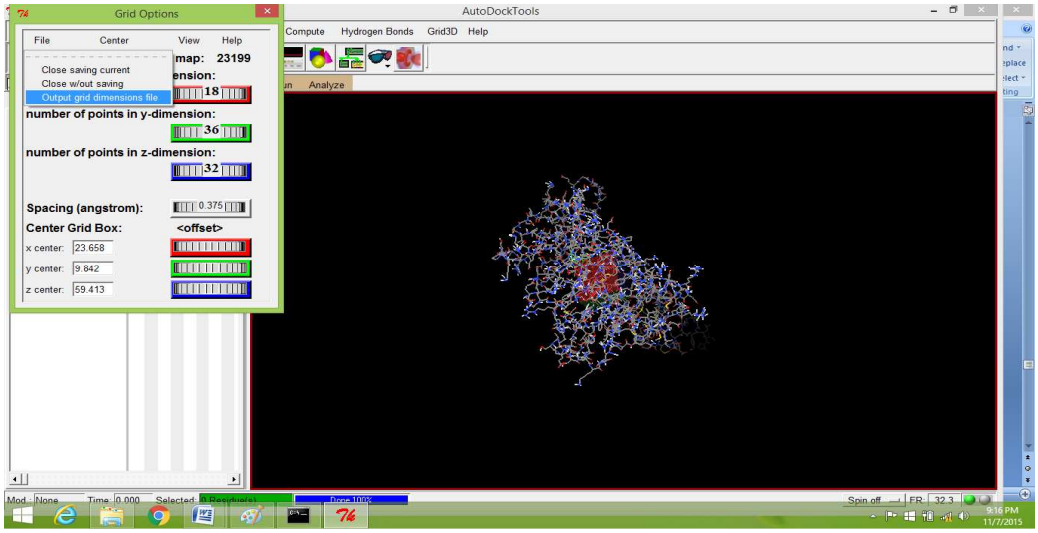


Figure 4. Preparation of 1m17 protein for molecular docking

Configuration file was prepared for ligand and its coordinate based on grid output txt file and molecular docking was performed by "Command Prompt" and saving the output file as log.txt. Thereafter all the newly designed ligands are prepared by ChemDraw Ultra and converted to 3D structure [18]. Geometry of all designed ligands was optimized by semiemperical MM2 method and saved to pdb files. Molecular docking was performed on optimized structure of protein and compared with erlotinib ligand on the basis of binding affinity of output file (Figure 5).

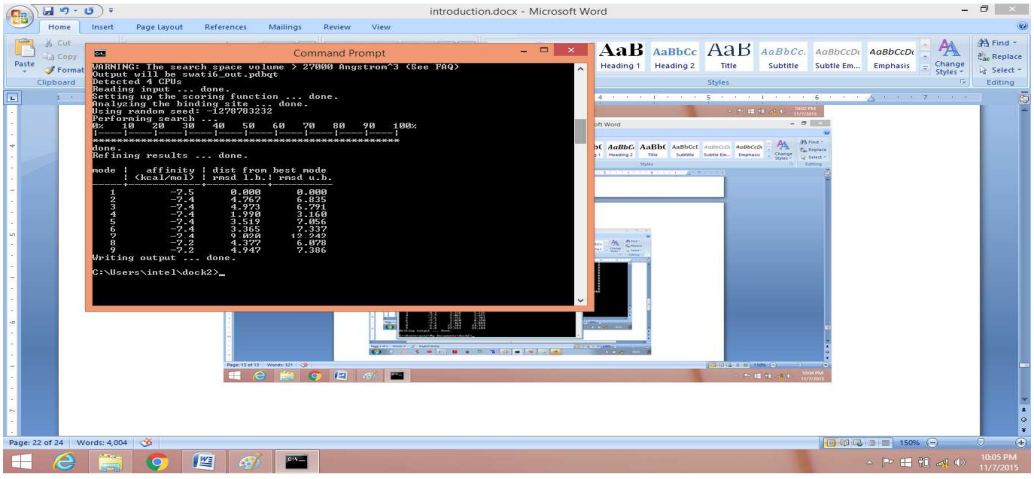
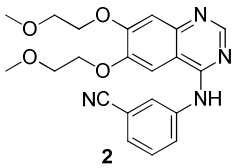
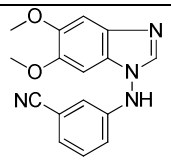
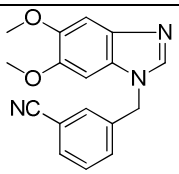
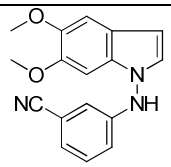
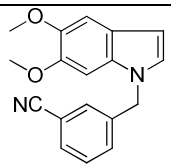
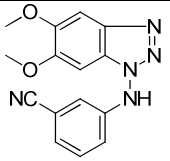
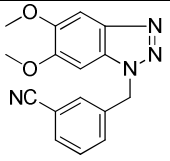


Figure 5.Output file of Autodock Vina molecular docking

Table 2. Comparison of estimated free energy of binding of the investigated ligands

S.No.	Ligand	Binding affinity (Kcal/mol)
1	 **2** Erlotinib	-6.9
2	 **3**	-7.7
3	 **4**	-7.5
4	 **5**	-8.0
5	 **6**	-7.9
6	 **7**	-7.4
7	 **8**	-7.7

RESULTS AND DISCUSSION

The designed molecules were analyzed by molecular docking and identified as potential EGFR inhibitors as shown in table 2. Modification of ring B lead to benzimidazole (**3,4**), indole (**5,6**) and triazole (**7,8**) analogue of erlotinib. All the newly designed molecules (Entries 2-7, table 2) showed the better binding affinity than the erlotinib (Entry 1,

table 2) on selected EGFR protein (1m17) at the binding site [19-21]. Results obtained through this study revealed some inportetn structural features for potential EGFR inhibitors such as quinazoline ring of erlotinib can be modified to other ring as the bioisosteric replacement. The binding affinity represnted in ascending manner as indole ligand > benzimidazole ligand > benzotriazole ligand > quinazoline ligands which means indole ligand (**5**) are better ligand with high affinity whereas benzotriazole ligand (**8**) has low affinity in the series bt still higher than marketed drug erlotinib (**2**).

For in-depth study of the binding interaction, we have selected the best posed ligand (**5**) which showed highest binding affinity -8.6 Kcal/mol (Entry 4, table 2). The interactions are visualized by pymol visualiser and studied at the binding site of the EGFR receptor (1m17) and depicted in figure 6 (Overlay ligand **5** and erlotinib); figure 7 (binding interaction of ligand **5** with binding site of 1m17 protein) and figure 8 (posed ligand at protein 1m17). Overly in figure **5** showed the perfect orientation of ligand (**5**) similar to erlotinib at the binding site. Both methoxy of ring A showed hydrophobic interaction with Val702, and Leu694 amino acid residues whereas CN at ring C forms hydrogen bonds with Asp831 and Lys731 amino acid residues (Figure 7 and 8).

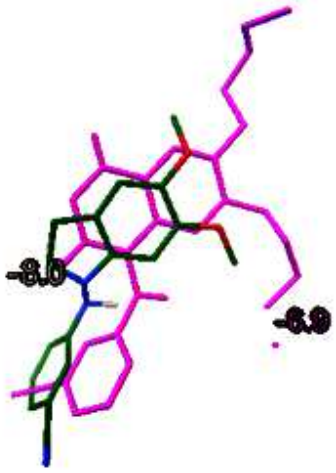


Figure 6. Overlay of docked ligand (5; green in colour) and erlotinib (pink in colour)

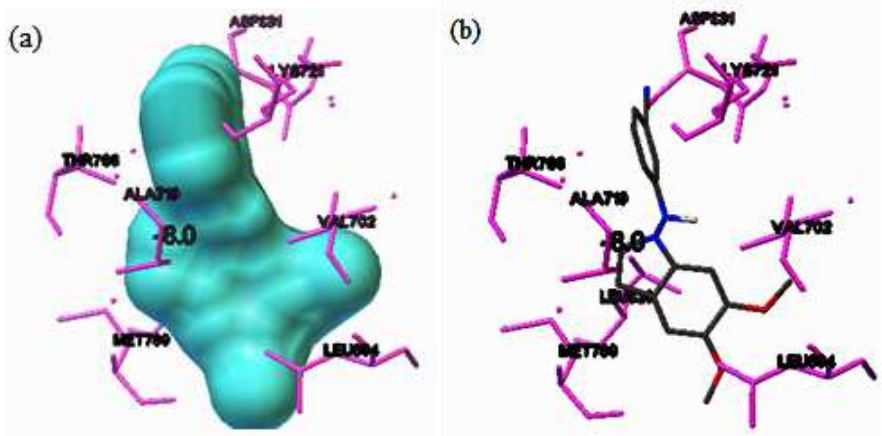


Figure 7. Binding interaction of ligand (5) at the binding site of 1m17 protein (EGFR receptor) (a) showing molecular surface of ligand (5), (b) showing ball and stick model of ligand (5)

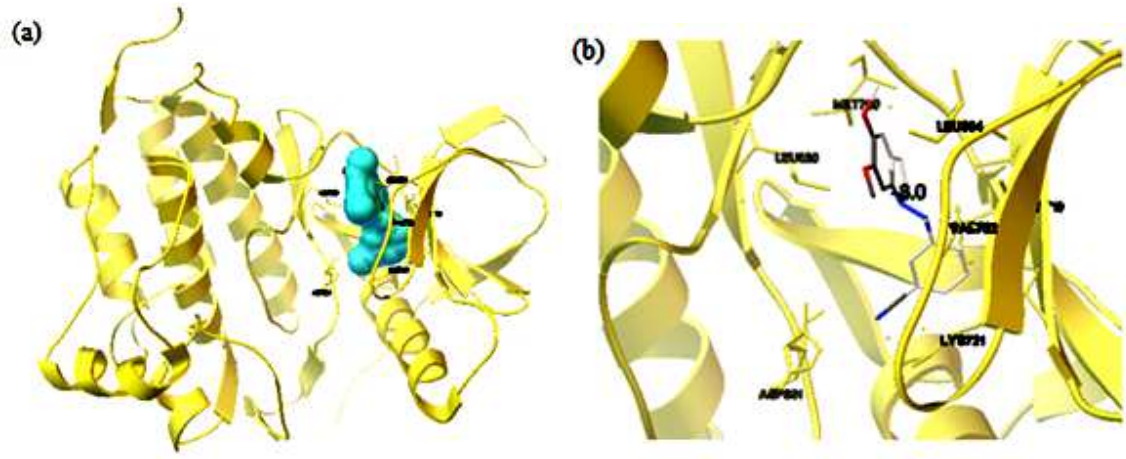


Figure 8. Docked ligand in the binding site of 1m17 protein (a) showing molecular surface of ligand (5) and ribbon structure of protein (b) showing close contact of ligand (5) and ribbon structure of protein

All the ligands are binding at the same site but possess different binding affinity, among all the ligands; indole ligand (**5**) has better affinity with -8.0 kcal/mol. This may be due to its lower molecular surface which fits better than ligand tested. These results suggested that substitution on ring A could be smaller such as methoxy as an electron donating functional group for hydrophobic interactions is necessary (Entry 1-7; table 2). Looking at the other results such as comparison of size of ring B which suggested that it could be modified to five membered rather than a six membered ring to improve binding affinity (Compare entry with and 2-7; table 2), whereas C ring topography remain same for biological activity. Further investigation on these newly designed ligands may provide the better solution in current cancer chemotherapy y targeting EGFR as potential target.

CONCLUSION

The utility of epidermal growth factor receptor (EGFR)-blocking agents is limited to a small number of tumor types. Several reports suggested that head and neck squamous cell carcinoma (HNSCCs) is due to over growth of tumor cells as well as premalignant epithelial cells. EGFR inhibitor generally inhibits the induced cancer cell proliferation. First generation EGFR like TKIs, which reversibly bind to kinases, had limited success after prolonged application i.e. patients have developed TKI resistance because of secondary mutations. To overcome this resistance, second generation drugs were developed which are currently being investigated. Hence we designed the novel ligand which could serve as a lead for drug discovery and development. The results from our study showed that the molecular docking method would be able to generate novel ligand with better binding affinity than erlotinib as the EGFR inhibitor. With the increased understanding of binding mode of the investigated ligands, we have been identified small molecule with good binding affinity indole ligand (**5**). Further it can be optimized and studied for biological evaluation to develop an alternate drug with better profile.

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