Insilco Molecular Docking Study Of Natural Compounds On wild and Mutated Epidermal Growth Factor Receptor

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In silico molecular docking study of natural compounds on wild and mutated epidermal growth factor receptor

Pushpendra Singh · Felix Bast

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Abstract The role played by overexpression of tyrosine kinase epidermal growth factor receptor (EGFR), the transmembrane receptor central to numerous cellular processes comprising cell migration, adhesion, apoptosis, and cell proliferation, has been highlighted in various cancers such as prostate, breast, lung, and ovarian cancers as well as in mutations in the EGFR kinase domain. Although many therapeutic approaches have targetted EGFR, the mutations occurring in the EGFR kinase domain including L858 EGFR and T790/L858R had led to the amplification of EGFR signals, consequently leading to increased cell proliferation and cell growth. The strategies involving the inhibition of EGFR L858 and T790M have been accredited with limited achievement in addition to being associated with unwanted adverse effects as a result of crosstalk of wild-type EGFR. All current EGFR tyrosine kinase inhibitors have been identified as ATP competitive inhibitors of wild-type EGFR possessing aniline and quinazoline moiety on the ligands skeleton. Our results obtained by performing molecular docking study on Maestro 9.3 molecular docking suite indicated that CID5280343 possesses better energy conformation against wild-type EGFR as well as two mutated EGFR. Moreover, it was discovered in this study that the natural compounds CID72276, CID5280445, CID441794, and CID72277 and InterBioScreen's library STOCK1N-78657, STOCK1N-78976, and STOCK1N-78847 have better binding conformation against gatekeeper T790M mutated EGFR concluded to be brought about by means of flexible ligands/receptor-based molecular docking protocol. Miraculous features of these compounds are their various pharmacokinetic and pharmacodynamic parameters which were found to be satisfactory as drug-like molecules. This molecular docking study also summarizes docking free energy, protein–ligands interaction profile, and pharmacokinetic and pharmacodynamic parameter of lead molecules which were tremendously helpful in enhancing the activity of these natural compounds against EGFR.

Keywords Cancer · Epidermal growth factor receptor · Mutation · Natural compound · Maestro 9.3 (Schrodinger 2012)

Introduction

Tyrosine kinase EGFR, a 170 kDa glycoprotein, is the first member of the Her receptor family playing a conspicuous role in numerous signal transduction processes including the apoptosis and cell proliferation. EGFR is expressed on a variety of epithelial cells such as skin, cervix, prostate, epithelial bronchi, bladder, and breast myoepithelial cells (Guérin *et al*., 2010). Meanwhile, cell surface EGFR is overexpressed in a number of human cancers, including non-small-cell lung cancer, head and neck cancer, ovarian, colon, bladder, kidney, breast, and prostate cancers, which ultimately results in increased metastatic potential and neoangiogenesis (Di Lorenzo *et al*., 2002; Paule and Brion, 2003; Traish and Morgentaler, 2009). Furthermore, literature has also outlined the association of the numerous known oncogenes and proto-oncogenes activation on cancer cells with tyrosine kinase (Kobayashi *et al*., 2005).

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Activation of EGFR signaling requires the binding of this receptor to growth factors such as EGF, TGFα, and heparin-binding EGF, which leads to the formation of homo- or hetero-dimers with other receptors of Her family (Salomon *et al*., 1995). The autophosphorylation and transphosphorylation of receptor's cytoplasmic tyrosine residues resulted in the recruitment of intracellular adaptor protein molecules such as Grb2 and c-Src, which in turn initiated a cascade of intracellular signaling events prompting diverse biological responses such as metabolism, cell proliferation, cell differentiation, cell survival, and cell growth (Bianco *et al*., 2006). Deregulation of EGFR expression and signaling pathways, occurring as a result of EGFR gene amplification, overexpression of EGFR, overexpression of EGF, and genetic mutation on EGFR, leads to many complications on the cell growth, development, cell migration, and metastasis. In addition, literature has also evidenced correlation between increased expression of EGF and its receptor EGFR in patients with metastatic prostate cancer with disease progression to the point of castration-resistant prostate cancer (Zhu and Kyprianou, 2008). EGFR activation leads to activation of the mitogen-activated protein kinases (MAPK) pathway in addition to phosphatidylinositol 3-kinases (PI3K) including non-small-cell lung cancer, breast, and prostate cancers (Abreu-Martin *et al*., 1999; Saini and Piccart-Gebhart, 2010; Sos *et al*., 2009; Wong *et al*., 2010). Activating EGFR mutations have been associated with increased sensitivity to gefitinib and erlotinib. Moreover, an increased EGFR copy number is associated with improved survival in non-small-cell lung cancer patients, suggesting that increased expression of mutant or wild-type EGFR molecules could be molecular determinants of responses to gefitinib (Lynch *et al*., 2004; Ono and Kuwano, 2006). The most common frameshift mutation in exon 19 and a missense mutation at 858 in exon 21 of EGFR resulted in an arginine to leucine substitution (L858R). Therapeutic agents targeting the EGFR signaling pathway, including two EGFR kinase inhibitors—gefitinib and erlotinib, are clinically effective in treating lung cancer patients harboring these EGFR activating mutations (Lin and Bivona, 2012; Lynch *et al*., 2004; Mitsudomi *et al*., 2010; Sharma *et al*., 2007; Yatabe and Mitsudomi, 2007). Mutations in the ATP-binding pocket of the EGFR-TK domain on non-small-cell lung cancer modulate the EGFR functions and mechanism of action in response to EGFR inhibitor. The non-small-cell lung cancer cell mutations in exons 18–21 leads to increased EGFR activity through overactivation of PI3K signaling pathways and decreased sensitivity to gefitinib or erlotinib (Lynch *et al*., 2004; Pao and Miller, 2005). FDA approved the first antibody/drug (ado-trastuzumab emtansine) conjugate for the treatment of people with Her2-positive metastatic breast cancer on February 22nd, 2013 (http://www.gene.com). Monoclonal antibodies and tyrosine kinase inhibitors are the two main categories of molecules currently available for the rational targeted EGFR drug discovery. The best known agents targeting EGFR with the most advanced clinical development include cetuximab, gefitinib, and erlotinib (Baselga, 2001; Bonomi, 2003; Fukuoka *et al*., 2003; Herbst, 2003; Herbst and Hong, 2002; Ranson *et al*., 2002). Unfortunately, current clinical results are disappointing due to mutations on the EGFR kinase domain which are associated with a number of human cancers, including lung, head and neck, prostate cancers, and brain tumors. Thus, the development of novel EGFR tyrosine kinase inhibitors against EGFR-mutated tumors is required.

As the majority of anticancer drugs are of natural origin, natural products play an important role in the development of novel treatment amenity for cancer. It is reported that natural products derived from medicinal herbs, food sources, and marine organisms are able to inhibit EGFR signaling (Efferth, 2011; Sertel *et al*., 2010). Receptor-based molecular docking of EGFR-TK against the selected natural compounds were carried out since the desired information could not be obtained from the in silico approach. Therefore, all the selected natural product compounds were docked with the X-ray crystal structure of PDB: 1M17, 4I20, and 3W2Q retrieved from the Protein Data Bank using Maestro 9.3 (Schrodinger 2012).

Methodology

Selection and preparation of ligands

Candidate ligand molecules were selected in our study and divided in two groups: (a) InterBioScreen's updated natural compounds library (IBS, 905 Compounds, February 2013) and (b) Natural compounds that have been reported to possess anticancer potential in the published literature (Cho and Park 2008, da Rocha *et al*., 2001; Hillman, 2012; Phosrithong and Ungwitayatorn, 2010; Sarkar and Li, 2006; Sunil, 2012), represented in supplementary table 1. These compounds were subjected into ligand preparation by Ligprep wizard application of the Maestro 9.3 (Schrodinger 2012). Ligprep performed many corrections on the ligands such as the addition of hydrogens, 2D to 3D conversion, corrected bond lengths and bond angles, low energy structure, stereochemistries, and ring conformation followed by minimization and optimization in optimized potential for liquid simulations (OPLS 2005) force field (Jorgensen *et al*., 1996; Jorgensen and Tirado-Rives, 1988; Shivakumar *et al*., 2010). Subsequently, one conformation for each ligand was generated. Aside from that, other parameters such as ionization did not change, tautomers were not generated, and retain specified chiralities and

Table 1 Lowest binding energy for the ligand–EGFR (PDB, 1M17) protein interaction as detected by GLIDE molecular docking

Ligand type	Compounds ID	GScore	Lipophilic EvdW	Bond	Electro	Protein–ligands interaction
EGFR tyrosine kinase inhibitors (control)	CID176870	−8.5	−5.36	−1.29	−0.27	Met 769 and Cys 773
	CID10184653	−8.5	−5.56	−1.25	−0.43	Met 769 and Cys 773
	CID208908	−7.1	−6.02	−0.66	−0.3	
	CID5328779	−6.94	−2.82	−2.29	−1.37	Asp 831, Lys 721, and Met 769
	CID123631	−6.84	−5.49	−0.62	−0.17	Met 769
Anticancer natural compounds	CID5281672	−10.47	−3.49	−4.49	−1.42	Asp 831, Lys 721, and Met 769
	CID5280343	−9.88	−3.32	−3.57	−1.88	Lys 721 and Met 769
	CID5280445	−9.25	−3.31	−3.42	−1.4	Asp 831, Lys 721, and Met 769
	CID5280863	−8.98	−4.15	−2.55	−0.88	Glu 738 and Met 769
	CID65064	−8.59	−2.81	−5.51	−1.19	Cys 773, Lys 721, and Met 769
Updated natural compounds library (IBS)	STOCK1N-78849	−9.31	−3.59	−4.32	−1.91	Phe 771, Thr 830, Lys 721, and Met 769
	STOCK1N-78986	−8.91	−5.33	−1.35	−0.66	Phe 699, Asp 831, and Met 769
	STOCK1N-78978	−8.8	−6.31	−1.39	−0.27	Met 769
	STOCK1N-79043	−8.63	−5.87	−1.18	−0.34	Met 769
	STOCK1N-79165	−8.46	−5.76	−1.33	−0.34	Met 769

generate at most one per ligands were used as default parameters in Maestro 9.3.

Preparation of protein molecules

The X-ray crystal structure of mutant L858R EGFR (PDB; 4I20), mutant T790M/L858R (PDB; 3W2Q), and EGFR (PDB; 1M17) were retrieved from the Protein Data Bank (Gajiwala *et al*., 2012; Sogabe *et al*., 2012; Stamos *et al*., 2002). Maestro 9.3 protein preparation wizard application executes the correction of raw PDB structure, where amendments such as addition of hydrogen atoms, assigning bond orders, creating zero order bonds to metal, creating disulfide bonds, fixing of the charges, and orientation of groups were incorporated.

Receptor grid formation

GLIDE molecular docking needs one ligand to bind with X-ray crystal structure of protein for determining active site receptor grid. Receptor grid-based molecular docking assists the ligands to bind in more than one possible conformation. The scaling factor and partial charge cutoff of van der Waals radius scaling are 0.25 and 1 Å, respectively. Other parameters such as sites, constraints, rotatable groups, and excluded volume, which are the default setting of the Maestro 9.3 are used (Friesner *et al*., 2006; Repasky *et al*., 2007).

GLIDE molecular docking

After preparing the ligand and protein, and defining the grid on active site of the protein, molecular docking procedures were carried out. GLIDE molecular docking tools use systematic computational simulation method for evaluating particular poses and ligand flexibility. GLIDE systematic method, a new approach for rapid, accurate molecular docking, and its output GScore, which is an empirical scoring function, are a combination of various parameters. The GScore is calculated in kcal/mol and it includes ligand–protein interaction energies, hydrophobic interactions, hydrogen bonds, internal energy, π–π stacking interactions, and root mean square deviation (RMSD) and desolvation. GLIDE module of the XP visualizer analyzes the specific ligand–protein interactions. The selected ligands were docked with the X-ray crystal structure of EGFR (PDB; 1M17), mutant L858R EGFR (PDB; 4I20), and mutant T790M/L858R (PDB; 3W2Q) using GLIDE. The best fit compounds were chosen for each target by thermodynamic optimal energy value, types of interactions, potential of bonding, and conformations (Friesner *et al*., 2004; Halgren *et al*., 2004).

ADME properties studies

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/T) properties of the selected best docked ligands molecules were predicted using QikProp tool of Schrodinger 2012. It predicted properties such as QP log $P_{o/w}$, QP log BB, overall CNS activity, Caco-2, MDCK cell permeability, logKhsa for human serum albumin binding, percentage of human oral absorption, etc. (Jorgensen and Duffy, 2002; Lu *et al*., 2004).

Results and discussion

Receptor EGFR tyrosine kinase is known to play a fundamental role in numerous processes such as cell growth,

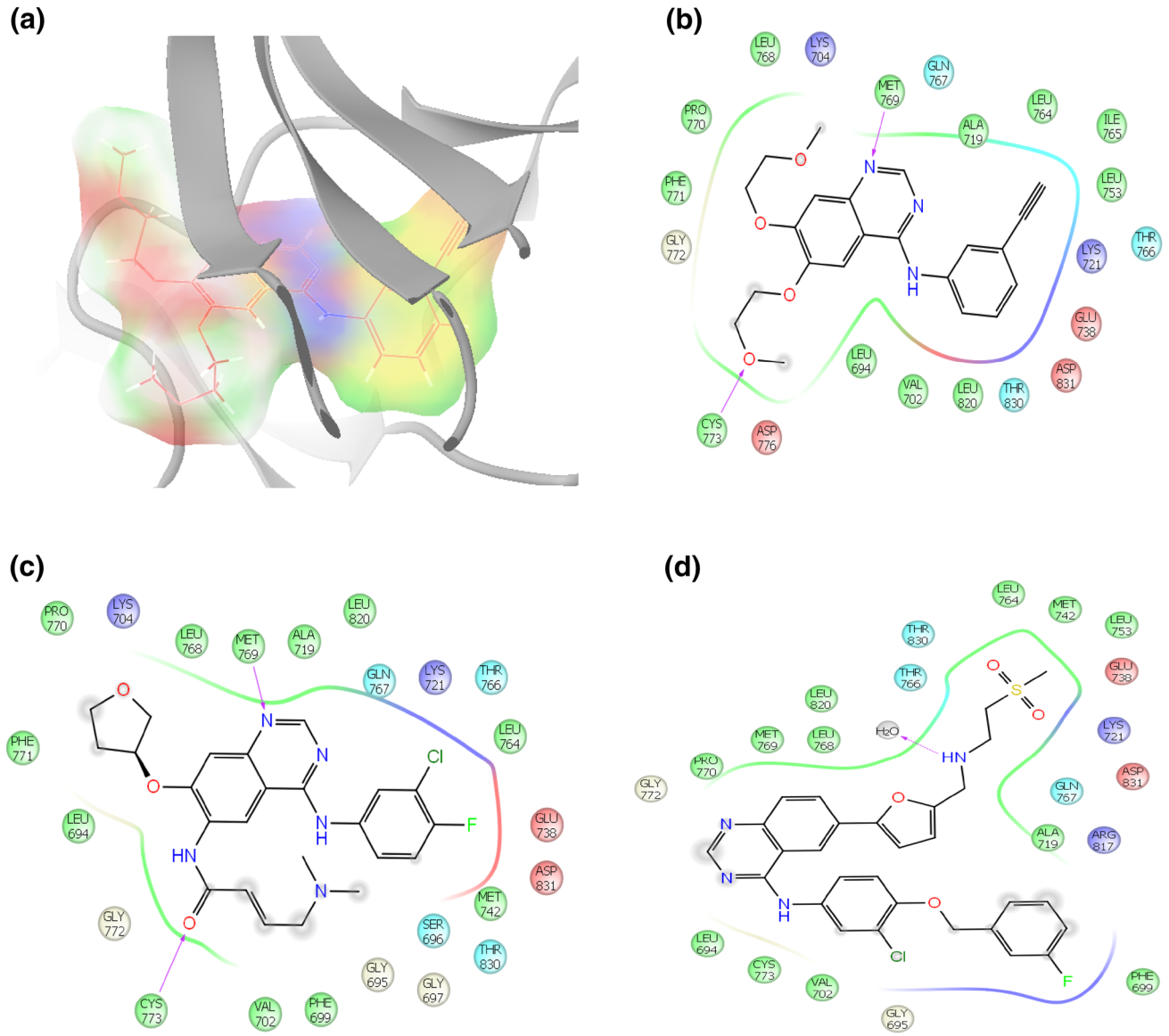

Fig. 1 **a** Ribbon presentation of EGFR (PDB; 1M17) protein molecule and EGFR tyrosine kinase inhibitor CID176870. **b** Protein–ligands interaction profile of 1M17 with CID176870, **c** protein–ligands interaction profile of 1M17 with CID10184653, and **d** protein–ligands interaction profile of 1M17 with CID208908 (control)

cell proliferation, and metabolism. Deregulation of EGFR through activation of oncogene and suppression of tumor suppression gene can result in an abnormal cascade of signaling pathways. High levels of EGFR activity and mutation in the EGFR are observed in a variety of cancers that correlate with poor prognosis and resistance to chemotherapy. Recently, receptor-based molecular docking of EGFR-TK against natural compounds has been carried out. All the selected natural compounds were docked with the X-ray crystal structures of PDB: 1M17, 4I20, and 3W2Q retrieved from the Protein Data Bank using Maestro 9.3 (Schrodinger 2012). Molecular docking procedure identifies the thermodynamic optimal energy value, types of interactions, potential of bonding, and conformations against these receptor protein molecules. The crystal structure of the kinase domain of the EGFR has been determined with 2.6-Å resolution, both with and without an EGFR-specific inhibitor, erlotinib (Stamos *et al*., 2002). Protein–ligand interaction of 1M17 with EGFR-specific inhibitor and anticancer agent, erlotinib, revealed that Met 769 forms hydrogen bond with tyrosine kinase inhibitor, and Leu 820, Leu 768, Gly 772, Met 769, and Leu 694 involved in hydrophobic interaction with tyrosine kinase inhibitor, erlotinib.

A pharmacophore model of the ATP-binding pocket of EGFR was proposed by Traxler and Furet in 1999 (Traxler

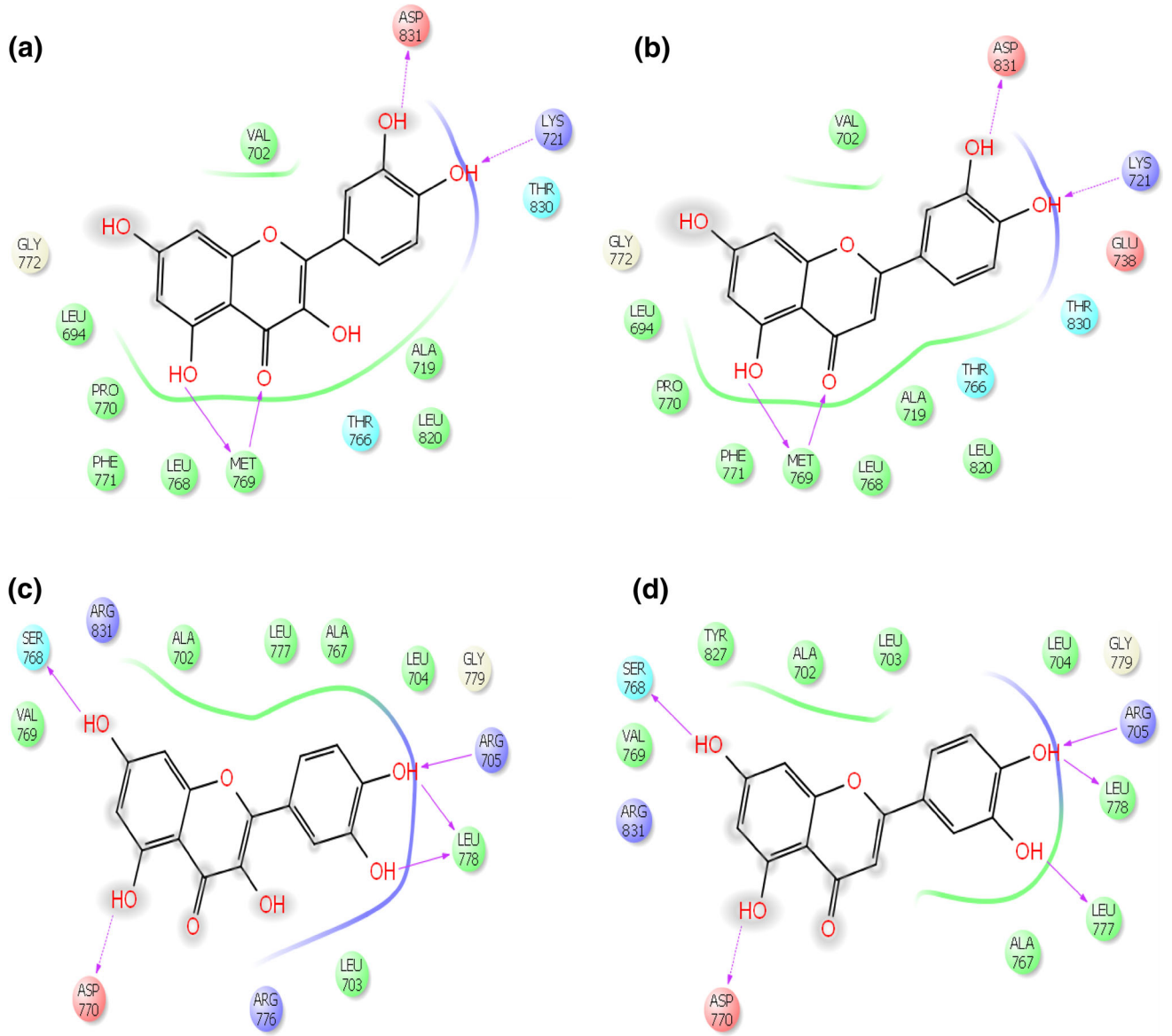

Fig. 2 Protein–ligands interaction profile EGFR (PDB; 1M17) protein molecule with **a** CID5280343 and **b** CID5280445. Protein–ligands interaction profile of EGFR kinase domain T790/L858R mutant (PDB, 3W2Q) protein molecule with **c** CID5280343, and **d** CID5280445

and Furet, 1999), which consisted of five "regions" conserved throughout the protein kinase catalytic domains: (1) hydrophobic region I, (2) adenine region, (3) hydrophobic region II, (4) sugar packet, and (5) phosphate-binding region. An EGFR kinase domain consists of an N-terminal lobe, a C-terminal lobe, and a hinge region connecting the two lobes. Amino acid T790 is present in the hinge region, whereas residue L858 is in the activation loop of the EGFR, its Cα atom being 12 Å away from the bound erlotinib. The ATP-binding cleft is composed of the hinge region of the EGFR (Liu *et al.*, 2006).

The structure of the EGFR-TK complexed with erlotinib highlighted the lipophilic, electrostatic, and hydrogen bond interaction to be the main contributor in protein–ligand interaction. The aniline moiety of erlotinib is inserted into the hydrophobic pocket of Val 702, Met 742, and Leu 764 denoted as hydrophobic region I. Hydrophobic amino acids such as Leu 694, Leu 768, Pro 770, Phe 771, and Leu 820 are associated with hydrophobic region II, and also contributed to protein–ligand interaction. Wild and mutated EGFR docked with the selected ligand molecules and five high-rank compounds (each group) were analyzed by protein–ligand interaction module in Maestro 9.3.

Molecular docking result of 1M17 against the natural compounds revealed that CID5281672, CID5280343, CID5280445, CID5280863, and CID65064 compounds have better Gscore −10.47, −9.88, −9.25, −8.98, and −8.59 kcal/mol, respectively. Protein–ligands interaction

Table 2 Lowest binding energy for the ligand–oncogenic mutant L858 EGFR kinase domain (PDB, 4I20) interaction as detected by GLIDE molecular docking

Ligand type	Compounds	GScore	Lipophilic EvdW	HBond	Electro	Protein–ligands interaction
EGFR tyrosine kinase inhibitors (control)	CID5328779	−6.58	−2.35	−2.56	−1.09	Asp 837, Asn 842, and Arg 841
	CID10184653	−5.98	−4	−1.17	−0.58	Lys 745, Leu 718, and Met 793
	CID123631	−5.7	−3.87	−0.96	−0.52	Lys 745, Gly 721, and Met 793
	CID208908	−5.19	−4.67	−0.7	−0.24	Ser 720
	CID176870	−3.82	−3.23	−1.23	−0.79	Lys 745
Anticancer natural compounds	CID5281672	−7.6	−3.0	−3.4	.9	Asp 831 and Met 769
	CID5281670	−7.58	−3.25	−3.03	−0.89	Gln 791 and Arg 841
	CID5281612	−7.4	−3.14	−3.01	−0.84	Gln 791, Asn 842, and Met 793
	CID5280343	−7.31	−2.98	−3.02	−0.91	Asn 842 and Met 793
	CID72276	−7.16	−2.91	−2.75	−1.09	Asn 842 and Met 793
Updated natural compounds library (IBS)	STOCK1N-78642	−7.0	−4.3	−1.3	−0.7	Lys 745, Gln 791, Asn 842, and Arg 841
	STOCK1N-78527	−6.69	−4.3	−0.67	−0.49	Lys 745 and Lys 745
	STOCK1N-78610	−6.27	−3.71	−2.38	−1.07	Asn 842 and Gln 791
	STOCK1N-79002	−6.27	−2.15	−2	−0.72	Gln 791 and Met 793
	STOCK1N-78478	−6.13	−5.06	−0.58	−0.25	Met 793

profile revealed that Lys 721, Glu 738, Met 769, and Asp 831 amino acids involve in the hydrogen bond and π–π stacking interaction in addition to hydrophobic interaction. Data in Table 1 also show that IBS natural compounds STOCK1N-78849, STOCK1N-78986, STOCK1N-78978, STOCK1N-79043, and STOCK1N-79165 have better energy scores of −9.31, −8.91, −8.8, −8.63, and −8.46 kcal/mol, respectively. Furthermore, docking results also revealed that the main interaction force of the candidate compounds with the EGFR-TK active site is hydrophobic interaction. STOCK1N-78849 interacts with amino acid residues, Lys 721, Met 769, Phe 771, and Thr 830, by

Table 3 Lowest binding energy for the ligand–EGFR kinase domain T790/L858R mutant (PDB, 3W2Q) interaction as detected by GLIDE molecular docking

Ligand type	Compounds	GScore	Lipophilic EvdW	HBond	Electro	Protein–ligands interaction
EGFR tyrosine kinase inhibitors (control)	CID5328779	−3.99	−1.89	−1.76	−0.72	Leu 703 and Arg 776
	CID10184653	−3.86	−2.92	−0.67	−0.34	Leu 778
	CID176870	−2.88	−2.21	−1.04	−0.43	Ala 767 and Arg 705
	CID123631	−2.43	−2.32	−1.25	−0.3	Arg 705 and Leu 703
	CID208908	0.15	−3.33	−0.74	−0.2	Arg 705 and Arg 776
Anticancer natural compounds	CID72276	−7.15	−1.89	−4.17	−1.11	Ala 767, Val 769, Arg 705, and Leu 778
	CID5280445	−6.82	−2.44	−3.45	−0.99	Arg 705, Leu 778, Leu 777, and Ser 768
	CID441794	−6.73	−1.66	−4.25	−1.03	Val 769, Arg 776, Leu 778, and Leu 777
	CID5280343	−6.71	−2.34	−3.33	−1.15	Arg 705, Leu 778, Asp 770, and Ser 768
	CID72277	−5.8	−2.1	−2.51	−1.05	Arg 776, Asp 770, Leu 777, and Leu 778
Updated natural compounds library (IBS)	STOCK1N-78657	−5.14	−1.92	−1.04	−0.54	Arg 705, Ala 767, Leu 703, and Arg 776
	STOCK1N-78976	−5.09	−1.79	−2.65	−1.03	Ser 768, Ala 767, Arg 776, and Leu 777
	STOCK1N-78847	−5.04	−1.23	−2.44	−0.81	Val 769
	STOCK1N-78759	−4.33	−3.38	−1.24	−0.76	Arg 705
	STOCK1N-79222	−4.21	−2.99	−1.22	−0.53	Leu 703 and Arg 705

Ligand CID, Pubchem IDs of the lead molecules; Ligand STOCK, updated natural compounds library InterBioScreen's library (IBS); GScore, glide extra precision scores (kcal/mol); Lipophilic EvdW, Chemscore lipophilic pair term and fraction of the total protein–ligand vdw energy; HBond, hydrogen-bonding term; Electro, electrostatic rewards; Protein–ligands interaction; π–π stacking, π–cat interaction, and hydrogen bond between the ligands and protein

hydrogen bond and π–π stacking interaction. STOCK1N-78986 interacts with amino acid residues, Phe 699, Met 769, and Asp 831 (Table 1; Figs. 1, 2).

A number of reports confirm that CID5280343 (Quercetin) is an efficient anticancer agent that induces apoptosis and decreases cell proliferation in EGFR overexpressing oral cancer cells (Huang *et al.*, 2013). Furthermore, it was also observed that Quercetin may induce apoptosis and decrease cell proliferation on HeLa cells via AMPK-induced HSP70, and downregulation of EGFR (Jung *et al.*, 2010). Interestingly, it is reported that Quercetin has higher potency among Delphinidin, Quercetin, and Epigallocatechin-3-gallate (EGCG) for inducing apoptosis and decreasing cell proliferation on colon carcinoma cell line (HT29) and human vulva carcinoma cell line (A431) with the suppression of EGFR activity (Fridrich *et al.*, 2008). As evident in several reports, CID5281672 (Myricetin) flavonoid myricetin induced apoptosis by decreasing the activity of PI3K on the primary and metastatic pancreatic cancer cell lines. In silico results highlighted that Myricetin, Quercetin, and Luteolin have a better Gscore when docked with PI3K protein molecules (Singh and Bast, 2013). Furthermore, in vivo treatment of Myricetin on orthotopic pancreatic tumors increased the tumor regression and decreased metastatic spread (Phillips *et al.* 2011). Myricetin induced apoptosis by modulating the PI3K and MAPK signaling pathways on human T24 bladder cancer cells and HepG2 cells (Sun *et al.*, 2012; Zhang *et al.*, 2013). Myricetin attenuated tumor promoter-induced activation of activator protein-1 (AP-1), sturdily inhibited MEK1 kinase activity and suppressed EGF-induced phosphorylation of extracellular signal-regulated kinase (ERK), and ultimately led to inducing apoptosis and decreasing cell proliferation (Lee *et al.*, 2007). CID5280445 (Luteolin), a naturally occurring flavonoid, is a potent stimulator of degradation of Her2 tyrosine kinase expression, which effectively inhibited cell proliferation and induced apoptosis in Her2 overexpressing cancer cells. Furthermore, Luteolin sensitizes cancer cells to induced apoptosis and decreases cell proliferation by inhibiting PI3K signaling pathways (Lin *et al.*, 2008; Zhang *et al.*, 2013). CID5280863 (Kaempferol) reduces the cell survival through downregulation of cMyc in promoting apoptosis of ovarian cancer cells (Luo *et al.*, 2010). Quercetin, Kaempferol, and Myricetin inhibited tyrosine kinase receptor signaling in a medulloblastoma cell line (Labbé *et al.*, 2009). CID72276 (Catechin) green tea catechins reduce the cell proliferation and sensitize the cell for apoptosis ultimately leading to cancer cell growth inhibition on colorectal and hepatocellular carcinoma cells, by blocking the activation of the EGFR (Khan *et al.*, 2006; Shimizu *et al.*, 2008, 2011).

Molecular docking result of oncogenic mutant L858 EGFR kinase domain (PDB, 4I20) against the natural compounds indicate that there were not much differences among the previously available tyrosine kinase inhibitors and the selected natural compounds with respect to the docking score (Table 2). Development of EGFR-TKI resistance due to secondary mutation of the EGFR gene was identified in 2005, which compromises drug therapy (Kobayashi *et al.*, 2005; Pao *et al.*, 2005). T790M mutation builds up drug resistance in spite of the occurrence of drug-sensitive activating mutations. Somatic activating mutations of the EGFR increase tumor responses and positive clinical outcomes with these agents in patients with non-small-cell lung cancer. Crystal structure of mutant T790M/L858R (PDB; 3W2Q) has revealed that T790 is located in the ATP-binding pocket of the catalytic region and plays a critical role in binding of erlotinib and gefitinib. T790M mutations do not significantly affect the binding affinity between EGFR and EGFR-TKI but increase the binding affinity between EGFR and ATP, causing a comparative decrease in binding with EGFR-TKI. An in vitro

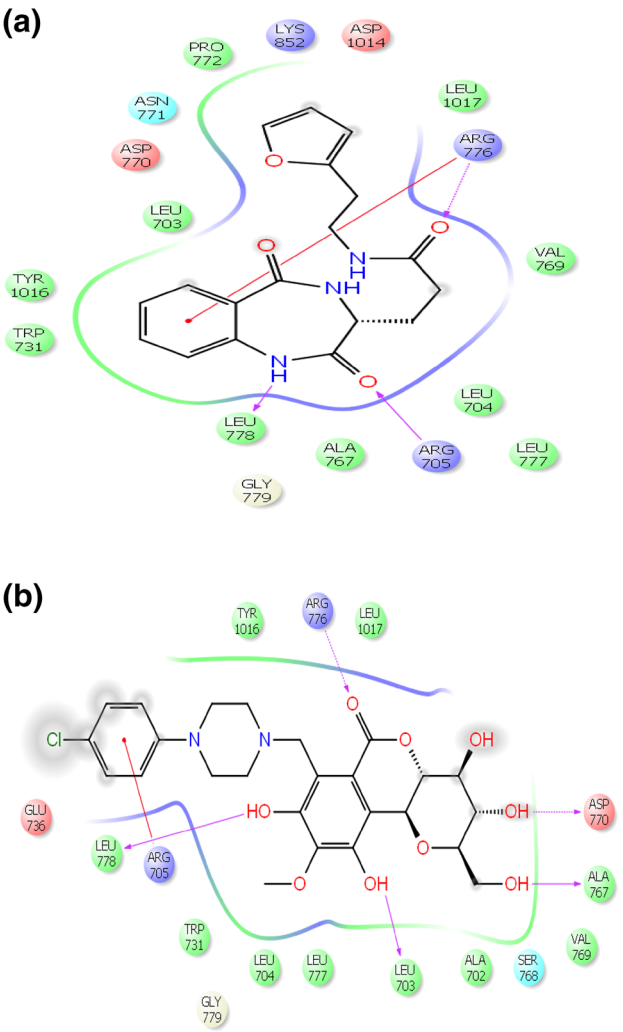

Fig. 3 Protein–ligands interaction profile of EGFR kinase domain T790/L858R mutant (PDB, 3W2Q) protein molecule with **a** natural compounds library STOCK1N-78657, and **b** STOCK1N-78976

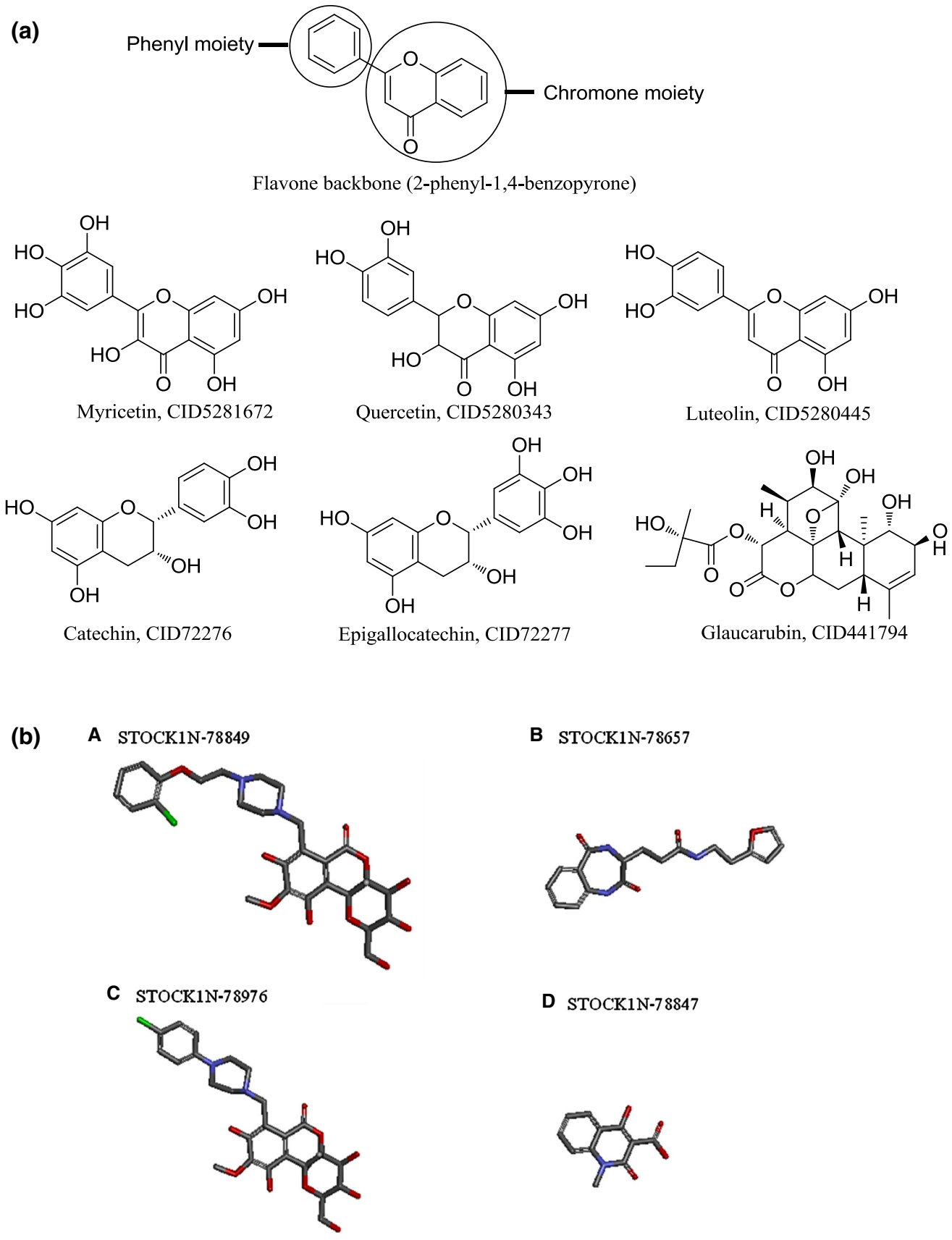

Fig. 4 Chemical structures of **a** lead natural compounds, and **b** lead InterBioScreen's library (IBS) compounds

Table 4 Evaluation of drug-like properties of the lead molecules by Qikprop Maestro 9.3 molecular docking suite (Schrodinger 9.3)

S.N.	Compounds	Q P log $P_{o/w}$ (−2.0 to 6.5)	Q P log HERG (acceptable range: above −5.0)	QPP Caco (nm/s) <25-poor >500-great	Q P log BB (−3 to 1.2)	QPP MDCK (nm/s) <25-poor >500-great	Q P log Kp (−8.0 to −0.1)	Q P log Khsa (acceptable range: −1.5 to 1.5).	Percentage of human oral absorption; (<25 % is poor and >80 % is high)
1	CID72276	−4.675	55.168	−1.847	21.591	−4.689	−0.405	61.012	61.01
2	CID72277	−4.577	20.038	−2.345	7.225	−5.575	−0.546	36.292	
3	CID5280343	−5.356	21.055	−2.418	7.623	−5.353	−0.316	53.688	53.68
4	CID5280445	−5.051	42.097	−1.946	16.119	−4.851	−0.19	61.636	
5	CID5281672	−4.869	7.525	−2.827	2.507	−6.323	−0.491	28.028	28.08
6	CID441794	−3.576	157.53	−1.547	67.111	−4.155	−0.24	72.97	
7	STOCK1N-78849	−0.018	−7.558	5.179	−1.704	4.742	−7.77	−0.775	13.7
8	STOCK1N-78657	1.176	−5.201	111.988	−1.789	78.063	−3.186	−0.514	70.50
9	STOCK1N-78976	0.328	−6.256	21.159	−1.675	20.939	−6.172	−0.602	26.6
10	STOCK1N-78847	1.522	−2.067	58.872	−0.958	29.458	−3.907	−0.667	67.53

Ligand CID, Pubchem IDs of the lead molecules; Ligand STOCK, Updated natural compounds library InterBioScreen's library (IBS); Predicted IC_{50} value for blockage of HERG K+ channels; (acceptable range: above −5.0); QPP Caco, predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells is a model for the gut-blood barrier; (nm/s) <25-poor >500-great; Q P log BB, predicted brain/blood partition coefficient; QPP MDCK, predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier; (nm/s) <25-poor >500-great; Q P log KP, predicted skin permeability; Q P log K_{hsa}, prediction of binding to human serum albumin; (acceptable range: −1.5 to 1.5); Percentage of human oral absorption; (<25 % is poor and >80 % is high)

experiment and results of cell lines transfection showed that resistance to gefitinib and erlotinib is evident when T790M mutation is present (Kosaka *et al*., 2011).

Recently, Yun *et al*. proposed that the resistance in T790M mutation causes drug resistance by restoring affinity for ATP (Azam *et al*., 2008; Suda *et al*., 2009; Yun *et al*., 2008; Zhou *et al*., 2009), thus, leading to the hypothesis that T790M mutation in EGFR kinase increases the affinity for ATP by extrastabilization of hydrophobic spine. Results of molecular docking of EGFR mutant kinase domain T790/L858R (PDB, 3W2Q) against the natural compounds showed that the natural compounds CID72276, CID5280445, CID441794, CID5280343, and CID72277 have better energy scores −7.15, −6.82, −6.73, −6.71, and −5.8 kcal/mol, respectively. The foremost IBS natural compounds STOCK1N-78657, STOCK1N-78976, and STOCK1N-78847 have better binding conformation and docking score (Table 3; Figs. 3, 4).

ADME properties

The pharmacokinetic and pharmacodynamic properties of the natural compounds with the best conformations were assessed through the Qikprop application of Maestro 9.3. According to "Lipinski's Rule of five," top five lead molecules with the best scores were selected which were observed to possess drug-like properties and are represented in Table 4. CID5280343, Quercetin, has the best docking interaction profile among all the three selected receptor molecules. Pharmacokinetic and pharmacodynamic parameters of this compound were found to be satisfactory as in drug-like molecules. Q P Log P, Q P log HERG, QPP Caco, Q P log BB, QPP MDCK, Q P log Kp, Q P log Khsa, and percent human oral absorption of compound CID5280343 were found to be −5.356, 21.055, −2.418, 7.623, −5.353, −0.316, 53.688, and 53.68, respectively; these values are satisfied by "Lipinski's Rule of five" as in drug-like molecules. Our result also indicates that STOCK1N-78657 and STOCK1N-78847 (IBS) are novel dual-effective inhibitors for wild-type EGFR and mutated EGFR with better pharmacokinetic and pharmacodynamic properties.

Conclusion

Epidermal growth factor receptor plays an indispensable role in various signal transduction processes encompassing apoptosis and cell proliferation. Overexpression and deregulation of EGFR transmembrane cell surface receptor, as well as the mutations of the EGFR kinase domain have been emphasized in majority of cancers including breast, lung,

ovarian, and anal cancers, and head/neck and brain tumors. Owing to their dominant role in cancer, molecular docking of EGFR-TK and mutated EGFR-TK against the natural compounds was carried out. Our results indicated that CID5280343 possesses better energy conformation against wild-type EGFR as well as two mutated EGFR. Four of them—CID5281672, CID5280445, CID5280863, and CID72276—were identified as dual-effective inhibitors for wild-type EGFR and mutated EGFR by means of flexible ligands/receptor-based molecular docking protocol. Natural compounds CID72276, CID5280445, CID441794, CID 5280343, and CID72277 and natural compounds library STOCK1N-78657, STOCK1N-78976, and STOCK1N-78847 were observed to possess better binding conformation against gatekeeper T790M mutated EGFR. The docking studies further revealed that amino acids Ala 719, Lys 721, Glu 738, Leu 764, Thr 766, Gln 767 Leu 768, Leu 820, Thr 830, and Asp 831 were involved in a hydrophobic, lipophilic, and van der Waals interaction and amino acids Leu 694, Met 769, Pro 770, Phe 771, and Gly 772 are involved in electrostatic force. Further in vitro and in vivo experimental work is required for validation of our in silico results, as well as identification wild and Mutated EGFR inhibitor.

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