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In-silico Identification of the Novel Anti EGFR Compounds from Ginger Through Virtual Screening and Molecular Docking Analysis

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Abstract **Introduction:** The EGFR receptor tyrosine kinase is revealed to be the critical biomarker involved in cancer metastasis and proliferation. The FDA approved drugs have shown an outstanding result in cancer treatment but these drugs suffer a lot of side effects, so there is a need to identify the novel phytochemicals that may have anti-EGFR activity. **Methodology:** The protein EGFR was retrieved from the PDB along with the hetero atoms attached with the crystal structure. The chosen 86 ginger compounds were downloaded from TIP database in 3D sdf format. Using the PyRx virtual screeing tool the target protein and ligand set were docked and then the Dockthor server was used to find docking score of the potential compounds. The docking score of all the compounds along with the standard compound, Erlotinib was obtained and analysed after the execution of docking program. Moreover, the Pharmacokinetic study was performed for the potential compounds. **Results:** The molecular docking study of our selected top compounds, TIP012988, TIP009544 and TIP013002 with higher binding affinity score than standard compound and good pharmacokinetic profile reveal that the selected ginger compounds are potent in obstructing the EGFR activity. **Conclusion:** The EGFR tyrosine kinase is found to be critical in the proliferation and metastasis of cancer. The identified top three ginger compounds through computational approaches exhibit higher potential in targeting EGFR activity in comparison to standard, Erlotinb, as the binding energy of the standard is less than the identified potential top three compounds. Moreover, the identified potential compounds possess good pharmacokinetic features indicating their characteristic of being safe for human consumption. The results obtained can be further validated through in-vitro approaches. The in-vitro validation is important as it will ensure that our findings are fruitful and synergetic for the cancer patients who possess EGFR lead cancer progression. Enhancing synergy with EGFR inhibitors and optimizing drug delivery could improve efficacy, leading to potential preclinical and clinical development of plant-derived EGFR-targeted cancer therapies.

Key Words EGFR Inhibition, ginger phytochemicals, molecular docking, virtual screening, cancer metastasis

INTRODUCTION

Cancer remains a significant global concern, with substantial social, public health and economic implications, responsible for approximately one in six deaths worldwide. The EGFR protein plays a crucial role in cell signaling pathways associated with cell proliferation and development and its deregulation is implicated in cancer progression. Epidermal growth factor receptor represents the ErbB family of RTKs (receptor tyrosine kinases) [1]. That is usually over expressed or mutated in various cancer types therefore is considered as the critical therapeutic target to treat cancer patients [2]. The signal-based activation of EGFR leads to the activation of multiple pathways those results in the activation of genes accountable for proliferation, survival and differentiation [3]. The signaling pathways associated with the EGFR in the phenomenon of cancer include PI3K-mTOR, MAPK-AKT, JAKSTAT and PLC-Y. The PI3K-mTOR pathway leads to cell proliferation and MAPK pathway supports cell survival. Whereas, STAT and PLC-Y pathways are accountable for migration and progression of the cancer cells respectively [4]. The EGFR suffers many mutations in various domains but the kinase domain as shown in the Figure 1, is said to be very

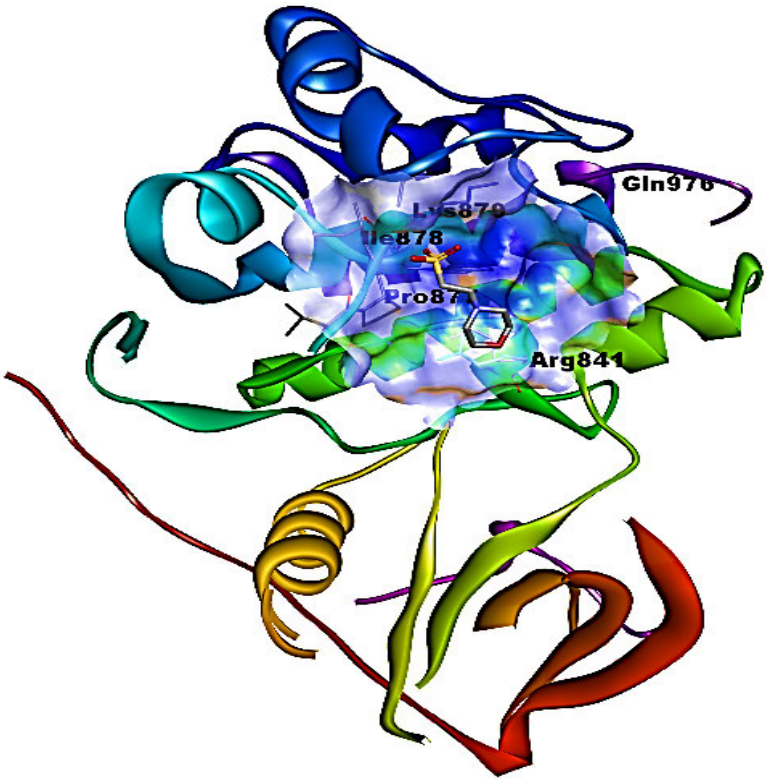

Figure 1: 3-Dimensional representation of Kinase Domain of EGFR showing the active site or binding pocket of the protein shown by surface color

prone to most of the mutations like L858R (exon-19 deletion), T790M and C797S etc. these mutations has resulted in resistance to various anti-EGFR approved drugs like Erlotinib, Afatinib, Dacomitinib, Osmeritinib and 176870. The EGFR is highly up-regulated in various types of cancers like glioblastoma, head and neck cancer etc. The exon-21 L858R substitution and exon-19 deletion are considered as the common mutation types that accounts for 90% of mutations found in NSCLC. The other uncommon mutations that occurred in EGFR include G719X in exon-18, L858R in exon-21, S768I, in exon-18 etc. that are completely understood till day [5-7]. The first-generation drugs like, Erlotinib and Gefitinib proven to be beneficial for the patients harbouring substitution mutations. The Afatinib and Dacomitinib has also proven to act as first line treatment to the patients with classical EGFR mutations [8,9]. Similarly, for the patients with uncommon mutations like G719X, L861Q, S768I and complex mutations upon treatment with second generation TKIs, outstanding outcomes have been revealed [10]. The exon-20 mutation on the other hand is considered to be intensive to anti-EGFR drugs [11-13]. The complex mutations in the EGFR reported so far accounts for 3-18% of all EGFR positive mutations approximately [13-15]. The first generation anti-EGFR drug, Gefitinib, was designed to inhibit the EGFR actively by binding to the ATP site at the intracellular level competitively [16]. It has been revealed that Gefitinib significantly had longer Progression Free Survival (PFS). Thus, Gefitinib gained approval as being the sophisticated anti-EGFR drug. The other two first generation drugs like Erlotinib and Icotinib were also developed. But with the course of time, the NSCLC patients start developing resistance within 9-14 months of treatment against first generation drugs. Therefore the need to develop, second generation drugs arouse [17]. These secondary mutations include T790M that is accountable for sustaining the ATP binding potential of EGFR TKI's. The second generation TKIs include Dacomitinib and Afatinib that were specifically designed to hinder L858R-T790M activity. The second generation TKI's exhibit better potency but suffers implications like mucositis and diarrhea in NSCLC patients [18,19].

Patients harboring T790M and L858R mutations in the EGFR gene often develop resistance to second-generation tyrosine kinase inhibitors (TKIs), rendering these treatments less effective over time. The T790M mutation, commonly referred to as the "gatekeeper mutation," alters the ATP-binding pocket of EGFR, leading to reduced drug binding and persistent cancer cell survival. Similarly, the L858R mutation enhances EGFR activity, contributing to aggressive tumor progression. To counteract this acquired resistance, researchers have developed fourth-generation EGFR inhibitors, specifically aminopyrimidine derivatives, designed

to selectively target mutant EGFR while minimizing off-target effects. These aminopyrimidine-based compounds exhibit improved binding affinity and irreversible covalent interactions with the mutant EGFR, effectively overcoming drug resistance. Unlike earlier-generation TKIs, fourth-generation inhibitors are engineered to maintain potency against resistant cancer cells while reducing toxicity and adverse effects. Their development represents a significant advancement in precision oncology, offering new hope for patients with EGFR-mutated cancers who have exhausted previous treatment options. The third-generation drug like Osimertinib, has shown efficacy to hinder the activity of T790M EGFR activity in NSCLC patients. It has been revealed that T790M vectors while administered into the cells exhibit drug- resistance to gefitinib a second generation drug [19,20]. The third generation TKIs, Osimertinib has revealed better treatment efficacy against L858R, exon-19 and T790M patients but has shown meagre efficacy against the wild type EGFR tyrosine kinase [21]. In case of Osimertinib, the fourth-generation drug, a new type of mutation namely (C797S) devolved and simultaneously, the concerned drug lost its potential to inhibit the activity of EGFR. The other reasons that weaken the efficacy of 176870 include HER2 or MET amplification, signal bypassing, KRAS, BRAF and AlK mutation [22]. Erlotinib is said to be the first-generation EGFR inhibitor. There are reports which revealed that Erlotinib has remained ineffective due to evolution of mutations in the kinase domain. So, there is a need to search for the novel potential lead compounds that can inhibit cancer pathways by targeting the EGFR activity and can may have good pharmacokinetic profile. Natural resources are considered a good source of anti-cancer compounds and are considered safe and cost effective. The ginger (Zingiber officinale) is one of most widely used natural plant to treat ailments like nausea, respiratory issues, gastric problems, loss of appetite etc. [23].The ginger belongs to the family of Zingibaracae and contains 400 different compounds [24]. The major compounds include lipids (3-8%), carbohydrates (50-60%) and terpenes and phenolic compounds [25].

Terpenes include zingiberenes, β-bsibolene, α-farnesene, β-sesquiphellandrene and α-curcumine. The phenolic compounds include gingerols, paradols and shagaol. The aminoacids, raw fiber, ash, protein, phytosterols, vitamins and minerals are also present in the ginger [23-25]. It has been reported from various studies that the active compounds of ginger suppress the growth of various cancers and have the capability of induce apoptosis. The anti-cancer activity of the ginger has been shown in skin, ovarian cancer, colon cancer, breast and cervical cancer [26]. This research is focused on investigating ginger constituents for anti-cancer activity by binding to the kinase domain of EGFR. The ginger constituents were obtained from the TIP DB and were docked with the active site along with the standard, ERLOTINIB. After docking execution, the binding affinity score was analyzed to achieve the best hit. We obtained three best hits, the binding energies of which were greater than the binding energy of the standard. The top three compounds were also checked for pharmacokinetic profile and it was found that our selected lead compounds were following Lipinski rule of 5 and possess good HIA, safe BBB profile, etc. So based on the above findings we can say that our newly identified hits may synergistically target the activity of EGFR tyrosine kinase to halt the cancer progression and metastasis.

METHODS

Target Retrieval and Preparation

We retrieved the target protein EGFR with PDB ID: 4WRG form the Protein Data Bank in pdb format. The retrieved protein has a resolution of 1.9 angstrom with attached ligand MRS and sodium ion, the target ion prepared by removing the unnecessary components like attached ligand and metal ion using the Biovia Discovery Studio software, the protein in this sense was prepared and made ready for molecular docking. The target was prepared by using a protein preparation wizard.

Ligand Retrieval and Preparation

The selected ligands were retrieved from the TIP database which includes all the compounds of ginger. The ginger is linked to the family of ginger ales. We retrieved about 86 compounds from the selected database along with the control, 176870 (Erlotinib). All the compounds were prepared and minimized energetically by applying uff (universal force fields) force field.

ADME Prediction (Pharmacokinetic Study)

It is important to know the ADME profile of the compounds before they are administered. The ADME prediction was performed for our selected top compounds and standard, Erlotinib by online server, SwissADME ". The ADME profile was checked for parameters like, GI absorption, BBB permeant, Log Kp (skin permeation), Pgp substrate and Lipinski.

RESULTS

Molecular Docking Analysis

In this study we retrieved selected 86 phytochemicals from TIP database in .sdf format and the control drug, ERLOTINIB from the PubChem database. The target protein, EGFR was retrieved form the Protein Data Bank (PDB) along with the attached heteroatoms. The resolution of the retrieved protein is 1.90 Å that is considered good structure for study purpose. The attached peptide chain as ligand atom was used to determine the active site of the target using the academic version of the Biovia discovery visualizer tool. The binding pocket residues of the target under study include; A_718 A_726 A_743 A_745 A_762 A_766 A_775 A_788 A_790 A_791 A_793 A_796 A_797 A_800 A_841 A_842 A_844 A_854 A_855. We firstly carried out the docking based virtual screening of 86 ginger compounds and obtained leads

and molecular docking process using the online AI based server Dockthor was utilized to dock the top leads to cross check the docking score obtained earlier. The process of active site molecular docking begins with the preparation of the target protein using the protein preparation wizard module, the missing residues and terminal residues acting as a cap terminus were added to the protein. The UFF force fields were used to minimize the target protein energetically so as to make it ready for docking with the selected compounds or molecules. The PyRx software and Dockthor tool provide all the required modules that accept the generated target grid file in .pdf format to execute the docking process with the selected 86 ginger compounds and control drug, Erlotinib. After the docking execution the binding score of each compound is obtained and the compounds are ranked on the basis of binding affinity scores. After analyzing the docking scores and comparing binding affinity score of each compound with that of the control drug, we found six potential compounds having binding affinity score greater than control, Erlotinib (B.E = -5.543 kcal/mol) as shown in Table 1. The six compounds includes, TIP012988, TIP009544, TIP013002, TIP012969, TIP012974 and TIP012970 with binding energy of -7.697 kcal/mol , -7.973 kcal/mol, -6.593 kcal/mol, -6.455 kcal/mol, -5.933 kcal/mol, -5.892 kcal/mol respectively as shown in the Table 1. There are top three compounds out of six compounds that possess higher binding affinity towards the target of interest than standard drug, Erlotinib and that are; TIP012988, TIP009544, TIP013002. So we selected the same compounds of interest in our study and laid more interest on these ginger constituents.

Molecular Interaction Analysis of Standard ERLOTINIB and the Top Compounds with the Active Site Residues of the Target, EGFR

In our research study we observed that docked complexes of our compounds with the target, EGFR and our analysis revealed that the docked complexes establish various types of interactions like h-bond interaction, Pi-cation, Pi-alkyl, Sulphur bond and van der waals interactions. In the Erlotinib -Target complex, three hydrogen bonds are formed between the ligand atoms and target residues Lys 745 and Asp855. In TIP009544-target complex we observed 2 hydrogen bonds with Met793 and one H-bond with Glu762 and another one with Asp855. Similarly, the compound TIP012988 establishes only on H-bond with the residue Met793 whereas the compound TIP013002 generates one H-bond with Met793 and another one with Leu718. Besides these H-bond interactions the same compounds were able to establish Van Der Waals, Pi-alkyl, Pi-Cation, sulphur bonding with the active site residues of the target EGFR as shown in Figure 1 and 2.

Analyzing the interactions the compounds made with the protein target we revealed that our selected top three compounds are able to liberate greater energy than as liberated by the control, Erlotinib. The greater number of

Table 1: Docking score of the top three compounds along with the standard, Erlotinib and the interacting residues of the active site of the target protein

Compound ID's	Score	Total energy	No. of H-bonds	H-bond distance (Å)	Interacting residues
Docking score of top ginger constituents					
Erlotinib	-5.543	59.902	A:ARG135:HE - LIG2:O	1.85494	Leu93,Arg97,Arg135
			A:ARG135:HH22 - LIG2:O	2.02332	Lys167,Val168,Pro169
			A:LYS171:HZ1 - A:ILE210:O	1.91457	Ile170,Lys171,Trp172
			A:LYS171:HZ2 - A:TYR207:O	2.19573	Glu198,Lys205,Pro206
			A:LYS171:HZ3 - LIG2:O	1.84326	Tyr207,Asp208,Gly209
			A:ILE210:H - A:TYR207:O	2.44905	
TIP012988	-7.697	13.317	A:SER720:H - A:GLY724:O	2.24972	Leu718, Gly719
			A:VAL726:H - A:GLY719:O	1.803	Ser720, Val726
			A:MET793:H - :UNK900:O1	2.11981	Ala743' Thr790
			A:GLY796:H - A:MET793:O	2.21457	Gln791' Leu792
					Met793, Pro794
					Gly796, Leu844
TIP009544	-7.978	37.787	A:LYS745:HZ1 - :GLU762:OE1	1.87462	Leu718, Val726
			A:LYS745:HZ1 - A:GLU762:OE1:B	1.64236	Ala743, Lys745
			A:MET793:H - :UNK900:O3	1.89896	Glu762, Met766
			A:GLY796:H - A:MET793:O	2.21457	Cys775, Leu788
			A:ASP855:H - A:THR854:OG1	2.29032	Thr790, Gln791
			A:ASP855:H - :UNK900:O1	2.07925	Leu792, Met793
			A:PHE856:H - A:GLU762:OE2	2.00113	Gly796, Leu844
			:UNK900:H5 - A:GLU762:OE2	1.66236	Thr854, Asp855
			:UNK900:H14 - A:MET793:O	2.29564	Phe856
TIP013002	-6.593	18.267	A:VAL726:H - A:GLY719:O	1.803	Leu718, Gly719
			A:CYS775:HG - A:ILE853:O	2.42591	Ser720, Val726
			A:MET793:H - :UNK900:O1	2.08405	Ala743, Met766
			A:GLY796:H - A:MET793:O	2.21457	Cys775, Thr790
			A:ASP855:H - A:THR854:OG1	2.29032	Gln791, Leu792
			:UNK900:H23 - A:LEU718:O	2.1643	Met793, Gly796
					Leu844, Thr854

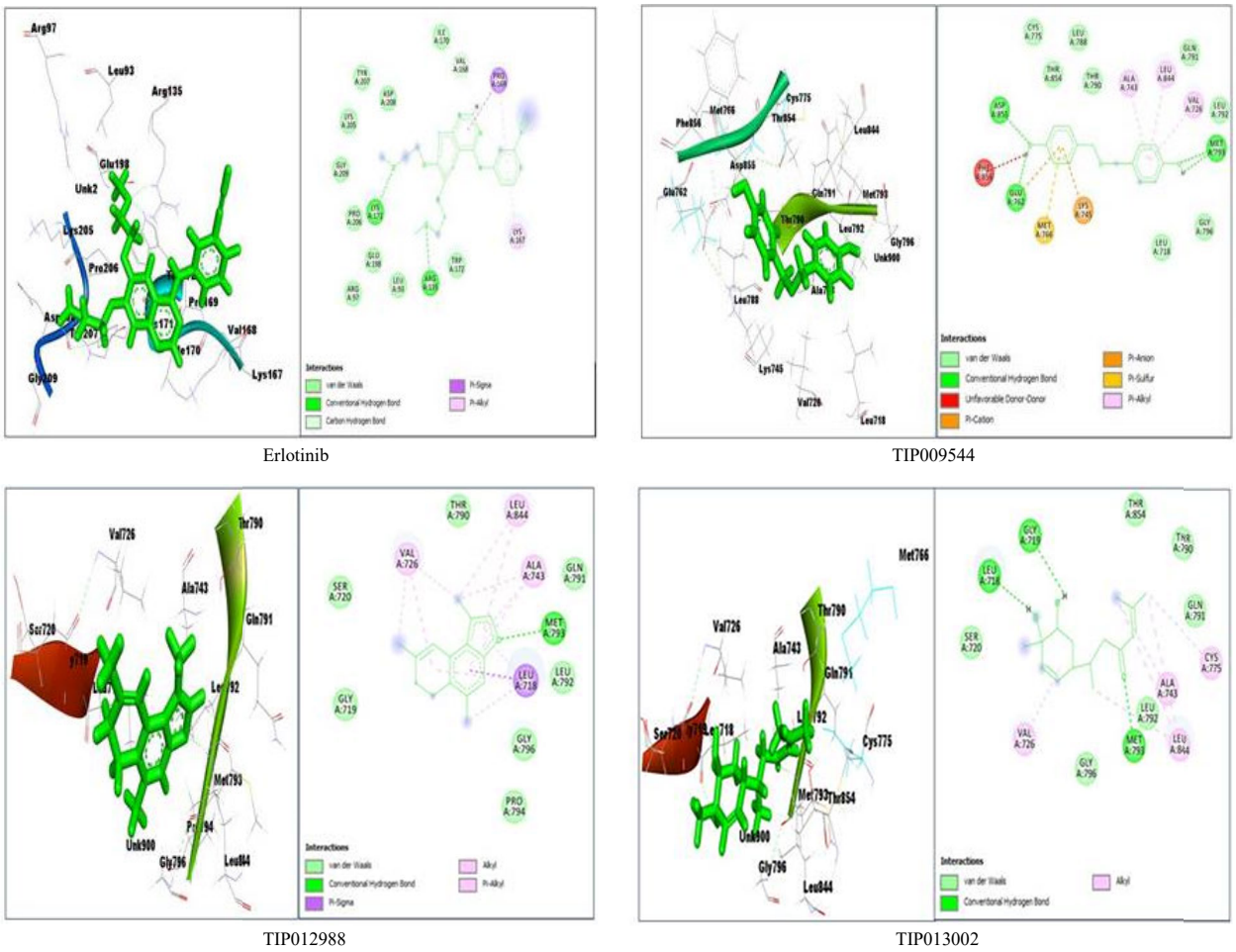

Figure 2: 3D and 2D representation of the potential selected compounds indicating the interaction pose of the ligands and the number and types of residues involved in the generating interaction types with the ligand molecules

Table 2: Prediction of binding affinity score and drug-likeliness showing that our potential compounds behave more effectively than the standard, Erlotinib

	Drug-likeliness parameters				
Compound ID's	H-bond Acceptor	H Bond donor	Molecular weight	TPSA (angstrom)	Xlogp
Erlotinib	7	1	393.443	111.0	3.405
TIP012988	1	0	212.12	13.14	4.437
TIP009544	3	2	230.094	49.69	2.644
TIP013002	2	2	252.173	57.53	1.793
TIP012969	7	2	384.121	102.29	2.507
TIP012974	2	0	232.146	26.3	2.905
TIP012970	2	0	194.131	34.14	1.67

interactions the compound shows with the target residues, more the complex is said to be stable and greater is the chance that a specific compound can modulate the activity of the target involved in the cancer progression (Figure 3).

Physiochemical Properties of Potential Compounds and Standard Drug, Erlotinib

The natural compounds we selected for our study exhibits drug like properties by following Lipinski rule of five parameters i.e., MW<500 Da, HBD<5, HBA<10 and Xlogp<5. The selected compounds also possess good ADME and Toxicity profile that we predicted by using Pre-ADMET server. The drug-likeness features and ADMET profile of the compounds have been shown in the Table 1. The potential ADMET and drug like features of the compounds indicates the compounds have the potential to be selected for further study and investigation in an *in vitro* analysis (Table 2).

Prediction of ADME Profile

The ADME profile predicted result given in Table 3 for our top compounds and the standard, Erlotinib exhibits good value for each parameter with high GI absorption, moderate

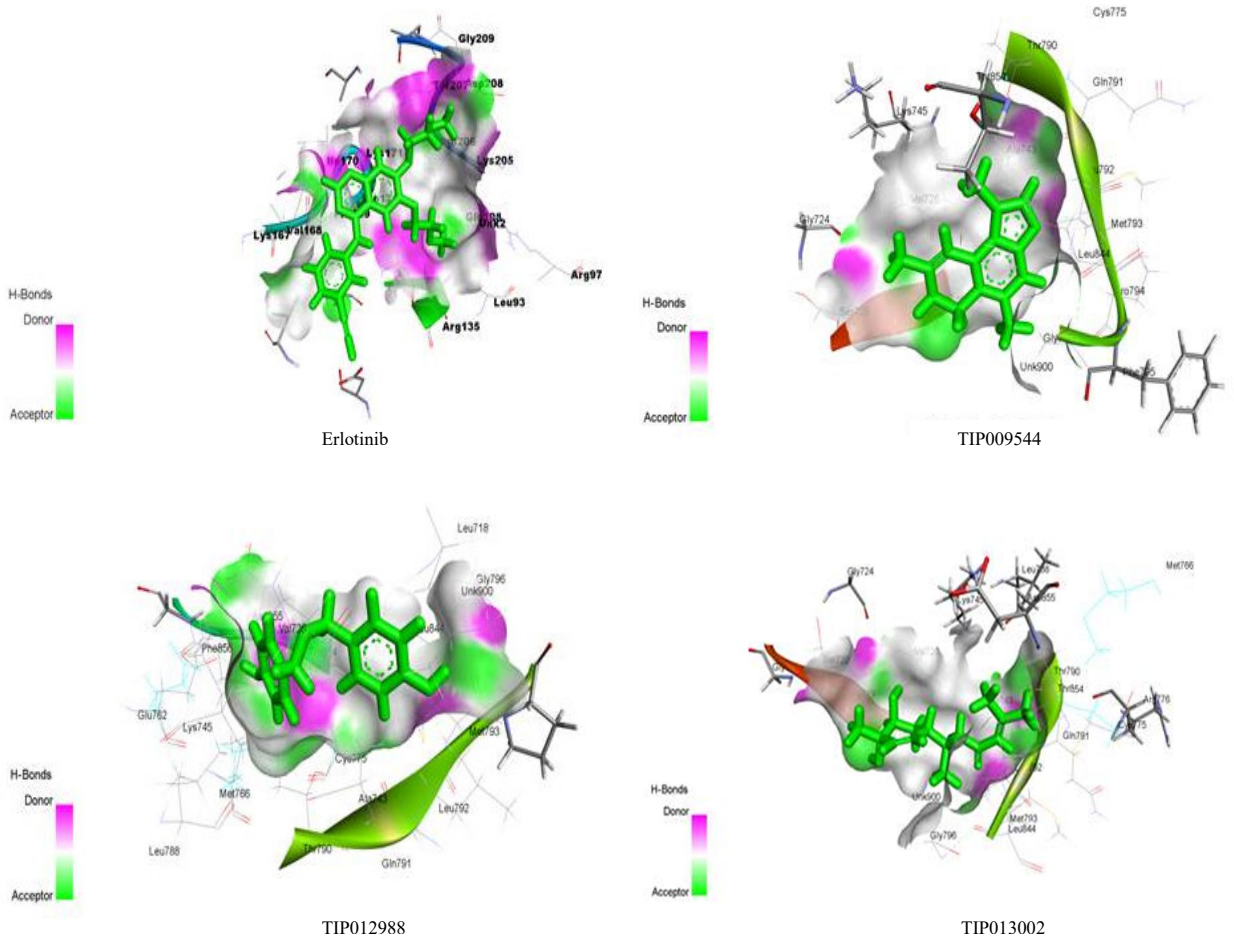

Figure 3: 3D surface representation of the top leads indicating the interaction pose of the ligands and the number and types of residues involved in the generating interaction types with the ligand molecules

Table 3: ADME profile of the top compounds and standard, Erlotinib

S. no.	Compound ID	GI absorption	BBB permeant	Log K_p (skin permeation)	P-gp substrate	Lipinski
1	Erlotinib	High	yes	-6.35 cm/s	No	Yes; 0 violation
2	TIP009544	High	Yes	-6.03 cm/s	No	Yes; 0 violation
3	TIP012988	High	Yes	-4.72 cm/s	yes	Yes; 0 violation
4	TIP013002	High	yes	6.50 cm/s	No	Yes; 0 violation

sin permeation, non-inhibitor of PgP and acceptable value for Lipinski rule. Figure 4 shows that the compounds show good pharmacokinetic property and can be used for further studies to understand their potential against cancer progression and proliferation.

DISCUSSION AND CONCLUSION

Epidermal growth factor receptor being a critical target is predominantly involved in cancer progression, proliferation and metastasis. Various pathways like PI3K-mTOR, MAPK, JAKSTAT etc. are activated through EGFR dimerization that leads to cancer cell growth, proliferation, differentiation and metastasis. Being the critical target to treat cancer, various FDA approved drugs have been used to inhibit the cancer associated activity of the receptor tyrosine kinase, EGFR. The TKI's like Erlotinib, Dacomitinib, Afatinib, Gefitinib and Osimeritininb have shown better efficacy and progression free survival but due to emerging mutational resistance, side effects and bypassing signaling, the approved drugs exhibit failure to inhibit EGFR. So, there is need for the identification of the novel TKIs inhibitors that can target EGFR with good pharmacokinetics profile. The natural substances seem to be a good resource to obtain the new anti-EGFR chemical species. In this research work we have selected ginger plant constituents to get insights into their possible inhibiting activity f or EGFR to treat cancer progression and metastasis. The ginger contains a huge number of anti-cancer compounds. The terpenes and phenolic compounds have shown anti-proliferation and cancer- suppression features in various cancer cell lines. In our study we retrieved 86 ginger

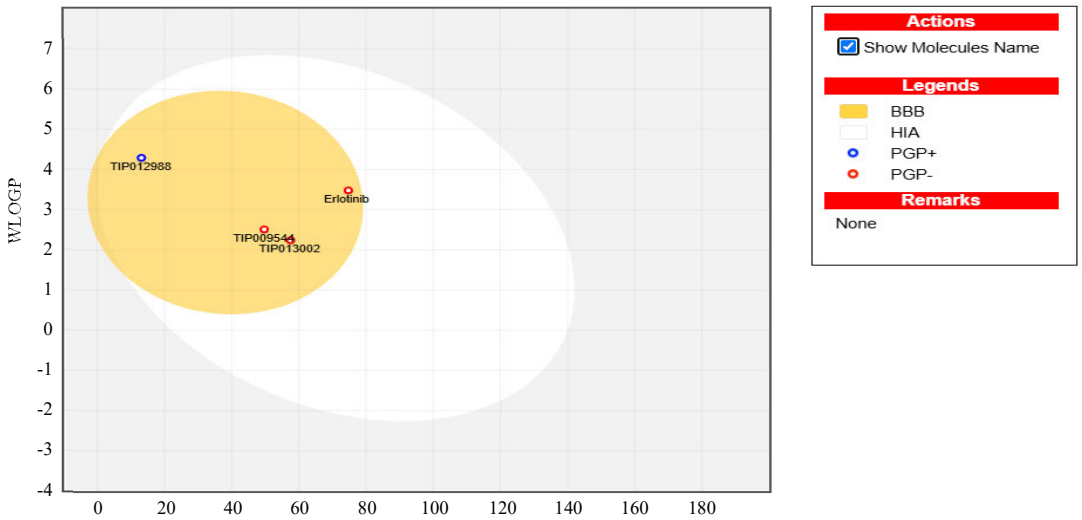

Figure 4: Boiled egg diagram of our top compounds and standard, Erlotinib using SwissADME Tool indicating their ADME features

constituents from TIP database in .sdf format. The standard drug, Erlotinib was obtained from the PubChem database in 3D .sdf format. The retrieved ginger-derived compounds were docked into the active site of the EGFR protein, which was obtained from the Protein Data Bank (PDB). Molecular docking was carried out using the PyRx tool, which facilitated ligand preparation and preliminary docking, followed by further refinement and scoring using the Dockthor server. These tools provided a comprehensive approach for evaluating ligand-protein interactions, ensuring precise grid generation, docking process execution and ranking of potential inhibitors based on their binding affinities. This approach enabled the identification of compounds with strong interactions and potential EGFR inhibitory activity. The ligands were docked with the active site of the receptor protein, EGFR and the binding affinities of all the compounds along with the standard, Erlotinib were obtained and analyzed for best hit identification. After comparing the binding affinities ginger constituents with the binding affinity score of the standard we found 6 compounds having higher binding affinities than standard. From the docking analysis, we selected the top three ginger-derived compounds-TIP012988, TIP009544 and TIP013002-for further pharmacokinetic evaluation based on their strong binding affinities with the EGFR active site. These compounds demonstrated docking scores of -7.244 kcal/mol, -6.983 kcal/mol and -6.557 kcal/mol, respectively, indicating their potential as EGFR inhibitors. The selection criteria were based on their superior docking performance compared to the standard compound, Erlotinib. To assess their drug-like properties, pharmacokinetic studies were performed, focusing on absorption, distribution, metabolism and excretion (ADME) characteristics. The results suggest that these compounds exhibit favorable pharmacokinetic profiles, indicating their potential safety and efficacy for human consumption. Given their strong interaction with EGFR, these compounds may effectively inhibit EGFR-mediated signaling pathways, thereby impeding cancer cell proliferation and metastasis. These findings highlight their potential as novel therapeutic candidates for EGFR-targeted cancer therapy, warranting further experimental validation to confirm their synergistic anticancer activity.

Conflicts of Interest

The author declare that no conflict of interest in this work.

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