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RESEARCH LETTER

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Epidermal growth factor receptor (EGFR) structure-based bioactive pharmacophore models for identifying next-generation inhibitors against clinically relevant EGFR mutations

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Present work elucidates identification of next generation inhibitors for clinically relevant mutations of epidermal growth factor receptor (EGFR) using structure-based bioactive pharmacophore modeling followed by virtual screening (VS) techniques. Three-dimensional (3D) pharmacophore models of EGFR and its different mutants were generated. This includes seven 3D pharmacophoric points with three different chemical features (descriptors), that is, one hydrogen bond donor, three hydrogen bond acceptors and three aromatic rings. Pharmacophore models were validated using decoy dataset, Receiver operating characteristic plot, and external dataset compounds. The robust, bioactive 3D e-pharmacophore models were then used for VS of four different small compound databases: FDA approved, investigational, anticancer, and bioactive compounds collections of Selleck Chemicals. CUDC101 a multi-targeted kinase inhibitor showed highest binding free energy and 3D pharmacophore fit value than the well known EGFR inhibitors, Gefitinib and Erlotinib. Further, we obtained ML167 as the second best hit on VS from bioactive database showing high binding energy and pharmacophore fit value with respect to EGFR receptor and its mutants. Optimistically, presented drug discovery based on the computational study serves as a foundation in identifying and designing of more potent EGFR next-generation kinase inhibitors and warrants further experimental studies to fight against lung cancer.

KEYWORDS
computer-aided drug design, epidermal growth factor receptor, erlotinib, next-generation EGFR inhibitors, non-small cell lung cancer, pharmacophore, virtual screening

The development of tyrosine kinase inhibitors (TKIs) targeting the epidermal growth factor receptor (EGFR) provide an important insight on oncogenic therapy.[1–4] TKIs, gefitinib, and erlotinib inhibit reversibly by competitively binding at the ATP pocket of the EGFR tyrosine kinase domain. These drugs represent a promising therapeutic strategy in non-small cell lung cancer (NSCLC) patients. The key mutations of this functional kinase domain include: exon 21 mutations causing L858R substitution and exon 19 mutations causing in-frame deletions of amino acids 747–750. Remaining EGFR point mutations are seen in exon 18 and 20 regions, respectively. However, these first line TKIs developed resistance due to the secondary EGFR substitution mutation T790M (gatekeeper mutation) and thereby downstream oncogenic signaling pathway was not very effective.[5–8]

Abbreviations: 3D, three-dimensional; A, hydrogen bond acceptor; BA, binding affinity; D, hydrogen bond donor; EGFR, epidermal growth factor receptor; H, hydrophobic; NMR, nuclear magnetic resonance; N, negative ionization; NSCLC, non-small cell lung cancer; P, positive ionization; PDB, protein data bank; R, aromatic ring; ROC, receiver operating characteristic; SP, simple precision; TKIs, tyrosine kinase inhibitors; VS, virtual screening; XP, extra precision.

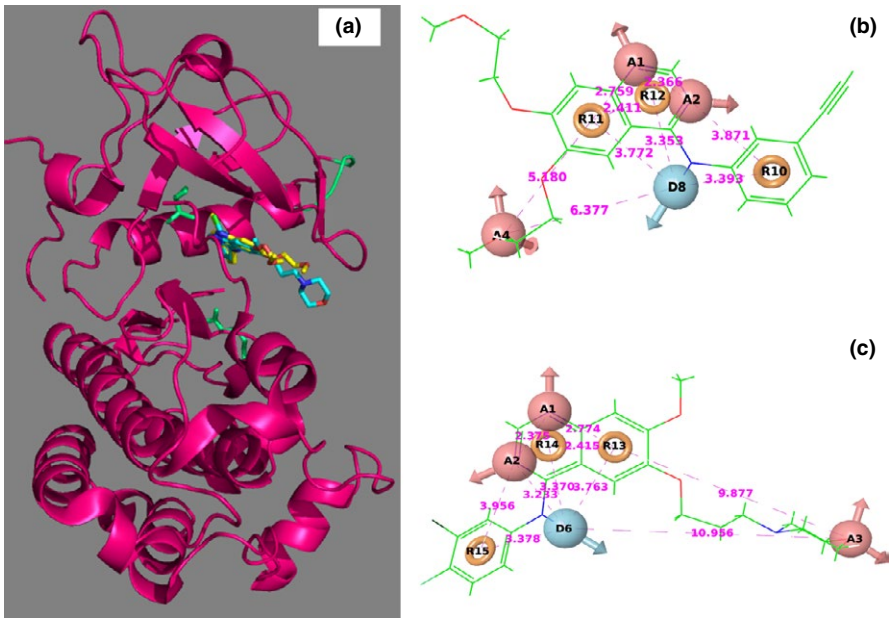


FIGURE 1 The crystal structure of EGFR (PDB ID: 4I22) with mutations L858R, T790M bound to Gefitinib (pink color) and the superimposed crystal structure of Erlotinib (yellow color) bound to EGFR (PDB ID: 1M17) (right). The mutated residues are shown in stick format. EGFR crystal structure with L858R mutation is shown in stick format (green color) and deletion region of residues 746–750 shown in green color (a). 3D structure-based Pharmacophore model of EFGR wild type (b) and EGFR point mutant (c) [Colour figure can be viewed at wileyonlinelibrary.com]

The main motivation of the present work involves developing a computational model to provide key insight on the EGFR resistance arising from its FDA approved drugs, and its clinical outcome of the patients tested at the Amrita Institute of Medical Sciences (AIMS), Kochi, routinely. We collected EGFR-mutation test performed on 54 patients at AIMS, Kochi. Among these, majorities of patients showed substitution mutation L858R at Exon 21 and deletion mutation at Exon 19 (delE746-750). Lichun et al. created EGFR mutant structural database by collecting information of 942 NSCLC patients toward 112 mutation types which includes: insertion, deletion, duplication, modification, and substitution. Clinical data showed that the large number of patients have L858R mutation followed by Exon 19 and Exon 21 mutations, respectively.[9]

Three dimensional (3D) structural information of a protein is very useful to understand the mode and mechanism of inhibitor binding at its functionally important active site residues. Structure-based pharmacophore modelling, molecular docking, and molecular dynamics simulation techniques are well established in computer-aided drug design. Different types of weak interactions between protein–drug complexes at the atomic level lead to the complex stability and molecular mechanisms of drug action and its resistance toward particular disease.[10,11] The degree of drug resistance can be quantitatively addressed by measuring the binding free energy (or affinity) between the drug and its mutant proteins. 3D structural details are available for the wild type and few EGFR mutants from the Protein Data Bank (PDB) using X-ray crystallography and nuclear magnetic resonance (NMR) techniques.[12] These 3D structures provide valuable insight on the kinase-inhibitor interaction mechanism and its resistance arising due to mutations. Further, the free energy index depends mainly on the EGFR mutant 3D structure and the bioactive conformational mode of interactions with its TKIs. Zhou et al. computationally predicted EGFR mutation induced drug resistance based on the TKIs binding free energy calculations toward its active sites.[1]

In this study, we employed an in silico modeling approach to develop effective EGFR 3D structure-based bioactive pharmacophore model for identifying inhibitors against the clinically significant EGFR mutations. Energy-based pharmacophore modeling methodology was computed using structure-based drug-protein complexes by mapping the energetic terms from the Glide extra precision (XP) docking score function onto atom centers of the EGFR active site residues in complex with its drugs (erlotinib or gefitinib). Pharmacophore 3D sites were predicted by providing the results from Glide XP docking energy descriptors.[13,14] The pharmacophoric sites were ranked based on the interaction energies of the TKIs ligand atoms with the EGFR target protein. Phase module of Schrödinger (PHASE, v.4.1) software suite was used for the automatic generation of pharmacophoric sites using the EGFR-drug interactions descriptors to develop its 3D structure-based bioactive pharmacophore model. Phase module consists of six different types of pharmacophoric chemical features to describe the protein-ligand contacts. This includes hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic (H), aromatic ring (R), positive ionization (P), and negative ionization (N), respectively.

We developed structure-based 3D pharmacophore models using crystal structures of EGFR in complex with its inhibitors gefitinib and erlotinib. Point mutation (L858R and T790M) in complex with Gefitinib (PDB id: 4I22, resolution 1.71 Å) and wild type in complex with Erlotinib (PDB id: 1M17, resolution 2.6 Å)[15,16] crystal structure shown in

Figure 1a were used to build the pharmacophore models. These two 3D models showed seven pharmacophoric features AAADRRR, with three different chemical features—three hydrogen bond acceptors (A), three aromatic rings (R), and one hydrogen bond donor (D), respectively. These pharmacophoric features are located in 3D space measured in terms of chemical feature distances and angles. They represent the chemical groups responsible for its EGFR inhibitory activity. Interestingly, these 3D measurements varied in our two different pharmacophore models as mentioned above, showing the specificity toward designing EGFR wild type and its resistant mutants inhibitor, and is presented in Figure 1b,c. This 3D pharmacophoric features in turn guide us in designing new EGFR-specific inhibitors.

The 3D pharmacophore model robustness was validated using decoy dataset, receiver operating characteristic (ROC) plot, and enrichment factor (EF). Results showed promising true positive result with high goodness of fit value. This model was then used for an effective virtual screening (VS) of the four different focused small compound databases to identify putative EGFR inhibitors (or repurposed compounds), and thereby addressing its drug resistance.[17]

The main objectives of the present work include: (i) Development of 3D structure-based pharmacophore models with specific chemical features using favorable interaction energy descriptors from gefitinib and erlotinib (ii) Sequential VS using both wild type and mutant forms of the EGFR specific 3D e-pharmacophore models for identifying common hits with similar pharmacophoric chemical features, to address its drug resistance. Hits obtained from the VS technique should be capable of making key molecular interactions at the active site of the EGFR wild type and mutant receptors.

Pharmacophoric fitness value is one of the key parameters used to rank and identify the potent EGFR inhibitors. The fitness value determines how well an inhibitor fits or satisfies the 3D spatial requirements of the model. We employed sequential VS techniques using four different small compound databases by employing various computational techniques. These include (i) 3D e-Pharamcophore model-based VS using PHASE module, (ii) Simple precision (SP) followed by an XP-based molecular docking to filter compounds on the basis of docking score or binding affinity (BA in unit of kcal/mol) using Glide module to identify novel and putative EGFR specific lead compounds, and (iii) ADMET-based VS using the Qikprop module.

The efficiency of 3D structure-based pharmacophore model for VS depends mainly on the specificity and accuracy of its model query. It is well-known that the VS procedure does have limitations of false positive (or false negative) results. This could be assessed by employing the decoy dataset test and other statistical parameters, namely sensitivity, goodness of hit and enrichment factor. We constructed a decoy library containing 1,012 compounds with 1,000 decoys and 12 known EGFR inhibitors as actives.[18] The decoys have almost similar molecular weight, LogP, hydrogen bond donors/acceptors, but with differences in the chemical and topological differences so that they may not bind to the EGFR active sites. The efficiency of the 3D Pharmacophore model was computed using the statistical formula

$$\mathrm{EF} = \frac{H_a/H_t}{A/N} \tag{1}$$

$$\mathrm{GH} = \left[\frac{H_a\left(3A + H_t\right)}{4H_tA}\right]\left[1 - \frac{H_t - H_a}{N - A}\right] \tag{2}$$

where EF = enrichment factor, GH = goodness of hit, N = total number of compounds in the data set, A = total number of actives in the dataset, H_t = total hits and H_a = active hits. Further, to standardize the VS protocol, the obtained results were evaluated by EF with 1% and Boltzmann enhanced discrimination of the ROC curve, and is presented in Figure 2a,b.

VS of these decoy dataset compounds were performed using the PHASE generated pharmacophore models for different EGFR crystal structures in complex with drugs. The computed statistical values obtained for two different pharmacophores were significant showing its high VS efficiency, and is presented in Table S1. These models were further confidently used for VS of four different small compound databases.

In computer-aided drug designing, VS is an important technique to retrieve and discover new lead like compounds for any druggable target.[17] Pharmacological structural space was defined as the protein (receptor) key binding sites where its potential ligands make the bioactive conformation for its mechanism of action. VS involve the search for a compound in 3D chemical space and which are complementary to the pharmacological space for the protein of interest involved in the diseased conditions. We adopted sequential VS strategies using three different focused small molecule databases to reduce the search space for potential drug candidates by filtering out compounds unlikely to interact with the EGFR and its mutant structures. Three different small compound databases containing 1,826 FDA approved drugs, 545 investigational drugs, and 386 anticancer drugs were used for sequential VS.[a,b] Most of these compounds are FDA approved or under clinical trials and can be considered as repurposed drugs in our VS strategy. Different steps adopted for sequential VS strategy are explained below, and its flowchart is depicted in Figure 2c.

In the first step, EGFR and its mutants specific 3D pharmacophore models having seven chemical features were used as a query for VS. On the basis of pharmacophore feature site matching and its fit values, 2,757 compounds from three different focused databases were prioritized, as mentioned earlier. Two different pharmacophores were generated which include- (i)

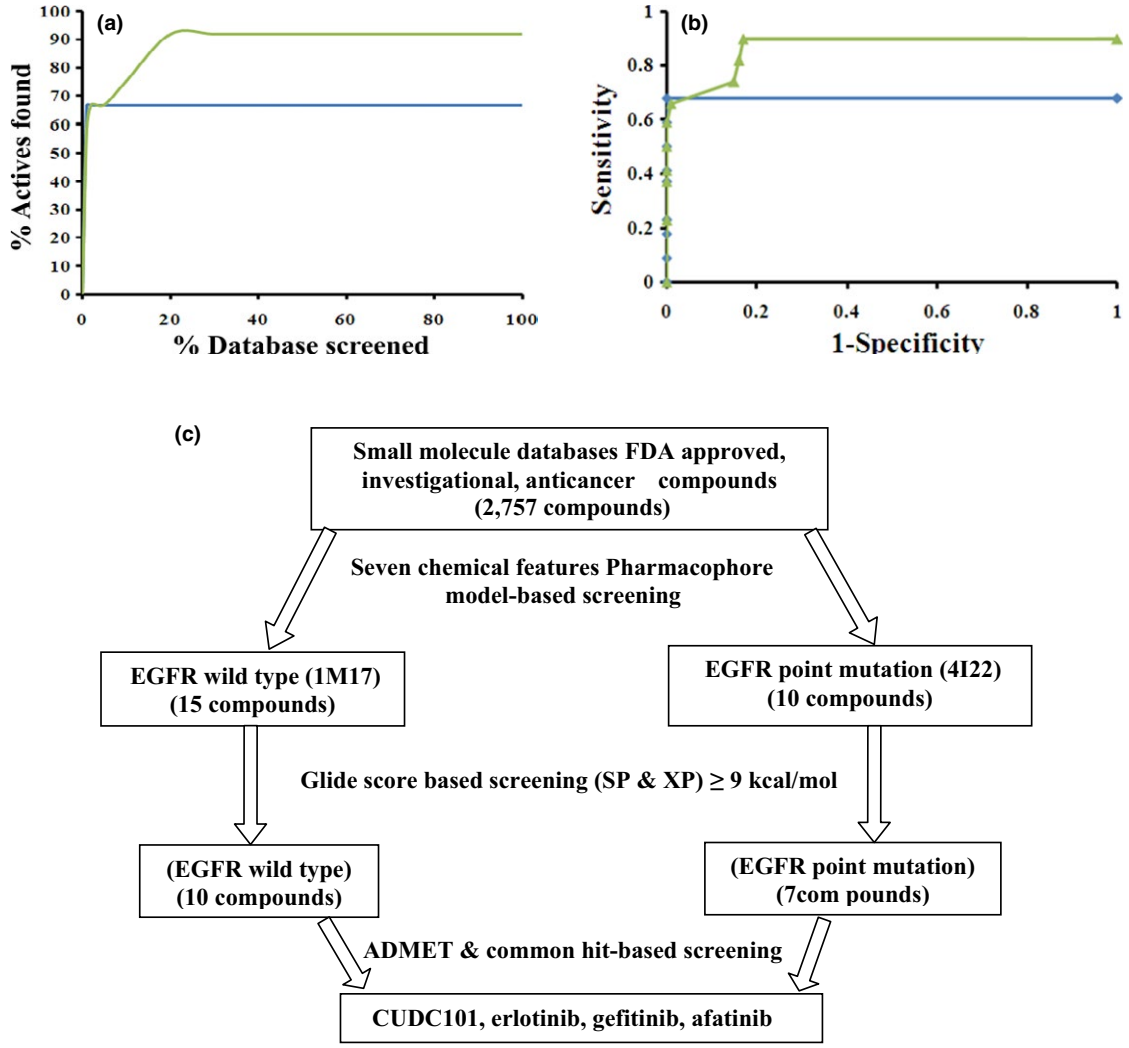


FIGURE 2 The percentage recovery rate of known actives from the constructed decoy and ranked database (a) ROC curve (b) obtained from three EGFR specific pharmacophore models. The curves shown in green color belong to wild-type EGFR and red color belongs to point mutant EGFR. A flowchart showing in silico sequential virtual screening strategy of the present studies using 3D pharmacophore model, molecular docking, and ADMET techniques (c) [Colour figure can be viewed at wileyonlinelibrary.com]

First pharmacophore model obtained from the EGFR wild type retrieved 15 best fit compounds, (ii) and second pharmacophore model obtained from the EGFR point mutation retrieved 10 best fit compounds. This stage of the 3D e-pharmacophore model-based VS process was very effective on the basis of the best 3D pharmacophoric chemical feature matching and fitness value (Table S2) in retrieving 25 compounds from these databases. The VS results are presented in Figure 2c.

A molecular docking technique using Glide module was used as the second step of sequential VS. It scores possible kinase-ligand interactions according to their best pose and computed BAs (kcal/mol) for VS,[19] and is illustrated in Figure 2c. We have chosen Glide score (or BA) ≥9 for XP filtering and which further takes care of filtering false positive compounds from the small compound databases. 25 compounds from our first stage of VS were then further prioritized. 17 compounds were selected on the basis of the best glide score (or BA) and its mode of atomic level interactions with the EGFR and its mutants structure (Table S2).

The last stage of our sequential VS is the ADMET properties-based filtering using QikProp module.[20] Initially, Lipinski's rule of 5 was applied and the drugs which do not follow this rule were rejected. Further, QPlogPo/w, QPlogHERG, and human oral absorption values were calculated. The best four compounds from two different pharmacophores were selected, which include CUDC101, Afatinib, Erlotinib, and Gefitinib (Figure 2c). It is interesting to know that our pharmacophore models were able to retrieve the correct set of compounds (including FDA approved drugs) from different small molecule databases showing its robustness and importance to address the drug resistance. The ADMET properties of some of these most common hits are shown in Table S3. The best compound CUDC101 retrieved from the databases were tested for two different pharmacophores

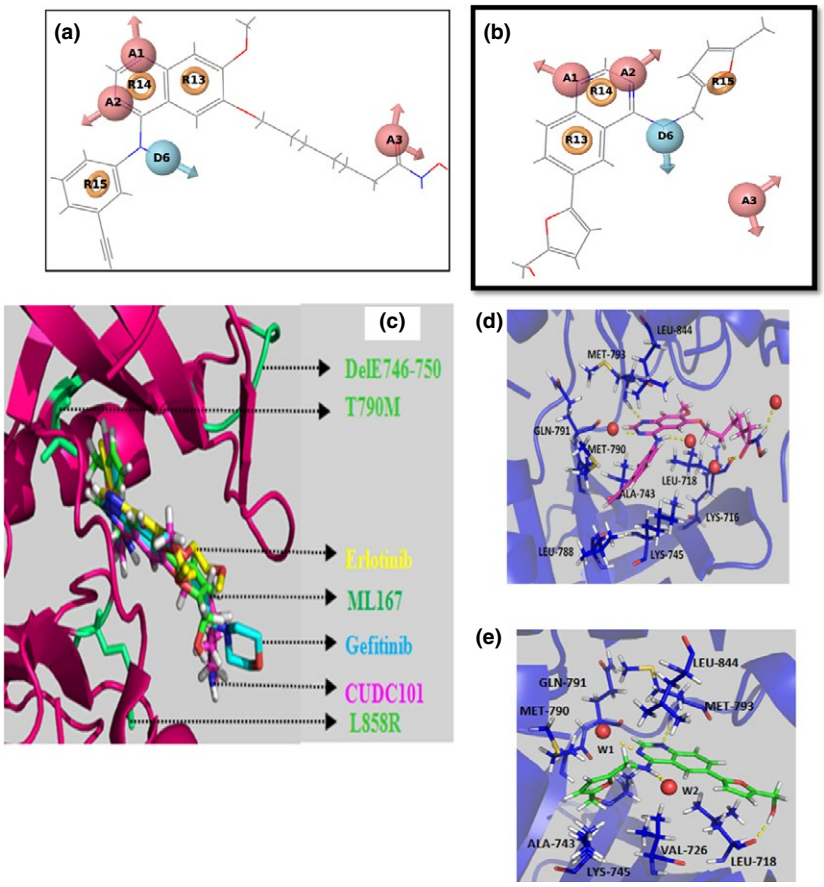


FIGURE 3 3D structure-based pharmacophore model map fitting of CUDC101 compound (a) and ML167 (b) in complex with EGFR point mutant obtained using Phase and Glide module. The crystal structure of EGFR with point mutations (T790M and L858R) and deletion region (Del E746-A750) are shown in lime green color along with bound inhibitor Erlotinib (yellow), Gefitinib (blue), CUDC-101 (pink), ML167 (green) (c). Interaction of CUDC-101 and ML167 inhibitor with the active site residues in (d, e) EGFR point mutation. Water molecules are shown as orange spheres [Colour figure can be viewed at wileyonlinelibrary.com]

map fitting. The corresponding fitness value was 1.756 (wild type) and 2.124 (point mutation-Figure 3a) respectively.

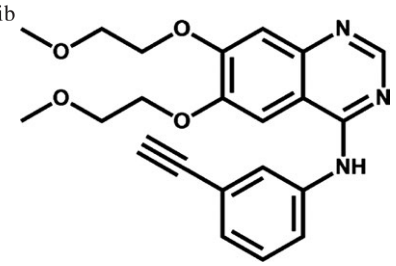
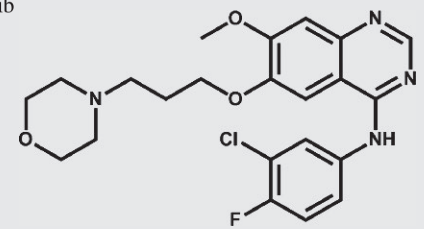
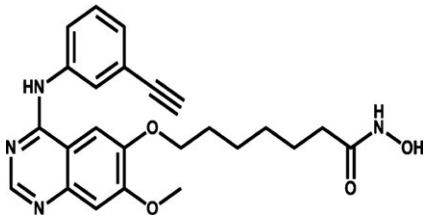
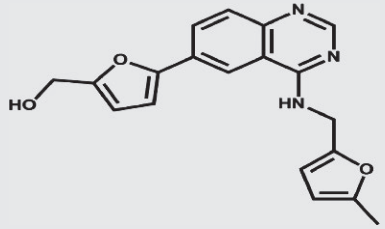
To retrieve new scaffold-based compounds from these 3D e-pharmacophore models using other small compound databases, we completely failed in the VS process to filter (identify) common VS hits. This was based on the best 3D pharmacophoric fit values and BAs, respectively. So, we relaxed to six chemical features in our seven featured 3D pharmacophore model-based VS that is two hydrogen bond acceptors (A), three aromatic rings (R), and one hydrogen bond donor (D), respectively. This new EGFR and its mutants specific 3D e-pharmacophore model having six chemical features was used as a query for VS on the basis of pharmacophore feature site matching and its best fit values. This modified chemical hypothesis allowed us to retrieve new scaffold-based compounds in the chemical space.

Two different pharmacophore models were generated using the above mentioned six chemical features (AADRRR) for VS from SelleckChem database containing 2,084 bioactive compounds. The VS hits of (i) first pharmacophore obtained from EGFR wild type (1M17) retrieved 286 best fit compounds, and (ii) second pharmacophore obtained from EGFR point mutation (4I22) retrieved 132 best fit compounds. This stage of the VS was very effective to retrieve 418 total compounds from 2,084 bioactive compounds showing 3D pharmacophore fit value ≥ 1.

Our next stage of VS uses molecular docking technique using Glide module. The interaction energy of VS compounds with the EGFR active sites was measured and BA of each compound was computed. This VS stage retrieved 17 compounds from the XP docking methodology on the basis of its BA values. Among these, the best overlapping VS hit was ML167 compound. This compound showed good pharmacophoric fitness value of 1.784 and BA of −9.33 kcal/mol for EGFR wild type, and pharmacophoric fitness value of 2.151 and BA of −11.12 kcal/mol for EGFR point mutation, and is presented in Table 1, Figures 3b and S1. These results are comparable with the well-known FDA approved drugs gefitinib and erlotinib obtained from the present 3D e-pharmacophore and docking studies. Table 1 shows the key interacting (hydrogen bonding and hydrophobic) residues of EGFR wild type and its mutants for the best two compounds CUDC101 and ML167 in comparison to that of FDA approved drugs gefitinib and erlotinib, along with its binding affinity and pharmacophore fit value. Thus, the probability of repurposed compound ML167 to succeed as a potential EGFR and its mutant inhibitor was promising. However, experimental in vitro and in vivo validation is urgently required to succeed in this drug discovery endeavor.

Our seven chemical featured pharmacophore model-based VS retrieved CUDC101 compound as the promising common hit. This compound showed multitargeted inhibition toward

TABLE 1 Interacting residues of wild type and mutant EGFR proteins to known FDA approved drugs (Erlotinib and Gefitinib) and best two hits obtained by virtual screening with their respective binding affinity (docking score) and pharmacophoric fitness value

Ligand	Protein	EGFR protein interacting residues	Binding affinity (kcal/mol)	Pharmacophore fit value
Erlotinib 	Wild type EGFR protein	MET769[a], PRO770[a], GLY767[a], LYS721[b], ALA719[b], MET769[b], LEU82[b], LEU694[b]	−9.586	2.52
	Mutant EGFR protein	MET793[a], ALA743[a], LEU788[a], LEU718[a,b], ASP800[a], GLN791[a], VAL726[b], LYS745[b], MET790[b], LEU844[b]	−10.793	2.52
Gefitinib 	Wild type EGFR Protein	MET793[a,b], LYS745[a,b], LEU718[a,b], PRO794[a], GLN791[a], LEU788[b], ALA743[b], LEU844[b], VAL726[b]	−9.434[c]	1.21
	Mutant EGFR protein	MET793[a], LEU718[a,b], ASP800[a], GLN791[a], ALA743[b], LYS745[b], LEU788[b], MET790[b], VAL726[b], ALA743[b], LEU844[b]	−11.463[c]	2.44
CUDC-101 	Wild type EGFR protein	ASP831[a], MET769[a], GLN767[a], LEU694[a,b], LYS721[b], LEU764[b], ALA719[b], LEU820[b], VAL702[b], LYS721[b]	−10.378	1.76
	Mutant EGFR Protein	MET793[a], GLN791[a], ALA743[a,b], LEU788[a], LYS745[a], LEU788[b], MET790[b], LEU718[b], LEU844[b], VAL726[b]	−12.743	2.12
ML167 	Wild type EGFR protein	MET769[a], THR830[a], GLN767[a], ALA719[b], LYS721[b], LEU694[b], LEU820[b], VAL702[b]	−9.330[d]	1.78
	Mutant EGFR protein	LEU718[a,b], MET793[a,b], GLN791[a], LEU792[a], MET790[b], ALA743[b], LYS745[b], LEU844[b], VAL726[b]	−11.125[d]	2.15

[a]Hydrogen bond.
[b]Hydrophobic bond.
[c]Gefitinib binding affinity is more for L858R mutant than the wild type supporting the experimental evidence.[24]
[d]ML167 inhibitor obtained using six features 3D pharmacophore model and showed better BA toward L858R mutant than that of its wild-type EGFR.

EGFR, histone deacetylase and HER2.[21] So, our pharmacophore model will be highly significant with meaningful 3D chemical features and can be confidently used as a control for further VS of new compound databases. Other promising common hits obtained by sequential VS include afatanib, erlotinib, and gefitinib FDA approved drugs shown in Table S2. Further, CUDC101 displayed potent antiproliferative and pro-apoptotic activities against cultured and implanted tumor cells that are sensitive or resistant to several single-targeted drugs.[22] The second most common promising hit retrieved from the Bioactive database (SelleckChem) was ML167 using six chemical feature pharmacophore model. This compound was already known to be a potent CLK4 inhibitor.[23]

The next step includes the molecular recognition analysis of CUDC101 and ML167 clinical compounds with the active site of EGFR protein and its mutants. Both these repurposed compounds showed promising hydrogen bonding, hydrophobic, and other weak interactions (van der Waals, π–π, and π–σ) with the EGFR wild type and its mutant protein. Molecular superimposition of these compounds (gefitinib, erlotinib, CUDC101, and ML167) showed similar mode of binding toward the active sites of EFGR mutant crystal structure, presented in Figure 3c. Interacting residues of CUDC101 with wild-type EGFR protein and its point mutants are presented in Table 1, Figures 3d, and S2. Water molecules showed promising hydrogen bonding interaction with CUDC101, which further stabilized its interaction with EGFR and its mutants. Gefitinib also showed interaction with residues MET793, LEU718, LEU788, LEU844, PRO794, ALA743, GLN791, and LYS745. CUDC101 binds with more affinity than the native FDA approved drugs viz. Gefitinib and Erlotinib, as shown in Table 1. Propyl morpholino ring of Gefitinib and 2-methoxyethoxy group of Erlotinib lie outside the EFGR kinase active site cavity without making any atomic interactions. While the hydroxamic end of CUDC101 completely fits within the active site of EGFR (Figure 3). Another key insight from the present computational study was, gefitinib drug has a better binding affinity (−11.463 kcal/mol) and pharmacophoric fit value (2.45) with the active site cleft of EGFR mutant (L858R and T790M) structure, in comparison with that of the EGFR wild type (−9.434 kcal/mol and 1.21) shown in Table 1. Further, the crystal structure of EGFR mutant (L858R and T790M) shows that due to mutation, the A-loop gets disordered, and the active site region increased by changes in the conformation in the side chain of Phe723 residue. This in turn leads to less ATP binding affinity at the kinase active site with respect to that of the gefitinib binding. Our docking studies also clearly predicted increased gefitinib binding in this mutant case (Table 1), and which is in excellent agreement with that of the in vivo results reported by Yoshikawa et al.[24]

It is now well established that the quinazoline moiety-based small compounds such as erlotinib, gefitinib, lapatinib etc. have precedence as intracellular kinase inhibitors.[23] Some of these FDA approved drugs are currently used for NSCLC and pancreatic cancers. The molecular mechanism of action of these drugs is well known. Further, our best VS compound ML167 has quinazoline moiety acting as CLK family inhibitors.[23] Thus, the present work provided significant insight on the 3D binding modality and the mechanistic action of ML167 toward EGFR and its mutants for predicting its drug resistance. Further, the study provided necessary structural features important for wild type and mutant EGFR inhibition (Table 1 and Figure 3). The deletion mutant (Exon 19 → delE746-750) of the EGFR crystal structure was not yet solved experimentally (X-ray or NMR), which hindered us to develop the 3D e-pharmacophore model specific to this deletion mutation. However, we presume that the present developed pharmacophore model has the ability to deal with the EGFR resistance mutations effectively.

Some of the significant insight obtained from the present study includes: (i) BA and pharmacophoric fit values can be considered as an important quantitative parameter for identifying new compound of interest by VS technique with increased understanding toward its resistance mechanism; (ii) CUDC101 and ML167 can be taken forward for experimental testing to address the EGFR inhibition and other mutants causing resistance; (iii) Gefitinib BA is increased due to the point mutation L858R in comparison to that of its wild-type EGFR (Table 1). This is in accordance with the in vivo (NIH3T3 cell line) experiments[24]; (iv) Our point mutant EGFR (L858R and T790M) pharmacophore model showed better BA for final VS hits in comparison to that of its wild-type EGFR (Table 1). These results revealed that our point mutant model can address the drug resistance by having a better BA of CUDC101 and ML167 inhibitors (Table 1), than that of its natural ligand ATP. Experimentally, it is proven that the T790M mutation in the EGFR kinase causes drug resistance by increasing the BA toward ATP[25,26] (iv) Present 3D e-pharmacophore models can be used for VS of new scaffolds containing compounds from different databases for EGFR wild type and its mutant targeted drug discovery program.

In summary, we report an in silico modeling approach to identify new lead compounds for designing EGFR specific inhibitors and its resistance. PHASE generated 3D e-pharmacophore models were developed using the wild-type EGFR and its mutants. These models are validated using the decoy dataset, EF and GH calculations, and were found to be statistically significant. Sequential VS of small compound databases from these pharmacophore models filtered best hit CUDC101 with high BA and pharmacophore fitness value. These modeled parameters are better than that of the FDA approved drugs, Gefitinib and Erlotinib toward EGFR and its mutants. Compound ML167, which was known to be a potent CLK4 inhibitor was also discovered from the present

computational approach to be a putatively effective EGFR inhibitor. The developed 3D pharmacophore models and structural information presented in this study is expected to provide tools to design next-generation EGFR kinase inhibitors, optimize currently available structures for more potency and specificity, and repurposing-based study using known drugs. Thus, the present study provides a key guidance to overcome the drug resistance mechanisms to qualify them for preclinical and clinical development from the known FDA approved drugs.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

ENDNOTES

[a]www.specs.net.

[b]www.fda.gov.

REFERENCES

[1] D. D. Wang, W. Zhou, H. Yan, M. Wong, V. Lee, *Sci. Rep.* **2013**, *3*, 2855.
[2] F. A. Shepherd, Pereira J. Rodrigues, T. Ciuleanu, E. H. Tan, V. Hirsh, S. Thongprasert, *N. Engl. J. Med.* **2005**, *353*, 123.
[3] S. V. Sharma, D. W. Bell, J. Settleman, D. A. Haber, *Nat. Rev. Cancer* **2007**, *7*, 169.
[4] A. J. Barker, K. H. Gibson, W. Grundy, A. A. Godfrey, J. J. Barlow, M. P. Healy, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1911.
[5] H. A. Yu, M. E. Arcila, N. Rekhtman, C. S. Sima, M. F. Zakowski, W. Pao, *Clin. Cancer Res.* **2013**, *19*, 2240.
[6] B. W. Murray, N. Miller, *Mol. Cancer Ther.* **2015**, *14*, 1975.
[7] A. Michalczyk, S. Kluter, H. B. Rode, J. R. Simard, C. Grutter, M. Rabiller, *Bioorg. Med. Chem.* **2008**, *16*, 3482.
[8] S. Giroux, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 394.
[9] L. Ma, D. D. Wang, Y. Huang, H. Yan, M. P. Wong, V. H. Lee, *BMC Bioinform.* **2015**, *16*, 85.
[10] T. E. Balius, R. C. Rizzo, *Biochemistry* **2009**, *48*, 8435.
[11] Z. W. Cao, L. Y. Han, C. J. Zheng, Z. L. Ji, X. Chen, H. H. Lin, *Drug Discov. Today* **2005**, *10*, 521.
[12] L. W. Yang, E. Eyal, C. Chennubhotla, J. Jee, A. M. Gronenborn, I. Bahar, *Structure* **2007**, *15*, 741.
[13] Schrödinger, LLC. Glide v6.5. Schrödinger, LLC, USA. **2014**.
[14] Schrödinger, LLC, PHASE v4.1, Schrödinger, LLC, New York, NY **2014**.
[15] K. S. Gajiwala, J. Feng, R. Ferre, K. Ryan, O. Brodsky, S. Weinrich, *Structure* **2013**, *21*, 209.
[16] J. Stamos, M. X. Sliwkowski, C. Eigenbrot, *J. Biol. Chem.* **2002**, *277*, 46265.
[17] S. Gupta, A. Fallarero, P. Jarvinen, D. Karlsson, M. S. Johnson, P. M. Vuorela, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1105.
[18] M. M. Mysinger, M. Carchia, J. J. Irwin, B. K. Shoichet, *J. Med. Chem.* **2012**, *55*, 6582.
[19] H. Zhao, D. Huang, A. Caflisch, *ChemMedChem* **2012**, *7*, 1983.
[20] Schrödinger, LLC, Qikprop v4.2., Schrödinger, LLC, USA. **2014**.
[21] T. Shimizu, P. M. LoRusso, K. P. Papadopoulos, A. Patnaik, M. Beeram, L. S. Smith, *Clin. Cancer Res.* **2014**, *20*, 5032.
[22] C. J. Lai, R. Bao, X. Tao, J. Wang, R. Atoyan, H. Qu, *Can. Res.* **2010**, *70*, 3647.
[23] P. Wu, T. E. Nielsen, M. H. Clausen, *Trends Pharmacol. Sci.* **2015**, *36*, 422.
[24] S. Yoshikawa, M. Kukimoto-Niino, L. Parker, N. Handa, T. Terada, T. Fujimoto, *Oncogene* **2012**, *32*, 27.
[25] C. H. Yun, K. E. Mengwasser, A. V. Toms, M. S. Woo, H. Greulich, K. K. Wong, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2070.
[26] S. Wang, S. Cang, D. Liu, *J. Hematol. Oncol.* **2016**, *9*,34.

SUPPORTING INFORMATION

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