Original Article

Exploring Novel ALK Inhibitors Using Energy Based Pharmacophore Mapping and High Throughput Virtual Screening

Nivya James, Raunaq Surana, Isha Thigale, B. Preethi, V. Shanthi, K. Ramanathan.

Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute Of Technology, Vellore, Tamil Nadu, INDIA.

ABSTRACT

Introduction: Recent years has witnessed major paradigm shifts in the treatment of NSCLC with the emergence of biomarkers and targeted therapies. Most importantly, ALK is a validated biomarker and its inhibition using targeted therapies have had significant effects on patients suffering from NSCLC. However, emergence of drug resistance has limited the usage of these agents in the patients. **Objective:** In the present objective, e-pharmacophore based virtual screening was employed to discover potent ALK inhibitors from a natural compound database, TIPdb. **Result:** Screening of TIPdb using the e-pharmacophore model (DDRRR) retrieved 1000 hits. The docking procedures and ADME analysis led to the identification of piperphilippinin VI as the best hit. Further interaction analysis showed that piperphilippinin VI exhibited crucial interactions with kinase domain of the protein, which is the major targeting site for ALK inhibitors. Note that, PASS prediction analysis highlighted the presence of anti-neoplastic activity of the hit molecule towards lung cancer in particular NSCLC. **Conclusion:** The comprehensive analysis of the present study shows that, piperphilippinin VI has higher binding affinity, low toxicity profiles and increased CNS activity. We believe that these observations are of immense importance in the rational designing of a novel and potent ALK inhibitors from natural sources.

Key word: ALK, E-Pharmacophore model, TIPdb database, PHASE, GLIDE.

INTRODUCTION

Lung cancer is one of the fatal malignancies that has reached an epidemic proportion in the past few years. For instance, it was considered to be a rare disease in the begin of 20th century but has become the cause for around one-fifth of the total fatalities globally, in the year 2012.[1] On the basis of their histology, lung cancer is further classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) in which NSCLC accounts for about 80% to 85% of all lung cancer cases.[2] Previously, cytotoxic chemotherapy was the commonly used treatment modality for NSCLC.[3] However, it was found that this therapy preferentially selects rapidly proliferating cells thus posing a threat to all cells of the body.[4] In recent years the understanding of biological and molecular mechanisms underlying cancer led to new and efficacious therapies specifically targeted therapies. Targeted drugs have the ability to target specific molecules or pathways that are known to play a role in the growth and development of cancer cells.[5,6] It was discovered that signaling pathways regulated by protein tyrosine kinases play an important role in cancer particularly against ALK tyrosine kinases. The interest on ALK was considerably increased after it was discovered that ALK fusions are present in 3-5% of patients with NSCLC.[7,8] The formation of ALK fusion oncogene occurs when the 3'-end of *ALK* gene fuses with the 5' end of EML4

Submission Date: 12-10-2017;
Revision Date: 24-01-2017;
Accepted Date: 17-05-2018

DOI: 10.5530/ijper.52.4.82

Correspondence:
Prof. K. Ramanathan,
Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute Of Technology, Vellore – 632014, Tamil Nadu, INDIA.
Phone: +91 4162202538;
Fax: +91 4162243092
E-mail: kramanathan@vit.ac.in

www.ijper.org

(echinoderm microtubule-associated protein-like 4) gene.[2,9] This oncogene will result in the activation of several key cellular processes like cell survival, cell migration, cellular proliferation thus leading to tumor formation.[2,9] These studies suggests that EML4-ALK fusion protein can be a susceptible target in targeted therapies. Thus, personalized treatment options for patients with advanced ALK-positive NSCLC is given through ALK inhibitors. In particular, Crizotinib was granted FDA approval because of its rapid and durable responses observed in ALK-positive NSCLC patients.[10] However, crizotinib acquires resistance after it is exposed to the tumor. It is either because of manipulation of signaling pathways, alterations in the drug target (i.e secondary mutations).[11,12] The common adverse effects include visual impairment, nausea, fatigue, vomiting.[11] Additionally, poor CNS (Central Nervous System) activity of crizotinib restricts its use. CNS is a frequent site of relapse in ALK-positive lung cancer patients, yet CNS involvement of crizotinib was found to be minimal in patients.[12] These problems associated with the drug has necessitated the search for more potent ALK inhibitors with reduced side effects. Of note, plants are considered as the natural reservoir of bioactive chemicals with therapeutic values and fewer side effects. Therefore, to overcome these problems, we would be focusing on finding new inhibitors particularly from a plant database having plant compounds from indigenous plants of Taiwan.

Computer-aided techniques or *in silico* approaches has given important contributions to the field of drug discovery in recent years.[13] It has opened a path for the quick and cost-effective development of small molecules within the context of pharmaceutics. Particularly, techniques such as virtual screening and e-pharmacophore modelling techniques are being applied to pharmacology for the rapid development of drugs. In the recent years, virtual screening has been used as an important tool to access novel drug-like compounds from a large database.[13,14] Moreover, e-pharmacophore modelling has been used in a variety of studies such as in the synthesis of a New Class of Heat Shock Protein 90 Inhibitors,[15] as well as for the virtual Screening of Hepatitis C Virus NS5B Polymerase Inhibitors.[16] E-pharmacophore modelling has also found importance in cancer studies. Recently it was used for the discovery of nicotinamide phosphoribosyl transferase inhibitors[17] and for inhibitor designing for c-Jun-NH2 terminal kinase (JNK).[18] Also, it was employed for identifying potent and selective inhibitors for P90 Ribosomal S6 Kinase (RSK)[19] as well as for the identification of novel inhibitors for Estrogen Receptors 1 (ESR1).[20] Therefore, in this paper, we are focusing on the e-pharmacophore modelling for the drug discovery of potential ALK inhibitors.

MATERIALS AND METHODS

Dataset

The X-ray structure of the native ALK protein was extracted from Brookhaven Protein Data Bank (PDB).[21] The PDB code of native protein was 2XP2 and had a resolution of 1.9 Å. The ALK inhibitors reported in literature such as Alectinib, Brigatinib, Entrectinib, ASP-3026, CEP-28122, X-394, CEP-37440, Ceritinib, Crizotinib, Lorlatinib, TAE684 and AZD3463[22-25] were retrieved from PubChem. The Tipdb database consisting of about 88000 compounds[26] were extracted in SDF format and utilized for the virtual screening (VS) protocol.

Phase database creation

A phase database was created using the SDF files of molecules extracted from the TIPdb database. It contains three-dimensional structures of biologically active phytochemicals from indigenous plants of Taiwan.[26] Prior to using the molecules for the study it is recommended to pre-process the structures.[27] Thus, the ligand structures were prepared, assigned proper ionization and tautomeric states using Epik.[28] This pre-processing ensure that the ligands will have appropriate bond orders, tautomeric states and protonation states.[29,30] Further, conformers were generated for each ligand using ConfGen by an OPLS_2005 force field.[31] Moreover, the molecules were pre-filtered during the database creation based on 'Lipinski's rule of five' to eliminate compounds without drug-likeness properties.[32]

Protein preparation

The protein structure imported from PDB was prepared using the protein preparation wizard of Maestro software package for use in molecular docking experiments.[33] During the preparation process bond orders were assigned, tautomers were generated, missing hydrogens were added to heavy atoms, and hydrogen bonds in the protein were optimized to pH 7.[34,35] Further, water molecules were removed from the protein before docking to prevent the disruption of the hydrogen network needed for ligand binding and to prevent the protein from collapsing in unphysical ways.[36] Finally, Optimized Potentials for Liquid Simulations (OPLS) 2005 force field was used to minimize energy where heavy atoms were converged to 0.30Å average root mean square deviation (RMSD) value.[37]

Molecular docking

E-pharmacophore modelling is a combination of both structure based and ligand based methods.[16] Therefore, the twelve inhibitors were initially docked onto the native protein using Glide module of the Schrödinger suite.[38] Prior to docking a grid was generated around the active site of the protein. The grid size of 20Å size was generated to accommodate the complete set of binding residues such as V1130, N1254, L1196, L1122, M1199, G1202, G1269, A1148, A1200, R1253, K1150, G1201, L1198, L1256, E1197 and D1203.[39,40] Subsequently, the ligands were docked onto the catalytic binding site of the protein in Glide Extra Precision (XP) mode. In particular, XP docking was employed because of its ability to rank the ligands based on its interaction with a specific binding conformation of the protein more accurately than other precision modes.[41] Finally, the docked files were utilized for e-pharmacophore generation.

E-pharmacophore generation

E-pharmacophore generation was performed using the Schrodinger PHASE Version 4.3.[42] Using Phase 4.3, the pharmacophoric sites were generated based on six default chemical features - Aromatic ring (R), hydrophobe (H), hydrogen-bond acceptor (A), positive ionizable (P), negative ionizable (N) and hydrogen-bond donor (D).[43] During e-pharmacophore generation, the Glide XP energy descriptors of each atom present in the pharmacophoric sites are added up.[43,37] For instance, various interactions like π−π stacking, hydrophobic enclosure, electrostatic rewards, hydrophobically packed correlated hydrogen bonds, π−cation and others are a part of these Glide XP descriptors. Since there were 12 ligands, fragment-based hypothesis generation approach was utilized.[44] Finally, the e-pharmacophore model was constructed which was used as a query to retrieve compounds from chemical databases with novel chemical features.

Insilico screening

The virtual screening procedures were carried out using the GLIDE software.[45] Glide is one of the standard molecular docking tools that has been useful in the process of screening large databases during a drug development phase.[45] Glide based *insilico* screening includes three steps namely high throughput virtual screening (HTVS), Standard Precision (SP) and XP. Since HTVS is a fast process helping in rapid screening, this protocol is used as the initial step in docking studies. Subsequently, 50% of the resultant molecules were passed on to the next stage of SP docking where screening was further carried out. Finally, 50% of the top scoring molecules were passed on to XP docking, which is a much more stringent method to remove all the false positives.[46,47] The results of the docking protocols were examined based on their binding energies and Glide energies.[48]

Absorption, distribution, metabolism and excretion (ADME)

The most important strategy of the pharmaceutical industry to overcome the failure rates during drug discovery is to focus on the molecular properties like absorption, distribution, metabolism and excretion (ADME).[49] ADME property determination is essential to avoid the candidate molecule failure in the clinical trials, as it reflects the efficiency of a drug in the living system.[50] QikProp Program of the Maestro interface was used in the prediction of both pharmaceutically pertinent properties along with the physically significant descriptors.[51] Various parameters like brain/blood partition co-efficient (QPlogBB), water solubility (QPlogS), octanol/water partition co-efficient (QPlogPo/w) and percentage of human oral absorption which are critical for the estimating the absorption and distribution of a hit compound within the body[52] were determined using this program. In addition, the Lipinski rule of five which is based on property values such as the number of hydrogen bond acceptors and donors, logP and molecular weight were also calculated to judge the overall potential of the hit molecule to qualify as a potential drug candidate.[52] Moreover, CNS activities were also predicted for the hits. The recommended range for the Central Nervous System activity is in the range of -2 (inactive) to 2 (active).[53] Likewise, the #stars property was also predicted since it is a major factor that helps in determining the drug- likeness of each hit.[54] The higher the number of stars, the lesser is the drug-likeness of the molecule.[54] Thus the chemical, physical and pharmacokinetic properties of the hits were analyzed.

RESULTS

E-pharmacophore model generation:

An e-pharmacophore hypothesis was generated in the Maestro workspace using PHASE module. Prior to e-pharmacophore generation, the twelve ALK inhibitors were docked on to the protein in the Glide XP precision mode. The docked poses of the inhibitors containing both its ligand and structural aspects were used to create the pharmacophore model. Using the "Docking post processing" in the Maestro interface, "e-pharmacophore" option was specified. Further, the ligand-receptor complex file is required as an input for the generation of hypoth-

esis. Since there were twelve ligands, fragment mode was chosen in the settings and pharmacophoric sites were generated. These sites were generated based on the Glide XP energetic terms which were mapped onto the atoms of the ligands. Keeping all the other parameters as default, a pharmacophore hypothesis was written in which the features were ranked on the basis of their energy contributions to binding. The top 5 features having an energy score >1.0 kcal/mol were included in the hypothesis (Table 1).[55] Subsequently, a five point e-pharmacophore model (DDRRR) was generated with the chosen sites. The model had three aromatic rings (R) and two hydrogen bond donor groups (D) (Figure 1). The generated e-pharmacophore was then utilized for screening of phase database where it was able to pick a good number of active molecules which in return explains the effective screening of molecules from the database.

Table 1: The E-pharmacophore features and its energy scores.

S. No.	Feature Table	Score (kcal/mol)
1	D65	-2.20
2	R176	-2.05
3	D53	-2.04
4	R164	-1.40
5	R165	-1.04

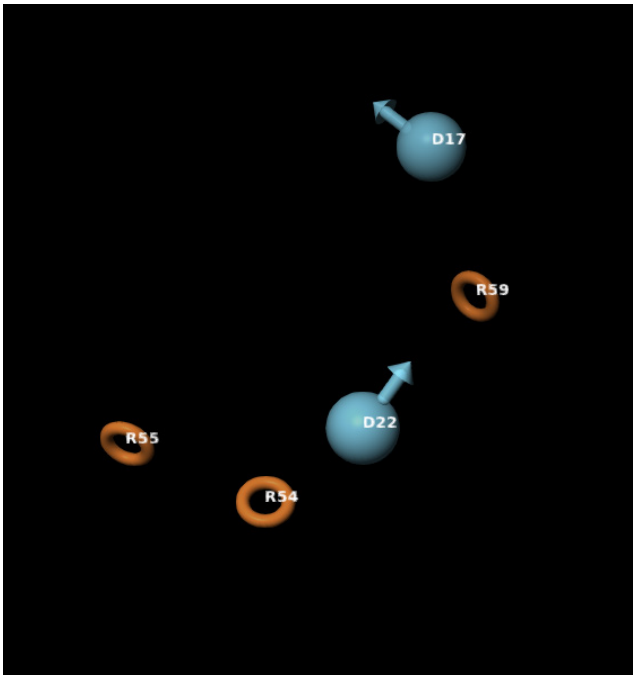


Figure 1: The five feature Pharmacophore model DDRRR generated using PHASE illustrating hydrogen bond donor group (D17, D22; blue) and aromatic ring group (R54, R55, R59; orange).

Database screening using e-pharmacophore model

The e-pharmacophore model (DDRRR) was used to screen the phase database to retrieve a diverse set of hits capable of inhibiting ALK. Since conformers of the ligands were generated using ConfGen option during the phase database creation, on-the-fly conformer generation was not carried out.[56] These conformers were then searched against the e-pharmacophore model to identify those ligands that match the features. Additionally, reorientation of the ligand conformers was also allowed to determine if they match the pharmacophoric sites or not. Further, the basic criteria for matching of ligand sites with the e-pharmacophore model were pre-defined to 4 out of 5 sites so that any potential molecule is not lost.[57] Finally, 'Return at most 1000 hit molecules' were specified in the "hit treatment" option while screening the database. After the process was run, PHASE retrieved 1000 hit molecules which could superimpose with the template hypothesis. All the 1000 hit molecules were then subjected to virtual screening procedures.

Virtual screening using glide

The virtual screening strategy was carried out to identify potent ALK inhibitors with higher glide scores and energies. During the virtual screening process, the compounds were screened through three steps. Initially, the 1000 hit molecules obtained from database screening were screened through HTVS. Subsequently, 50% of the screened hits (500 molecules) were taken to the next docking stage using SP mode. Finally, the next 50% (250 molecules) of the top scoring ligands obtained from SP were subjected to XP docking stage to refine good ligands. After the docking protocol, the hits were analyzed for their docking scores. Since docking scores show the measure of the affinity of each ligand to its receptor, higher docking scores of ligands than the reference molecule confirms higher affinity and thus possible higher inhibition rate than the available drug molecule. Keeping this in mind, 23 hits that showed higher docking scores than the reference ligand crizotinib (>5.112 kcal/mol) were retrieved. The docking scores of the hit molecules along with the crizotinib score is illustrated in Table 2. Further, these 23 hits were analyzed for their ADME properties.

ADME property analysis

The 23 hits were analyzed for their ADME properties using the QikProp module of the Schrödinger software.[58]

Table 2: Docking scores of screened hit molecules and crizotinib against ALK.

S. No	Ligands	Glide gscore (kcal/mol)	Glide energy (kcal/mol)
1	Crizotinib	-5.112	-43.376
2	TIP001103	-10.324	-47.26
3	TIP001834	-9.862	-45.968
4	C00037818.cdx	-8.622	-46.568
5	TIP001834	-8.603	-39.952
6	C00007203.cdx	-8.6	-43.294
7	TIP001834	-8.541	-38.46
8	TIP001838	-8.535	-36.226
9	TIP001736	-8.455	-39.871
10	TIP001853	-8.292	-43.772
11	TIP001834	-8.272	-37.837
12	TIP000871	-8.138	-37.697
13	TIP001125	-7.978	-37.332
14	C00031799.cdx	-7.961	-29.239
15	TIP001853	-7.97	-37.902
16	C00007062.cdx	-7.954	-55.602
17	TIP001887	-7.808	-47.481
18	C00043053.cdx	-7.733	-33.267
19	TIP001125	-8.576	-35.12
20	C00007942.cdx	-7.6	-37.91
21	TIP002168	-7.554	-38.121
22	C00007060.cdx	-7.536	-36.966
23	TIP001838	-7.417	-41.06
24	C00047809.cdx	-7.44	-43.719

In particular, The CNS descriptors were one of the main descriptors considered in our analysis. CNS determine whether the molecules have the ability to cross the blood-brain barrier. Thus 2 hit molecules such as TIP001125 and TIP002168 with CNS values -1 was selected. In addition, the 2 hit molecules were screened for their #stars value. It was found that only 1 (TIP001125) hit possessed the least #stars value 0. Further, TIP001125 (piper philippinin VI) was also found to have 100% HOA property. Moreover, piper philippinin VI was analyzed for its bioavailability, absorption, solubility, distribution, plasma protein binding, membrane penetration properties. Table 3 shows the ADME properties of the screened hit molecule. The results indicate that piperphilippinin VI satisfies all the ADME descriptors.

Interaction studies of piperphilippinin VI

The interaction pattern of piperphilippinin VI was analysed using the Ligand Interaction Diagram (LID) of Schrödinger suite to gain insight into the binding pattern. The results are illustrated in Figure 2. The purple lines indicate the presence of hydrogen bonds between the protein and the ligand. The atomic interaction details of piperphilippinin are shown in Table 4. It can be visualized that piperphilippinin is found to possess hydrogen bond interactions with the crucial Met1199 and Glu1197 residues of the protein. Additionally, piperphilippinin exhibited interactions with the Asp1203 residue of the protein. Interestingly, piper philippinin was found to possess similar interactions as that of the FDA approved drug crizotinib. Of note, the interacting residues were found to have crucial roles in tyrosine kinase activity as well as in triggering the downstream signalling processes.[59] The superimposed image of piper philippinin VI with the binding site of ALK and with the hypothesis are illustrated in Figure 3 and 4 respectively. The ball and stick model of piper philippinin VI along with the plant source information are illustrated in Figure 5.

Biological activity prediction using PASS algorithm

The biological activity of piper philippinin was further examined using PASS algorithm. For PASS prediction, the "SMILES" of the compound was provided as the input. Subsequently the Pa and Pi values of piperphilippinin VI against each activitiy were retrieved. In particular, piperphilippinin VI was searched for its anti-cancer properties. The results of PASS prediction are depicted in Table 5. It was found that piperphilippinin VI exhibited Pa value of 0.365, 0.296 and 0.332 against lung cancer, lymphoma and solid tumors. Most importantly, it exhibited a Pa value of 0.180 and Pi value of 0.107 towards NSCLC. Additionally, it had a Pa value of 0.279 and Pi value of 0.047 towards SCLC. Thus, PASS predicted a significant amount of anti-cancer property of piperphilippinin VI which can provide the basis for further experimental studies.

DISCUSSION

ALK-EML4 rearrangements belongs to a small but significant molecular subtype of NSCLC and has been described as a strong therapeutic target in previous studies. A recent transformation in the therapeutic landscape of ALK positive NSCLC was made by the advancement of targeted therapies. These therapies, as compared with chemotherapy has shown great promise in patients with an improved survival rate. Even though it is reported that CNS is the most frequent site of relapse in ALK-positive patients, the efficiency of chemotherapy to cross the impenetrable blood brain

Table 3: ADME analysis of screened hit compounds.

TIPdb ID	MW[a] (Da)	SASA[b]	FOSA[c]	donor HB[d]	accptHB[e]	QPlogPoct[f]	QPlogPw[g]	QPlogPo/w[h]	QPlogS[i]	QPlogBB[j]	QPlogKp[k]	CNS[l]	HOA[m]	#stars[n]
001834	362.422	696.183	235.777	5	7.35	22.995	16.001	2.027	-3.634	-2.491	45.226	-2	2	0
00037818	424.465	723.189	225.432	4	9	23.685	16.467	2.02	-3.82	-3.268	2.382	-2	2	1
001834	362.422	695.782	240.563	5	7.35	23.012	15.964	2.06	-3.628	-2.434	51.186	-2	2	0
00007203	374.39	623.645	275.718	3	6.75	19.981	12.644	2.385	-3.705	-1.535	119.071	-2	2	0
001834	362.422	696.209	235.632	5	7.35	23.044	16.002	2.026	-3.634	-2.492	45.217	-2	2	0
001838	358.39	638.727	215.157	3	7.65	20.938	14.522	1.943	-4.045	-1.717	69	-2	2	1
001736	329.352	630.532	161.631	4	6.45	21.18	14.432	2.399	-3.599	-1.845	92.9	-2	3	0
001853	360.406	641.944	222.264	4	7.35	22.124	15.452	1.964	-3.914	-1.756	71.82	-2	2	0
001834	362.422	695.273	241.687	5	7.35	23.14	15.952	2.067	-3.62	-2.415	53.178	-2	2	0
000871	288.299	532.09	123.815	1	3.95	13.217	7.367	2.853	-3.27	-1.1	272.289	-2	3	0
001125	360.406	519.017	245.049	3	6.4	17.286	11.103	2.261	-1.841	-0.941	422.705	-1	3	0
00031799	300.354	592.958	96.885	4	4.9	18.925	12.999	2.331	-3.33	-1.793	84.744	-2	3	0
001853	360.406	648.625	207.64	4	7.35	22.556	15.613	2.002	-4.017	-1.835	61.368	-2	2	0
00007062	340.375	600.649	176.253	3	5.95	19.999	12.762	2.354	-3.855	-1.511	89.025	-2	3	0
001887	486.52	867.157	171.097	3	6	25.026	13.536	4.986	-7.059	-3.277	17.118	-2	1	4
00043053	440.492	797.241	322.871	4	6.45	24.831	14.65	3.387	-6.168	-3.019	14.036	-2	1	2
00007942	290.272	529.043	57.146	3	3.75	15.36	10.304	1.408	-2.836	-2.38	12.762	-2	2	0
002168	374.39	613.122	302.946	3	7.15	20.275	13.638	2.398	-4.092	-0.914	299.678	-1	3	1
00007060	340.375	631.658	203.379	2	4	16.897	9.257	3.513	-4.882	-1.554	123.602	-2	3	0
001838	358.39	651.176	217.022	3	7.65	21.295	14.624	2.01	-4.241	-1.794	63.026	-2	2	1
00047809	374.346	673.675	144.378	4	7	21.918	15.268	1.979	-3.998	-3.17	1.81	-2	2	1
001103	343.379	667.907	277.986	3	5.5	19.153	11.584	3.018	-4.47	-1.686	152.053	-2	3	0

[a]Molecular Weight
[b]Solvent accessible surface area
[c]Hydrophobic solvent accessible surface area
[d]Donor Hydrogen Bond
[e]Acceptor Hydrogen Bond
[f]Predicted octanol/gas partition coefficient
[g]Predicted water/gas partition coefficient
[h]Predicted water/octanol partition coeffient
[i]Predicted aqueous solubility
[j]Predicted brain/blood partition coefficient
[k]Predicted skin permeability
[l]Predicted central nervous system activity on a -2 (inactive) to +2 (active) scale
[m]Human Oral Absorption
[n]Number of property or descriptor values that fall outside the 95% range of similar values for known

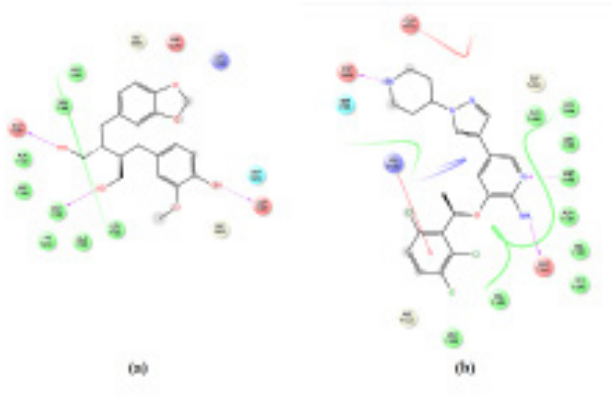


Figure 2: The LID of the reference molecule and screened hit molecules a crizotinib b piperphilippinin VI.

Table 4: Analysis of interaction of screened hit molecule with ALK protein.

S. No.	TIPdb ID	Number of H-bonds	Interacting atoms of protein-ligand complex	Distance (Å)
1	001125	3	Met 1199…Lig(O)	1.86492
			Glu 1197…Lig(O)	2.12271
			Asp 1203…Lig(O)	1.64162

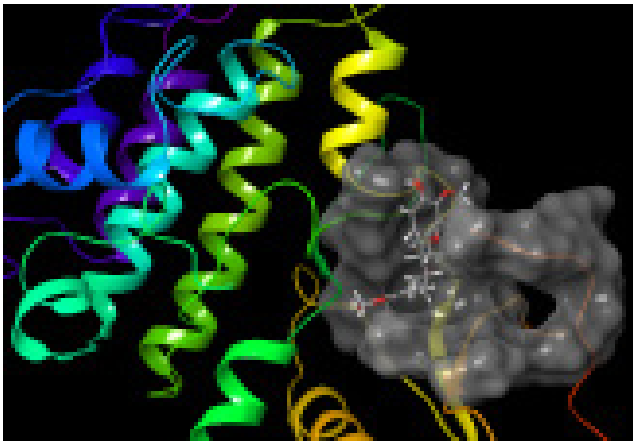


Figure 3: The superimposed image of piperphilippinin VI to the binding site of the receptor.

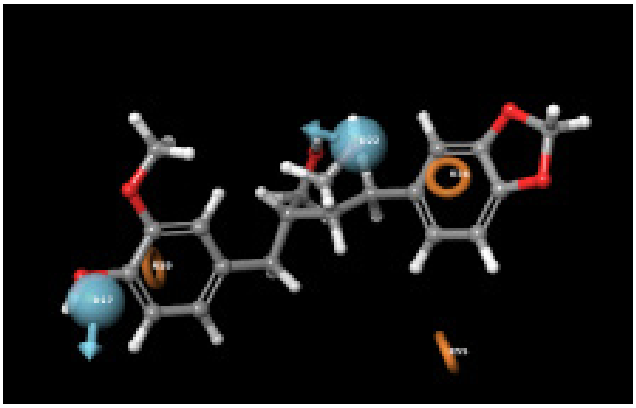


Figure 4: The hit molecule piperphilippinin VI superimposed with the e-pharmacophore hypothesis generated.

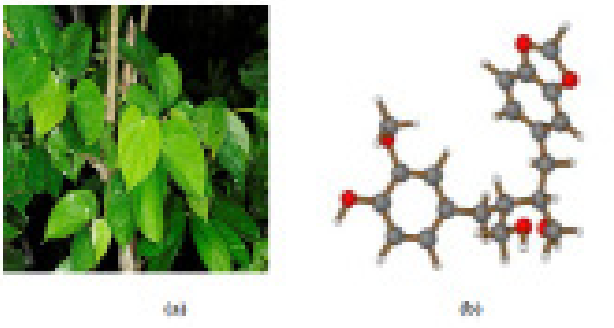


Figure 5: (a) The Plant Piper Philippinum (b) Ball and Stick Model of the chemical compound piperphilippinin VI.

Table 5: Biological activity prediction of piper philippinin VI using PASS algorithm.

S. No	TIPdb ID (Name)	Biological activity	Pa value	Pi value
1	001125 (piperphilippinin VI)	Antineoplastic (lung cancer)	0.365	0.028
		Antineoplastic (lymphoma)	0.296	0.024
		Antineoplastic (solid tumors)	0.332	0.067
		Antineoplastic (non-small cell lung cancer)	0.180	0.107
		Antineoplastic (small cell lung cancer)	0.279	0.047

barrier is controversial. Moreover, the reported response rate in patients to chemotherapy is just 15-30%.[60] Whereas, the response rate of crizotinib, was found to reach 60-80% denoting the success of these agents in treating brain metastasis. However, despite its initial success, the use of crizotinib faces limitations due to its insufficient CSF (Cerebrospinal Fluid) concentration and the occurrence of bypass-mediated resistance pathways. [60] Additionally, the present tyrosine kinase inhibitors for ALK such as crizotinib, ceritinib, alectinib etc pose the threat of side effects on the patients. [60] Considering the aforementioned aspects, the present study is strengthened by the fact that the inhibitor is obtained from natural source thus it may lack side effect issues produced by chemical compounds. Of note, Plants have been considered as a rich supply of conventional medication for many years because they have bioactive molecules with a wide spectrum of biological activities. Moreover, natural compounds are considered as valuable sources of lead structures that could be used as new pharmaceutical agents. Therefore, the present study was undertaken to discover natural targeted agents

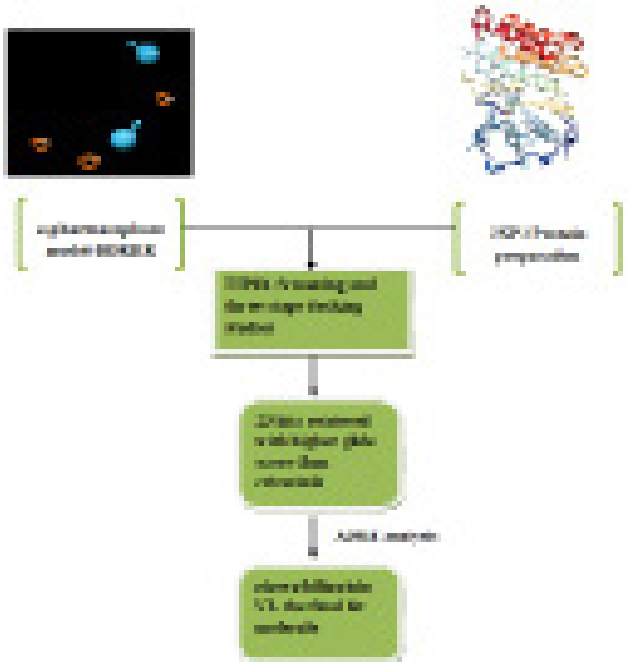


Figure 6: The virtual screening workflow followed throughout the study.

with ALK inhibitory activity from a database of Taiwan indigenous plants, TIPdb.

Initially, a five-point e- pharmacophore hypothesis (DDRRR) was generated from 12 ALK inhibitors using the PHASE module. Keeping the hypothesis as the template the phase database prepared from TIPdb was screened to identify hit molecules that could superimpose with the hypothesis. The 1000 hit molecules were analysed using the HTVS, SP and XP docking protocols. It is evident that 23 hit molecules emerged with higher glide score than the reference ligand. With the resultant 23 hit molecules, ADME property analysis was performed. Moreover, the compounds possessing CNS activity was given more priority. Accordingly, 2 hit molecules with CNS activity were identified and then taken for further ADME property analysis. The second property that was taken into consideration was #stars. #stars are a major factor that helps in determining the drug-likeness of each hit molecules. Evidence suggest if a molecule has lesser #stars it is more likely to be a drug like compound.[54] Finally, one hit molecule piper philippinin VI with the least #stars value 0 was chosen. This hit was further analysed for their QPlogPw, QPlogPoct, QPlogKp, QPlogPo/w, QPlogS, QPlogBB and HOA properties and were found to be in the acceptable range. The workflow of the present study is illustrated in Fig. 6.

Furthermore, interaction analysis was carried out to rationalize the inhibitory property of the hit compound, piper philippinin VI. The binding mode analysis of the hit showed the presence of essential interactions required by an ALK inhibitor. Of note, it was observed that the hit compound binds with Met1199 of the hinge region.[59] The hinge region helps in coordinating ATP for its efficient catalysis and thus triggering of the subsequent downstream signalling leading to cell proliferation and cell survival.[61] Of note, it is the key recognition motif of any kinases which is targeted by a majority of kinase inhibitors. Additionally, piper philippinin VI was also found to possess interactions with another hinge residue, Glu1197.[59] Furthermore, piper-philippinin VI showed interaction with Asp1203 which is found at the bottom of the ATP binding site.[62]

Finally, piper philippinin VI was examined for its possible anticancer properties by employing PASS algorithm. PASS algorithm has been employed as a potential tool for the prediction of biological activity spectra of synthetic as well as natural products.[63] It predicts the activity of the compound on the basis of probable activity (Pa) and probable inactivity (Pi) values. The Pa and Pi values ranges from 0.000 to 1.000. In essence, a compound can be interpreted as having a biological activity only if the Pa > Pi.[63] Thus, the results (Table 5) shows that piper philippinin VI is found to possess antineoplastic activities towards lymphoma and solid tumours. Most importantly, it was found to possess antineoplastic activity towards lung cancer especially NSCLC.

Overall, we found that the hit molecule piper philippinin VI has better CNS ability than crizotinib. Also, it was found that piper philippinin VI binds to the hinge region residues which will hamper the ATP catalysis process resulting in the tumor growth inhibition. It is interesting to note that piper philippinin VI has an interaction profile as that of type I ALK inhibitor in particular crizotinib[62] thus strengthening the fact that it can cause to ALK inhibition. Moreover, PASS algorithm was able to predict the hidden pharmacological potential of piper philippinin VI which can be explored further using *in vivo* as well as *in vitro* studies. Thus, it could be concluded from our results that piper philippinin VI extracted from *Piper Philippinum* plant would be a promising alternative for the development of ALK inhibitors with better CNS activity, improved binding efficiency and minimal side effects.

CONCLUSION

Findings from the current study shows that piper philippinin VI extracted from *Piper Philippinum* plant would be a promising alternative for the development of ALK inhibitors with better CNS and improved binding

efficiency. In the present study, piper philippinin VI was found to have higher glide scores than crizotinib (first FDA approved drug generally used for treatment of ALK positive NSCLC) denoting its higher binding affinity towards ALK and thus its possible capacity for more potent ALK inhibition than crizotinib. The ADME property of piper philippinin VI also supported this notion by revealing the good pharmacokinetic property, better CNS activity and 100% HOA possessed by it. Further interaction analysis of piper philippinin VI showed the presence of crucial Met1199 and Glu1197 interactions with the kinase domain of ALK and also with Asp1203 present at the floor of ATP binding site. Furthermore, confirmation on the possible anti-tumour activity was obtained from PASS prediction values which showed anti-neoplastic activity towards NSCLC making it an accessible lead for the disease. Thus, this study provides first evidence on the anticancer activity of piperphilippinin VI specifically towards ALK positive NSCLC. Further *in vitro* and *in vivo* studies are necessary to validate its possible therapeutic implications towards ALK positive NSCLC.

ACKNOWLEDGMENT

The authors are grateful to Department of Science and Technology - Science and Engineering Research Board (DST-SERB) for funding the research project (File No: EMR/2016/001675) and the management of Vellore Institute of Technology, Vellore for providing the facility to carry out this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

NSCLC: Non-Small Cell Lung Cancer; **ALK:** Anaplastic Lymphoma Kinase; **GLIDE:** Grid-Based Ligand Docking with Energetics; **HTVS:** High Throughput Virtual screening; **SP:** Standard Precision; **XP:** Extra Precision**ADME:** Absorption, Distribution, Metabolism and Excretion; **PASS:** Prediction of Activity Spectra for Substances.

REFERENCES

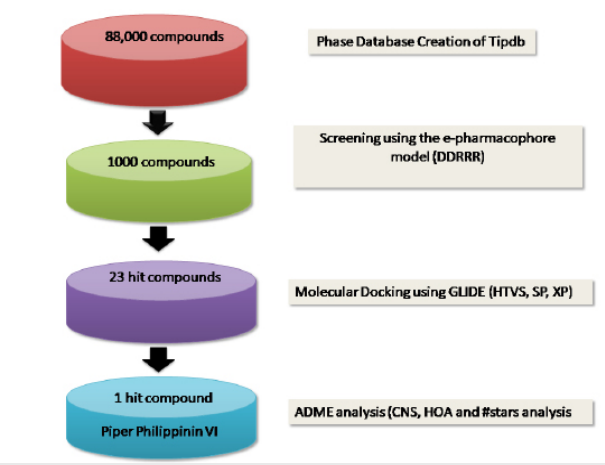
1. Vieira AR, Abar L, Vingeliene S, Chan DS, Aune D, Navarro-Rosenblatt D, *et al*. Fruits, vegetables and lung cancer risk: A systematic review and meta-analysis. Ann Oncol. 2016;27(1):81-96.
2. Kumar A, Shanthi V, Ramanathan K. Computational investigation and experimental validation of crizotinib resistance conferred by C1156Y mutant anaplastic lymphoma kinase. Mol Info. 2015;34(2-3):105-14.
3. Ettinger DS, Bepler G, Bueno R, Chang A, Chang JY, Chirieac LR, *et al*. Non-small cell lung cancer: Clinical Practice Guidelines in Oncology™. J Natl Compr Canc Netw. 2006;4(6):548-82.
4. Gerber DE. Targeted therapies: A new generation of cancer treatments. Am Fam Physician. 2008;77(3):311-9.
5. Sakorafas GH, Tsiotos GG. Molecular biology of pancreatic cancer: Potential clinical implications. Bio Drugs. 2001;15(7):439-52.
6. Stoffel A. Targeted therapies for solid tumors: Current status and future perspectives. Bio Drugs. 2010;24(5):303-16.
7. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer. 2008;8:11-23.
8. Wu J, Savooji J, Liu D. Second- and third generation ALK inhibitors for non-small cell lung cancer. J Hematol Oncol. 2016;9(1):19.
9. Francesco F, Marcello T, Di Massimo M, Paolo G, Emilio B, Giulio R, *et al*. Tackling ALK in non-small cell lung cancer: The role of novel inhibitors. Transl Lung Cancer Res. 2016;5(3):301-21.
10. Blackhall F, Cappuzzo F. Crizotinib: From discovery to accelerated development to front-line treatment. Ann Oncol. 2017;27(3):iii35-41.
11. Pluzanski A, Piórek A, Krzakowski M. Crizotinib in the treatment of non-small-cell lung carcinoma. Contemp Oncol (Pozn). 2012;16(6):480-4.
12. Wu J, Savooji J, Liu D. Second- and third-generation ALK inhibitors for non-small cell lung cancer. J Hematol Oncol. 2016;9(1):19.
13. Lionta E, Spyrou G, Vassilatis DK, Cournia Z. Structure-based virtual screening for drug discovery: Principles, applications and recent advances. Curr Top Med Chem. 2014;14(16):1923-38.
14. James N, Ramanathan K. Discovery of Potent ALK Inhibitors Using Pharmacophore-Informatics Strategy. Cell Biochem Biophys. 2017;6:1-4.
15. Lauria A, Abbate I, Gentile C, Angileri F, Martorana A, Almerico AM. Synthesis and Biological Activities of a New Class of Heat Shock Protein 90 Inhibitors, Designed by Energy-Based Pharmacophore Virtual Screening. J Med Chem. 2013;56(8):3424-8.
16. Therese PJ, Manvar D, Kondepudi S, Battu MB, Sriram D, Basu A, *et al*. Multiple e-Pharmacophore Modeling, 3D-QSAR, and High-Throughput Virtual Screening of Hepatitis C Virus NS5B Polymerase Inhibitors. J Chem Inf Model. 2014;54(2):539-52.
17. Clark DE, Waszkowycz B, Wong M, Lockey PM, Adalbert R, Gilley J, *et al*. Application of virtual screening to the discovery of novel nicotinamide phosphoribosyltransferase (NAMPT) inhibitors with potential for the treatment of cancer and axonopathies. Bioorg Med Chem Lett. 2016;26(12):2920-6.
18. Katari SK, Natarajan P, Swargam S, Kanipakam H, Pasala C, Umamaheswari A. Inhibitor design against JNK1 through e-pharmacophore modeling docking and molecular dynamics simulations. J Recept Sig Transd. 2016;36(6):558-71.
19. ECE A. E-Pharmacophore Mapping Combined with Virtual Screening and Molecular Docking to Identify Potent and Selective Inhibitors of P90 Ribosomal S6 Kinase (RSK). Turk J Pharm Sci. 2016;13(2):241-7.
20. Munir A, Azam S, Mehmood A, Khan Z, Mehmood A. Structure-Based Pharmacophore Modeling, Virtual Screening and Molecular docking for the Treatment of ESR1 Mutations in Breast Cancer. Drug Des. 2016;5(3):1000137.
21. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al*. The protein data bank. Nucleic Acids Res. 2000;28(1):235-42.
22. Sullivan I, Planchard D. ALK Inhibitors in Non-Small Cell Lung Cancer: The Latest Evidence and Developments. Ther Adv Med Oncol. 2016;8(1):32-47.
23. Mezquita L, Besse B. Sequencing ALK Inhibitors: Alectinib in Crizotinib-Resistant Patients, a Phase 2 Trial by Shaw *et al*. J Thorac Dis. 2016;8(11):2997-3002.
24. De la Bellacasa RP, Karachaliou N, Estrada-Tejedor R, Teixidó J. Costa C. Borrell JI. ALK and ROS1 as a Joint Target for the Treatment of Lung Cancer: A Review. Transl Lung Cancer Res. 2013;2(2):72-86.
25. Wang Y, Wang L, Guan S, Cao W, Wang H, Chen Z, *et al*. Novel ALK Inhibitor AZD3463 Inhibits Neuroblastoma Growth by Overcoming Crizotinib Resistance and Inducing Apoptosis. Sci Rep. 2016;6:19423.
26. Tung CW, Lin YC, Chang HS, Wang CC, Chen IS, Jheng JL, *et al*. TIPdb-3D: The three-dimensional structure database of phytochemicals from Taiwan indigenous plants. Database (Oxford). 2014;13.
27. Glaab E. Building a virtual ligand screening pipeline using free software: A survey. Brief Bioinform. 2016;17(2):352-66.

28. Schrödinger Release 2015-2: Epik v Schrödinger. Schrödinger, LLC, New York, NY. 2005.
29. Kalliokoski T, Salo HS, Lahtela-Kakkonen M, Poso A. The effect of ligand-based tautomer and protomer prediction on structure-based virtual screening. J Chem Inf Model. 2009;49(12):2742-8.
30. Sadowski J, Rudolph C, Gasteiger J. The generation of 3D models of host–guest complexes. Anal Chim Acta. 1992;265(2):233-41.
31. Watts KS, Dalal P, Murphy RB, Sherman W, Friesner RA, Shelley JC. Conf Gen: A conformational search method for efficient generation of bioactive conformers. J ChemInf Model. 2010;50(4):534-46.
32. Muralidharan AR, Selvaraj C, Singh S, Nelson Jesudasan CA, Geraldine P, Thomas P. Virtual screening based on pharmacophoric features of known calpain inhibitors to identify potent inhibitors of calpain. Med Chem Res. 2014;23(5):2445-55.
33. Palakurti R, Sriram D, Yogeeswari P, Vadrevu R. Multiple e-Pharmacophore Modeling Combined with High-Throughput Virtual Screening and Docking to Identify Potential Inhibitors of β-Secretase (BACE1). Mol inform. 2013;32(4):385-98.
34. Veeramachaneni GK, Raj KK, Chalasani LM, Bondili JS, Talluri VR. High-throughput virtual screening with e-pharmacophore and molecular simulations study in the designing of pancreatic lipase inhibitors. Drug Des Devel Ther. 2015;9:4397-412.
35. Milletti F, Vulpetti A. Tautomer preference in PDB complexes and its impact on structure-based drug discovery. J Chem Inf Model. 2010;50(6):1062-74.
36. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aided Mol Des. 2013;27(3):221-34.
37. Sindhu T, Srinivasan P. Identification of potential dual agonists of FXR and TGR5 using e-pharmacophore based virtual screening. Mol Biosyst. 2015;11(5):1305-18.
38. Small-Molecule Drug Discovery Suite 2015-2: Glide v Schrödinger (2015) Schrödinger, LLC, New York, NY.
39. Miller BR, Roitberg AE. Design of e-pharmacophore models using compound fragments for the trans-sialidase of Trypanosoma cruzi: Screening for novel inhibitor scaffolds. J Mol Graph Model. 2013;30;45:84-97.
40. Kumar A, Ramanathan K. Exploring the structural and functional impact of the ALK F1174L mutation using bioinformatics approach. J Mol Model. 2014;20(7):2324.
41. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, *et al*. Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem. 2006;49(21):6177-96.
42. Small-Molecule Drug Discovery Suite 2015-2: Phase v Schrödinger (2015) Schrödinger, LLC, New York, NY.
43. Salam NK, Nuti R, Sherman W. Novel method for generating structure-based pharmacophores using energetic analysis. J Chem Inf Model. 2009;49(10):2356-68.
44. Kalyaanamoorthy S, Chen YP. Energy based pharmacophore mapping of HDAC inhibitors against class I HDAC enzymes. Biochim Biophys Acta. 2013;1834(1):317-28.
45. Sándor M, Kiss R, Keseru GM. Virtual fragment docking by Glide: A validation study on 190 protein-fragment complexes. J Chem Inf Model. 2010;50(6):1165-72.
46. Rajput VS, Mehra R, Kumar S, Nargotra A, Singh PP, Khan IA. Screening of antitubercular compound library identifies novel shikimate kinase inhibitors of Mycobacterium tuberculosis. Appl Microbiol Biotechnol. 2016;100(12):5415-26.
47. Afzal, Kumar S, Kumar R, Firoz A, Jaggi M, Bawa S. Docking based virtual screening and molecular dynamics study to identify potential monoacylglycerol lipase inhibitors. Bioorg Med Chem Lett. 2014;24(16):3986-96.
48. Elancheran R, Saravanan K, Choudhury B, Divakar S, Kabilan S, Ramanathan M, *et al*. Design and development of oxobenzimidazoles as novel androgen receptor antagonists. Med Chem Res. 2016;25(4):539-52.
49. Wang NN, Dong J, Deng YH, Zhu MF, Wen M, Yao ZJ, *et al*. ADME properties evaluation in drug discovery: Prediction of Caco-2 Cell permeability using a combination of NSGA-II and boosting. J ChemInf Model. 2016;56(4):763-73.
50. Wang Y, Xing J, Xu Y, Zhou N, Peng J, Xiong Z, *et al*. In silico ADME/T modelling for rational drug design. Q Rev Biophys. 2015;48(4):488-515.
51. Jorgensen WL, Duffy EM. Prediction of drug solubility from structure. Adv Drug Deliv Rev. 2002;54(3):355-66.
52. Ramar V, Pappu S. Exploring the inhibitory potential of bioactive compound from Luffa acutangula against NF-κB-A molecular docking and dynamics approach. Comput Biol Chem. 2016;62:29-35.
53. Shukla S, Srivastava RS, Shrivastava SK, Sodhi A, Kumar P. Synthesis, characterization, *in vitro* anticancer activity, and docking of Schiff bases of 4-amino-1, 2-naphthoquinone. Med Chem Res. 2013;22(4):1604-17.
54. Das N, Dhanawat M, Shrivastava SK. Benzoxazinones as Human Peroxisome Proliferator Activated Receptor Gamma (PPARγ) Agonists: A Docking Study Using Glide. Indian J Pharm Sci. 2011;73(2):159-64.
55. Cappel D, Dixon SL, Sherman W, Duan J. Exploring conformational search protocols for ligand-based virtual screening and 3-D QSAR modeling. J Comput Aided Mol Des. 2015;29(2):165-82.
56. Dixon SL, Smondyrev AM, Knoll EH, Rao SN, Shaw DE, Friesner RA. PHASE: A new engine for pharmacophore perception, 3D QSAR model development, and 3D database screening: 1. Methodology and preliminary results. J Comput Aided Mol Des. 2006;20(10-11):647-71.
57. Guasch L, Ojeda MJ, González-Abuín N, Sala E, Cereto-Massagué A, Mulero M, *et al*. Identification of novel human dipeptidyl peptidase-IV inhibitors of natural origin (part I): virtual screening and activity assays. PloS one. 2012;7(9):e44971.
58. Small-Molecule Drug Discovery Suite 2015-2: QikProp v Schrödinger (2015) Schrödinger, LLC, New York, NY.
59. Roskoski R. Anaplastic lymphoma kinase (ALK) inhibitors in the treatment of ALK-driven lung cancers. Pharmacol Res. 2017;117:343-56.
60. Dempke Wc, Edvardsen K, Lu S, Reinmuth N, Reck M, Inoue A. Brain metastases in NSCLC-are TKIs changing the treatment strategy?. Anticancer Res. 2015;35(11):5797-806.
61. Lee CC, Jia Y, Li N, Sun X, Ng K, Ambing E, *et al*. Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain. Biochem J. 2010;430(3):425-37.
62. Roskoski R. Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. Pharmacol Res. 2016;103:26-48.
63. Goel RK, Singh D, Lagunin A, Poroikov V. PASS-assisted exploration of new therapeutic potential of natural products. Med Chem Res. 2011;20(9):1509-14.

SUMMARY

- The developed e-pharmacophore hypothesis retrieved potential compounds from Tipdb database (88000) which were then subjected to ADME analysis resulting in piper philippinin VI as the potential hit.
- Piper philippinin VI had increased CNS activity, 100% HOA. It also exhibited Met1199, Glu1197 and Asp1203 with the kinase domain of ALK.
- Piper philippinin VI showed promising results in silico and provides a strong outlook for the future designing and discovery of novel ALK inhibitors.

PICTORIAL ABSTRACT



About Authors

Dr. K. Ramanathan, Professor, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu.

Dr. V. Shanthi, Associate Professor, Department of Biotechnology,School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu.

Nivya James, Research Associate, Medical Informatics Lab, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu.

B. Preethi, Junior Research Fellow, Medical Informatics Lab, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu.

Raunaq Surana, B.Tech Student, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu.

Isha Thigale, B.Tech student, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore- 632014, Tamil Nadu.

Cite this article: James N, Surana R, Thigale I, Preethi B, Shanthi V, Exploring Novel ALK Inhibitors Using Energy Based Pharmacophore Mapping and High Throughput Virtual Screening. Indian J of Pharmaceutical Education and Research. 2018;52(4):707-17.