PHARMACOPHORE-BASED DATABASE SEARCHING OF KINASE-INHIBITOR MIMETIC MOLECULAR HITS

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ABSTRACT

Protein phosphorylation catalysed by kinases is involved in the regulation of most aspects of cell life, whereas deregulation of kinase activity has emerged as a major mechanism by which cancer cells evade normal physiological constraints on growth and survival. Protein kinases have emerged as one of the major drug target classes that are amenable to the development of small molecule inhibitors. They share a conserved structural similarity in the region of the ATP binding site, where most inhibitors interact. Pharmacophore feature is defined by a set of chemical structure patterns having the active site of drug like molecule. Pharmacophore can be used to assist in building hypothesis about desirable chemical properties in drug molecule which can be used to refine and modify drug candidates. Using a pharmacophore approach, we have developed pharmacophore models that represent five different kinase-inhibitor datasets. Each model was used for virtual screening of small molecules to find compounds that show activity against their respective kinases which could serve as potential leads to discover and characterize new kinase inhibitors.

Keywords: Kinase-inhibitors, pharmacophore, database searching, ATP-mimetics, lead compounds

No: of Tables :10 **No:of Figures:5** **No:of References:47**

Introduction

With the increase of computing power, computer aided drug discovery (CADD) has emerged as an indispensable application in drug discovery and development programs. CADD provides valuable insights regarding experimental observations and is used in various stages of drug discovery processes. Numerous compounds discovered using CADD methods have reached the level of clinical trials and have become approved drugs (Clark 2006 and Talele *et al*., 2010). The CADD methods use virtual screening techniques, which perform fast computational screening of large chemical compound databases to identify compounds that can actively bind to a specified protein target. In the absence of the structural information of the target proteins, pharmacophore search is an established and effective mechanism of virtual screening of new potent inhibitors (Spitzer *et al*., 2010 and Horvath, 2011). It represents the three dimensional representation of molecular features necessary for the recognition of a ligand by a protein in order to carry out its function. These features include hydrophobic, aromatic, hydrogen bond acceptor, hydrogen bond donor, cation, anion, and may be a combination of these features. Pharmacophore modeling has been used as an essential technique in the discovery of new drugs (Mason, 2001; Guner, 2000; Langer and Krovat, 2003 and Leach *et al*. 2010). Many successful works have been reported regarding the implication of pharmacophore modeling approaches in inhibitor discovery (Kubinyi, 2006; Deng *et al*., 2008 and Mustata *et al*., 2009). Pharamacophore models can also be used in docking methods (Claussen *et al*. 2004), lead optimization (Sun, 2008; Leach *et al*., 2010; Yang, 2010; and Gao, Yang & Zhu, 2010), multitarget drug design (Wei *et al*., 2008), activity profiling (Steindl *et al*., 2006) and target identification (Rollinger *et al*. 2004). Many commercial softwares such as Catalyst (Accelrys) (Kurogi, and Guner, 2001), DISCO (Martin, 1993), LigandScout (Inte:Ligand) (Wolber & Langer, 2005), MOE (Chemical Computing Group), Phase (Schrodinger) (Dixon *et al*. 2006), and free resources like Pharmapper (Liu et *al*. 2010), PharmaGist (Duhovny *et al*. 2008), ZincPharma (Koes and Camacho, 2012) are available for pharmacophore detection. However, pharmacophore modeling techniques still face issues such as ligand flexibility, computational time cost and lower hit rates. Hence, pharmacophore methods that produce more accurate, optimal models and algorithms that perform efficient molecular alignments are required (Yang, 2010).

Protein kinases have emerged as one of the most important research subjects of pharmaceutical interest due to their crucial role in cellular mechanism. Since they share a conserved ATP binding site (Hanks and Hunter, 1995 and Hanks, Quinn and Hunter, 1988) a number of

small molecule inhibitors have been developed to bind to this site (Liao, 2007). A study on pharmacophore mapping of 220 small molecule protein kinase-inhibitor complexes to identify common features required for a kinase binding ligand has been reported (McGregor, 2007). This commonality enables the kinase-specific screening of chemical compounds (Muegge and Enyedy, 2004 and Schürer, Tyagi and Muskal, 2005) but poses the problem of cross reactivity (Dancey and Sausville, 2003 and McGovern and Shoichet, 2003).

Pharmacophore models describe the features of small molecules and enable the screening of molecules with the required features to bind to a specific target (McGregor, 2007). Hence, in the present work, five different pharmacophore models for five individual kinase-inhibitor datasets have been obtained. On the basis of the pharmacophore models, we have performed database searching of small molecular hits that can develop into potential lead compounds specific to their target kinases.

Materials and Methods

20 kinase-inhibitors with IC_{50} values less than 200 nM were chosen from each of the five kinase families, cyclic AMP-dependent protein kinase, cell division protein kinase2, mitogen-activated protein kinase 14, proto-oncogene tyrosine protein kinase LCK and serine/threonine-protein kinase Chk1 from the Protein Data Bank (Berman *et al*., 2000). The details of the dataset of kinase-inhibitor complexes, PDB ID, Hetero ID, IC_{50} (nM) and ligand name are provided in Supplementary file.

PharmaGist

PharmaGist (Duhovny *et al*. 2008) is a webserver for searching pharmacophore from a set of ligand molecules that are known to bind to a target receptor. For each input ligand molecule, this server lists the number of atoms, spatial and physicochemical features such as aromatic rings, hydrophobic regions, hydrogen bond donors and acceptors. The method efficiently searches for possible pharmacophores, generates 3D visualization of candidate pharmacophores detected by multiple flexible alignments of the input ligands and performs virtual screening based on pharmacophore detection.

ZINCPharmer

ZINCPharmer (http://zincpharmer.csb.pitt.edu/) (Koes & Camacho, 2012) is a web interface for searching pharmacologically related compounds in the ZINC database (http://zinc.docking.org/) (Irwin & Shoichet, 2005), which is a public resource of over 35 million three-dimensional compound structures used for virtual screening applications.

The inhibitor dataset for each kinase family was given as input in the PharmaGist web server in Mol2 format. The output lists the input molecules with their atom counts and assigned physico-chemical features. It also provides the

highest scoring pharmacophore model based on the number of input molecules. The top scoring pharmacophore model was loaded into ZINCPharmer database as a query to search the ZINC database to search for small molecular hits. For obtaining more reliable hits, the ZINCPharmer interface provides filter options, and the criteria set for retrieving the molecular hits for the pharmacophore models of five kinase families in the present work are as follows:

- Maximum of 2 oritentations returned for each conformation
- Maximum of 1 conformation returned for each compound
- Maximum of 10 hits returned
- Maximum permitted root mean square deviation (RMSD) of 0.6Å with pharmacophore query points
- Permitted range molecular weight: 450 to 550 (in Daltons)
- Permitted number of rotatable bonds: 8 to 10

Molinspiration server

Molinspiration server (http://www.molinspiration.com/cgi-bin/properties) requires SMILES (simplified molecular-input line-entry system) or SDfile structures of molecules to be tested on-line for bioactivity. The server uses a virtual screening protocol based on Bayesian statistics to compare structures of active ligands of a particular target with structures of inactive molecules and to identify substructure features essential for active molecules from which a fragment-based model is developed and the bioactivity of screened molecules is calculated as a sum of activity contributions of fragments in these molecules. This provides a molecule activity score (ranging from -3 to 3). Molecules with the highest activity score have the highest probability to be active. The druglikeness score are predicted towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets.

The SMILES of the hit compounds obtained in the present study were given as input in the Molinspiration server to predict kinase activity.

Results and Discussion

The input of the 20 inhibitors for each of five dataset kinases loaded into the PharmaGist server gives pharmacophore models of the input molecules along with their scores and pharmacophoric features. The high scoring pharmacophore model was loaded into ZINCPharmer database and 10 hit compounds have been retrieved for each of the five kinase families. The ten molecular hits were tested for kinase activity.

Pharmacophore-based search results of cell division kinase2 protein-inhibitors

The atom numbers, spatial and other pharmacophoric features of 20 cell division protein kinase2-inhibitors obtained from the PharmaGist server. Based on the features of 20 cell division protein kinase2-inhibitors, the PharmaGist

server gave a high scoring pharmacophore model (score = 38.184) with assigned features shown in figure 1. The pharmacophore model contains two aromatic features, one donor and one acceptor feature. The model was loaded into the ZincPharmer interface and 10 hit compounds were obtained and the predicted kinase activity of the hit compounds is presented in Table 1. Compounds 1 and 5 searched from the ZINC database show predicted kinase activity score of 0.34 and 0.01 respectively.

Pharmacophore-based search results of cyclic AMP-dependent kinase-inhibitors

The pharmacophore features of 20 cyclic AMP-dependent kinase-inhibitors provided the best pharmocophore model (score = 34.369) and its features for 20 cyclic AMP dependent protein kinase-inhibitors are shown in figure 2. The model has two aromatic features and one acceptor feature. The pharmacophore model loaded into the ZincPharmer interface returned 10 hit compounds and the predicted kinase activity of the hit compounds are shown in Table 2. The compounds 1and 3 with predicted activity scores, 0.07 and 0.27 respectivity show activity for kinase targets.

Pharmacophore-based search results of mitogen activated kinase 14 protein-inhibitors

The PharmaGist server output of 20 mitogen activated kinase 14 protein-inhibitors provided the high scoring model (score = 36.000) obtained by aligning 20 mitogen activated protein kinase 14-inhibitors and their features are presented in figure 3. The best candidate pharmacophore model containing two aromatic and two acceptor features was used to search the ZINC database for obtaining ten molecular hits and the kinase activity predicted for the hit compounds are shown in Table 3. Compound 6 has shown a positive predicted activity score (0.29) to inhibit kinase targets.

Pharmacophore-based search results of proto oncogene tyrosine protein kinase LCK-inhibitors

The output obtained from the PharmaGist server for 20 proto oncogene tyrosine protein kinase LCK-inhibitors provide the high scoring pharmacophore model (score =30.923) and their features obtained by aligning 20 proto oncogene tyrosine protein kinase LCK -inhibitors are presented in Figure 4. The model constitutes of two aromatic features and one acceptor feature. A list of ten chemical compounds was obtained by database searching based on the top ranking pharmacophore model and the predicted kinase activity of the chemical compounds is shown in Table 4.

Pharmacophore-based search results of serine threonine protein kinase Chk1-inhibitors

The pharmacophoric features of 20 serine threonine protein kinase Chk1-inhibitors are provide the pharmacophore alignment of 20 serine threonine protein kinase Chk1-inhibitors

provided a best scoring model (score: 37.108) and its features are shown in Figure 5. Two aromatic features, one donor and one acceptor features are assigned to the representative pharmacophore model. The model was used to search database to retrieve ten hit compounds and their predicted kinase activity are listed in Table 5. The compounds 2 and 6 has shown activity for kinases with predicted activity scores 0.03 and 0.04 respectively.

The ultimate aim of pharmacophore detection is to identify common features that are required for a molecule to bind specifically to its target. Pharmacophore modeling based on kinase families can be of significant importance in addressing the issue of target selectivity (McGregor, 2007). Hence the pharamcophore modeling was carried out for five different kinase-inhibitors and the features were analysed in the present study.

A five-point pharmacophore for kinase 'frequent hitters' has been identified to discriminate between frequent hitters and selective ligands which can be used for database searching of small molecular hits, that have the potential to develop as lead molecules for specific kinase families (Aronov & Murcko, 2004). A study on the pharmacophore map of small molecule protein kinase inhibitors (McGregor, 2007) has analysed the six most common pharmacophore types, H-bond acceptor, H-bond donor, hydrophobic, negative charge, positive charge, and aromatic shared by 220 kinase-inhibitors. The author has reported that H-Bond acceptor to be the most common feature of all kinases and is made by every ligand in the dataset analyzed; the aromatic features are the major determinant of selectivity in kinase ligands; positively charged groups are quite common compared to the negatively charged ones. In the present study, two aromatic features are shared by all the pharmacophores representing the five kinase families taken for analysis. Atleast one acceptor feature is present in all the five high-scoring pharmacophore. These aspects of our present work reflect the findings of the reported studies in kinase-inhibitor pharmacophore mapping.

Pharmacophore-based virtual screening to identify potent inhibitors has been reported for KDR kinase (Yu *et al*. 2007), spleen tyrosine kinase (Xie *et al*. 2009) and VEGFR-2 kinase (Lee *et al*. 2010) targets. In the present work, the ZINC database was searched for potent chemical compounds and totally seven compounds that show activity against kinase targets have been obtained.

Table 1 Details of the ten molecular hits obtained using the top scoring cell division protein kinase2-inhibitor -based pharmacophore model and their predicted kinase activity scores (Compounds 1 and 5 show predicted kinase activity)

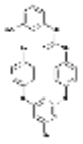
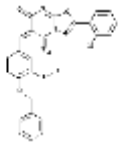
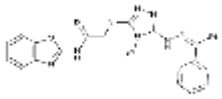
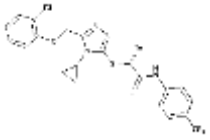
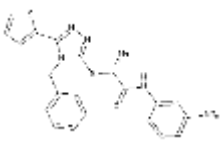
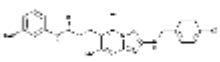
COMPOUND NO	ZINC ID	CHEMICAL NAME	2D STRUCTURE	RMSD	MASS	ROTATABLE BONDS	PREDICTED KINASE ACTIVITY SCORE
1.	ZINC11027544	3-(3-methoxyphenyl)-1-[4-[4-methyl-6-(p-tolylamino)pyrimidin-2-yl]aminophenyl]-urea		0.306	455	8	**0.34**
2.	ZINC26607791	(6E)-6-[(4-benzyloxy-3-ethoxy-phenyl)methylene]-2-(2-chlorophenyl)-5-imino-[1,3,4]thiadiazolo[3,2-a]		0.554	517	8	-0.58
3.	ZINC21322753	2-[[4-amino-5-[(2Z)-2-(1-phenylpropylidene)hydrazinyl]-1,2,4-triazol-3-yl]sulfanyl]-N-(1,3-benzothiazol-2-yl)acetamide		0.512	453	9	-0.67
4.	ZINC33273436	N-(1,3-benzodioxol-5-ylmethyl)-3-[2-[(3,4-dimethylphenyl)carbamoyl-methylamino]ethyl]-1,2,4-oxadiazole-5-carboxamide		0.594	451	9	-0.02
5.	ZINC71768578	N-[(4-isopropylphenyl)methyl]-1-[3-(3-phenyl-1,2,4-oxadiazol-5-yl)-2-pyridyl]imidazole-4-carboxamide		0.594	465	8	**0.01**
6.	ZINC09330612	2-[[4-benzyl-5-(2-fluorophenyl)-1,2,4-triazol-3-yl]sulfanyl]-N-(2-isopropylpyrazol-3-yl)-propanamide		0.470	465	10	-0.68
7.	ZINC09414130	(2R)-2-[[5-[(2-chlorophenoxy)methyl]-4-cyclopropyl-1,2,4-triazol-3-yl]sulfanyl]-N-[4-(trifluoromethyl)phenyl]propanamide		0.459	497	9	-0.70
8.	ZINC35532559	N-(4-butylphenyl)-2-[oxo-[[(2S)-tetrahydrofuran-2-yl]methyl]BLAHyl]sulfanyl-acetamide		0.485	498	9	-0.89
9.	ZINC36859547	(2R)-2-[[4-benzyl-5-(2-thienyl)-1,2,4-triazol-3-yl]sulfanyl]-N-(3-nitrophenyl)propanamide		0.459	466	8	-0.85
10	ZINC21804106	3-[2-[(4-chlorophenyl)methylamino]-5-methyl-7-oxo-1H-[1,2,4]triazolo[1,5-a]pyrimidin-6-yl]-N-(3-methoxyphenyl)propanamide		0.495	467	9	-0.41

Table 2 Details of the ten molecular hits obtained using the top scoring cyclic AMP dependent protein kinase-inhibitor-based pharmacophore model and their predicted kinase activity scores (Compounds 1and 3 show activity)

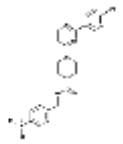
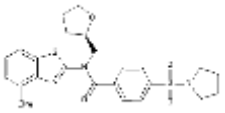
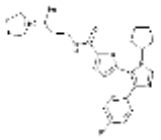
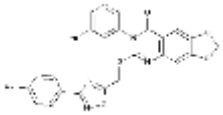
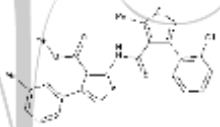
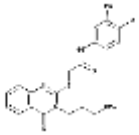
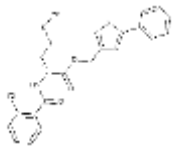
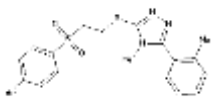
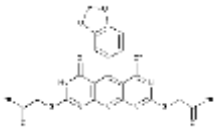
COMPOUND NO	ZINC ID	CHEMICAL NAME	2D STRUCTURE	RMSD	MASS	ROTATABLE BONDS	PREDICTED KINASE ACTIVITY SCORE
1.	ZINC85649307	N-[[4-(diethylamino)phenyl]methyl]-1-[2-(p-tolyl)pyrimidin-4-yl]piperidine-4-carboxamide		0.175	458	10	**0.07**
2.	ZINC10873495	N-(4-methoxybenzothiazol-2-yl)-4-pyrrolidin-1-ylsulfonyl-N-(tetrahydrofuran-2-ylmethyl)benzamide		0.526	502	9	-0.42
3.	ZINC65055002	5-[3-cyclopentyl-5-(4-fluorophenyl)imidazol-4-yl]-N-[(3R)-3-pyrrolidin-1-ylbutyl]furan-2-carboxamide		0.147	466	8	**0.27**
4.	ZINC33279826	6-[[3-(4-ethylphenyl)-1,2,4-oxadiazol-5-yl]methylsulfanyl]-7-(m-tolyl)-[1,3]dioxolo[4,5-g]quinazolin		0.560	499	8	-0.27
5.	ZINC02329543	(4R)-5-(4-fluorobenzyl)-3-(2-hydroxy-3,5-dimethyl-phenyl)-4-(4-hydroxy-3-methoxy-phenyl)-1,4-dihydro		0.126	473	10	-0.36
6.	ZINC02089605	ethyl 2-[[3-(2-chlorophenyl)-5-methyl-1,2-oxazole-4-carbonyl]amino]-4-(3-methylphenyl)thiophene-3-carboxylate		0.125	481	9	-0.45
7.	ZINC09412718	N-(4-bromo-3-methyl-phenyl)-2-[3-(3-methoxypropyl)-4-oxo-quinazolin-2-yl]sulfanyl-acetamide		0.555	476	9	-0.62
8.	ZINC09587140	(2-phenylthiazol-4-yl)methyl		0.153	461	10	-0.51
9.	ZINC11390611	3-[2-(4-bromophenyl)sulfonylethylsulfanyl]-4-methyl-5-(o-tolyl)-1,2,4-triazole		0.155	452	8	-0.68
10	ZINC08426318	5-(1,3-benzodioxol-5-yl)-2,8-bis[(2-oxopropyl)sulfanyl]pyrimido[5',4':5,6]pyrido[2,3-d]pyrimidine-4,6(3H,7H)-dione		0.577	512	9	-0.34

Table 3 Details of the ten molecular hits obtained using the top scoring mitogen activated protein kinase 14- inhibitor-based pharmacophore model and their predicted kinase activity scores (Compound 6 show positive activity score)

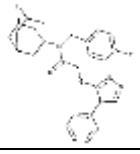
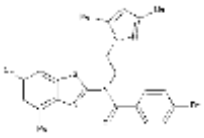
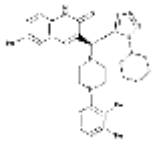
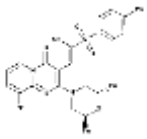
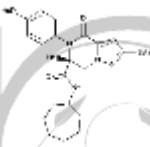
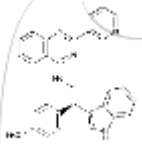
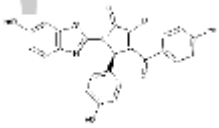
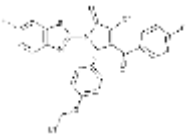
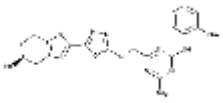
COMPOUND NO	ZINC ID	CHEMICAL NAME	2D STRUCTURE	RMSD	MASS	ROTATABLE BONDS	PREDICTED KINASE ACTIVITY SCORE
1.	ZINC11426773	N-(1-adamantyl)-N-[(4-fluorophenyl)methyl]-2-[(4-phenyl-1,2,4-triazol-3-yl)sulfanyl]acetamide		0.394	477	8	-0.57
2.	ZINC38902835	4-bromo-N-(6-chloro-4-methyl-1,3-benzothiazol-2-yl)-N-[2-(3,5-dimethylpyrazol-1-yl)ethyl]benzamide		0.325	504	9	-0.41
3.	ZINC19941328	3-[(S)-(1-cyclohexyltetrazol-5-yl)-[4-(2,3-dimethylphenyl)piperazin-1-yl]methyl]-6-methyl-1H-quinolin-2-one		0.512	512	8	-0.40
4.	ZINC13120109	(E)-3-[2-(2,6-dimethylmorpholin-4-yl)-9-methyl-4-oxopyrido[1,2-a]pyrimidin-3-yl]-2-(4-methylphenyl)sulfonylprop-2-enenitrile		0.330	479	8	-0.56
5.	ZINC21852704	2-tert-butyl-N-cycloheptyl-5-(4-methoxyphenyl)-6-methyl-4-oxo-7H-pyrazolo[1,5-a]pyrazine-6-carboxamide		0.439	453	9	-0.31
6.	ZINC13096395	N-[(2R)-2-(1H-indol-3-yl)-2-(4-methoxyphenyl)ethyl]-2-(3-pyridyl)quinazolin-4-amine		0.523	472	8	**0.29**
7.	ZINC33537664	3-hydroxy-5-(4-hydroxyphenyl)-1-(6-methoxybenzothiazol-2-yl)-4-(4-methylbenzoyl)-5H-pyrrol-2-one		0.461	472	8	-0.77
8.	ZINC09333864	1-(6-fluorobenzothiazol-2-yl)-4-[(4-fluorophenyl)-hydroxy-methylene]-5-(4-propoxyphenyl)-pyrrolidine		0.500	506	8	-0.79
9.	ZINC09866670	2-N-(2-methoxyphenyl)-6-[[5-[(5S)-5-methyl-4,5,6,7-tetrahydro-1-benzothiophen-2-yl]-1,3,4-oxadiazol-2-yl]sulfanylmethyl]-1,3,5-triazine-2,4-diamine		0.279	482	10	-0.18
10	ZINC15019095	2-[[4-ethyl-5-[(1S)-1-(3-oxo-1,4-benzoxazin-4-yl)ethyl]-1,2,4-triazol-3-yl]sulfanyl]-N-(4-methylphenyl)acetamide		0.576	452	9	-0.94

Table 4 Details of the ten molecular hits obtained using the top scoring proto oncogene tyrosine protein kinase LCK - inhibitor-based pharmacophore model and their predicted kinase activity scores

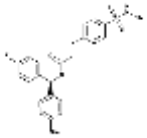
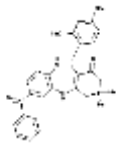
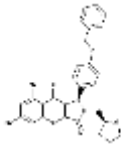
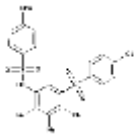
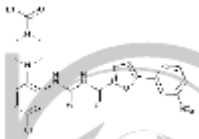
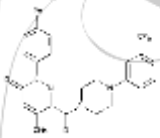
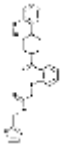
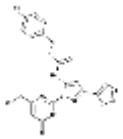
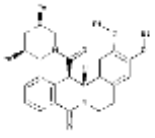
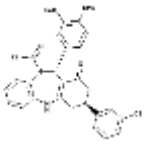
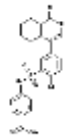
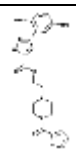
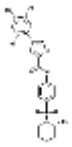
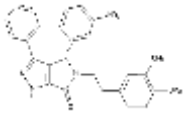
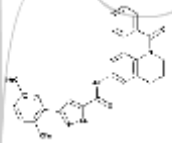
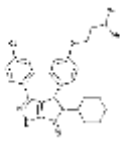
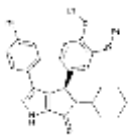
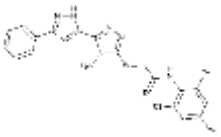
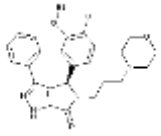
COMPOUND NO	ZINC ID	CHEMICAL NAME	2D STRUCTURE	RMSD	MASS	ROTATABLE BONDS	PREDICTED KINASE ACTIVITY SCORE
1.	ZINC72028721	N-[(S)-(4-fluorophenyl)-(4-methoxyphenyl)methyl]-3-[4-(methylsulfamoyl)phenyl]propanamide		0.079	457	10	-0.29
2.	ZINC18143042	7-benzoyl-11-(2,4-dimethoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydro-1H-dibenzo[b,e][1,4]diazepin-1-one		0.262	483	9	-0.84
3.	ZINC38592716	6,8-dimethyl-2-(oxolan-2-ylmethyl)-1-(4-phenylmethoxyphenyl)-1H-chromeno[2,3-c]pyrrole-3,9-dione		0.086	496	8	-0.84
4.	ZINC01071922	N-[5-(4-chlorophenyl)sulfonyl-4-hydroxy-2,3-dimethyl-phenyl]-4-methoxy-benzenesulfonamide		0.558	482	10	-0.44
5.	ZINC59620445	N-[[5-chloro-2-(4-propanoylpiperazin-1-yl)phenyl]carbamothioyl]-5-(3-nitrophenyl)furan-2-carboxamide		0.429	542	9	-0.38
6.	ZINC09573783	5-methoxy-2-(p-tolyl)-6-[4-[3-(trifluoromethyl)phenyl]piperazin-1-yl]carbonyl-pyridazin-3-one		0.547	472	8	-0.14
7.	ZINC12791364	2-[2-[4-(2-ethoxyphenyl)piperazine-1-carbonyl]phenyl]sulfanyl-N-(2-furylmethyl)acetamide		0.520	480	10	-0.56
8.	ZINC12007317	2-(4-chlorophenoxy)-N-[5-(2-furyl)-2-(4-oxo-6-propyl-3H-pyrimidin-2-yl)pyrazol-3-yl]acetamide		0.532	454	8	-0.48
9.	ZINC20155421	13-(3,5-dimethylpiperidine-1-carbonyl)-2,3-diethoxy-5,6,13,13a-tetrahydroisoquinolino[2,1-b]isoquinolin-8-one		0.183	477	10	-0.49
10	ZINC09043139	1-[(3-chlorophenyl)-(3,4-dimethoxyphenyl)-hydroxy-BLAHyl]propan-1-one		0.513	517	9	-1.03

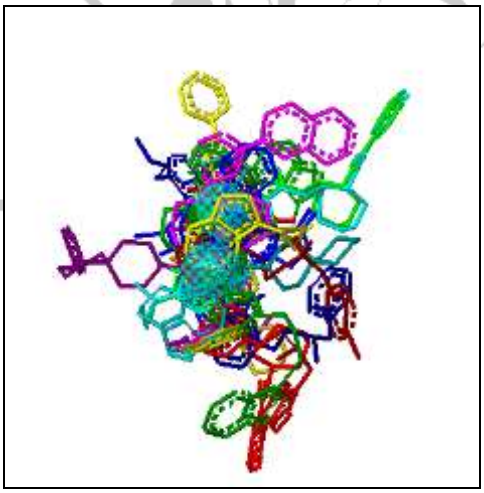
Table 5 Details of the ten molecular hits obtained using the top scoring serine threonine protein kinase Chk1-inhibitor-based pharmacophore model and their kinase activity scores (Compounds 2 and 6 has shown activity)

COMPOUND NO	ZINC ID	CHEMICAL NAME	2D STRUCTURE	RMSD	MASS	ROTATABLE BONDS	PREDICTED KINASE ACTIVITY SCORE
1.	ZINC45900691	methyl 4-[[2-ethyl-5-(4-oxo-5,6,7,8-tetrahydro-3H-phthalazin-1-yl)phenyl]sulfonylamino]benzoate		0.203	468	9	-0.35
2.	ZINC44226834	3-(2,5-dimethoxyphenyl)-N-[2-[4-(thiophene-2-carbonyl)piperazin-1-yl]ethyl]-1H-pyrazole-5-carboxamid		0.095	470	10	**0.03**
3.	ZINC38704408	(5Z)-5-(3,4-dimethyl-6-oxocyclohexa-2,4-dien-1-ylidene)-N-[4-(2-ethylpiperidin-1-yl)sulfonylphenyl]-1,2-dihydropyrazole-3-carboxamide		0.096	483	9	-0.19
4.	ZINC40336565	5-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-nitrophenyl)-3-phenyl-1,4-dihydropyrrolo[3,4-c]pyrazol-6-one		0.085	485	10	-0.44
5.	ZINC59850034	4-[2-[4-(4-benzylpiperazin-1-ium-1-yl)butylamino]-2-oxoethyl]-5-(3-methylphenyl)-1,2,4-triazole-3-thiolate		0.072	480	10	-0.41
6.	ZINC49506194	N-(1-benzoyl-3,4-dihydro-2H-quinolin-6-yl)-3-(2,5-dimethoxyphenyl)-1H-pyrazole-5-carboxamide		0.095	483	8	**0.04**
7.	ZINC40343666	3-(4-chlorophenyl)-5-cyclohexyl-4-[4-(3-methylbutoxy)phenyl]-1,4-dihydropyrrolo[3,4-c]pyrazol-6-one		0.084	478	9	-0.34
8.	ZINC40341179	(4R)-5-cyclohexyl-4-(3,4-diethoxyphenyl)-3-(p-tolyl)-1,4-dihydropyrrolo[3,4-c]pyrazol-6-one		0.086	460	10	-0.37
9.	ZINC09447235	2-[[4-amino-5-(3-phenyl-1H-pyrazol-5-yl)-1,2,4-triazol-3-yl]sulfanyl]-N-(2-chloro-4,6-dimethylphenyl)acetamide		0.179	454	8	-0.29
10	ZINC40337796	4-(3-ethoxy-4-hydroxyphenyl)-5-(3-morpholin-4-ylpropyl)-3-phenyl-1,4-dihydropyrrolo[3,4-c]pyrazol-6-one		0.086	463	10	-0.31

Figures

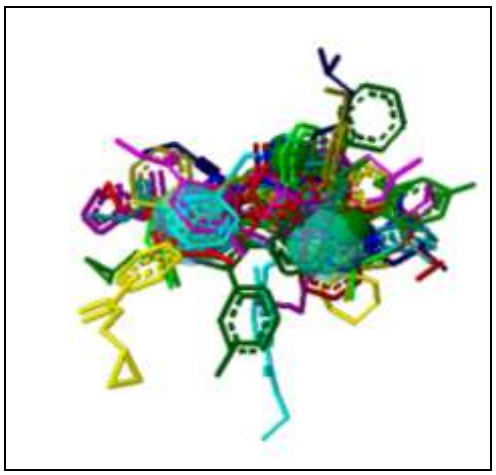
Score	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
38.184	4	4	2	0	1	1	0	0

Figure 1 Top scoring candidate pharmacophore model with assigned features of 20 cell division protein kinase2-inhibitors



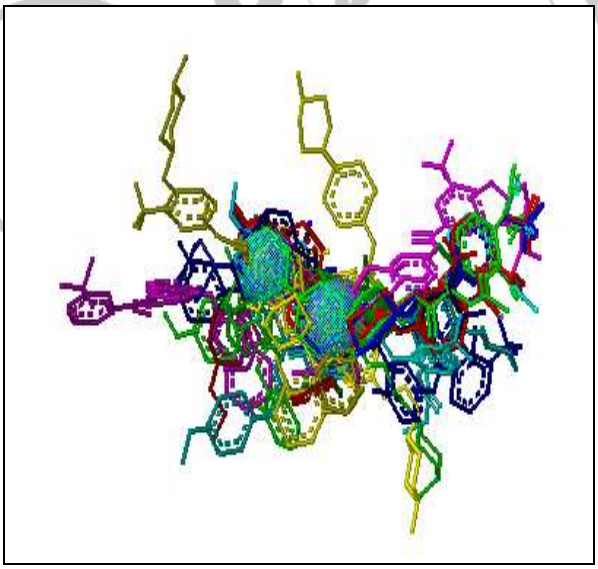
Score	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
34.369	3	3	2	0	0	1	0	0

Figure 2 Top scoring candidate pharmacophore model with assigned features of 20 cyclic AMP dependent protein kinase-inhibitors



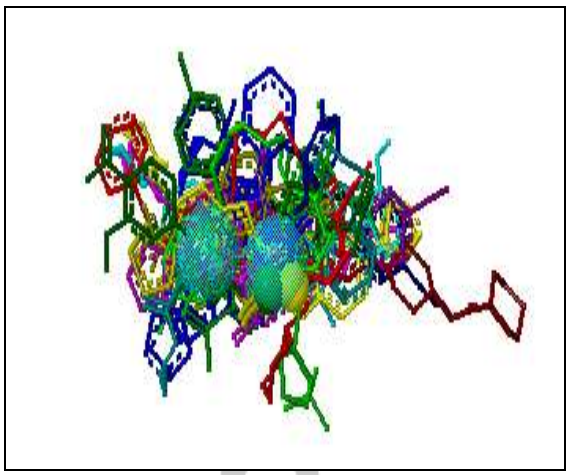
Score	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
36.000	4	4	2	0	0	2	0	0

Figure 3 Top scoring candidate pharmacophore model with assigned features of 20 mitogen activated protein kinase 14-inhibitors



Score	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
30.923	3	3	2	0	0	1	0	0

Figure 4 Top scoring candidate pharmacophore model with assigned features of 20 proto oncogene tyrosine protein kinase LCK –inhibitors



Score	Features	Spatial Features	Aromatic	Hydrophobic	Donors	Acceptors	Negatives	Positives
37.108	4	4	2	0	1	1	0	0

Figure 5 Top scoring candidate pharmacophore model with assigned features of 20 serine threonine protein kinase Chk1-inhibitors

Conclusion

Pharmacophore modeling of five different kinase-inhibitors belonging to five individual kinase families in the present work, highlights the presence of aromatic features in all of the kinases studied, thereby resembling the aromatic ring of the ATP molecule and ensuring the aromatic interactions. The hydrogen bond acceptor feature, too, is commonly found in all the five kinase-inhibitor classes. The similarities in the structural features of the kinase-inhibitors reflect their specificity to their respective targets and the differences highlight the selectivity between the kinase families. Certain compounds obtained by the pharmacophore-based searches show activity for kinase targets and could be used for docking studies. These hit compounds can be used as starters for kinase-inhibitor discovery. The methodolgy ought to be further refined for yielding improved results.

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