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Identification of novel inhibitors of human Chk1 using pharmacophore-based virtual screening and their evaluation as potential anti-cancer agents

Vikash Kumar · Saman Khan · Priyanka Gupta · Namrata Rastogi · Durga Prasad Mishra · Shakil Ahmed · Mohammad Imran Siddiqi

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Abstract Kinases are one of the major players in cancer development and progression. Serine threonine kinases such as human checkpoint kinase-1 (Chk1), Mek1 and cyclin-dependent kinases have been identified as promising targets for cancer treatment. Chk1 is an important kinase with vital role in cell cycle arrest and many potent inhibitors targeted to Chk1 have been reported and few are currently in clinical trials. Considering the emerging importance of Chk1 inhibitors in cancer treatment there is a need to widen the chemical space of Chk1 inhibitors. In this study, we are reporting an integrated in silico approach to identify novel competitive Chk1 inhibitors. A 4-features pharmacophore model was derived from a co-crystallized structure of known potent Chk1 inhibitor and subjected to screen Maybridge compound library. Hits obtained from the screening were docked into the Chk1 active site and filtered on the basis of docking score and the number of pharmacophoric features showing conserved interaction within the active site of Chk1. Further, five compounds from the top ranking hits were subjected to in vitro evaluation as Chk1 inhibitor. After the kinase assay, four compounds were found to be active against human Chk1 (IC_{50} range from 4.2 to 12.5 μM). Subsequent study using the cdc25-22 mutant yeast cells revealed that one of compound (SPB07479; $IC_{50} = 4.24$ μM) promoted the formation of multinucleated cells, therefore overriding the cell cycle checkpoint. Validation studies using normal and human cancer cell lines, indicated that SPB07479 significantly inhibited proliferation of cervical cancer cells as a single agent and chemosensitized glioma and pancreatic cancer cell lines to standard chemotherapy while sparing normal cells. Additionally SPB07479 did not show significant cytotoxicity in normal cells. In conclusion we report that SPB07479 appear promising for further development of Chk1 inhibitors. This study also highlights the role of conserved water molecules in the active site of Chk1 for the successful identification of novel inhibitors.

Keywords Pharmacophore-based screening · Chk1 inhibitor · Molecular docking · Anti-cancer agents · Molecular modelling

Vikash Kumar and Saman Khan have contributed equally to this article.

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V. Kumar · S. Khan · S. Ahmed · M. I. Siddiqi (✉)
Molecular and Structural Biology Division, CSIR-Central Drug Research Institute, Lucknow 226031, India
e-mail: imsiddiqi@yahoo.com

P. Gupta · N. Rastogi · D. P. Mishra
Endocrinology Division, CSIR-Central Drug Research Institute, Lucknow 226031, India

D. P. Mishra · S. Ahmed · M. I. Siddiqi
Academy of Scientific and Innovative Research, New Delhi, India

Introduction

Cancer is one of the most deadly diseases in the world. Each year millions of deaths are reported worldwide [1]. Large numbers of protein targets have been reported to be involved in cancer initiation and progression [2, 3] of which kinases are the most attractive [4, 5]. Inhibitors of tyrosine kinases such as epidermal growth factor receptor (EGFR) are marketed as drugs [6]. In recent years, interest has shifted to serine threonine kinases, many of which have been proved to play an

important role in carcinogenesis [7]. Serine threonine kinases are characterized by phosphorylation of serine/threonine residue on the substrate protein. Currently Chk1 [8], Mek1 [9], Pim1 [10], and CDKs [11] have been reported as some of the attractive targets for the development of inhibitors towards prevention of cancer progression.

Among them, Chk1 is a 476 amino acid long serine threonine kinase, which gets activated after DNA damage. N-terminal kinase domain harbour ATP binding pocket [12], which offers binding of competitive inhibitors. Chk1 inhibitors have been found to be promising anti-cancer agents when used with DNA damaging agents [13]. After DNA damage, cells relies on checkpoint proteins for initiation of DNA repair. Chk1 is one of such proteins which become activated after DNA damage response. Among serine threonine kinases, residues that are involved in ATP binding are highly conserved and it raises the selectivity issue during drug design and discovery [14]. However, few Chk1 inhibitors have been reported to show selectivity over other kinases [15, 16]. A Chk1 inhibitor, UCN-01 entered into the clinical trial but failed to demonstrate high level of clinical activity in combination of chemotherapeutic agents [17]. Recently, an allosteric site [18] has been characterized for Chk1 inhibition, and a series of thioquinazolinone allosteric inhibitors were identified using a HTS approach [19]. However, the reported inhibitors were found to be less potent as compared to the other reported competitive Chk1 inhibitors. Therefore, novel Chk1 inhibitors are urgently required, that have the potential to block cancer progression.

In silico techniques have helped in search and design of many potent anti-cancer agents. Particularly virtual screening is a widely used method to screen a large database in very short time. One approach to virtual screening is through pharmacophoric query. In previous studies reported in literature [20, 21] different sets of Chk1 inhibitors have been used to propose the pharmacophore models and subsequently these models were used for virtual screening. Pharmacophore models reported by these groups have been well described in terms of requirements of interactions for the potency and selectivity. However, the identified hits using these models were not evaluated experimentally. In the present study, we have used pharmacophore-based virtual screening and molecular docking studies, subsequently followed by biological evaluation of selected molecules to identify novel inhibitors of Chk1 with potential anti-cancer activity.

Materials and methods

3D-pharmacophore model generation

Generally ligand-based 3D-pharmacophore construction involves the alignment of more than one diverse, active molecules, to identify key features that are involved in binding to the receptor. Pharmacophore can be also built using a receptor structure, a method called as a receptor-based pharmacophore modelling. When the crystal structure of the target with its ligand is available, then both ligand and receptor-based approach can be exploited to derive a more useful pharmacophore model. Here, we have considered a co-crystallized conformation of a potent competitive Chk1 inhibitor [22, 23] for 3D-pharmacophore generation. Crystal structure of Chk1 with compound 1 (PDB entry code 3PA5) was retrieved from PDB and its interaction with ATP binding pocket was thoroughly investigated using Sybyl7.1 software [25].

Apart from this, we also looked into a reported thienopyridine (TP) series [23], for the presence of common pharmacophoric features required for Chk1 inhibition. 3D-pharmacophore model was finally generated manually using bound conformation of compound 1 with the help of Unity module provided in Sybyl7.1 [24]. Further, spatial and distance constraints were applied to the generated pharmacophore.

Pharmacophore-based screening

We utilised the constructed 3D-pharmacophore for virtual screening against Maybridge database. The database contains approximately 56,000 drugs like compounds that can be procured for biological evaluation. The 3D-pharmacophore search was performed by using the flexible search protocol available in Unity module [24], with all options set as default. In the Unity search, the conformations of the screening database were generated on the fly by means of the Directed Tweak method [25].

Molecular docking

To further narrow down the number of screened hits, molecular docking was carried out using FlexX [26, 27]. FlexX uses an incremental approach [26] to build the conformation of ligand in the binding pocket and the poses are scored based on a variety of different scoring functions. Performance of docking protocol was validated by re-docking of compound 70 (PDB entry code: 3PA3) into the active site of Chk1. Conserved water molecules were also considered during docking process. After successful re-docking of bound conformation of compound 70 with rmsd <1 Å, all the 1,067 screened hits were docked using the same receptor parameters. For each hit a total 30 docked conformations were generated.

Cell cycle checkpoint assay

The survival assay of *cdc25-22* mutant was performed to find out loss of survival at higher temperature in the

presence of different compounds. Mid log phase cultures were grown at 25 °C and then one thousand cells were spread on plates containing 20 μg/ml of each compound. The plates were incubated to 36 °C for different time interval before shifting to 25 °C for 4–5 days. The colonies were counted manually and their percent survival was calculated. The relative percent survival was plotted as bar graph. For passing mitosis experiment the mid log phase culture of *cdc25-22* mutant cells were shifted to 36 °C in the presence or absence of compound for 12 h. Cells were fixed with 70 % ethanol, nuclei were stained with DAPI and visualized using a fluorescence microscope. About 200 cells were counted and percentage of bi or multi nucleated cells was calculated as a measure of cells passing mitosis.

In vitro kinase assay

ADP-Glo + CHK1 Kinase Assay System (cat.#V1941) were purchased from Promega. Compounds were screened at different concentrations (from 1 to 10 μM) using Kinase enzyme system protocol as described by manufacturer. In brief, 1× reaction buffer containing 1 μg Chktide, 50 μM ATP, 50 μM DTT and 0.1 μg Chk1 were added sequentially to 96 well assay plate with 0–10 μM concentration of compound in a total volume of 10 μl. The plate was incubated at room temperature for 1 h. 10 μl of ADP Glo reagent was added and plate was further incubated at room temperature for 40 min. Finally 20 μl Detection reagent was added and plate was incubated at room temperature for 30-60 min. The readings were recorded by GloMax® 96 Microplate Luminometer.

Cell culture

The glioma (A172, U87MG and T98G) and pancreatic (MiPaCa, PANC-1 and BxPC-3) cancer cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The glioma and pancreatic cells were maintained in MEM and RPMI-1640 media respectively supplemented with 10 % fetal bovine serum both from Gibco (Carlsbad, CA, USA) along with 1 % penicillin and streptomycin from Sigma (St. Louis, MO, USA) in a humidified incubator at 37 °C with 5 % CO_2.

Cell viability assay

Assessment of chemosensitizing activity in term of cell viability was performed by using the PE Annexin V apoptosis detection kit I (BD Pharmingen, CA, USA) according to manufacturer's protocol. Briefly cells were seeded in six well plates and treated with indicated dose of compound D alone or in combination with MEK ½ inhibitor CI-1040 (PD184352) (1 μM) and Gecitabine (1 μM) for 48 h. Thereafter, cells were harvested with trypsinization and washed twice with ice cold PBS. Cell pellet was dissolved in 100 μl of 1X binding buffer containing 5 μl of Annexin-PE and 5 μl of 7-AAD. Cells were incubated for 15 min in dark at the room temperature. After incubation, 400 μl of 1X binding buffer was added to each sample and analyzed using the flow cytometer (FACS Caliber, BD Biosciences).

Ligand efficiency (LE) and chemical similarity comparison with known Chk1 inhibitors

Experimental hits were compared with few known potent Chk1 inhibitors in terms of LE and chemical similarity. For LE calculation we used the given formula [30–32].

$$LE = 1.37(PIC_{50})/HAC$$

where PIC_{50} is inverse logarithm of IC_{50} and HAC is heavy atoms count.It is common practice to substitute the Kd value with IC_{50}, because later is known in most of cases [32]. We have also calculated the binding efficiency index (BEI), which gives the relation between binding energy of ligands with their corresponding molecular weight [33].

$$BEI = PIC_{50}/\text{molecular weight}$$

Chemical similarity between pair of molecules can be measured both quantitatively and qualitatively. Tanimoto coefficient (T_C) is most widely used to measure the pairwise similarity of molecules. 3D structures of known Chk1 inhibitors were constructed using Marvin Sketch program [http://www.chemaxon.com/products/marvin/marvinsketch/] and OpenBabel [http://openbabel.org/] command line utility was used to calculate the Tanimoto similarity. In OpenBabel, FP2 (default) and MACCS fingerprint [34] option were selected. For the given molecule A and B, T_C was calculated using following formula.

$$T_C = c/(a + b + c)$$

where c = count of bits on in both molecule A and molecule B, a = count of bits on in molecule A, b = count of bits on in molecule B.

Results and discussion

Pharmacophore modeling and virtual screening

Availability of inhibitor bound protein structure provides an excellent opportunity to harness the information related to their interaction and it will be advantageous to incorporate the derived information in structure-based drug design such as pharmacophore modeling and molecular docking. Moreover, it has been observed that in general

ligand bound protein structures (holo) show better enrichment of known actives than the apo proteins [35]. In such cases, consideration of inhibitor bound protein structures in virtual screening may increase the chance of getting active hits.

For construction of 3D-pharmacophore model in this work, we have adopted both structure and ligand based approaches. X-ray crystal structure of Chk1 in complex compound 1 (PDB entry code: 3PA5) was used for the generation of pharmacophore model. In this protein–ligand complex, (Fig. 1a) compound 1 makes direct H-bond interaction with residues Glu85, Cys87, Asp148, Asn135 and Glu134. In addition there are also water mediated hydrogen bonds with residues Glu91 and Ser147. The compound has two aromatic rings, one of them is a five-membered thiophene ring and other one is a phenyl ring, which occupy the hydrophobic cavity lined by residues Leu15 and Leu137. Among the reported series [23] of TP, which is based on compound 1, two of them (compound 2a and compound 70) have co-crystallized conformation (PDB entry codes 3PA4, 3PA3 respectively) with Chk1 [23]. Both the compound 2a and 70 are nanomolar inhibitors of Chk1. In the crystal structures, both the compounds occupy the same position in the active site of Chk1 and also show the interactions similar to that of compound 1. In all the three crystal structures, the selectivity pocket [28] has two conserved water molecules and are involved in direct hydrogen bonds with the bound inhibitors. These water molecules have been reported to play important role in inhibitor selectivity and potency [28, 29]. If we look into the TP series, all molecules have four features in common, i.e., two H-bond acceptors (HAA), one H-bond donor (HBD) and one aromatic ring (A). TP series has total of 56 molecules covering the wide range of activity (1–27143 nM). After looking into the reported structure activity relationship, we speculated that TP series requires minimal of four features to inhibit the Chk1 kinase activity up to the micromolar range. Based on above information, after mapping all four features on compound 1, we constructed a four features pharmacophore model (Fig. 1b). The pharmacophore model appears unique as one of acceptor feature, which we selected, was based on direct H-bond with conserved water molecule. Spatial and distance constraints were applied to make the pharmacophore model more realistic and restrictive. It is noteworthy that the developed 3D-pharmacophore model is restrictive and hence it can pick only those compounds fulfilling the features and constraints present in the model. As for the validation is concerned, we prepared a set of 560 decoys (Fig. S1, Please refer supplementary information for details) using DecoyFinder tool (www.urvnutrigenomica-ctns.github.io/DecoyFinder) and merged it into the WOMBAT (www.sunsetmolecular.com) dataset (containing 1,204 molecules). Decoys have similar features like actives, but they are assumed as inactives. The WOMBAT dataset contains active molecules for many pharmacological targets except Chk1. We also added the 56 active molecules belonging to the TP series to the validation dataset. 3D-pharmacophore model was then used as a query to search final validation dataset containing 1,820 molecules. The proposed pharmacophre model picked 55 active molecules (all from TP series) and none from the inactive subset (560 + 1,204) of validation dataset. After validation, pharmacophore-based screening of Maybridge database resulted in mapping of a total 1,067 hits on to the proposed pharmacophore model.

Molecular docking

Hits obtained from the virtual screening were based on feature matching and not necessarily represent the active conformation inside the ATP binding pocket. Many of these hits may have unfavourable interaction, so we applied docking simulation to discriminate the potential non-active hits. Although docking simulation does not efficiently distinguishes between actives and non-actives, but approach is widely used to reduce the size of hits as well as to identify and rank the potential binders (active molecules). All 1,067 hits were subjected to FlexX docking. In current docking study we have used one of previously mentioned three crystal structures of Chk1 kinase domain. This structure (PDB entry code 3PA3) has very good resolution and it has compound 70 in the ATP binding pocket. In redocking experiment, conformation of compound 70 was successfully reproduced, close to that of the co-crystallized conformation. We observed that during docking simulation, inclusion of two conserved water molecule, enhanced the reproducibility of experimental conformation, so we decided to include them during docking of all hits. Top 100 hits were selected on the basis of FlexX docking score. For each of selected hits, 30 docked conformations were analyzed for interaction with Chk1 active site residues. Interestingly most of docked conformations of hits occupied the same position as observed in the case of compound 70. After considering the number of pharmacophoric interactions retained during the docking simulation and overall binding of the compounds in the Chk1 active site, five compounds were further selected for biological evaluation from among the top ranking molecules. Structures and docking scores of the hits selected for biological evaluation are listed in Table 1.

Inhibition of kinase activity of Chk1 by novel compound and their effect on cell cycle progression

An in vitro kinase assay kit (ADP-Glo + CHK1 Kinase Assay System, Promega) was used to monitor phosphorylation of Chk1 substrate (Chktide) by purified Chk1 protein

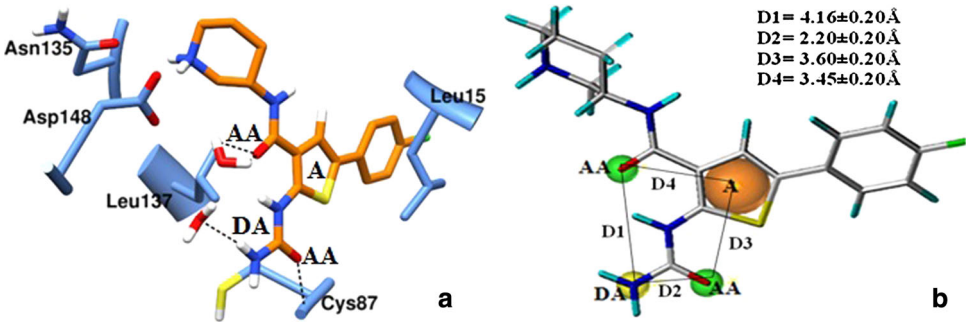


Fig. 1 **a** Conformation of compound 1 (*orange*) in the ATP binding pocket of Chk1 (PDB entry code 3PA5). Important interactions which were considered for pharmacophore development are shown. Two conserved crystallographic water molecules are shown. H-bonds are shown in black dotted lines. For clear representation only polar hydrogens are displayed. For image generation USCF Chimera was used. **b** Pharmacophoric features with spatial and distance constraints mapped on compound 1 (*AA* acceptor atom, *DA* donor atom, *A* aromatic ring)

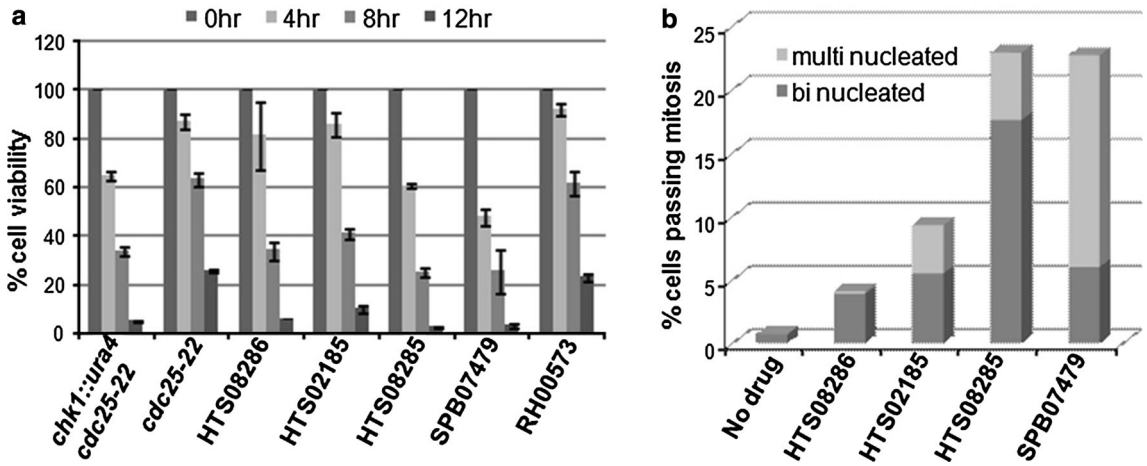
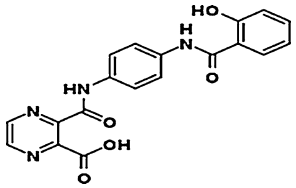
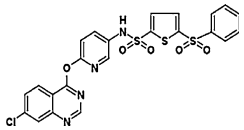
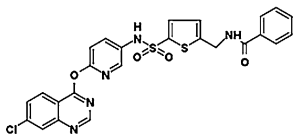
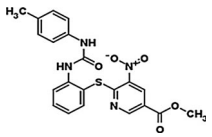
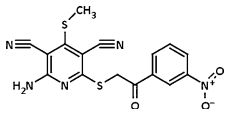


Fig. 2 The *cdc25-22* mutant cells treated with putative Chk1 inhibitors fail to arrest in G2 stage of the cell cycle. **a** The survival assay of *cdc25-22* cells in presence and absence of compound was monitored as a measure of Chk1 inhibition as described in "Materials and methods" section. **b** Percentage of cells that fail to arrest in G2 after treatment with compound was monitored by counting the number of cells having two or more nuclei. For each sample about 200 cells were counted and percentage was plotted

as described by manufacturer and detailed in "Material and method" section. Further we determined the effect of compounds that inhibited the kinase activity of Chk1 in vitro and percent inhibition was calculated. Interestingly, we found that four compounds out of five selected virtual screening hits showed micromolar inhibition of Chk1 activity. As shown in Table 1 the most potent inhibitor was compound SPB07479 with an IC_{50} of 4.2 μM. The compound HTS08286 and HTS 02185 had an IC_{50} of 5–6 μM range while IC_{50} of compound HTS08285 was 12.7 μM. The compound RH00573 did not give any significant inhibition at micromolar concentration.

Cdc25 is a phosphatase that activates cyclin dependent kinase by removing phosphate at tyrosine residues in the Cdk active site and hence acts as an inducer of mitosis. In fission yeast *S. pombe* the *cdc25-22* mutant cells arrest in G2 at the restrictive temperature because they cannot execute dephosphorylation of Cdk protein and hence prevent onset of mitosis. In response to DNA damage the checkpoint kinase Chk1 get phosphorylated and activates *cdc25* that in turn inhibit the mitotic entry [36–38]. Chk1 also phosphorylates Cdc25 at Serine 99 residue and inhibit its activity in an in vitro assay [39]. Cells having temperature sensitive mutant allele of *cdc25-22* arrest in G2 stage of the cell cycle when shifted at 36 °C, at permissive temperature (25 °C) these cells are able to resume their cell cycle progression. In absence of *chk1, cdc25-22* mutant cells fail to arrest at 36 °C and hence unable to form colonies when shifted back to normal temperature 25 °C (Fig. 2a). We reasoned that inhibition of Chk1 with the inhibitor should result in loss of survival with the same rate as in the case with *chk1* knockout cells. As presented in Fig. 2a the viability was reduced in *cdc25-22* mutant when these cells were treated with the compound except in case

Table 1 Selected hits for experimental evaluation

Sr. No.	Compound code	Structure	FlexX score	Chk1 kinase assay (IC_{50})
1.	HTS02185		−34.956	5.43 μM
2.	HTS08285		−33.591	12.7 μM
3.	HTS08286		−35.292	5.95 μM
4.	SPB07479		−26.89	4.24 μM
5.	RH00573		−26.846	ND

of compound RH00573 that has significant survival even after exposing the cells up to 12 h indicating that the inhibition of Chk1 kinase by these compound in *cdc25-22* mutant background could lead to the failure of G2 arrest and subsequently affect their viability. The defect in G2 arrest can lead to the premature mitosis with binucleated cells. We observed the appearance of bi or multi-nucleated cells as measure of defective G2 arrest in *cdc25-22* mutant cells after treatment with compounds. The mutant cells without compound treatment at 36 °C were arrested at G2 phase with single nuclei (Fig. 2b). The compound HTS08286 and HTS02185 exhibited about 4 and ninefold increase in multi nucleated cells respectively. The maximum failure of G2 arrest was observed in the cells treated with compound HTS08285 and SPB07479 having about 22 % multi nucleated cells (Fig. 2b).

Based on the results obtained we speculate that the compound SPB07479 looks more promising as it has low IC_{50} as well as exhibits cell cycle checkpoint defects that are related to Chk1 inhibition. Though the compound HTS08285S exhibit high level of checkpoint defects (22 % of multinucleated cells) but high IC_{50} (12.7 μM) associated with the compound indicates that the loss of survival might be due to the inhibition of other components of the cell cycle checkpoint pathway. The other two compound HTS08286 and HTS02185 exhibit comparable low IC_{50} and Chk1 dependent checkpoint defects indicating that these two compounds could also be a promising Chk1 inhibitor.

Compounds showing Chk1 inhibitory activity induce chemosensitization in cancer cells

The development of resistance to chemotherapeutic agents is one of the major bottle necks of cancer therapy, making it imperative to look for effective chemosensitizers. The Chk1 overexpression is associated with poor outcomes and may contribute to therapy resistance. The inhibition of Chk1 has been implicated in the potentiation of the cytotoxic actions of chemotherapeutic drugs in cancer cells [40, 41]. In continuation with earlier finding regarding the Chk1 inhibition, all the compounds selected for biological evaluation showed inhibition at different dosage and the compound SPB07479 demonstrated most potent chemosensitizing activity in glioma (Fig. 3a, b) and pancreatic cancer cells (Fig. 3c, d) to the standard chemotherapeutic drugs. Interestingly, this compound showed no significant cytotoxicity in normal cells. The chemosensitization effect of this compound in glioma and pancreatic cells appears to be mediated through its ability to modulate multiple cell-signaling molecules and pathways. These preliminary studies suggest that this compound may possibly act as a chemo-

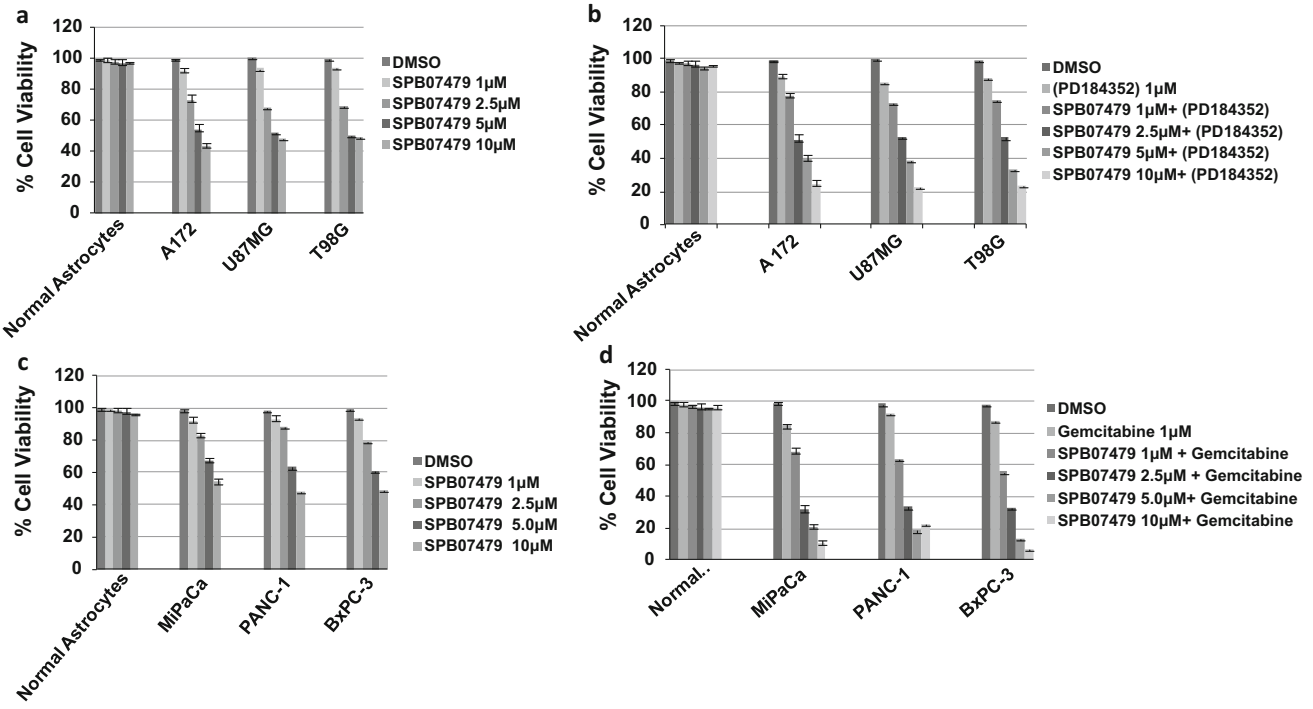


Fig. 3 Chk 1 inhibitor SPB07479 enhanced the efficacy of standard chemotherapeutic drugs cancer cell lines. **a** Glioma cell lines treated with various concentrations of SPB07479 alone or **b** in combination with 1 μM of the $MEK^{1/2}$ inhibitor (PD184352). **c** Pancreatic cancer cells treated with various concentration of SPB07479 alone or **d** in combination with 1 μM Gemcitabine. Cells were treated for 48 h and percentage of cell viability was calculated. Data represented as percent cell viability (mean ± SEM) and representative to three independent experiment

sensitizer and may improve the efficacy of standard chemotherapeutic drugs in glioma and pancreatic cancer cells.

Binding pose of experimental hits

Figure 4 shows binding mode predicted by FlexX for the compounds exhibiting biological activity. It has been observed that all of the compounds snugly occupy the ATP binding pocket and make interaction in a similar manner with the residues previously reported for binding of Chk1 inhibitors. SPB07479 (Fig. 4d) showed H-bonds with Tyr86, Cys87, Ser88 and W463. One of aromatic rings occupies the hydrophobic cavity lined by Leu15 and Leu137. Binding pose of HTS02185 (Fig. 4a) showed H-bond with Cys87, Glu17 and W441. Here also one of aromatic rings was observed to occupy the hydrophobic pocket.

Being similar in structure HTS08285 (Fig. 4b) and HTS08286 (Fig. 4c) showed many common interactions, i.e., both were involved in H-bonding with Cys87, Asn135, Glu17 and W463 and showed the aromatic ring placed in the hydrophobic pocket. Additionally HTS08285 also exhibited H-bond with Lys132 and W441. Similarly HTS08286 showed H-bond with Asp148. In the experimental assay, IC_{50} of HTS08285 was found to be less than that of HTS08286, which may be due to the steric effect caused by replacement of amide group by bulky—SO_2 group at the one end of molecule.

From above interaction study, we found that Cys87 plays an important role in inhibitor binding as all the experimentally evaluated compounds showed H-bonding with—NH group of main chain. Also in all the cases, we observed H-bond with the conserved water molecule/s and presence of an aromatic ring in the hydrophobic cavity. Hence predicted binding pose of experimental hits showed that at least three pharmacophoric features (two acceptors and one aromatic) are in good agreement with the proposed 3D-pharmacophore model (Supplementary fig. S2 and S3). Overall docked pose of hits appeared reasonable and supported our pharmacophore hypothesis.

LE and chemical similarity analysis of experimental hits

LE has become a useful parameter during hit to lead optimization and lead selection [31, 32]. When large number of hits are available then LE can be used to prioritize them on the basis of predicted docking score/

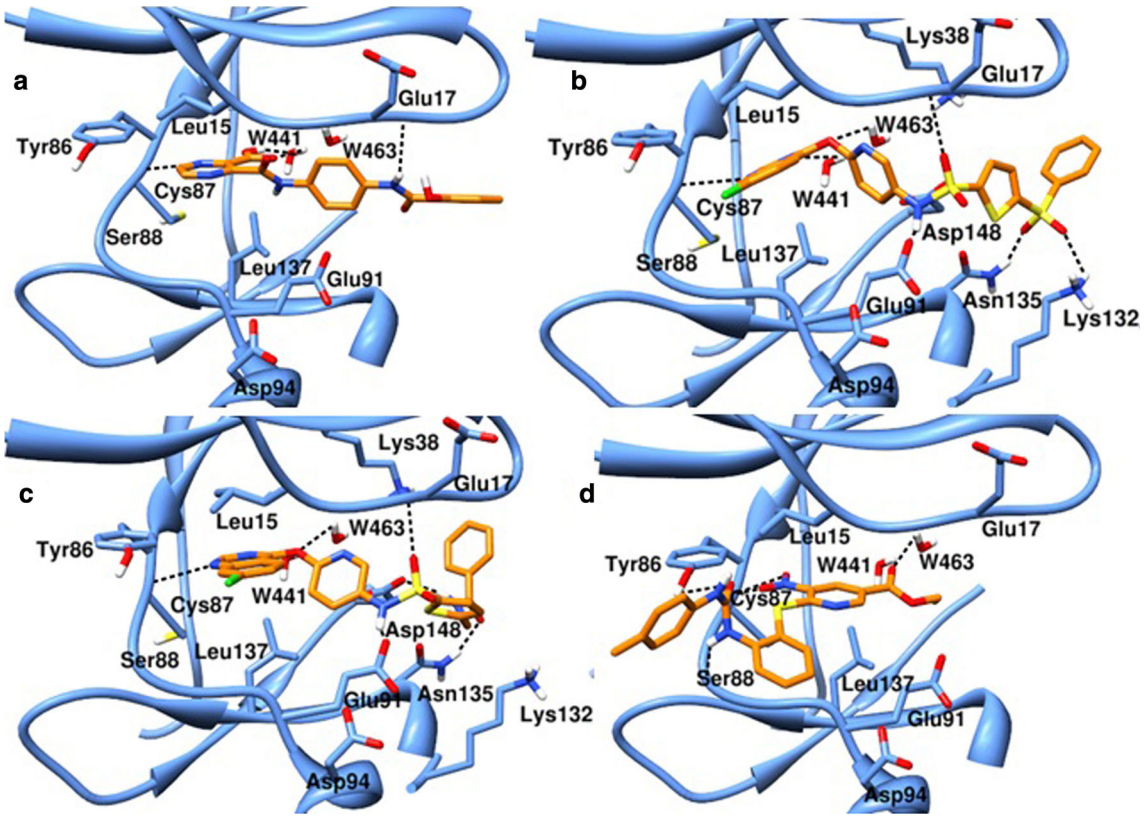


Fig. 4 **a** HTS02185 **b** HTS08285 **c** HTS08286 and **d** SPB07479 in the ATP binding pocket of Chk1. For image generation UCSF Chimera was used

binding energy (ΔG) and the number of heavy atoms. Interestingly, in the present study when FlexX score of identified hits was divided by their corresponding HAC and assumed as LE, we see the correlation between assumed LE and experimental IC_{50} value for three hits in the order of HTS02185 > HTS08286 > HTS08285. However, when we considered only the FlexX score, the above correlation was not observed. When binding energy data are not available then either IC_{50}, Ki or Kd values can be also used. For drug candidates the lower acceptable values of LE have been proposed to be ≥0.3 [32]. In contrast, hits obtained through virtual screening do not necessarily show good LE. In present study, we have calculated and compared the LE values of confirmed hits with the few known inhibitors of Chk1 (Table 2). HTS02185 and SPB07479 showed LE close to the acceptable value (0.3), while other two hits have very low LE. An extension to LE is BEI, which considers the types of heteroatoms in the compound in terms of molecular weight (kDa) [33]. All active hits showed low BEI value (Table 2). It is noteworthy that during hit to lead evolution, LE and BEI can show improvement [42]. Chemical similarity comparison (Table S1) with known Chk1 inhibitors revealed very low values of Tanimoto coefficient (<0.5), indicating that active hits are dissimilar to known Chk1 inhibitors and may provide novel scaffold for further optimization.

Conclusions

In this study, an integrated pharmacophore based virtual screening and molecular docking approach has been successfully applied, to identify novel inhibitors of human Chk1 in the micromolar range. In addition, we have shown that consideration of conserved water molecules during pharmacophore generation and molecular docking indeed helps in finding novel inhibitors. Moreover, our VS workflow clearly indicates that, if implemented rationally, in silico methods are highly efficient in identification of novel hits. Although active hits showed low LE and BEI, they can be further optimized to design potent inhibitors of Chk1. Experimental studies showed that one of the hits SPB07479 exhibited low IC_{50} against human Chk1 as well as multi-nucleation in yeast cell and demonstrated most potent chemosensitizing activity in glioma and pancreatic cancer cells to the standard chemotherapeutic drugs. In

Table 2 LE and BEI of active hits and known inhibitors of Chk1

Ligand name	Heavy atoms count	Molecular weight	PIC_{50}	LE	BEI	Ref.
HTS02185	28	378.09	5.272	0.257	13.94	–
HTS08285	36	557.98	4.896	0.186	8.77	–
HTS08286	37	551.04	5.225	0.193	9.48	–
SPB07479	31	438.09	5.372	0.237	13.08	–
SCH900776	23	375.08	8.522	0.506	22.72	[43]
LY2606368	27	365.16	8.823	0.446	24.16	[44, 45]
SAR-020106	27	382.13	7.876	0.398	20.61	[46]
Compound 1	25	380.10	8.698	0.475	22.88	[23]
Compound 60	26	386.09	5.339	0.281	13.82	[23]
Compound 5	25	343.28	4.872	0.266	14.19	[47]
Compound 15	27	351.14	3.692	0.187	10.51	[48]
Compound 25	31	439.17	10.301	0.455	23.45	[49]

addition SPB07479 showed no significant toxicity in normal cells. Above finding supports that SPB07479 can be further explored to design and develop more potent Chk1 inhibitors.

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