Discovery of Check Point Kinase 1 (Chk1) Inhibitors as Potential Anticancer Agents Using Ligand-Based Modelling and Virtual Screening

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Abstract
Check point kinase 1 (Chk1) is an important protein in the G2 phase required by cancer cells to maintain cell cycle and to prevent cell death. Accordingly, inhibitors of this kinase should have potent anti-cancer properties. Pharmacophoric space of 190 Chk1 inhibitors using seven diverse sets of inhibitors was explored to identify high-quality pharmacophores. Subsequently, genetic algorithm-based quantitative structure activity relationship (GA-QSAR) analysis was employed to select the best possible combination of pharmacophoric models and physicochemical descriptors that can explain bioactivity variation within the training inhibitors. Three successful orthogonal pharmacophores emerged in the optimum QSAR equation (r² = 0.54, r²c = 0.52, F = 20.27, r²PRESS against 38 test inhibitors = 0.50). The QSAR-selected pharmacophores were validated using receiver operating characteristic (ROC) curve analyses. Moreover, the QSAR-selected pharmacophores describe binding interactions comparable to those seen in crystallographic structures of bound ligands within Chk1 binding pocket. The three pharmacophoric models and associated QSAR equation were applied to screen the national cancer institute (NCI) list of compounds. The captured hits were tested in vitro and new anti-Chk1 hits were discovered.

Keywords: Check point kinase 1; Ligand based analysis; Serine-threonine kinase; Anticancer; Anti-inflammatory.

Introduction
Check point kinase 1 (Chk1) is a serine-threonine kinase that has an important role in repairing DNA damage and prevents cells from entering mitosis where DNA damage exists [1]. In fact, ionizing radiation and cytotoxic drugs along with many other DNA damaging reagents are expected to cause cell cycle arrest by various cell cycle checkpoints as a normal outcome in both normal and tumor cells [2]. Cell cycle checkpoints will temporarily stop the progression of cell cycle, to allow time for the repair of the DNA damage in order to maintain the genomic integrity and the survival of the cells. From a therapeutic point of view, Chk1-mediated S or G2 checkpoint arrests in tumor cells will limit the efficacy of radiation therapy and cytotoxic drugs leading to drug resistance. On the other hand, it has been suggested that Chk1 inhibition would preferentially sensitize tumors to DNA damaging agents, where 50% of the tumor cells are stated to be deficient in the protein P53 that causes DNA-damage-induced G1 arrest, but has minor role on the S or G2 checkpoints [3,4]. Cells depend normally on arrest at G1 phase for repair of DNA damage, while p53-mutated tumor cells depend on the cell cycle arrest and consequent DNA damage repair by S and G2 checkpoints action due to their incapability of G1 arrest. As a result, specific inhibition of S and G2 arrests through inhibition of Chk1 is expected to selectively drive p53-deficient tumor cells to enter mitotic catastrophe and finally apoptosis following DNA damage [5,6]. This assumption has been confirmed recently by the reported increase in effectiveness of DNA-damaging agents when given to p53-mutated tumor cells after knocking down their Chk1 using small interfering RNA (siRNA) or Chk1 antisense.
technique [7-9], or even if inhibited by a natural product such as 7-hydroxystaurosporine (UCN-01) [10,11], which proves Chk1 to be a target for selective chemosensitization. The continued interest in designing new Chk1 inhibitors prompted us to explore the possibility of developing ligand-based three-dimensional (3D) pharmacophore(s) integrated within self-consistent QSAR model. This approach avoids the pitfalls of structure-based techniques; furthermore, the pharmacophore model(s) can be used as 3D search queries to discover new Chk1 inhibitory scaffolds. We previously reported the use of this innovative approach towards the discovery of new inhibitory leads against glycogen synthase kinase-3, [12] bacterial MurF [13], protein tyrosine phosphatase [14], DPP IV [15], hormone sensitive lipase [16], β-secretase [17], influenza neuraminidase [18], migration inhibitory factor [19], Cyclin dependent kinase inhibitors (CDK1) [20], Heat Shock Protein 90 (Hsp90) [21] and Urokinase Plasminogen Activator (uPA) [22].

Method

Molecular modeling

Pharmacophore and QSAR modeling studies were performed using CATALYST (HYPOGEN module) [27] and CERIUS2 software suites implemented in Discovery Studio 2.5.5 from Accelrys Inc. (San Diego, California, www.accelrys.com). Structure drawing was performed employing ChemDraw Ultra 7.0 (Cambridge Soft Corp. (http://www.cambridgesoft.com), USA).

Data set and conformational analysis

The structures of 190 Chk1 inhibitors were collected from published literature [23-26]. The ligands’ structures list was carefully gathered to make sure that they were bioassayed under similar conditions, which should allow proper QSAR correlation. The in vitro bioactivities of the collected inhibitors were expressed as the concentration of the test compound that results 50% drop in the activity of Chk1 enzyme, i.e. IC_{50}. Figure 1 and Table 1 (under the Supplementary Materials) shows the structures and IC_{50} values of the collected inhibitors. The two-dimensional (2D) chemical structures of the inhibitors were sketched using ChemDraw Ultra and saved in MDL-molfile format. Subsequently, they were imported into Discover Studio 2.5.5, converted into corresponding standard 3D structures and energy minimized to the closest local minimum using the molecular mechanics CHARMm force field implemented in Discover Studio 2.5.5. The conformational space of collected each inhibitor (1-190, Figure 1, Table 1 under supplementary material) was explored adopting the “best conformer generation” option within Discover Studio 2.5.5 [27] based on the generalized CHARMm force field implemented in the program. Default parameters were employed in the conformation generation procedure of training compounds and screened libraries, i.e., a conformational ensemble was generated with an energy threshold of 20 kcal/mol from the local minimized structure at which has the lowest energy level and a maximum limit of 250 conformers per molecule [27,28].

Generation and assessment of binding hypotheses

All 190 molecules with their associated conformational models were grouped into a spreadsheet. The biological data of the inhibitors were reported with an “Uncertainty” value of 3, which means that the actual bioactivity of a particular inhibitor is assumed to be situated somewhere in an interval ranging from one-third to three-times the reported bioactivity value of that inhibitor [29,30]. Subsequently, seven structurally diverse training subsets were selected: subsets I-VII shown in Table 2 under Supplementary Materials. The selected training sets were utilized to conduct 28 modeling runs to explore the pharmacophoric space of Chk1 inhibitors. Table 3 under Supplementary Materials shows the training subsets and different parameters implemented for each pharmacophore exploration run. The exploration process included altering number and type of possible binding features (Hydrogen bond acceptors, hydrogen bond donors, aromatic rings, ionizable groups and hydrophobic features, positive ionizable and negative ionizable), feature spacing parameter (100 and 300 picometers) and the maximum number of allowed features in the resulting pharmacophore hypotheses. Pharmacophore modeling employing Hypogen proceeds through three succeeding steps: the constructive phase, subtractive phase and optimization phase (see Hypogen Modeling Algorithm under section SM-1 in Supplementary Materials) [27-33]. In the optimization phase, Hypogen attempts to minimize a cost function for each hypotheses consisting of three terms: Weight cost, Error cost and Configuration cost (see Hypogen Cost Analysis in Assessment of Generated Binding Hypotheses under section SM-2 in Supplementary Materials). HYPOGEN
cross-validates the resulting optimal pharmacophores using the Cat-Scramble module implemented in Discover Studio 2.5.5. This validation procedure is based on Fischer’s randomization test [34]. In this validation test; we selected a 95% confidence level, which instruct Hypogen to generate 19 random spreadsheets by the Cat-Scramble command. Subsequently, HYPOGEN is challenged to use these random spreadsheets to generate hypotheses using exactly the same features and parameters used in generating the initial unscrambled hypotheses. Success in generating pharmacophores of comparable cost criteria to those produced by the original unscrambled data reduces the confidence in the training compounds and the unscrambled original pharmacophore models [27,34,35]. Based on Fischer randomization criteria; 280 pharmacophores exceeded the 95% significance threshold for subsequent processing. Table 4 under supplementary materials shows different cost criteria and significance levels of representative pharmacophoric hypotheses (see pharmacophore clustering under QSAR modeling section).

**QSAR modeling**

QSAR modeling commenced by selecting a subset of 152 compounds from the total list of inhibitors (1-190, Figure. 1, Table 1 under Supplementary Materials) as a training set for QSAR modeling; the remaining 38 molecules (ca. 20% of the dataset) were employed as an external test subset for validating the QSAR models. The test molecules were selected as follows: all 190 inhibitors were ranked according to their IC_{50} values, and then every fifth compound was selected for the test set starting from the high-potency end. The selected test molecules should represent similar range of biological activities to that of the training set. The selected test inhibitors are marked with asterisks in Table 1 under Supplementary Materials. The logarithm of measured IC_{50} (µM) values was used in QSAR, thus correlating the data linear to the free energy change. Subsequently, we implemented genetic algorithm and multiple linear regression analyses to select optimal combination of pharmacophoric models and other physicochemical descriptors capable of self-consistent and predictive QSAR model. Section SM-3 under Supplementary Materials describes extensively the experimental details of QSAR modeling procedure [27,36].

**Addition of exclusion volumes**

To account for the steric constraints of the binding pocket we decided to complement pharmacophore from our QSAR-selected pharmacophore models (i.e., Hypo1/4, Hypo11/5 and Hypo28/6) with exclusion volumes employing HipHop-Refine module of Discover Studio 2.5.5. HipHop-Refine uses inactive training compounds to construct excluded volumes that resemble the steric constrains of the binding pocket. It identifies spaces occupied by the conformations of inactive compounds and free from active ones. These regions are then filled with excluded volumes [12-14,27]. Subset VIII (in Table 4 under Supplementary Material) was used to construct exclusion spheres around Hypo1/4, Hypo11/5 and Hypo28/6 pharmacophores. Section SM-4 under Supplementary Materials describes in details the Hip-Hop-Refine algorithm and settings implemented herein to decorate hypotheses with exclusion spheres. The resulting sterically-refined pharmacophores, as well as their unrefined versions, were validated by receiver operating characteristic curve analysis (ROC). [37-39]. Theoretical and experimental details of this procedure are as shown in section SM-5 under Supplementary Material.

**In Silico screening for new Chk1 inhibitors**

The sterically refined version of Hypo1/4, Hypo11/5 and Hypo28/6 pharmacophores were employed as 3D search query to screen the National Cancer Institute (NCI) database. The screening was done employing "Best Flexible Database Search" option implemented within Discover Studio 2.5.5. Captured hits were filtered according to Lipinski’s [40] and Veber’s [41] rules. Remaining hits were fitted against Hypo1/4, Hypo11/5 and Hypo28/6 using the "best fit" option within Discover Studio 2.5.5 via implementing equation (D) in section SM-2 under Supplementary Materials. The fit values together with the relevant molecular descriptors of each hit were substituted in the optimal QSAR equation. The highest ranking molecules based on QSAR predictions were acquired and tested in vitro.

**In vitro Experimental Studies**

**Materials**

All of the chemicals used in these experiments were of reagent grade and obtained from commercial suppliers. Captured hits samples were kindly provided by the NCI.

**Quantification of the anti-Chk1 bioactivities of different hits**

Briefly, recombinant Chk1 was obtained from Invitrogen (Carlsbad, CA). The Chk1 kinase assays were performed with the Invitrogen Z’-LYTE kinase assay kit - Ser/Thr 19 peptide. The assay was prepared for use with Chk1 as described in the Invitrogen kit protocol [42]. The Chk1 concentration was made to get the desired percent phosphorylation (30-50%) which implies the quality of an assay. The test was performed at final ATP and kinase concentrations of 50 µM and 7ng/10µl, respectively. Range of tested concentrations varied from 100 nM to 100 µM distributed log-linearly along the range of the concentration, with two data points at least from each concentration were collected. The IC_{50} value for each experiment was obtained using nonlinear regression of the log (concentration) versus percent inhibition values (Graph- Pad Prism 5.0).

**Results and Discussion**

**Exploration of Chk1 pharmacophoric space**

A total of 190 compounds were used in this study (1-190, see Figure. 1, Table 1 under supplementary material) [24-26]. We decided to explore the pharmacophoric space of Chk1 inhibitors through 28 HYPOGEN automatic runs and employing seven selected training subsets: subsets I-VII in Table 2 under supplementary material. The biological activity in the training subsets spanned from 3.0 to 3.5 orders of magnitude. The training compounds in these subsets were of maximal 3D diversity and continuous bioactivity spread over more than 3.0 logarithmic
cycles [32]. HYPOGEN was restricted to explore pharmacophoric models incorporating from zero to one PosIons, from zero to three HBA, HBD, Hbic, and RingArom features, as shown in Table 3 under supplementary material. The input features were reasonably selected based on visual evaluation of the training compounds and comparison between the structures of potent, moderate and inactive members. Furthermore, we instructed the software to explore only 4- and 5-featured pharmacophores, i.e., ignore models of lesser number of features (as shown in Table 3 under supplementary material). The reader is advised to see section Generation and Assessment of Binding Hypotheses in Method and sections SM-1 and SM-2 under Supplementary Materials more details about the HYPOGEN algorithm [28,29,32]. Eventually, 280 pharmacophore models emerged from 28 automatic HYPOGEN runs, exhibited Fisher randomization confidence levels ≥ 95%. These successful models were used in subsequent QSAR modeling. Interestingly, the representative models shared comparable features and acceptable statistical success criteria. Emergence of several statistically comparable pharmacophore models suggests the ability of Chk1 ligands to assume multiple pharmacophoric binding modes within the binding pocket. Therefore, it is quite challenging to select any particular pharmacophore hypothesis as a sole representative of the binding process.

**QSAR modeling**

Despite the excellent value of pharmacophoric hypotheses in probing ligand-macromolecule recognition and as 3D search queries to search for new biologically interesting scaffolds, their predictive value as 3D-QSAR models is generally hampered by steric shielding and bioactivity-enhancing or reducing auxiliary binding groups (e.g., the biological effects of electron-donating and withdrawing substitutions) [13-22]. Moreover, our pharmacophore exploration of Chk1 inhibitors furnished hundreds of binding hypotheses of comparable success criteria, which makes it very hard to select any particular pharmacophore as sole representative of ligand binding within Chk1. Accordingly, we were prompted to employ classical QSAR analysis to search for the best combination of pharmacophore(s) and other 2D descriptors capable of explaining bioactivity variation across the whole list of collected inhibitors (1-190, Figure 1, Table 1 under Supplementary Material). That is, we employed GFA-based QSAR as competition arena to select the best pharmacophore(s), i.e., among the resulting population of binding models, and supplement it(them) with two-dimensional (2D) descriptors to correct for the weaknesses of pharmacophore models (steric shielding and bioactivity-enhancing or reducing auxiliary binding groups). We employed genetic function approximation and multiple linear regression QSAR (GFA-MLR-QSAR) analysis to search for an optimal QSAR equation(s). The fit values obtained by mapping representative hypotheses (28 models) against collected Chk1 inhibitors (1-190, Figure 1, Table 1) were enrolled, together with around 319 other physicochemical descriptors, as independent variables in GFA-MLR-QSAR analysis [12,22,35,36]. We randomly selected 38 molecules (marked with asterisks in Table 1 under Supplementary Materials) and employed them as external test molecules for validating the QSAR models ($r^2_{\text{PRESS}}$). Additionally, all QSAR models were cross-validated automatically using the leave-one-out cross-validation (see sections QSAR Modeling under Method and section SM-3 under Supplementary Materials) [36]. Equation (1) shows the details of the optimal QSAR model. Figure 2 shows the corresponding scatter plots of experimental versus estimated bioactivities for the training and testing inhibitors.

$$\log(1/IC_{50}) = 1.85 \cdot 0.28 \times \text{AlogP} + 0.47 \times \text{ES\_count\_tOH} + 0.15 \times \text{ES\_sum\_tN} $$

$$- 0.34 \times x3 + 0.28 \times \text{Hypo1/4} + 0.23 \times \text{Hypo11/5} + 0.18 \times \text{Hypo28/6}$$

where, $r^2_{\text{LOO}}$ is the correlation coefficient against 152 training compounds, $r^2_{\text{LOO}}$ is the leave-one-out correlation coefficient, and $r^2_{\text{PRESS}}$ is the predictive $r^2$ determined for the 38 test compounds [35]. Hypo1/4, Hypo11/5 and Hypo28/6 (Table 1) represent the fit values of the training compounds against these pharmacophores as calculated from equation (D) in Supplementary Materials [36].
show the ROC results of our QSAR-selected pharmacophores (see SM-5 Receiver Operating Characteristic Curve Analysis under Supplementary Materials for more details). To correlate the binding features in Hypo1/4, Hypo11/5 and Hypo28/6 with ligand-receptor binding interactions anchoring inhibitors into Chk1’s binding pocket, we compared the pharmacophoric features of Hypo11/5 pharmacophore with the way the cocrystallized ligand within Chk1 protein (PDB codes: 2E9V, Resolution = 2.0 Å) [44] as in Figure 5, compares how cocrystallized training compound 132 (Table A under Supplementary Materials) maps Hypo11/5 hypotheses with the way the ligand bind within Chk1’s binding pocket. However, mapping 132 with hypo1/4 and Hypo28/6 was not successful. Clearly from the Figure 5A mapping the donor amidic nitrogen of 132 against donor feature in Hypo11/5 corresponds to hydrogen bond interaction connecting the same amidic group with the carboxylate of GLU85. The fact that the pentyl linker reside within a hydrophobic pocket of VAL23 and GLY16 correspond to fitting this group against Hbic feature in Hypo11/5. Similarly, the chlorophenyl moiety reside within a hydrophobic pocket of LEU137 corresponds to mapping the pheny ring with Hbic feature. Finally, RingArom feature in Hypo11/5 corresponds to the interaction of electron deficient cyanopyrimidine with the corresponding electron rich amino group of LYS38 (amino of LYS38 is not electron rich, size it’s positively ionized at physiological pH. Look at the binding site again and find another residue to complement ligand mapping).

Steric refinement, virtual screening and in vitro validation

Pharmacophores serve as useful 3D QSAR models and 3D search queries, however, they lack steric constrains necessary to define the size of the binding pocket. This liability renders pharmacophoric models rather promiscuous in some cases [15]. Therefore, we decided to complement the optimal pharmacophores with exclusion spheres employing the HipHop-Refine module implemented within Discovery Studio package [27]. Excluded volumes resemble sterically inaccessible regions within the binding site (see section SM-4: HipHop-Refine algorithm and employed settings under Supplementary Material for more details). We selected a diverse training subset for HipHop-Refine modeling (subset VIII in Table 4 under supplementary material). The training compounds were selected in such away that the bioactivities of weakly active compounds are explainable by steric clashes within the binding pocket. We assessed the success of steric refinement experiments through ROC analysis of the sterically refined pharmacophore versions. Table 2 shows the ROC results of the refined pharmacophores compared to their unrefined counterparts. Clearly from the table, steric refinement improved the classification power of the three pharmacophores. This effect was particularly evident with Hypo1/4, Hypo11/5 and Hypo28/6 pharmacophores which had their ROC areas under the curve (AUCs) increased from 91.1%, 75.1%, 99.8% to 97.4, 99.4 and 99.9%, respectively. Sterically-refined Hypo1/4, Hypo11/5 and Hypo28/6 (Figure 3D) were employed as 3D search queries against the NCI list of compounds (NCI, 238,819 structures). Table 3 summarizes the numbers of captured hits by stercically-refined versions of the pharmacophores [43]. Subsequently, captured
hits were filtered based on Lipinski’s and Veber’s rules, [40,41]. The remaining hits were fitted against Hypo1/4, Hypo11/5 and Hypo28/6 and their fit values, together with other relevant molecular descriptors, were substituted in QSAR equation (1) to predict their anti-Chk1 bioactivities. The highest-ranking hits were evaluated in vitro against human Chk1 [42]. Figure 9 and Table 4 show the most active hit, Figures 6-8 show how potent hit 191 maps against Hypo1/4, Hypo11/5 and Hypo28/6 and refined hypotheses [43]. By comparing this method with previously reported methods [46-48] we found that our method overcome the problem of modeling by proteins structure such as resolution, rigidity and predictability of Chk1 inhibitory activities. Our method clarifies the necessary of two dimensional and three dimensional features that are required for activity. However previous methods depend on protein docking and scoring which lacks the ability to calculate the predicted activity of the captured hits. Our method is statistically validated and it is applicable for predicting any future studies. It will be helpful for researchers
Table 1 Pharmacophoric features and corresponding weights, tolerances and 3D coordinates of Hypo1/4, Hypo11/5 and Hypo28/6.

<table>
<thead>
<tr>
<th>Model</th>
<th>Definition</th>
<th>HBA</th>
<th>HBD</th>
<th>Ring Arom</th>
<th>HBic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo1/4a</td>
<td></td>
<td>2.26</td>
<td>2.16</td>
<td>2.26</td>
<td>2.26</td>
</tr>
<tr>
<td>Tolerances</td>
<td>1.6</td>
<td>2.2</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Coordinates</td>
<td>X 2.61</td>
<td>1.153</td>
<td>-9.911</td>
<td>-7.73</td>
<td>-8.779</td>
</tr>
<tr>
<td></td>
<td>Y -5.34</td>
<td>-7.794</td>
<td>-4.715</td>
<td>-7.56</td>
<td>-3.45</td>
</tr>
<tr>
<td></td>
<td>Z -3.871</td>
<td>-4.797</td>
<td>2.34</td>
<td>1.3</td>
<td>1.653</td>
</tr>
<tr>
<td>Hypo11/5b</td>
<td></td>
<td>2.16</td>
<td>2.16</td>
<td>2.16</td>
<td>2.16</td>
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<tr>
<td>Tolerances</td>
<td>1.6</td>
<td>2.2</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Coordinates</td>
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<td>-3.422</td>
<td>-3.571</td>
<td>-3.692</td>
<td>3.428</td>
</tr>
<tr>
<td></td>
<td>Y -1.561</td>
<td>-3.841</td>
<td>0.442</td>
<td>1.375</td>
<td>-1.202</td>
</tr>
<tr>
<td></td>
<td>Z -1.199</td>
<td>-2.792</td>
<td>-0.49</td>
<td>-3.315</td>
<td>-0.834</td>
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<tr>
<td>Hypo28/6b</td>
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<td>2.298</td>
<td>2.298</td>
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<tr>
<td>Tolerances</td>
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<td>2.2</td>
<td>1.6</td>
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<tr>
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<td>1.907</td>
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<tr>
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<td>-1.552</td>
<td>-2.939</td>
<td>-4.56</td>
</tr>
<tr>
<td></td>
<td>Z -0.465</td>
<td>-2.794</td>
<td>2.297</td>
<td>4.552</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Hypo1/4: the 4th pharmacophore hypothesis generated in the 1st HYPOGEN run (table D under Supplementary Material).
Hypo11/5: the 5th pharmacophore hypothesis generated in the 11th HYPOGEN run (table D under Supplementary Material).
Hypo28/6: the 6th pharmacophore hypothesis generated in the 28th HYPOGEN run (table D under Supplementary Material).

Table 2 ROC curve analysis criteria for QSAR-selected pharmacophores and their sterically-refined versions.

<table>
<thead>
<tr>
<th>Pharmacophore Model</th>
<th>ROC*-AUCb</th>
<th>ACCc</th>
<th>SPCd</th>
<th>TPRe</th>
<th>FNRf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo1/4</td>
<td>0.911</td>
<td>0.985</td>
<td>0.998</td>
<td>0.131</td>
<td>0.002</td>
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<tr>
<td>Refine Hypo1/4</td>
<td>0.974</td>
<td>0.985</td>
<td>0.998</td>
<td>0.105</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypo11/5</td>
<td>0.751</td>
<td>0.980</td>
<td>0.986</td>
<td>0.947</td>
<td>0.014</td>
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<tr>
<td>Refine Hypo11/5</td>
<td>0.994</td>
<td>0.985</td>
<td>0.985</td>
<td>0.947</td>
<td>0.014</td>
</tr>
<tr>
<td>Hypo28/6</td>
<td>0.998</td>
<td>0.985</td>
<td>0.999</td>
<td>0.053</td>
<td>0.001</td>
</tr>
<tr>
<td>Refine Hypo28/6</td>
<td>0.999</td>
<td>0.985</td>
<td>0.999</td>
<td>0.052</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

*ROC: receiver operating characteristic curve.
*AUC: area under the curve.
*ACC: overall accuracy.
*SPC: overall specificity.
*TPR: overall true positive rate.
*FNR: overall false negative rate.

Table 3 Numbers of captured hits by sterically-refined versions of Hypo1/4, Hypo11/5 and Hypo28/6.

<table>
<thead>
<tr>
<th>Pharmacophore models</th>
<th>Databasea</th>
<th>Post screening filteringb</th>
<th>Sterically-Refined Hypo1/4</th>
<th>Sterically-Refined Hypo11/5</th>
<th>Sterically-Refined Hypo28/6</th>
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<tbody>
<tr>
<td>NCI</td>
<td>Before</td>
<td>1407</td>
<td>1490</td>
<td>88</td>
<td>42</td>
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<tr>
<td></td>
<td>After</td>
<td>1278</td>
<td>1351</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

*aNCI: national cancer institute list of available compounds (238,819 structures)
bUsing Lipinski’s and Veber’s rules.

Table 4 Experimental bioactivities of high-ranking hit molecules.

<table>
<thead>
<tr>
<th>Hitsb</th>
<th>Namea</th>
<th>Experimental %Inhibition at 10 µMc</th>
<th>IC50 (µM)d</th>
<th>R2</th>
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</thead>
<tbody>
<tr>
<td>191</td>
<td>80966</td>
<td>77.1</td>
<td>3.97</td>
<td>0.999</td>
</tr>
<tr>
<td>192</td>
<td>Staurosporine*</td>
<td>100</td>
<td>0.068</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Chemical structures as shown in figure 9.
*NCI number.
*Experimental percentage of inhibition determined at 10 µM inhibitor concentrations.
*IC50 values experimentally determined for most active hits. Each value represents the average of duplicate measurments.
*Reported inhibitory IC50 = 2.1 nM
who work on Chk1 inhibitor development or synthesis to find the optimum features and functional groups. It is also can be used as a guide for further Chk1 inhibitors optimization. This actually is inapplicable in previous studies.

Conclusions

As the understanding of signaling events at the molecular level leading to the checkpoint arrests during DNA damage started to emerge during the last few years, Chk1 has been considered as one of the most attractive targets for drug discovery of anticancer agents. Chk1 is currently considered as potential treatments for cancer. The pharmacophoric space of Chk1 inhibitors was explored via seven diverse sets of inhibitors and using CATALYST-HYPOGEN to identify high quality binding model(s). Subsequently, GFA and MLR analysis were employed to access optimal QSAR
Figure 8 (A) show Hypo28/6, (B) show refined Hypo28/6 (C) show Hypo28/6 fitted against hit 191 and (D) show refined Hypo28/6 fitted against hits 191.

Figure 9 (191) the most active NCI hit against Chk1, (192) standard Chk1 inhibitor.

model capable of explaining anti-Chk1 bioactivity variation across 190 collected Chk1 inhibitors. Three orthogonal pharmacophoric models emerged in one QSAR equation suggesting the existence of variable binding modes accessible to ligands within Chk1 binding pocket. The QSAR equation and the associated pharmacophoric models were experimentally validated by the identification of new scaffold Chk1 inhibitor retrieved via in silico screening, which illustrated micromolar potencies. Our results suggest that the combination of pharmacophoric exploration and QSAR analyses can be useful tool for finding new diverse Chk1 inhibitors.

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