Supporting Information for

Probing Binding Modes of Small Molecule Inhibitors to the Polo-Box Domain of Human Polo-like Kinase 1

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Supplemental Figures

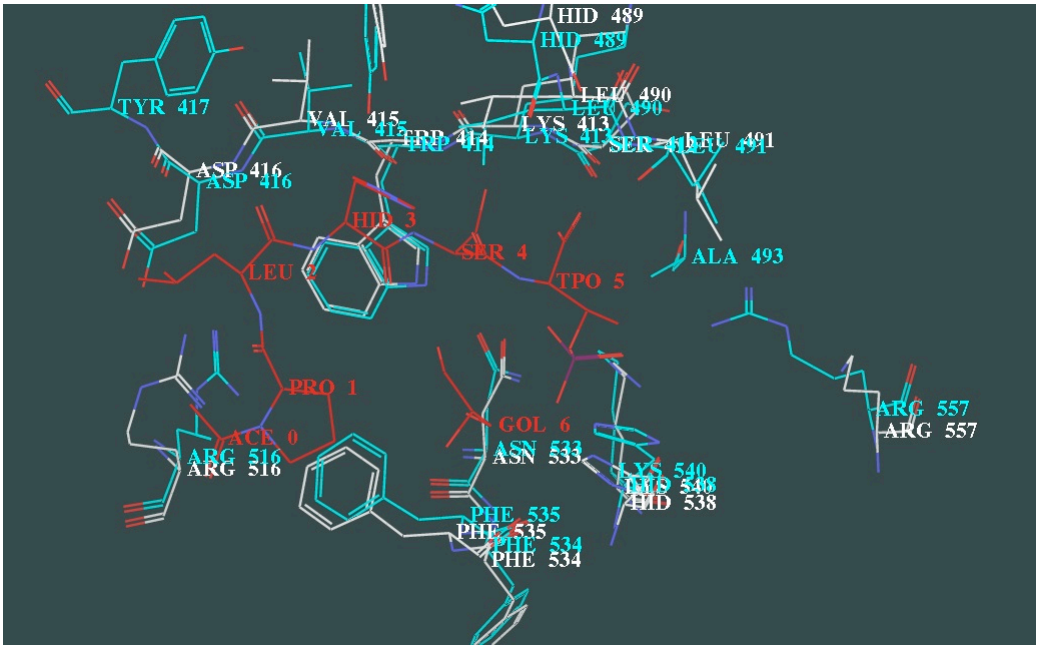
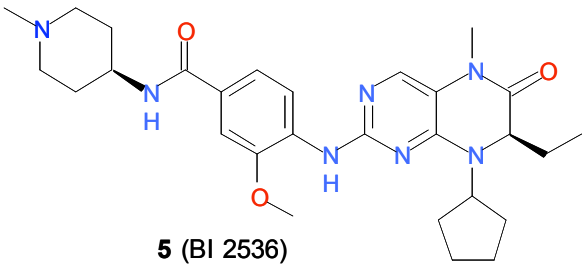


Figure S1. The movement of side chains upon binding of the phosphopeptide, PLHSpT, to the Plk1 PBD. The results were obtained by superimposing two PBD crystal structures, 3HIK[1] (white, in complex with PLHSpT) and 1Q4O[2] (cyan, with no ligand).



5 (BI 2536)

Figure S2. The chemical structure of BI 2536,[3] a highly potent Plk1 NCD inhibitor.

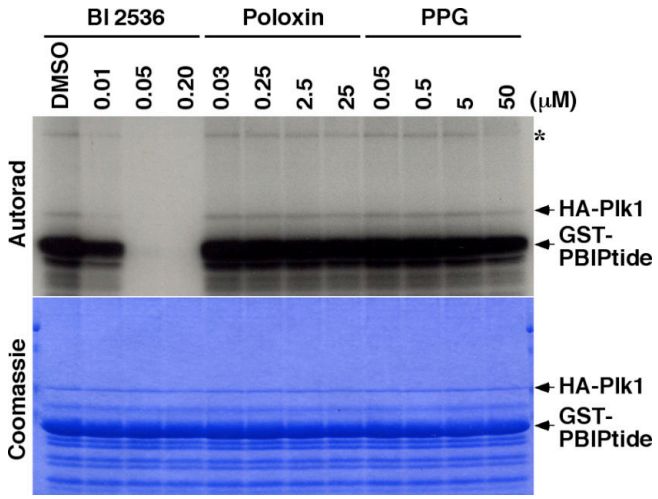


Figure S3. Both Poloxin and PPG do not influence the kinase activity of Plk1 *in vitro*. Plk1 kinase assay was carried out in the presence of the indicated amount of the compounds. Reactions were terminated and the samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After staining the gel with Coomassie, the gel was subjected to autoradiography (Autorad).

Experimental Procedures

Chemistry

The peptides were synthesized by using a 9-fluorenylmethoxycarbony (Fmoc)-based solid-phase method on Rink amide resin as described previously.[1] BI 2536 and Poloxin were gifts of Boehringer Ingelheim (Vienna, Austria) and Dr. Wolfgang Reindl (Max Planck Institute of Biochemistry, Martinsried, Germany), respectively. Purpurogallin (PPG) was purchased from Sigma-Aldrich (St. Louis, MO). The purity of these compounds (all >97%) were examined by using ^{1}H NMR and HPLC as illustrated below.

^{1}H NMR spectra were run at 300 MHz on a Bruker instrument using d6-DMSO as solvent; chemical shifts (δ) are reported in ppm. The >97% purity of all compounds was ascertained by a reversed-phase

high-performance liquid chromatography (RP-HPLC) on an analytical (10 μm, 4.6 × 250 mm) C18 Bondapak column using a water-acetonitrile gradient [10-70 %/35 min] containing 0.05% trifluoroacetic acid (TFA) (flow rate, 1.0 mL/min).

Molecular Modeling

Preparation of Crystal Structures. The X-ray crystal structures of the PBD in complex with PLHSpT (PDB ID: 3HIK)[1] was used in this study. This structure's resolution is 1.77 Å.

The starting structures for the proteins were prepared and fixed by the Protein Preparation Wizard,[4] a comprehensive protein preparation facility from Schrödinger. All water molecules were deleted; the bound ligand was adjusted manually; bond orders were assigned, and then hydrogen was added to all atoms. The orientation of the hydroxyl and amide groups of Asn and Gln, and the charge state of the His residues were optimized. The final step was a restrained minimization until the average root mean square deviation of the hydrogen atoms reached 0.3 Å, leaving heavy atoms in place.

Preparation of Ligands. The molecules of PPG, Poloxin and Thymoquinone were drawn in ISIS/Draw 2.5, and were imported into Maestro 9.0.[5] Conformational searches were performed using MacroModel 9.7[6] with default parameters. The global minima were used in further IFD studies.

Induced Fit Docking. The Schrödinger's IFD protocol[7] was employed in this study. IFD is based on Glide and the refinement module in Prime that accurately predicts ligand binding modes and concomitant structural changes in the receptor. This protocol models induced fit using the following steps: (1) Constrained minimization of the receptor with an RMSD cutoff of 0.18 Å. (2) Initial Glide docking of each ligand using a softened potential (van der Waals radii scaling). (3) One round of Prime side-chain prediction for each protein/ligand complex, on residues within a given distance of any ligand pose. (4) Prime minimization of the same set of residues and the ligand for each protein/ligand complex pose. (5) Glide redocking of each protein/ligand complex structure within a specified energy of the lowest energy structure. (6) Estimation of the binding energy (IFDScore) for each output pose. In our study, all docking calculations were run in the XP mode of Glide. The center of the grid box of the PBD

was defined by the selected Trp 414, His 538, and Lys 540 residues; the center of the grid box of the NCD was defined by the selected Cys 133 residue. Default values were used for all other parameters.

pK_a Values Calculation. ADME Boxes 4.9 from Pharma Algorithms, Inc.[8] and Marvin 5.1.4 from ChemAxon Ltd.[9] were used to calculate the pK_a values of PPG. Both of these two programs can calculate different kinds of pK_a values. For this study, only the predicting experimental pK_a values were calculated. They were run using the default options in the graphical user interface of a Windows XP machine.

Location of Binding Pockets. Potential binding pockets were identified using the default parameters in the program of SiteMap 2.3[10] from Schrödinger. A SiteMap calculation has three stages. First, a grid is set up, and the points are grouped into sets based on various criteria to define the sites. Second, the sites are mapped on another grid to produce files for the visualization of the maps. Finally, properties are evaluated and sites are written in a Maestro-readable form. Each stage is accomplished by running an Impact job.

Minimization of the Docking Complexes. Poloxin and Thymoquinone were docked into one pocket found by SiteMap 2.3 and were connected to the thiol group of Cys 544 manually. One double bond of each compound was changed to single bond. The formed complexes were minimized using MacroModel 9.7[6] in aqueous solution by employing the force field of OPLS_2005. The atoms beyond 20 residues of Poloxin and Thymoquinone were fixed during the minimization processes. The default values were taken for other parameters.

ELISA-based PBD-binding Inhibition Assay

One hundred microliter of a 0.3 μM biotin-conjugated p-T78 peptide dissolved in 1X coating solution (KPL Inc., Gaithersburg, MD) was immobilized onto a 96-well streptavidin-coated plate (Nalgene Nunc, Rochester, NY). Wells were washed once with PBS + 0.05% Tween 20 (PBST) and incubated with 200 μl of PBS + 1% BSA for 1 h to block unoccupied sites. For the source of Plk1, mitotic 293A lysates expressing HA-EGFP-Plk1[11] were first prepared in TBSN {20 mM Tris-Cl

(pH8.0), 150 mM NaCl, 0.5% NP-40, 5 mM EGTA, 1.5 mM EDTA, 0.5 mM Na_3VO_4, 20 mM *p*-nitrophenyl phosphate, and protease inhibitor cocktail (Roche, Nutley, NJ)} buffer. The resulting lysates (60 μg total lysates in 100 μl) were then applied onto the biotinylated peptide-coated ELISA wells immediately after mixing with the indicated amount of the competitors. After incubation with constant rocking for 1 h at 25°C, the ELISA plates were washed 4 times with PBST, and then incubated with 100 μl/well of monoclonal anti-HA antibody (0.5 μg/ml) for 2 h in the blocking buffer to detect the bound HA-EGFP-Plk1. After washing the plates 5 times, 100 μl of an HRP-conjugated secondary antibody diluted to 1:1000 in the blocking buffer was added onto each well and incubated for 1 h. The plates were then washed 5 times with PBST and incubated with 100 μl/well of 3,3',5,5'-Tetramethylbenzidine solution (TMB) (Sigma, St. Louis, MO) as substrate until a desired absorbance was reached. After terminating the reactions by the addition of 1N H_2SO_4, the optical densities for each sample were measured at 450 nm by using an ELISA plate reader (Molecular Device, Sunnyvale, CA).

Plk1 Kinase Assay

The kinase assay was carried out as described previously[12] using GST-PBIPtide as a Plk1-specific substrate[13]. Reactions were terminated, separated by SDS-PAGE, and then analyzed by autoradiography.

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