Electronic Supporting Information

Design and Synthesis of a New Orthogonally Protected Glutamic Acid Analog and Its Use in the Preparation of High Affinity Polo-like Kinase 1 Polo-box Domain – binding Peptide Macrocycles

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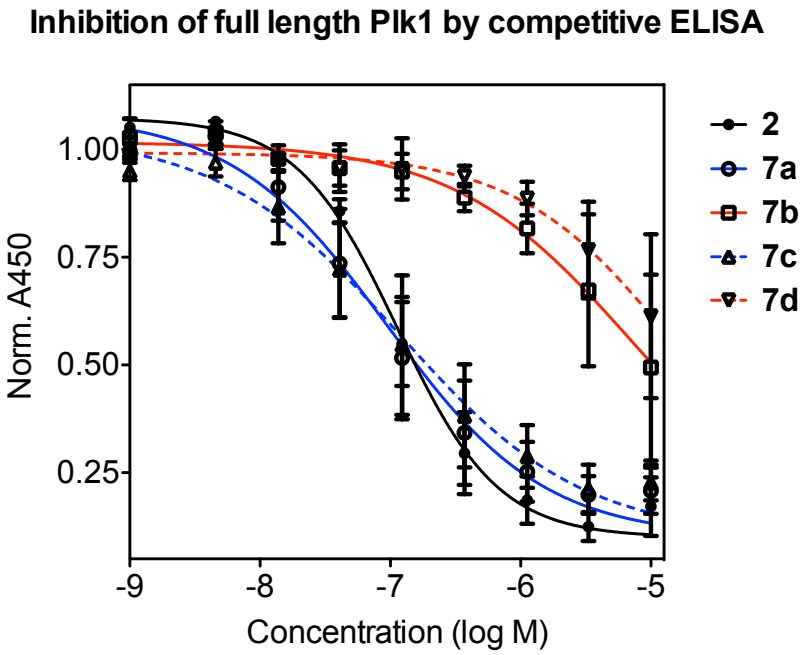

Fig. S1. ELISA-based competitive inhibition of binding to full length Plk1. Data points represent average ± SEM of normalized absorbance fit using non-linear regression in GraphPad Prism 7.

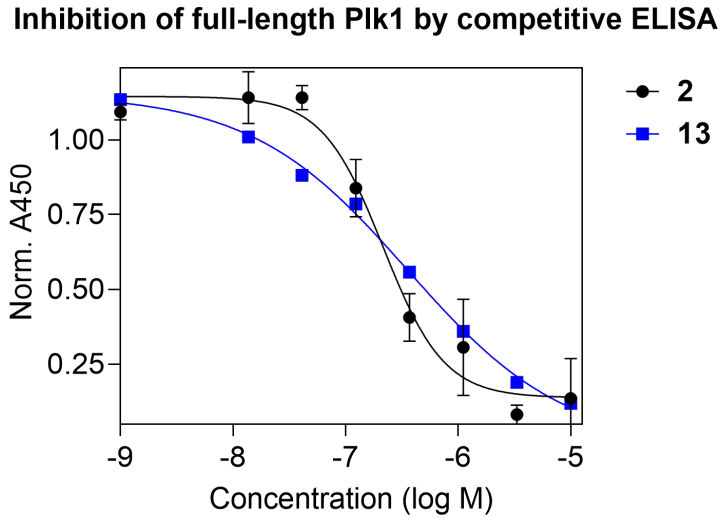

Fig. S2. ELISA-based competitive inhibition of binding to full length Plk1. Data points represent average ± SEM of normalized absorbance fit using non-linear regression in GraphPad Prism 7.

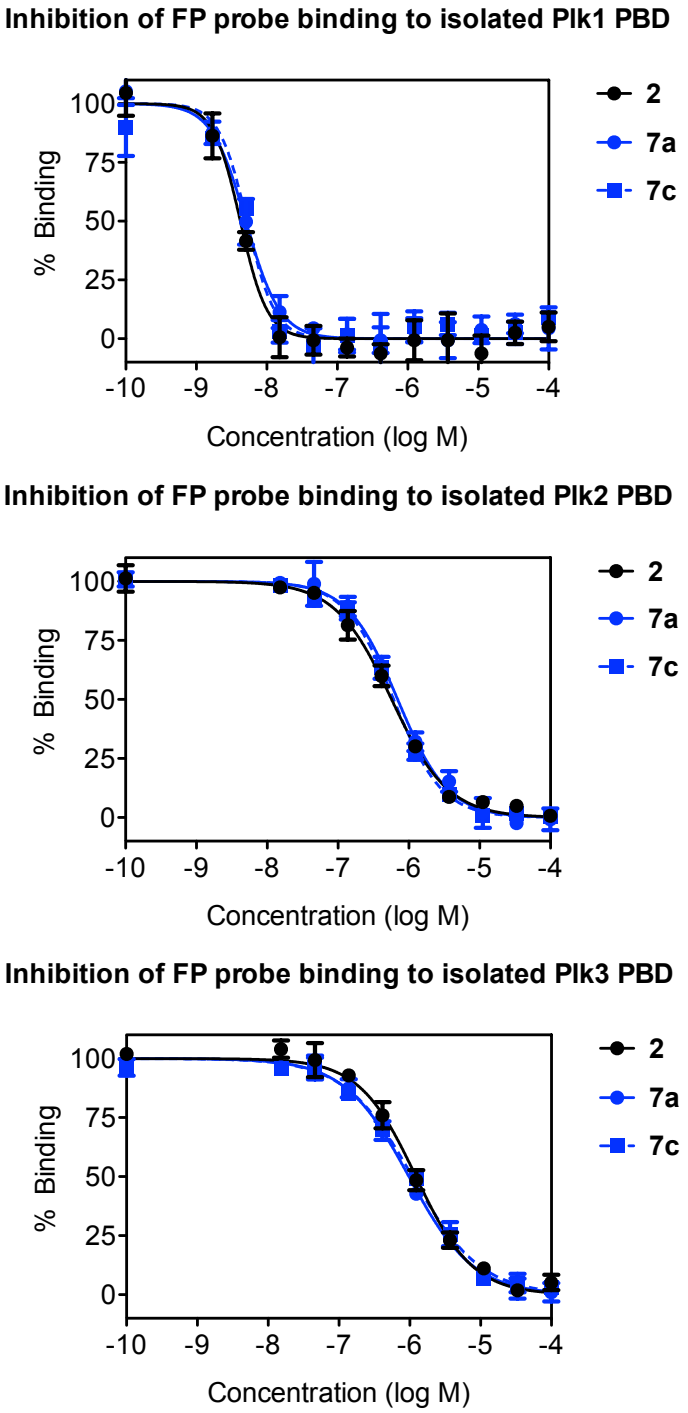

Fig. S3. Fluorescence polarization assay for competitive inhibition of the isolated PBDs of Plks 1 – 3 by macrocyclic ligands. Data points represent average ± SEM of % binding. normalized FP signal) from triplicate data points and fit using non-linear regression in GraphPad Prism 7.

Analytical Traces for Peptide Ligands

Agilent 1200 Series quaternary pump equipped with a Phenomenex Gemini-C_{18} (5 μm, 250 x 4 mm) analytical column, 1 mL/min flow rate, and UV detection at 210 nm. Solvent A = 99.9% H_2O +0.1% TFA / Solvent B = 99.9% MeCN + 0.1% TFA. Gradient condition: 95/5 A/B for 2 minutes, ramp to 100% B over 28 minutes.

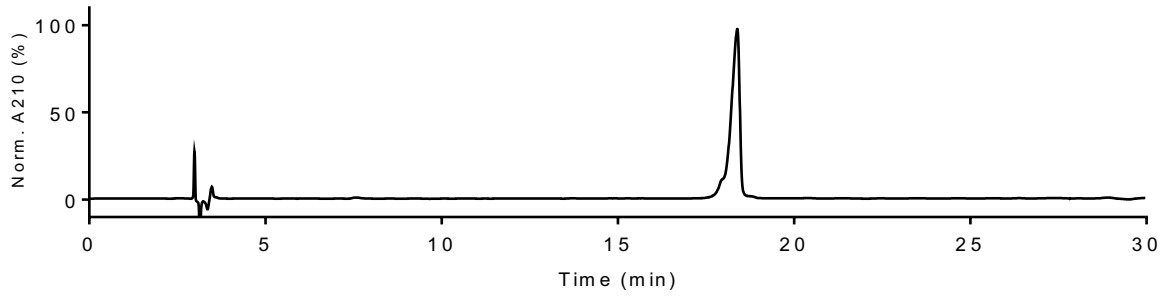

Fig. S4. Analytical HPLC trace of **7a**.

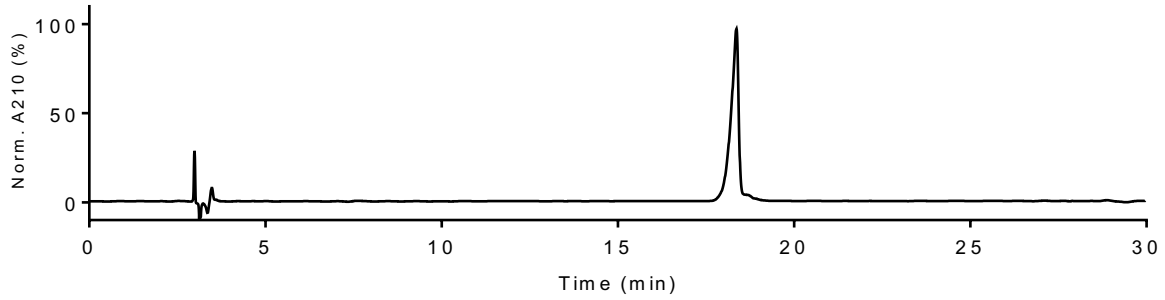

Fig. S5. Analytical HPLC trace of **7b**.

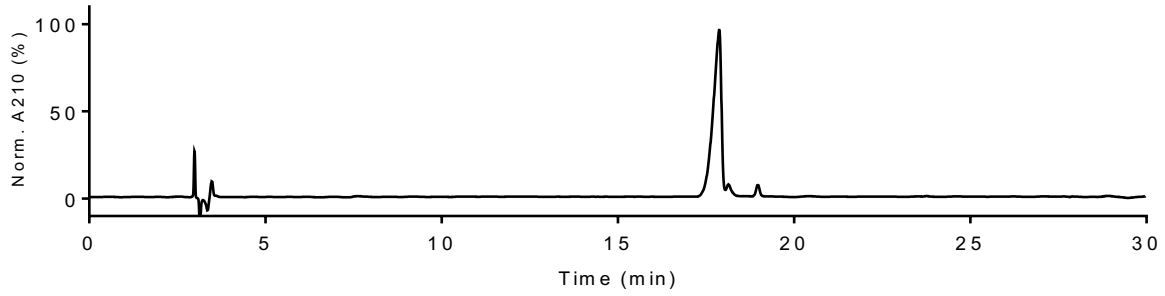

Fig. S6. Analytical HPLC trace of **7c**.

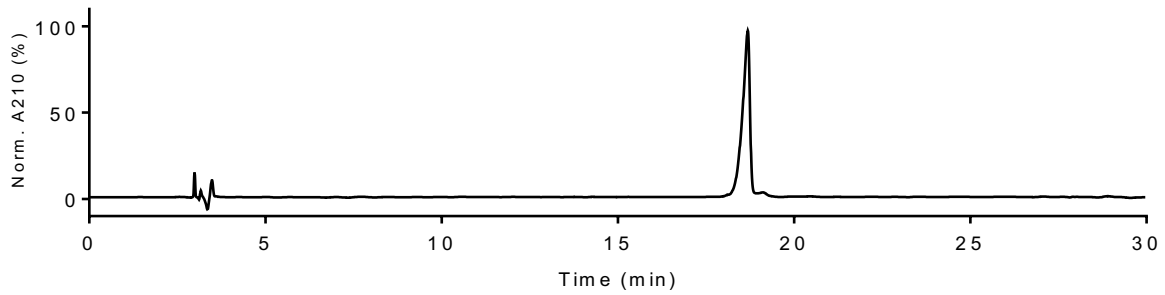

Fig. S7. Analytical HPLC trace of **7d**.

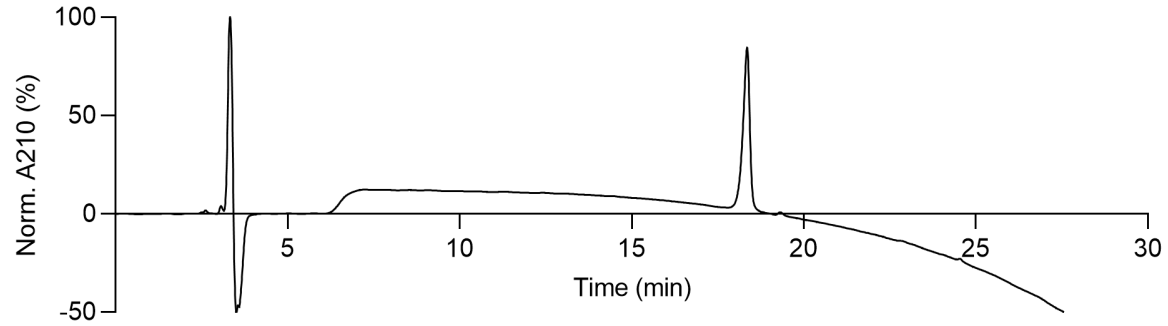

Fig. S8. Analytical HPLC trace of **13**.

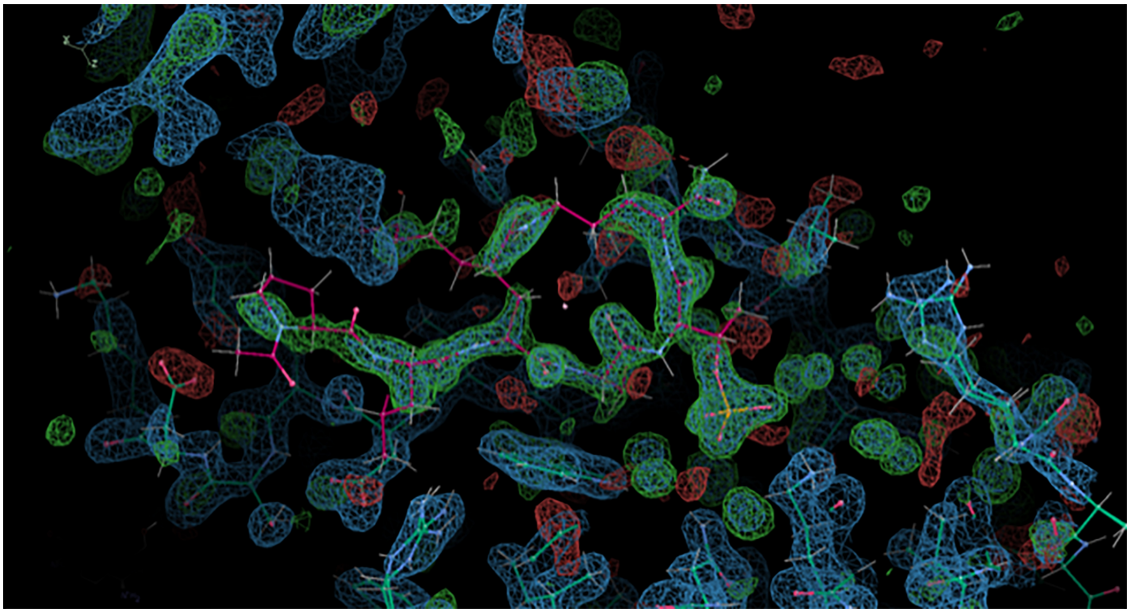

Fig. S9. Molecular replacement electron density. The electron density from the PHASER molecular replacement solution is shown with the 2Fo-Fc map (blue) contoured at 2.0 σ and the Fo-Fc difference density (green/positive, red/negative) contoured at 2.5 σ. The phasing model (consisting only of protein) is shown with carbons colored green. The final model for the **7a** macrocycle is shown with carbons colored magenta. The strong positive difference features clearly show the presence of the macrocycle and numerous water molecules that were not present in the search model.