Identifying novel covalent allosteric inhibitors of WEE1 protein kinase

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Introduction and Aim

WEE1 is a member of serine/threonine kinase family responsible for cell cycle regulation. Its effect specifically on the G2/M checkpoint is indicated by the termination of mitotic entry, promoting the repair of damaged DNA. Inhibition of this process allows the development of new anticancer drugs and increase the effectiveness of existing DNA-damaging response agents.

In this study, we investigated interactions at the potential allosteric site of WEE1 kinase by Covalent Docking and Molecular Dynamics, performed semi-manual selection of inhibitor candidates and performed an in vitro ADP-Glo assay to confirm the validity of bioinformatics approaches.

Computational techniques and selection of drug candidates

Since the catalytic site of most kinases has a similar structure, the development of allosteric inhibitors is becoming increasingly important [1]. It has been reported that CYS509 can be potential targetable amino acid for covalent compounds to obtain allosteric inhibition [2]. Our research group performed molecular docking, using Enamine covalent compounds library. Nine groups of different warheads active on cysteine included Acrylamides, BCBs, Cyanoacrylic esters, Allenes, But-2-ynamides, ChloroFluoroacetamides, Chloroacetamides, Cyanoacrylamides and NMe2Acrylamides.

Molecular docking analysis was carried out using Glide from Schrödinger Software with both attachment residue and the centre of grid box as CYS509 (later in MD – CYS223). Protein was taken from 5vc5 PDB model removing existing ligand, then prepared using Protein Preparation Wizard and Energy Minimization steps. Ligands were run through LigPrep to generate possible states in the pH range 7 ± 1.5. Output top included one thousand of complexes for each covalent ligand group. We examined the results manually, evaluating complexes using such criteria as docking score, number of bonds, type of bonds, interacting amino acids, size of the ligand and solvent exposure, and assigning a custom rating from 0 to 5. The most advanced 6 ligands according to the listed criteria were selected to perform 500 ns Molecular Dynamics analysis in Desmond.

Evaluating the RMSD values and basic characteristics of ligand-protein interaction, 3 complexes demonstrated high stability. Two of them revealed hit inhibitory properties in the *in vitro* kinase assay. RMSD, RMSF and Protein-Ligand Contacts data for the strong Cyanoacrylamide hit are shown in Fig. 1. RMSF values are slightly elevated due to fluctuating free loop (residues 151-170). The fluctuation is even higher in ligand-free WEE1 protein, that is seen in corresponding Molecular Dynamics data shown in Fig. 2. The presence of similar local changes of the apo-protein explains the validity of RMSF and RMSD values in the complexes with ligands. Protein-ligand contacts with ILE102, TYR106, ARG195, ALA227, and LEU230 have the most significant impact on the complex stabilization in analyzed biologically active compounds. LEU230 is particularly important, maintaining interaction >80% of simulation time.

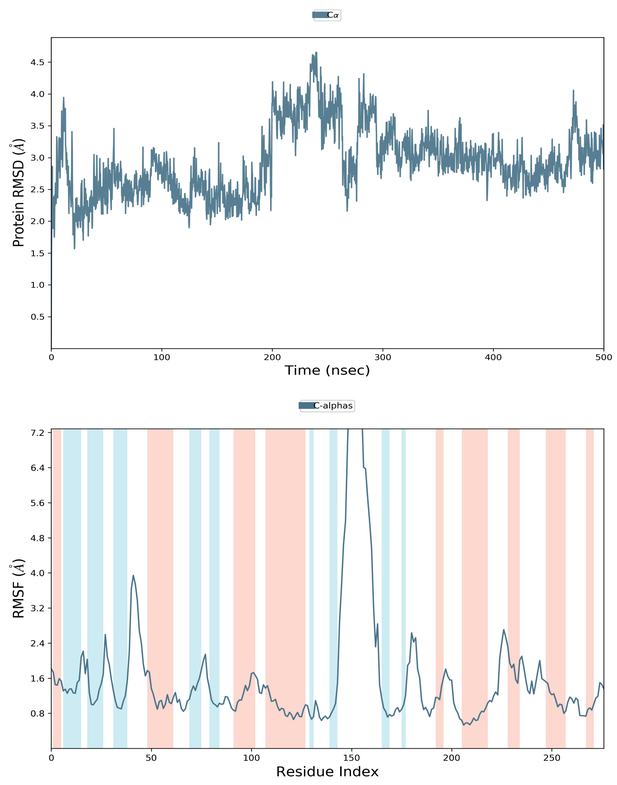

Figure 2. RMSD and RMSF of the WEE1 apo-protein.

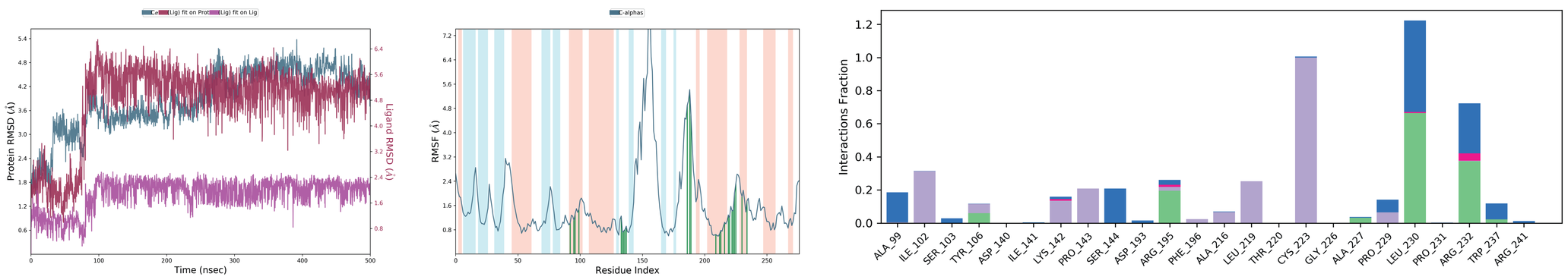

Figure 1. Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Protein-Ligand Contacts of the Cyanoacrylamide strong hit ligand with the docking score -5,041 kcal/mol and 63,7% WEE1 kinase inhibition at 50 μM. Colors on the diagram indicate H-bonds (green), Hydrophobic (violet), Ionic (pink) and Water Bridges (blue).

In vitro WEE1 kinase assay

The ADP-Glo assay was used to analyze the inhibition of WEE1 enzyme by the top 233 compounds according to the highest docking score and manual rating mentioned before. Enzymatic assays were performed in custom buffer (40mM Tris-HCl, 20mM MgCl2, 0.1mg/ml BSA, 50 uM DTT and 0.005% Brij-35 (pH 7.4)) in 384-well white polystyrene plates. Compounds were diluted in buffer to a final concentration 50 μM, mixed with WEE1 and preincubated for 90 minutes at 25°C to allow the covalent bonds to form properly. Adavosertib at 5 nM and 100 nM was used as a reference inhibitor to obtain 50 and 100% inhibition, respectively. The final concentration of DMSO in the assay was 1%. Due to WEE1 ability of autophosphorylation, ATP was used as a substrate (45 μM). Plates were read in luminescence mode using the SpectraMax Paradigm reader.

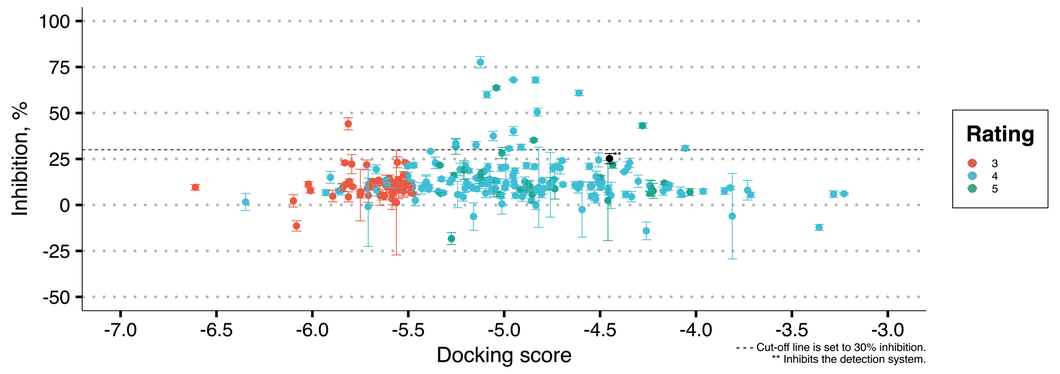

Figure 3. Percentage of WEE1 enzyme inhibition by selected compounds at 50 μM on y-axis versus docking score on x-axis. Manual rating (criteria mentioned in text) is marked by color, where 5 (green) is the highest evaluation of complex stability.

Conclusions

Our results demonstrated 18 hit compounds with the inhibition cut-off being 30% and higher (hit rate 7.73%). Two of the compounds previously selected to run Molecular Dynamics analysis demonstrated stronger (63.7%) and weaker (24.1%) inhibition. The most prominent covalent class appeared to be Cyanoacrylamides. The results indicate reliable criteria for selecting potential ligands with allosteric inhibitory activity. Further research will include dose-response WEE1 inhibition ADP-Glo assay, counter-screening hit validation and ADME/DMPK studies. Identification of the target contacting amino acids enables further Hit-to-Lead optimization.

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References

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