Triazolone inhibitors of Checkpoint Kinase I (Chk-1)

Introduction

The protein Checkpoint Kinase 1 (Chk-1) promotes arresting of the cell division cycle in the event of a DNA damage event, by triggering a cascade of reactions affecting the CDK cell cycle kinases. As such, its inhibition could force mutated cells to go into mitosis with catastrophic consequences (apoptosis and cell death). This is useful in the cancer therapy, e.g. to force radiotherapy-mutated malignant cells to go into mitosis and die. As such, there is been a lot of work in trying to find potent and selective inhibitors acting against this family of kinases. Staurosporine is a known kinase inhibitor that can act against Chk-1, however it is not very selective. As such, several high throughput screens (HTS) studies have been carried out to find new selective and potent Chk-1 inhibitors. One such study has been carried out in paper1 (Oza 2010) which you should read carefully. You should then should use PyMOL and other tools to fulfill the below presented tasks.

Exercises

1. Does Staurosporine have good drug-like properties ?... (use the MPROP tool to check it). Does staurosporine follow the Lipinksky's rule of 5 ? If not, what violations of the rule do occur ?

2. UCN-101 (7-Hydroxystaurosporine) is a modified staurosporine with an OH group at position 7. Examine its formula in pubbchem.org . Using the molprop molecular editor, change staurosporine into UCN-101 and recalculate the drug-like properties. What do you observe ?

3. Staurosporine is described as a promiscuous (non-specific) ligand of kinases. Let's check this using the RCSB Protein Databank: in the menu "Search" of the main page select the option "Ligand", and search for the name "staurosporine". How many structures come up ?...

4. There is the possibility that some of the proteins in complex with staurosporine are not kinases. Try to find out how many really are kinases, by using the option "Refine Query" on the left hand panel in the results page. How many structures contain staurosporine in complex with checkpoint kinase 1 (Chk-1) ?

5. Obtain the complex of staurosporine with Chk-1 from the PDB, with code `1nvr`. Open it in PyMOL.
    - Identify the ligand in the structure (code "STU"). Zoom into it with `zoom STU/`
    - Show the protein surface with `show surface`
    - Does the drug appear to fill the entire pocket? To check it, represent the staurosporine molecule as spheres (`show spheres, STU/`)
    - Change the protein representation to ribbon with `as ribbon`. Recover the ligand stick representation with `show sticks, STU/`
    - Identify full residues in the vicinity of the ligand using the command: `show sticks, byres all within 3.5 of STU/` (change the value 3.5 to other distances, to fewer or more residues around the ligand). Can you tell which residues may be forming interactions with atoms in the ligand (consider the distance and the hydrogen bond donors/acceptors).
    - To validate your previous answer, let's use the polar contact finder in PyMOL. Click on the stick representation of STU with the mouse left button - the molecule becomes selected. Now click on the right mouse button and a menu will pop up. Selecting "actions", then "find" then "polar contacts" then "to other atoms in the object". Dashed lines will show, indicating likely polar contacts between ligand and protein.

6. Find the Uniprot (www.uniprot.org) entry of Human Chk-1. Using the information in there, highlight the binding and active residues and the residues forming the "nucleotide binding site" in the structure loaded in PyMOL. Create a .pml command file to be able to apply those changes to futures Chk-1 structures (the teacher will explain how to create and manage ".pml" files).

7. A promising avenue of discovery started with a triazolone compound found in the HTS study (compound 3 in paper1, with an IC-50 of 0.8 uM). Using information in paper1, find the crystallographic complex between Chk-1 and this compound in the PDB. Using PyMOL, check which proteins residues may be interacting with the ligand (confirm this information using paper1). Can you find the water molecule obstructing the active site channel ? Which residue is it binding to ?...

8. Download the structure `5OOP` from the PDB (complex of Chk-1 with a the AMP-PNP competitive kinase inhibitor). Superimpose this structure on the one you have currently downloaded with the command `align 5oop, 2x8i` and identify the phosphate and sugar binding regions of the Chk-1 active site.

9. In an attempt to improve the binding affinity of triazolones to Chk-1, additional substituents were added to compound 3, seeking new interactions with the sugar binding pocket. Compound 4a is a 4-hydroxyphenyl triazolone which show a 10 fold increase in potency compared to compound 3. Based on information in paper 1, retrieve its crystallographic structure from the PDB and check what interactions in the substituent group may be responsible for the increased potency. Superimpose this structure with `2x8d` and compare the binding to the active site.

10. Following the work on paper 1, the best compounds in Tables 1 and 2 were tested for *in vivo* activity in cellular assays and found to have none. So a new series of 8-heterocylic substituted analogs was create (Table 3), where some compounds show extreme potency and significant activity in cell assays (**NOTE**: the article mistakenly refer to these compounds as being 7-substituted, but this is wrong). One such (compound 28) was design with the aim to access the conserved residue Lysine 38. The complex between compound 28 and Chk-1 was crystallized and deposited in the Protein Databank with code `2x8e`:

    - Retrieve the PDB file and load in in PyMOL, aligning it with the 2x8d structure.
    - What new contacts does compound 28 make with the protein structure ?
    - Compare the mode of binding of compound 28 and compound 4. Do you think this was the intended way for 28 to bind ?
    - Hide everything in `2x8e` except the ligand, with the command `hide everything, 2x8e and not x8e/`
    - Align the ligand `x8e` (compound 28) to ligand `x8i` (compound 4a) with the commmand `align x8e/, x8i/` (this moves `x8e` to its expected mode of binding to Chk-1). Find out which Chk-1 residues would interact with it in this mode of binding. Is this in agreement with the original design intent ?