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 use PyMOL and other tools to fulfill the tasks listed below.

Exercises

0. Goto to the Wikipedia page for "staurosporine" and on the bar on the right hand side, at the bottom, expand the SMILES code and copy it.
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 left side menu on the home page, select "Search", then "Chemical Sketch Tool" and then, at the bottom section namde "Load Molecule", select "SMILES" as the descriptor type and drop the staurosporine SMILES code in the box and press "Load". The staurosporine molecule should successfully load in the structure window. Now press the "Search" button at the bottom of the page to search for PDB entries containing staurosporine and similear compounds as ligands. In the result page, the first entry of code "STU" is staurosporine. Click on it to see how many entries do contain this ligand. Click on the number of entries shown to get the full list of PDB entries containgin the ligand STU. Doess the result suport the notion of staurosporine being a "promiscuos ligand" ?

4. Some of the proteins in complex with staurosporine listed in the previous exercise might not have been kinases. Try to find out how many really are kinases, using the option "Advanced Search Query Builder" on top of the page, selecting "Structure Title" and "contains word", then "kinase". This will combine a search for the word "kinase" in the structure title with the full list of structures having staurosporine as a ligand. Can we trust the obtained result ? Repeat the search using "Macromolecule Name" instead of "Structure Title". Are the results different ?

5. How many structures contain staurosporine in complex with human checkpoint kinase 1 (Chk-1) ? (There are seveeral ways to answer this question. One his to get the Uniprot code of Chk-1 at (https://www.uniprot.org) and use the "Acession Code(s)

    - Uniprot" on the RCSB advanced search.

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 that distance smaller than 3.0 A between hydrogen bond donors and acceptors generally indicate an H-bond).
    - 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 ligand 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 appear, indicating likely polar contacts between ligand and protein.
6. Go to the Uniprot web site (https://www.uniprot.org) and search for Human Chk-1 (entry code `CHK1_HUMAN`). Using the information listed in the entry, highlight with different colors the following regions in the PyMOL structure: ATP binding site, active site and nucleotide binding region. Create a `PyMOL` .pml command file with the coloring commmands,so that you may use it with future Chk-1 structures (the teacher will help you with this step).
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 μM). Using the 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 the AMP-PNP competitive kinase inhibitor). AMP-PNP is very similar to the ATP substrate and binds in the same way. Superimpose this structure on the one you have currently downloaded with the command `align 5oop, 2x8d` and identify the phosphate and sugar binding regions of the Chk-1 active site. Compare the way in which compound 3 and the substrate analogue fill the binding pocket.
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 created (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 designed 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` (In Fig. 4 of paper1 the PDB code is wrongly given as `2x8d`):

- 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 4a. 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 ?