Using Spark Reagent Databases to Find the Next Move

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Abstract

Cresset's Spark software was used to investigate new R groups that could be used to grow a fragment-like inhibitor of p38. The use of databases derived from available reagents ensured that the results could be tethered to molecules that were readily synthetically accessible. Using Spark resulted in R groups that were chemically diverse yet retained important shape and electrostatic properties. The results contain molecules that are similar to known inhibitors as well as novel suggestions, free from IP.

Introduction

Mitogen-activated protein kinases (P38) are a set of protein kinases with several isoforms which participate in signaling cascades to control cellular responses to stress stimuli and are involved in cell differentiation and apoptosis. Stress stimuli may include responses to cytokines, heat shock, osmotic shock and UV irradiation[1]. These kinases are recognized as important therapeutic targets: a P38-gamma inhibitor has recently been approved for idiopathic pulmonary fibrosis (IPF)[2], while P38-alpha inhibitors are potential anti-inflammatory agents (*e.g.*, PH-797804) currently in clinical trials[3] for COPD.

A large number of P38-alpha inhibitors are known, and for many of these, the X-ray crystal structures for the ligand complexes are available. P38-alpha is a particularly pliable protein to which inhibitors are known to bind to a variety of protein conformations.

This wealth of data includes large inhibitors, as well as smaller fragments. Three example P38-alpha inhibitors are shown in figure 1.

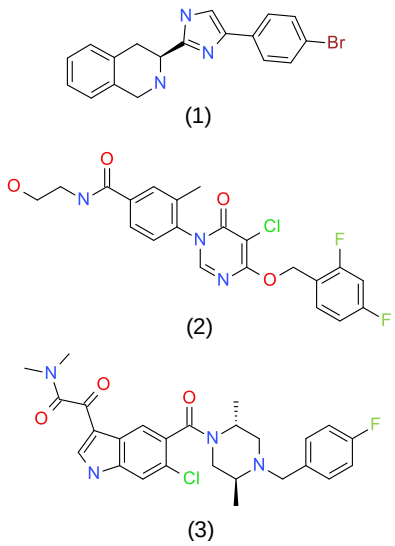

Figure 1: Example P38 alpha inhibitors. (1) PDB:3K3I; (2) PDB:3ROC; (3).

Compound 1 is a fragment which specifically binds to the inactive form of P38-alpha known as the 'DFG-out' kinase protein conformation. In this form, the activation loop is distorted and the catalytic residues are displaced from their usual position and are thus incapable of binding ATP (figure 2).

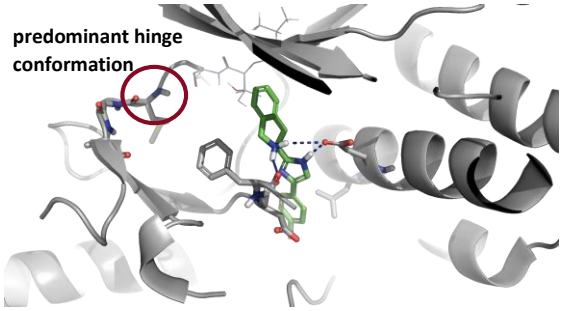

Figure 2: DFG-out P38 fragment inhibitor (PDB: 3K3I)[4].

Unfortunately, this attractive non-competitive form of inhibition, although relatively rare, is not unique; and inhibitors which bind to this conformation tend also to be inhibitors of other protein kinases which can adopt this conformation (*e.g.*, Imatinib inhibits both c-abl, c-kit and PDGF-R).

In contrast, inhibitors 2 and 3 are more selective for P38, despite being ATP competitive, and interact with an active conformation of P38. They are selective due to the plasticity of P38 in the hinge region where the hinge glycine residue allows a 180° flip of its peptide bond such that a switching of the H-bond donor/acceptor pattern is required by the inhibitor (Figure 3).

We have demonstrated in a previous Spark case study[7] a fragment growing protocol can be employed using compounds 1 and (2 or 3). The combination of this fragment with these selective inhibitors could potentially yield novel selective inhibitors which are non-ATP competitive. Growing towards the hinge region will also increase the potency of the fragment (compound 1).

Tethering Spark results to available synthetic chemistry

Spark's approach to fragment-growing uses Cresset's field-based technology[8] to identify viable replacements for a selected portion of a

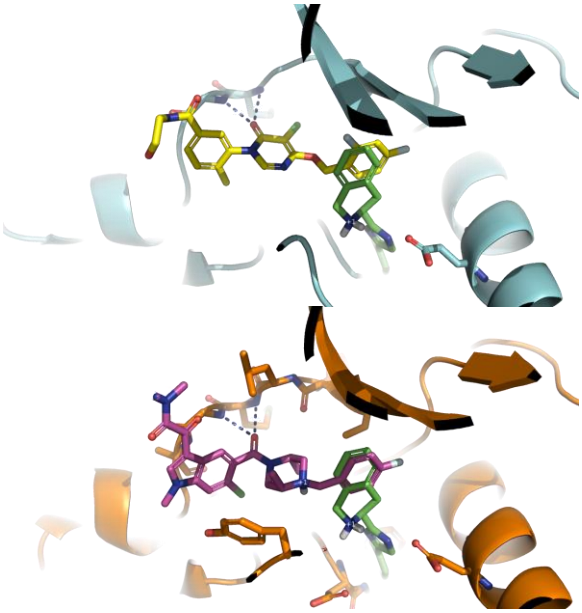

Figure 3: Selective P38 inhibitors (2, PDB: 3ROC)[5] and (3, PDB: 3HUB)[6] overlaid with the fragment (1).

reference compound using a series of fragment databases.[9]

In this case study we chose to use a database of fragments that was derived from available reagent pools. This gives the opportunity to rapidly search all R-groups that could be introduced at a selected position. Here we use the standard reagent databases supplied by Cresset which are based on the available chemicals directory. However, an optional Database Generator module enables the creation of fragment databases that are derived from corporate compound registries or inventory systems, linking your available chemistry directly to the Spark experiment.

Method

The fragment-growing protocol employs multiple reference compounds: a 'Starter' and 'Reference(s)'. The Starter compound is the one to which a modification will be made whilst the references are used to provide information about the region that is to be explored. New functional groups are scored against both the

starter and reference molecules with Spark offering control over how the different scores are combined. In this fragment growing example the score emphasis was placed on the reference to ensure that the additional regions of space that this described were fully explored in result molecules.

We took two co-crystalized inhibitors (3K3I and 3ROC) as starting points, and used our experiment to grow the smaller 3K3I DFG-out ligand using the 3ROC DFG-in hinge flipped ligand as a guide (reference). To set up the experiment, it was necessary to first align the protein crystal structures using a least squares fit of equivalent C-alpha atoms. This indicated that the optimal position to grow the 3K3I ligand was from the *ortho* position of the tetrahydro-isoquinoline part of the ligand. To this end a methyl group was added to the 3K3I ligand at the chosen attachment point as shown in figure 4.

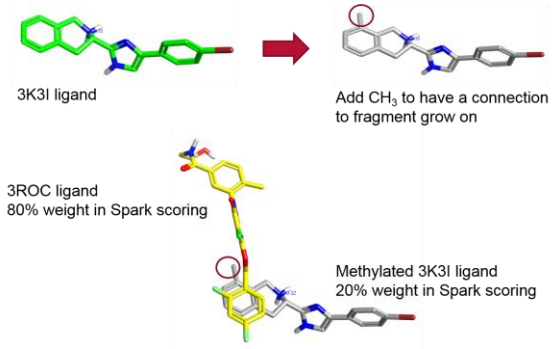

Figure 4: The structural set up for the Spark fragment growing experiment.

Using Spark's ability to have multiple references to score against, the 3K3I ligand was weighted at 20%, and the 3ROC ligand at 80%, to push the fragments onto the 3ROC ligand pattern.

In the current experiment, we attempt to exploit the synthetic chemistry used by Pfizer in making their PF-03715455 compound (figure 5).

This compound has been crystalized in P38 and hence it was decided to use this protein as an excluded volume as it has the desired DFG-out and Gly-flip conformation. The PDB 2YIS[10] protein was downloaded and superposed on to the 3K3I-3ROC proteins using a fit of equivalent C-alpha atoms.

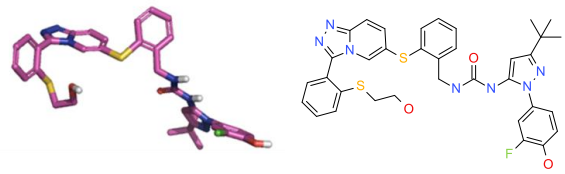

Figure 5: Pfizer compound PF-03715455, shown in its bioactive conformation from PDB:2YIS.

For this fragment growing experiment, we will use the fragments from the ZINC database of commercially available thiols, which should give results that are readily synthesizeable.

To ensure that results from the Spark experiment were significantly larger than the 3K3I starter molecule, the default restrictions on the size of the fragments that are acceptable was removed. Thus fragments were considered if their MW <250 or their rotable bond count <4. No other filters were placed on the thiol derived fragments.

Results and Discussion

Reviewing the results of this Spark experiment, a number of interesting compounds were identified. Shown in figure 6, is the first (top scoring) result, which fits nicely in the 2YIS pocket. Upon closer examination of the thiazolo-triazole moiety, it is observed to be taking advantage of the same H-bond interactions as the Pfizer compound.

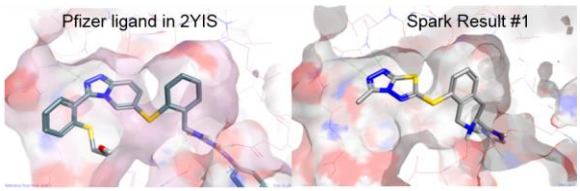

Figure 6: Shown on the left is Pfizer's COPD candidate in 2YIS pocket; on the right is shown result #1 from the Spark experiment. H-bond interactions are shown in light green lines.

Showing a couple more results (figure 7) from the Spark experiment, we see that these *in silico* designed molecules also potentially exploit the H-bond interaction described above.

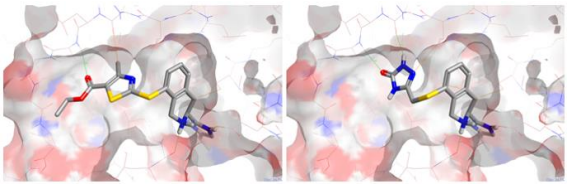

Figure 7: Representative results from Spark fragment growing experiment, showing exploitation of H-bond interactions with 2YIS pocket residues.

Conclusion

Having used Cresset's available thiols reagent pools, the same chemical transformation used by Pfizer to make their clinical candidate could be applicable. The search could also be expanded to exploit similar transformations including amines and alcohols, which may provide interesting results to test, however, would involve the use of different chemical transformations to synthesize.

In conclusion, using Spark's reagent databases can help enable quick profiling of accessible chemistry around a specific core.

References

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