

Displacing crystallographic water molecules with Spark

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Abstract

Cresset's Spark¹ software for bioisosteric replacement was used to carry out a water displacement experiment starting from the X-ray crystal structure of a selective inhibitor of Bruton's tyrosine kinase². The use of databases derived from available reagents ensured that the results could be tethered to molecules that were readily synthetically accessible. The availability of a sufficiently diverse source of reagents was crucial in demonstrating the feasibility of this approach.

Introduction

Bruton's tyrosine kinase (Btk) is a member of the Tec family of non-receptor tyrosine kinases. Recent literature findings² indicate that Btk inhibition could be an attractive approach for the treatment of autoimmune diseases such as rheumatoid arthritis, a progressive autoimmune disease characterized by swelling and erosion of the joints³.

A fragment-based drug design approach was recently² applied to the discovery of non-covalent, potent inhibitors of Btk inhibitors with Lck selectivity (Lymphocyte-specific protein tyrosine kinase, a target playing a key role in T-cell activation).

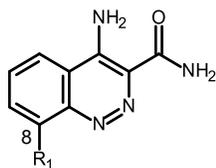
Among the most interesting hits identified with this approach, compound 2 (Table 1) was selected for further optimization. Position 8 of the cinnoline ring of fragment 2 was explored using the Suzuki–Miyaura⁴ synthetic methodology, starting from a series of monocyclic boronic acids/esters. This initial SAR exploration led to the discovery of compound 8

(Table 1), which shows improved potency and selectivity with respect to fragment 2.

The published X-ray crystal structure of compound 8 in the active site of Btk (PDB 4ZLZ) shows a water-mediated hydrogen bond from the pyridyl nitrogen to the P-loop backbone residues Phe413 and Gly414 of Btk² (Figure 1 – left). The replacement of 4-methylpyridin-3-yl in compound 8 with small bicyclic heterocycles displacing the water molecule and making direct H-bond interactions with the P-loop led to the discovery of compounds 10 and 11 (Table 1), with a 10-fold improved potency towards Btk.

The 3D structure of compound 8 and the bridging water molecule were used as the starting point for this Spark case study. The aim of this experiment is to verify whether our methodology is able to displace the bridging water molecule and correctly identify the same alternative indazole fragments.

Table 1. SAR exploration of fragment hit 2



Cmpd	R1	Btk IC50 (nM) ¹
2	H	3.5 μM
8		100±46
9		850±11
10		12±0.2
11		4.0±0.3

Spark reagent databases: accessing available chemical diversity

Spark's approach to scaffold hopping and R-group replacement uses Cresset's field-based technology^{5,6} to identify viable replacements for a selected portion of a reference compound using a series of fragments. In this case study we chose to use standard reagent databases⁷ supplied by Cresset which are based on the available chemicals directory. This gives the opportunity to rapidly search all R-groups that could be introduced at a selected position. 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 published X-ray crystal structure of compound 8 bound into to the active site of Btk (PDB 4ZLZ) was downloaded into Forge⁸. The structure of the ligand was minimized and then combined with the water molecule mediating the H-bond interaction with the P-loop backbone residues of Btk to make a single molecule entry. The merging of the two 3D structures was done using the 'combine selected pair into single molecule' feature available in Forge. The unique entry thus created (see Figure 1 - right) was used as the Starter molecule for the Spark experiment (Figure 2 - left).

In this water displacement experiment, we want the Spark search to be driven mainly by the electrostatic fields, rather than by the usual combination of fields and shape.

For this reason a constraint was added to the negative and positive field points of the water molecule using the Spark Field Constraints Editor (Figure 2 - right). This introduced a score penalty for those results that did not match the constrained field points.

Furthermore, the 'Normal' conditions for scoring the Spark search results were fine-tuned to 90% Field and 10% shape, using the Btk protein as a 'hard' excluded volume, to constrain the size of the potential replacement fragments. The gradient cutoff for minimization was set to 0.200 kcal/mol/Å, removing at the same time the automatic constraint of fragment size to ensure that the results of the search

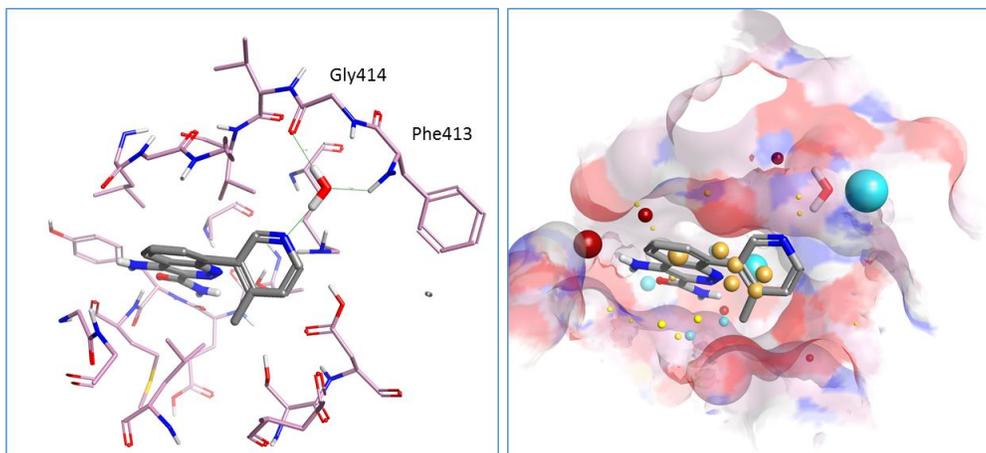


Figure 1. Left: X-ray crystal structure of compound 8 in the active site of Btk making a water mediated hydrogen bond with the P-loop backbone. Right: 3D structures and field points of compound 8 and of the bridging water molecule combined into a single entry.

Color coding of field points: blue = negative; red = positive; yellow = steric; gold = hydrophobic.

were not too biased by the size of the starter molecule.

Finally, to focus the experiment on small bicyclic heterocycles, monocyclic fragments were filtered out from the list of potential results using an appropriate SMARTS filter.

Two runs of Spark were carried out using the above conditions. The initial experiment was run on a database of 775 boronic acids to closely replicate the chemistry used in the original publication^{2,4}.

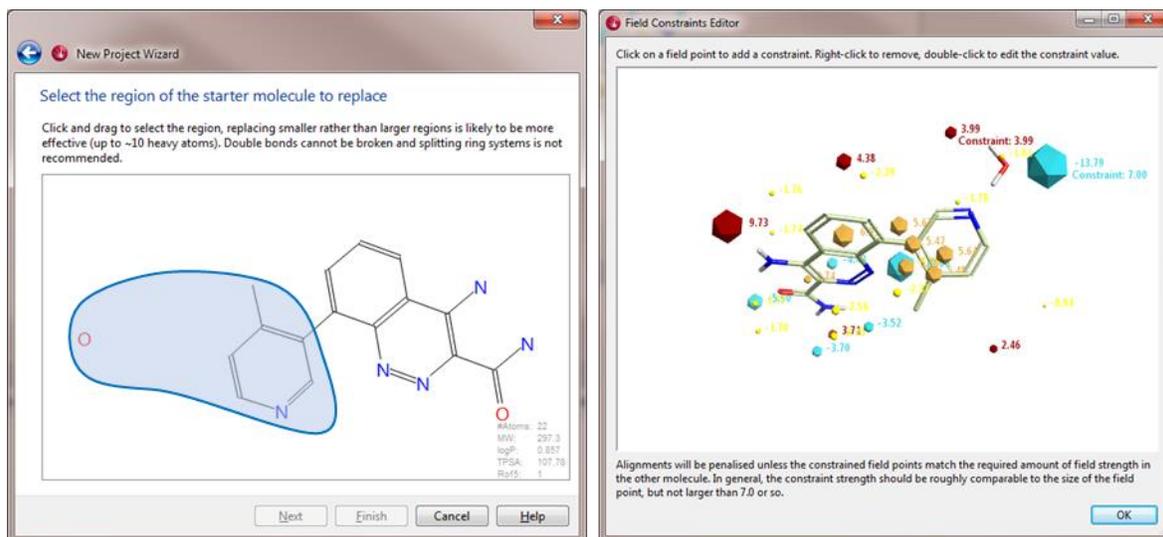


Figure 2. Left: the combined 3D structures of compound 8 and the bridging water molecule used as a starter molecule in the Spark experiment. Right: constraints associated to the field points of the water molecule.

Color coding of field points: blue = negative; red = positive; yellow = steric; gold = hydrophobic.

In the second experiment, the ZINC⁷ database of commercial aromatic halides (41K fragments) was also searched to explore a larger chemical diversity, starting from the assumption that the appropriate boronic acid/ester could be obtained from any interesting commercial aryl halide at the cost of an additional synthetic effort.

Results

The top scoring compound from the initial search (boronic acids only) is compound 10 (Table 1). As can be seen in Fig. 3 - right, this compound superimposes very well with the starter molecule and matches the constrained field points in a satisfactory manner. However, compound 11, which would presumably superimpose even better with the conformation of the *ortho*-methyl-pyridin-3-yl group of compound 8, was not found in this search, due to the limited chemical diversity of the database searched.

In the second Spark search, which was run on a much larger collection of reagents (boronic acids and aryl halides), compound 11 (Fig. 3 –

center and Fig. 5) is the top scoring result, while compound 10 ranks 4th in the list (Fig. 5).

The original paper² also reports the indole-substituted compound 9 (Table 1), quite similar in terms of 2D structure to the much more potent indazole compounds 10 and 11. This fragment is available in both the databases searched, but is not retrieved by Spark. The indole fragment in fact cannot match the constrained negative field point of the bridging water molecule, as shown in Fig. 6, where compound 9 is shown superimposed to the starter molecule in Forge. The lack of this relevant interaction explains the much lower potency of compound 9, with a Btk IC₅₀ = 850nM (Table 1).

Figure 4 shows a tile view of the 16 top scoring results from the second Spark experiment. Several different flavors of the indazole fragment carrying different substitution patterns are represented in this list. Alternative bicyclic fragments are also proposed, which may provide useful ideas for a further exploration of this target.

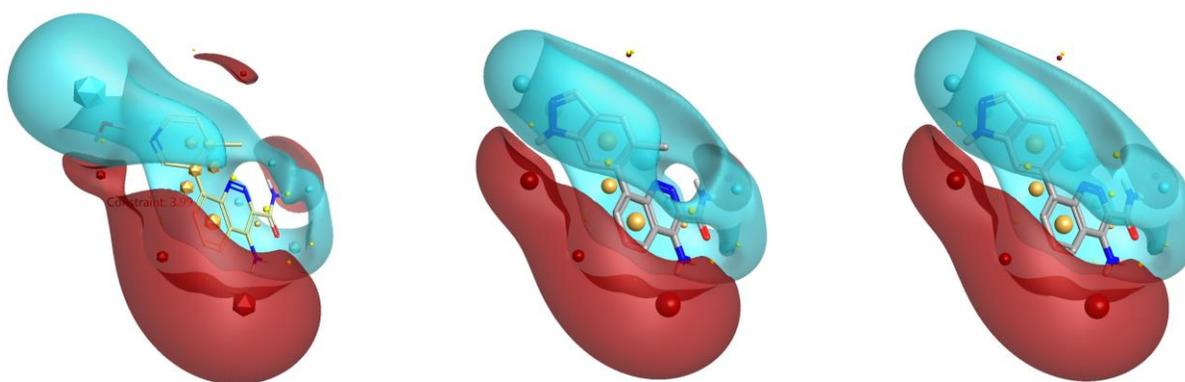


Figure 3. Left: Electrostatics of starter molecule. Center: compound 11 (Btk IC₅₀ = 4.0 nM). Right: compound 10 (Btk IC₅₀ = 12 nM)

Color coding of fields/field points: blue = negative; red = positive; yellow = steric; gold = hydrophobic.

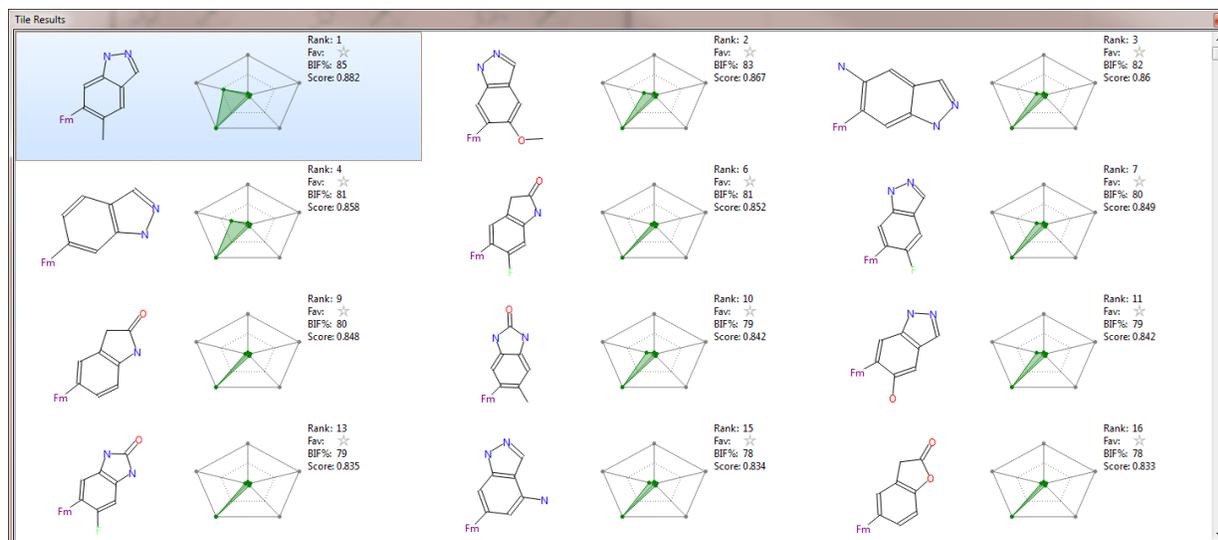


Figure 4. Tile view of the top scoring Spark results for the second experiment.

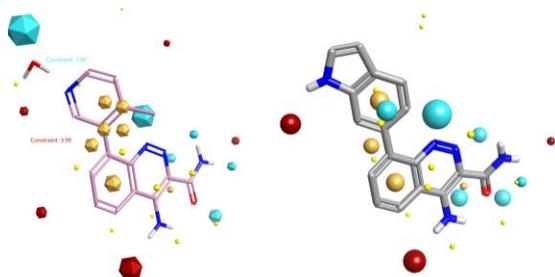


Figure 5. Compound 9 (right) superimposed to the starter molecule of the Spark experiment (left).

bridging the interaction between compound 8 and the P-loop of Btk, replacing it with small, synthetically accessible bicyclic heterocycles.

Availability of appropriate sources of chemical diversity is still a key factor in determining the success of any biososteric replacement experiment.

For this reason, the creation of fragment databases derived from corporate compound registries or inventory systems, linking your available chemistry directly to the Spark experiment, is highly recommended.

Conclusions

In this case study Spark successfully managed to displace the crystallographic water molecule

References and Links

1. <http://www.cresset-group.com/products/spark/>
2. Smith, C. R. *et al.*, *J. Med. Chem.* **2015**, 58, 5437–5444
3. Firestein, G. S., *Nature* **2003**, 423 (6937), 356–361
4. Miyaura, N., Suzuki, A. *et. al.*, *J. Am. Chem. Soc.* **1989**, 111 (1), 314–321.
5. *J. Chem. Inf. Model.*, **2006**, 46, 665–676.
6. <http://www.cresset-group.com/science/field-technology/>
7. Spark fragment databases come from commercial compounds, ChEMBL, ZINC and VEHICLE.
8. <http://www.cresset-group.com/products/forge/>