Using the Spark reagent databases to identify bioisosteric R-group replacements

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Abstract

The reagent databases[1] available with Cresset's Spark[2] software for bioisosteric replacement were used to identify alternative decorations for a series of triazolopyridazine and 8-fluorotriazolopyridine selective inhibitors of the c-Met Kinase. The use of databases derived from available reagents ensured that the results could be tethered to molecules that were readily synthetically accessible.

Introduction

The overexpression of c-Met and/or hepatocyte growth factor (HGF), the amplification of the MET gene, and mutations in the c-Met kinase domain can activate signaling pathways that contribute to cancer progression by enabling tumor cell proliferation, survival, invasion, and metastasis.[3,4] For these reasons, there has been significant interest in the discovery of small molecule c-Met inhibitors for the treatment of cancer. In particular, researchers at Amgen have recently published potent, selective, ATP-competitive and orally bioavailable small molecule inhibitors of c-Met belonging to the chemical classes of triazolopyridazine[3] and 8-F-triazolopyridine.[4]

The published X-ray crystal structure of compound 4[3] (an early representative of the triazolopyridazine series, see Table 1) bound to c-Met (PDB 3CD8), shows that this molecule adopts a "U-shaped" binding mode into the active site (Figure 1). A direct hydrogen bond is formed between the backbone NH of Met1160 (linker) and the quinoline nitrogen. A second hydrogen bonding interaction can be observed between N1 of the inhibitor and the backbone NH of Asp1222. The triazolopyridazine core makes a π-stacking interaction with Tyr1230. Finally, the aromatic C-H in position 7 makes an electrostatic interaction with the carbonyl of Arg1208.

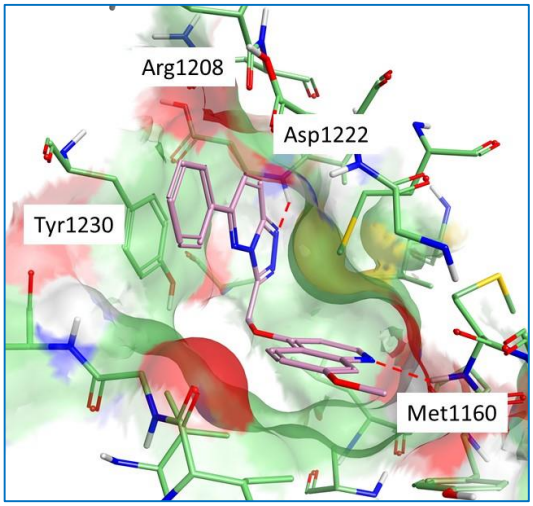
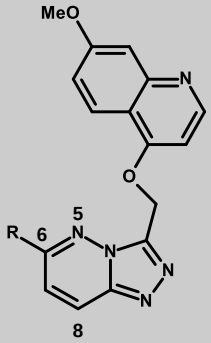
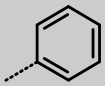
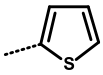
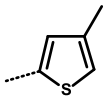
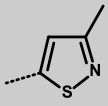
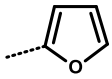
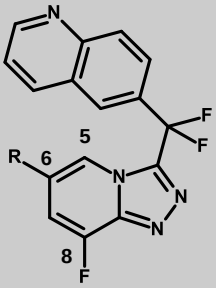
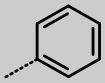
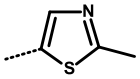
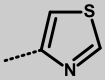
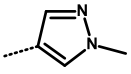


Figure 1. X-ray crystal structure of compound 4[3] in the active site of c-Met (PDB 3DC8).

Based on this experimental information, researchers at Amgen speculated that modifications of the C-6 phenyl group on the triazolopyridazine core would modulate the π-stacking interaction with Tyr1230 allowing for increased potency, and started a chemical exploration based on the synthesis of C-6 aryl and heteroaromatic analogues.[3]

Table 1. SAR of triazolopyridazine and 8-fluorotriazolopyridine compounds against c-Met.

Scaffold	Compound Number	R	c-Met IC50 (nM) a)	c-Met IC50 (nM) b)
triazolopyridazine[3] 	4[3]		9±2	46±11
	10j[3]		2±0.5	6±1
	10k[3]		4±0.1	29±13
	10l[3]		1±0.1	2±0.3
	10m[3]		3±0.1	2±0.3
	10n[3]		29±8	132±6
8-fluorotriazolo pyridine[4] 	4[4]		23	N/A
	10a[4]		12	32
	10b[4]		9	9
	10c[4]		11	35
	10d[4]		4	7
	10e[4]		22	15

a) Inhibition of c-Met kinase activity

b) Inhibition of HGF-mediated c-Met phosphorylation in PC3 cells

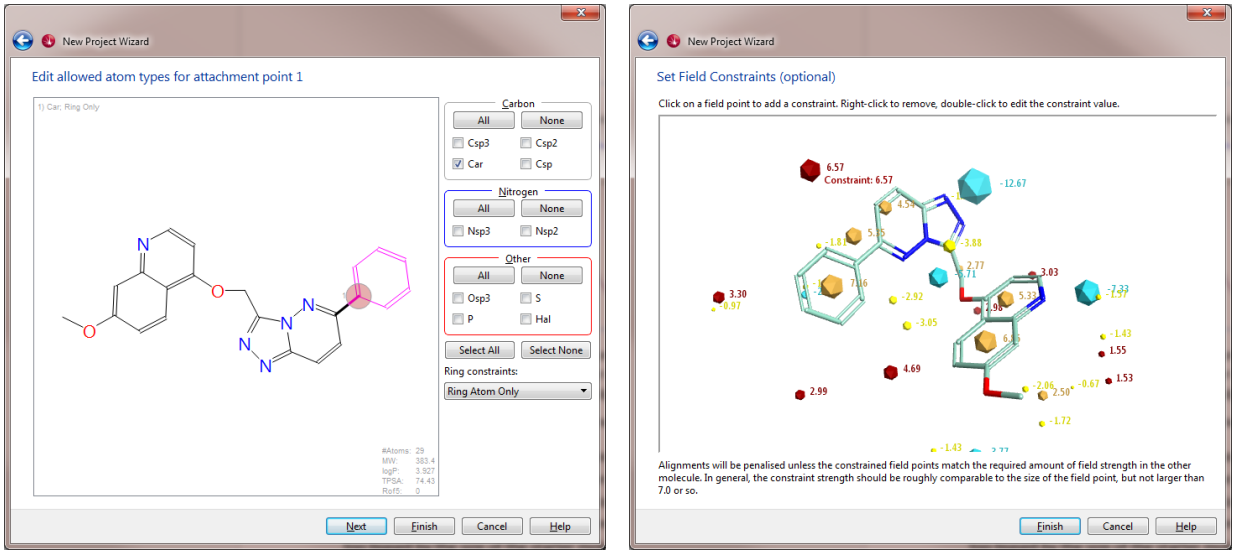


Figure 2. Left: starter molecule used in the Spark experiment. Right: constraint associated to the positive field point mapping the interaction of compound 4[3] with Arg1208 in c-Met.

The same strategy was applied to the exploration of 8-fluorotriazolopyridine compounds.[4]

The 3D structure of compound 4[3] was used as the starting point for this case study, where Spark was used in combination with the Cresset supplied reagent databases which are based on eMolecules building blocks.[5] The aim of this experiment is to verify whether our methodology could have facilitated the chemical exploration work at Amgen, correctly identifying, among the results of a single Spark run, the most active C-6 monocyclic heterocycles published in refs. 3, 4.

Method

The published X-ray crystal structure of compound 4[3] bound into to the active site of c-Met (PDB 3CD8) was downloaded into Forge.[6] The structure of the ligand was minimized and used as the Starter molecule for the Spark experiment (Figure 2 - left). The 'Accurate but slow' conditions for scoring the Spark search results were fine-tuned by setting the gradient

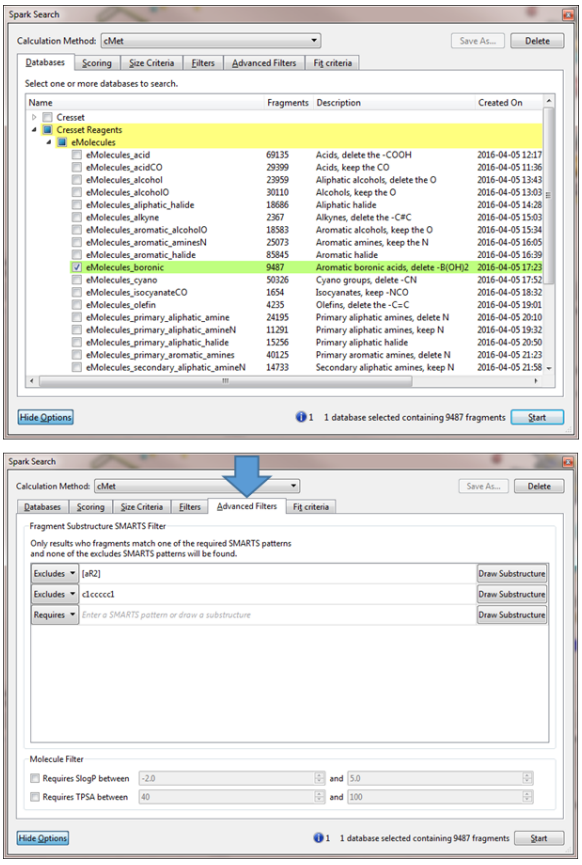


Figure 3. eMolecules reagent databases (top) and Advanced Filters options (bottom).

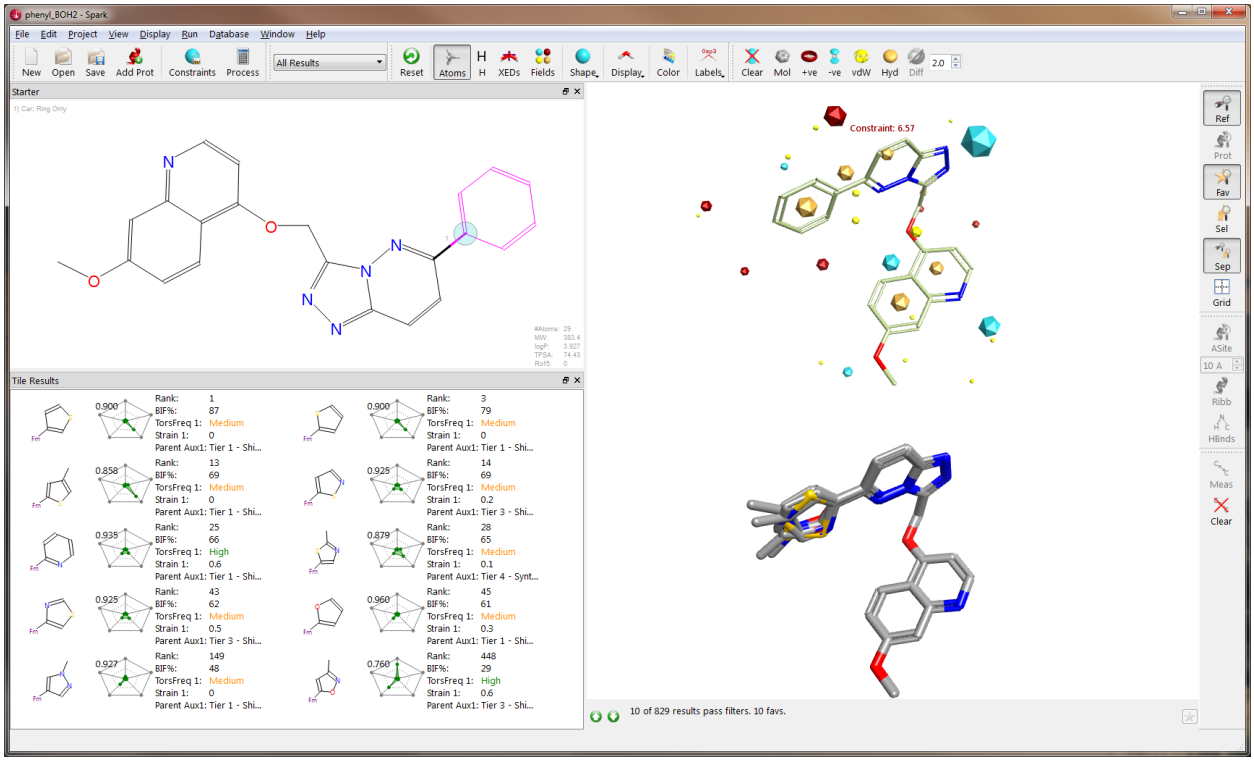


Figure 4. R-groups associated with known active inhibitors of c-Met found by Spark.

cutoff for minimization to 0.200 kcal/mol/A, and by setting a constraint on the positive field point mapping the interaction of compound 4[3] with Arg1208 in the c-Met kinase (Figure 2 - right). This introduced a score penalty for those results that did not match the constrained field point. Finally, to focus the experiment on small monocyclic heterocycles, bicyclic fragments and substituted phenyl fragments were filtered out during the search using an appropriate SMARTS filter using the 'Advanced Filters' panel options (see Figure 3).

The experiment was run on a database of 9.5K aromatic boronic acids derived from eMolecules (Figure 3) building blocks to closely replicate the chemistry used in the original publication.[3,4]

Results

As can be seen in Figure 4, the initial Spark experiment was able to identify the large majority of the monocyclic heterocycles used to explore the C-6 position of c-Met Kinase inhibitors published in ref. 3 (Table 1). In particular, 3-thienyl (10k), 2-thienyl (10j), 5-isothiazolyl (a close analogue of 3-methyl-isothiazol-5-yl used for compound 10m[1]), 4-methyl-2-thienyl (compound 10l), were correctly identified among the 15 top ranking Spark results.

The Spark experiment was also able to correctly identify C-6 heterocycles used in subsequent iterations of the project to explore the 8-fluorotriazolopyridine scaffold (Table 1). However, while 2-pyridyl (compound 10a), 4-

[1] 3-methyl-isothiazol-5-ylboronic acid is not available in the eMolecules database used for the Spark search.

thiazolyl (10d) and 2-methyl-5-thiazolyl (10c) rank reasonably high in the list of results, 1-methyl-4-pyrazolyl (10e) and 3-methyl-5-isoxazolyl (10b) are correctly retrieved, but with a lower rank.

This is disappointing, however, compound 4[3] is approximately 3-10 times less potent in terms of c-Met enzyme activity, and 20 times less potent in the cellular assay, than the most active heterocyclic compounds published in ref. 3 (10m and 10l). The Spark search was then repeated using 10m (which has a better pharmacokinetic profile than 10l[3]) as the starter molecule, to verify whether any improvement in the ranking of these two substituents could be achieved by starting from a more active compound, which is expected to even better fit the electrostatic and steric requirements of the c-Met binding site. The 3D conformation used for 10m was obtained by means of a field/shape alignment with the X-ray structure of compound 4[3] carried out within Forge.

The results of this second experiment are summarized in Figure 5. The ranking of 1-methyl-4-pyrazolyl was significantly improved, while no improvement was observed for 3-methyl-5-isoxazolyl.

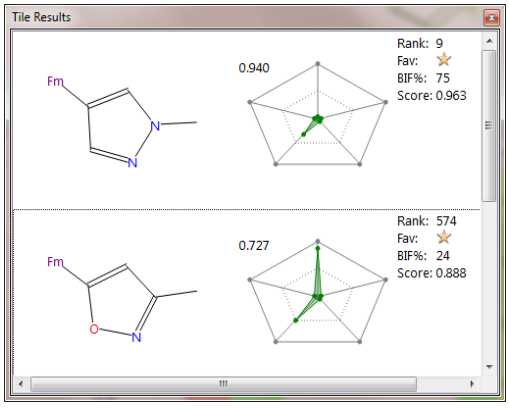


Figure 5. Ranking of 1-methyl-4-pyrazolyl and 3-methyl-5-isoxazolyl using 10m as the starter molecule.

A final Spark search carried out with compound 10m as a starter molecule on an expanded set of reagent databases (boronic acids and aromatic halides), suggested some interesting alternative small heterocycles which could have been tried, shown in Figure 6.

Strain and torsion frequency analysis

As reported in refs. 3,4, it was hypothesized by the Amgen authors that co-planarity would enhance potency towards c-Met, presumably due to an optimal configuration for π-stacking with Tyr1230. In evaluating the results of a Spark experiment for this target it is therefore important to ensure that potential replacement fragments can adopt a realistic planar conformation.

Two types of analysis are available in Spark to monitor the above. The first is a calculation of the strain of the newly formed bond from the potential replacement fragment and the scaffold. The strain is calculated by performing a 30 degree torsion scan for that bond in the result molecule, and calculating the energy difference between the torsion chosen by Spark in the result molecule and the lowest energy torsion found during the scan. Values lower than 2 are largely insignificant.

Additionally, the Torsion Library[7-9] method is used to assess the torsion associated with the newly formed bond, as well as the torsions associated with all rotatable bonds within the bioisostere fragment. The method is based on an analysis of the Cambridge Structural Database[10] (CSD), and reports the frequency with which a specific torsion is experimentally observed. Torsions associated with a low frequency are a possible cause for concern and should be further investigated.

Results

Rank	Structure	Radial Plot	BIF%	MW	SlogP	TPSA	Rof5	Tags	TorsFreq 1	TorsFreq Frag	Strain 1	Parent Aux1
6		0.960	84	373	2.9	88	0	novel	High		0	Tier 1 - Ships in 1-5 business days
56		0.885	68	387	3	103	0	novel	High	Medium	0	Tier 1 - Ships in 1-5 business days
176		0.900	60	374	2.3	100	0	novel	High		0.9	Tier 4 - Synthesis required; up to 12 weeks
181		0.900	60	385	2.7	100	0	novel	High		0.2	Tier 3 - Ships within 4 weeks
464		0.900	50	385	2.7	100	0	novel	High		0.8	Tier 1 - Ships in 1-5 business days

Figure 6. Novel potential replacement fragments identified by Spark.

As can be seen in Figure 4, all the fragments identified by the Spark experiment and reported in refs. 3,4 can adopt the required planar conformation, with no significant strain associated to the newly formed bond: torsional frequencies for this bond range from 'medium' to 'high', and should accordingly be realistic based on the experimental data in the CSD.

Figure 6 shows the strain and the torsional frequency for the potential novel decorations identified by Spark. In this case, there are no concerns associated with the conformations chosen by Spark.

Availability of reagents

Whenever the new eMolecules reagent databases are used in a Spark experiment, availability information is displayed in the results table (see Figures 4 and 6). This information is important for planning laboratory activity taking into account realistic delivery timelines. For example, for three of the fragments shown in Figure 6, shipment is to be expected within 1-5 days from order. Delivery times for 5-pyrimidinyl and 5-oxazolinyl boronic acids are longer: the former can be shipped within 4 weeks from order, while the latter needs to be synthesized and this may take up to 12 weeks.

Searching for the reagents of interest in the eMolecules site enables a check of real-time availability information.

Conclusions

In this case study, a Spark R-group replacement experiment successfully identified the majority of active monocyclic heterocycles used by Amgen in the discovery of new potent triazolopyridazine and 8-fluorotriazolopyridine inhibitors of c-Met kinase.

The results suggest that working in successive rounds of optimization, choosing for each Spark experiment the starter molecule with the best activity profile, is an excellent strategy to rapidly identify the R-groups associated with the highest activity or optimal overall profile.

Access to reagent availability information plays an important role in deciding which fragments should be included each round of optimization. Reagents with short delivery times should be preferred during the initial stages of the project to facilitate quick SAR information gathering, which will enable a more informed choice of fragments to explore in the successive rounds of lead optimization.

References and Links

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