Rapid and Accessible *In Silico* Macrocycle Design: Application to BRD4

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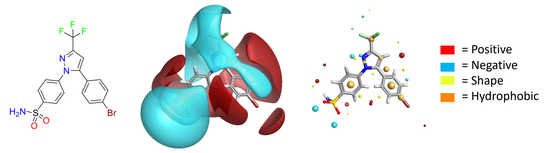
Background

Macrocyclization of pharmaceutical compounds plays an increasing role in drug discovery. With restricted conformational flexibility, macrocycles can contribute to improved binding affinity, selectivity and drug-like properties. Prediction of effective macrocyclization strategies prior to synthesis is crucial. Spark,[1] Cresset's bioisostere replacement and scaffold hopping tool, allows for rapid and accessible macrocyclization design that retains the bioactive conformation and electrostatic character of a known active molecule.

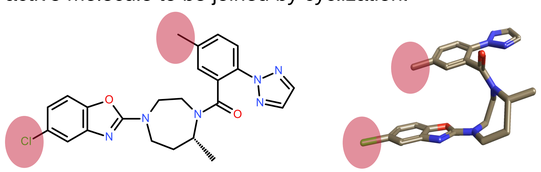
Cresset field technology[2] condenses the molecular fields down to a set of points around the molecule, termed 'field points'. Field points are the local extrema of the electrostatic, van der Waals and hydrophobic potentials of the molecule.



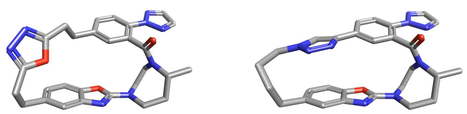
2D | 3D Molecular Electrostatic Potential (MEP) | Field Points

Spark macrocyclization workflow

The Spark macrocyclization approach uses a database of molecule fragments to suggest linker fragments that maintain the shape and electrostatic character of a known active molecule. The user identifies the two regions of a known active molecule to be joined by cyclization.



Distances and angles between any broken bonds are used to search a database of fragment conformations for linker fragments that match the geometry of the uncyclized molecules.

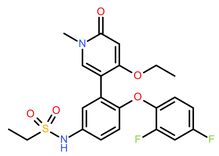


The product molecule is energy minimized and then scored as a replacement. Scoring is performed using an average of field and shape similarity on the product molecule.

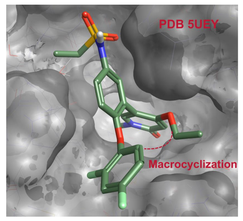
Application to BRD4

Spark macrocyclization wizard was used to design macrocyclization strategies for non-macrocyclic, pyridone BRD4 inhibitors and the results evaluated against experimental data reported by Wang *et al*.[3]

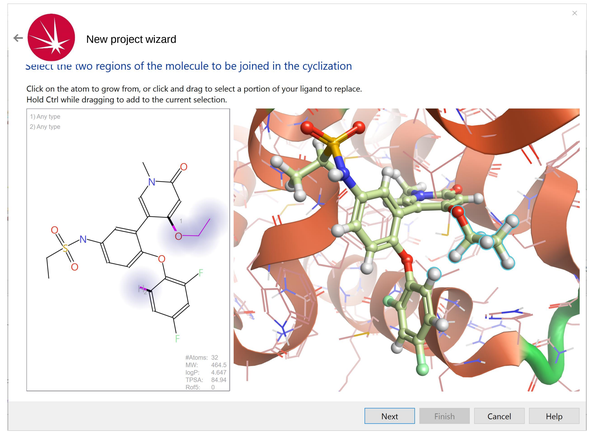
The macrocyclization wizard provides a quick and easy-to-use GUI workflow with dedicated Spark settings tailored for macrocycle designs. The X-ray structure of BRD4 in complex with the acyclic compound (1) (pdb 5UEY) was used as the protein, and compound (1) as the starter molecule.



Compound (1)
BRD4 K_i = 5.6 nM



The ethoxy group of the pyridone scaffold is in close proximity to the 2,4-difluorophenoxy ring and compound (1) is partially solvent exposed in this region of the binding site, offering sufficient space to accommodate additional linker atoms. The ethoxy moiety and the hydrogen atom at position 6 of the 2,4 difluorophenoxy ring were selected as the two regions of the molecules to be joined by cyclization.



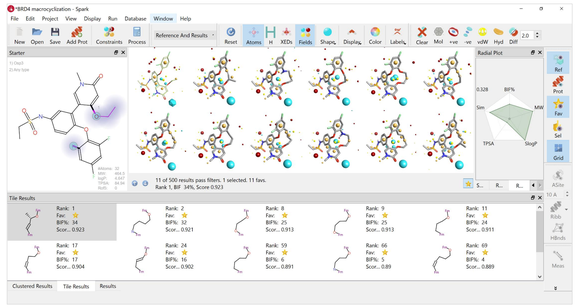
To bias linker design towards existing chemistry, the oxygen was specified as attachment point 1. The second attachment point was left unconstrained.

Additionally, the protein structure was used as an excluded volume to guide linker design and the field points of carbonyl and sulfonyl groups forming hydrogen bonds with the receptor were constrained.

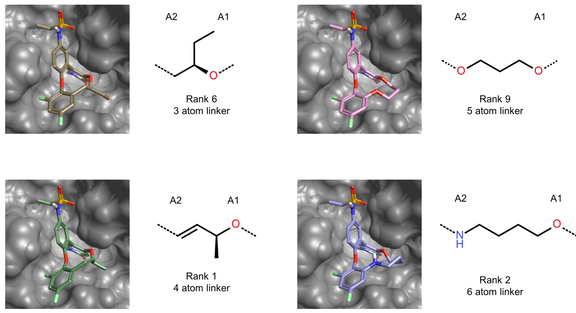
The experiment was run with the dedicated 'Ligand Joining / Macrocyclization' settings against the fragment databases CHEMBL_common, Common, and VeryCommon, containing altogether about 120K fragments.

Analysis

The macrocycle wizard experiment generated 500 rank-ordered macrocycles derived from fragments merged with the starter molecule.



Among the top 10 results Spark designed compounds with linker sizes between 3 to 6 atoms. The top ranking result for each linker size is shown below.

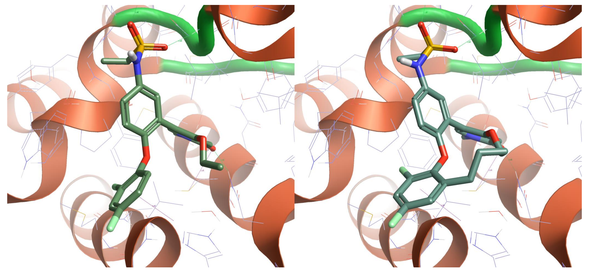


Linkers with 4 to 6 atoms were particularly enriched (Table 1). This finding is in good agreement with the experimental data reported by Wang *et al*., who found that short macrocycle linkers with only 3 atoms decrease the binding affinity by about 50x compared to a 5 atom linker. Four atom linkers were only slightly less potent than the 5 atom linkers, whereas 6 atom linkers showed a comparable binding affinity.

Table 1: Distribution of linker sizes in top10, top25, and top50 results from Spark (500 total).

Top N results	3 linker atoms	4 linker atoms	5 linker atoms	6 linker atoms	7 linker atoms
10	2	6	1	1	0
25	2	16	4	8	0
50	2	32	12	9	0

The X-ray structure of the reported and structurally validated macrocycle (2) (BRD4 K_i = 1.5 nM) shows that upon cyclization the molecule retains its key interaction with the BRD4 pocket, however, macrocycle (2) adapts a slightly different conformation, especially for the 2,4-difluorophenoxy ring.



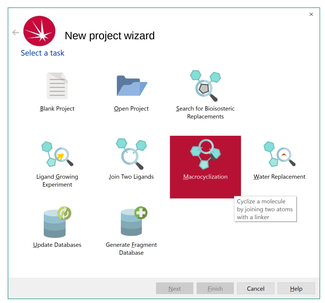
Compound (1) (5UEY) | Macrocycle (2) (5UEY)

The top scoring Spark results contained several macrocycles closely related to macrocycle (2), such as compounds with propan-1,3-diol (rank 9), 4-aminobutan-1-ol (rank 2) or butan-2-ol (rank 4). Macrocycle (2) was found at rank 59.

Conclusion

Spark successfully designed macrocycles that are identical or very similar to reported BRD4 macrocycle inhibitors.[3] The distribution of generated linker sizes was in good agreement with experimental SAR data.[3]

The Spark macrocyclization wizard is a quick and easy-to-use workflow that generates meaningful and diverse design ideas that can guide macrocycle drug discovery.



References

1. http://www.cresset-group.com/spark
2. Cheeseright, T.; Mackey, M.; Rose, S.; Vinter, A. Molecular Field Extrema as Descriptors of Biological Activity: Definition and Validation. *J. Chem. Inf. Model*. **2006**, 46 (2), 665-676
3. Wang L., Pratt J. K., Soltwedel T., Sheppard G. S., Fidanze S. D., Liu D., Hasvold L. A., Mantei R.A., Holms JH, McClellan WJ, Wendt MD, Wada C, Frey R, Hansen TM, Hubbard R, Park CH, Li L, Magoc TJ, Albert DH, Lin X, Warder SE, Kovar P, Huang X, Wilcox D, Wang R, Rajaraman G, Petros AM, Hutchins CW, Panchal SC, Sun C, Elmore SW, Shen Y, Kati WM, McDaniel KF,:. Fragment-Based, Structure-Enabled Discovery of Novel Pyridones and Pyridone Macrocycles as Potent Bromodomain and Extra-Terminal Domain (BET) Family Bromodomain Inhibitors. *Med. Chem.* **2017**, 60 (9), 3828–3850