Rapid approaches to new scaffold generation accelerate hit-to-lead

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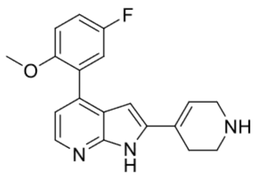
Introduction

This case study demonstrates a workflow which uses the Hit Expander[1] feature within Flare™ [2] to generate new molecules with small changes around a starting hit. These hits were triaged with Flare's relative free energy perturbation (FEP) calculations and the results were communicated to chemists using Torx®.

Cyclin-dependent kinase 9 (CDK9) is a type of serine/threonine kinase that plays a crucial role in regulating the elongation of transcription. Inhibiting CDK9 can have significant effects on the expression of short-lived proteins that are essential for tumor survival, such as the antiapoptotic protein MCL-1.

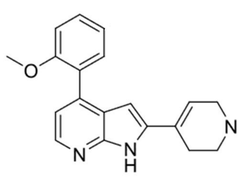
Tong *et al.*[3] identified orally efficacious azaindole-based inhibitors starting from cpd **1** (Figure1), which demonstrated reduced toxicity, adequate pharmacokinetic properties, and robust *in vivo* efficacy in mice upon oral dosing. The compounds can be found at the corresponding patent.[4]

1: starting cpd of Tong *et al.*



IC50 = 14nM / -10.7 kcal/mol

4PEP: our 'hit'



IC50 = 24nM /-10.4 kcal/mol)

Figure 1: Closely related to **1**, **4PEP** from the corresponding patent[4] has very slightly lesser activity.

The des-F analogue **4PEP** was used as reference for our studies: Known binding affinity; Charged pH 7; Reduced ring (double bond adds rigidity).

Methods

Hit Expander and Flare FEP, which are easy to use, fast and accurate methods in Flare reliably discover highly active molecules, which can be taken forward to experimental testing in less than 24 hours.

Hit Expander in **Flare™** generates 84 molecules from one hit **in seconds**

↓

Flare FEP calculates binding affinities of the 84 molecules **in ~ 6 hours**

↓

Flare FEP runs an 18 molecule, refined multi-connected production **in < 3 hours**

↓

Torx® distributes novel, promising designs to the medicinal chemists for synthesis **in seconds**

Figure 2: Proposed workflow: finding a predicted lead in less than a day.

Protein-ligand interactions

The **4PEP** ligand has an azaindole core and adopts a conformation, as shown in Figure 3.

Protein- Ligand interactions:

- Purple: Aromatic edge-to-face (PHE A103)
- Green: H-Bond (CYS A106)
- Gray: Hydrophobic contacts (LEU A156)

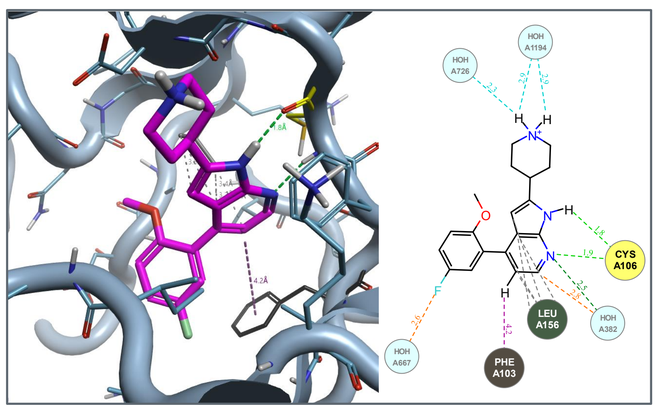


Figure 3: Left: 3D representation of the binding pose of the ligand. Right: Protein- ligand interactions.

Hit Expander and FEP calculations

Hit Expander enables swift exploration of the chemical properties of a single hit or lead compound. Its functionality involves introducing various small substituents at all feasible positions of the chosen compound, facilitating an efficient chemical exploration process. In our case, 84 new molecular design ideas were suggested (Figure 4).

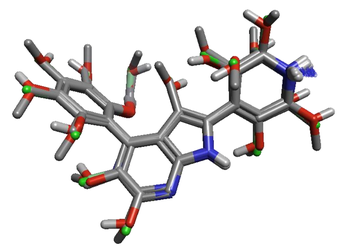


Figure 4: Hit Expander results for 4PEP, generating 84 new designs.

When a ligand has known activity and documented interactions with a protein, Free Energy Perturbation (FEP) calculations can be employed to determine the relative activity of structurally similar ligands to the reference ($\Delta G_{binding}$). Firstly, Flare FEP was used to benchmark the accuracy of the molecular system. The statistics of our model (R^2 = 0.46, MUE = 0.49 kcal/mol) showed that our FEP model is accurate and reliable for further calculations.

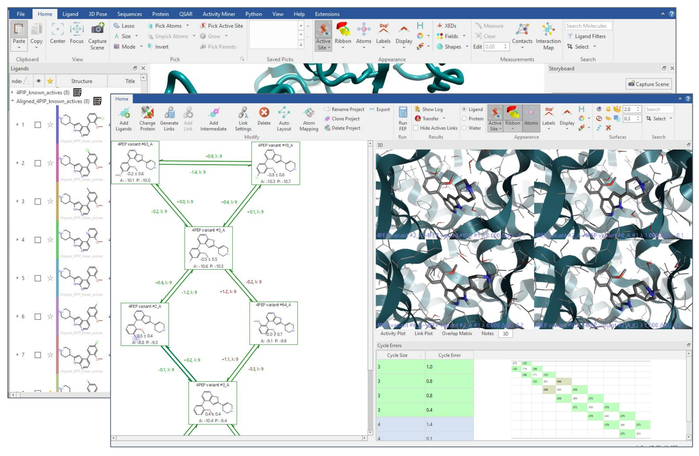


Figure 5: FEP calculations can be run easily in the user-friendly interface of Flare FEP.

Flare FEP was then used to predict the activities of the 84 novel ligands. The ligands were graphically organized using the Minimum Spanning Tree network, with the reference ligand with the known activity (**4PEP**) positioned in the center and the remaining molecules connected around it.

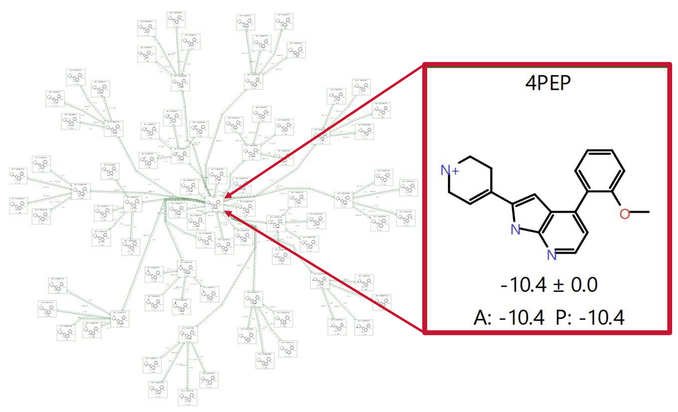


Figure 6: A Minimum Spanning Tree, linking the 84 molecules suggested from Hit Expander.

Based on the results, we identified the top 17 compounds exhibiting the highest affinity (ranging from -12.6 to -10.9 kcal/mol) and constructed a multi-connected Free Energy Perturbation (FEP) graph (Figure 7), which enables for a more precise and reliable calculation. The top predicted binder was **Variant #20**, our new 'lead', which shows high activity and a low error: $\Delta G_{binding}$ = -12.2 ± 0.4 kcal/mol.

Ligand	ΔG (kcal/mol)	IC50 (nM)
Variant #20	-12.2	1.15
Variant #53	-12.0	1.55
Variant #24	-11.90	1.95
Variant #5	-11.90	2.00
Variant #51	-11.70	2.82

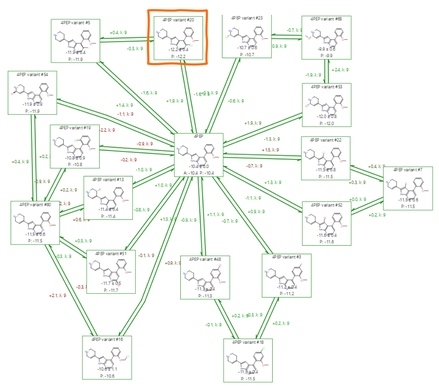


Figure 7: Multi-connected FEP graph of top 17 compounds branching from 4PEP with known activity.

Discussion on the FEP results

The difference between **4PEP** and **Variant #20** is the 5-position Cl. Analyzing the interactions (Figure 8), we observe that a common azaindole compound engages with the kinase hinge by forming a hydrogen bond with the carbonyl group, while also accepting a bond from the amine of a CYS residue.

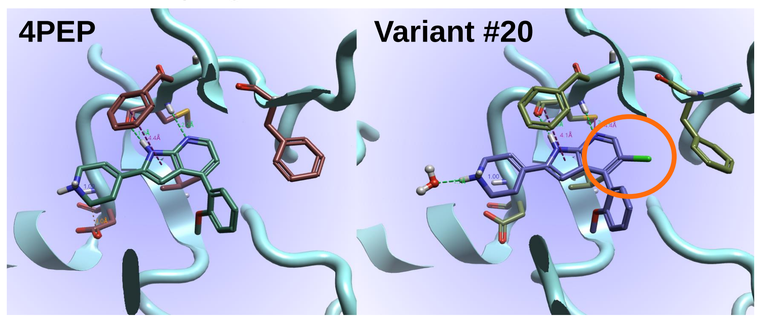


Figure 8: **4PEP** vs **4PEP variant #20**. Substitution at 5-azaindole position with Cl improved binding affinity.

Effects of the Cl addition on Protein- **Variant #20** interactions:

- Exclusion of water molecules from a hydrophobic area
- Formation of a bond between the lone pair on the nitrogen atom of the azaindole and the sulfur atom of the CYS residue
- More favorable orientation of the azaindole in relation to the same PHE residue
- Coordination of protonated tetrahydropyridine with water prevents clashes with ASP residue located below
- Maintained 56-degree torsional twist on the ortho-methoxy group (crucial for CDK2 binding, as observed in PDB: 7M2F)

Torx: Share the prioritized designs with the medicinal chemists

Having obtained the strongest binders from the FEP project, the synthesis of these compounds in the laboratory can be initiated. This process is seamlessly facilitated using Torx[5], a cloud-native web-platform that enables real-time data sharing during the DMTA (Design, Make, Test, Analyze) cycle. Torx enables its users to access all relevant information about any project they are working on and track the compound synthesis from design to testing, enhancing productivity and efficiency.

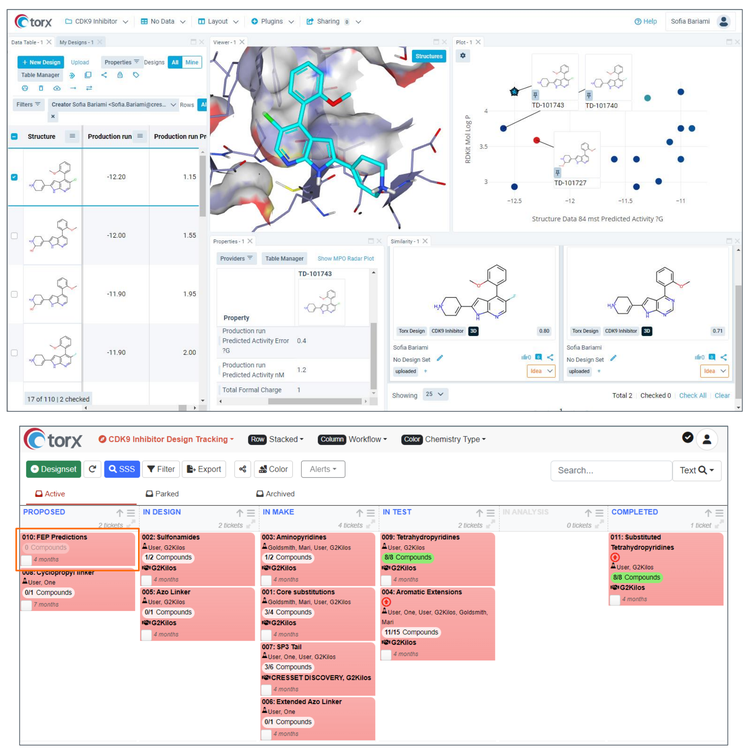


Figure 9: Torx enables you to track compound synthesis from start to finish with ease.

In this case, the results from the FEP run in Flare were sent directly to Torx Design (Figure 9 up) to enable discussion with medicinal chemistry colleagues. Promising compounds will be progressed to Torx Make (Figure 9 down) for synthesis.

Conclusions

- In under 24 hours, we efficiently identified molecules with highly reliable binding affinity predictions.
- FEP swiftly generates accurate predictions, showing its applicability in various drug discovery projects.
- This streamlined process using Flare and Torx saves both time and resources by avoiding unnecessary investments in inappropriate molecules.

References

1. Muegge *et al.*, *J. Med. Chem.* **2020**, 63, 17, 8956–8976 | 2. Flare™, version 7, Cresset®, Litlington, Cambridgeshire, UK; http://www.cresset-group.com/flare/; 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; Bauer M. R., Mackey M. D.; Electrostatic Complementarity as a Fast and Effective Tool to Optimize Binding and Selectivity of Protein–Ligand Complexes *J. Med. Chem.* **2019**, 62, 6, 3036-3050; Maximilian Kuhn, Stuart Firth-Clark, Paolo Tosco, Antonia S. J. S. Mey, Mark Mackey and Julien Michel Assessment of Binding Affinity via Alchemical Free-Energy Calculations J. *Chem. Inf. Model.* **2020**, 60, 6, 3120–3130 | 3. Tong, *et al.*, *ACS Med. Chem. Lett*. **2021**, 12, 7, 1108–1115 | 4. United States Patent US9796708, Oct. 24, **2017** | 5. Torx®, version 2.0, Torx Software®, Litlington, Cambridgeshire, UK; http://www.torx-software.com; 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