

Development of a docking strategy for the generation of kinase-focused libraries

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Kinase enzymes play a crucial role in signal transduction and cellular proliferation and differentiation, and their inhibition has proved useful for new therapies in cancer or inflammation. Different approaches have been used until now for the identification of new promiscuous kinase scaffolds and the synthesis of kinase-focused libraries¹. In this context, we have synthesized a kinase-focused library based on medicinal chemistry knowledge. We have also developed a docking and scoring strategy using crystal 3D structures of three kinases from different families.



A virtual screening approach based on docking

Initially, a systematic docking of the compounds from a set of well-known kinase ligands has been performed on three different enzymes receptors extracted from the Protein Data Bank (PDB). We have used the module "LigandFit" from Cerius2® (V 4.10) and followed for each compound the same procedure: selection of residues around the co-crystallized ligands, deletion of co-crystallized ligands and water molecules, optimization of the sites, generation of ligand conformations for each compound, selection of conformations according to their adaptation to the shape of the site, orientation into the binding site and energy optimization.



Six scoring functions implemented in LigandFit were calculated for each pose of each compound: PLP1, PLP2, LigScore1, LigScore2, PMF, Jain. For each known kinase ligand the highest scoring values (including all poses) were selected, and the "global" lowest values were chosen as cutoffs.

Finally, the same docking and scoring procedure was also applied to the compounds included in BioPrint and a kinase-focused library, and the above obtained cutoffs were applied as "filters" in order to discard compounds that were not able to pass them.

The kinase crystal 3D structures

Kinase enzymes co-crystallized with well known kinase inhibitors have been obtained from the Protein Data Bank:

- Abelson Tyrosine Kinase - Abl². Co-crystallized with Imatinib.
- Epidermal Growth Factor Receptor kinase - EGFR³. Co-crystallized with Erlotinib.
- Cyclin Dependent Kinase 2 - CDK2⁴. Co-crystallized with Purvalanol B.

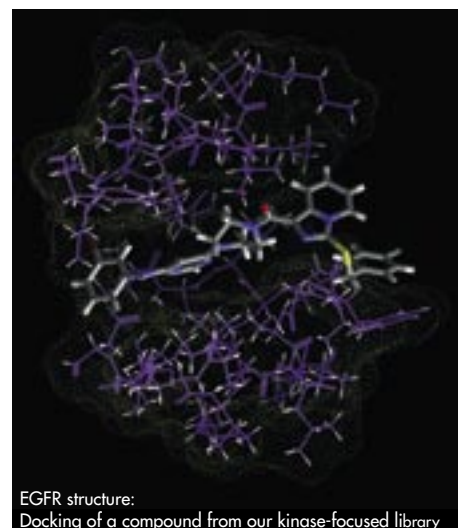
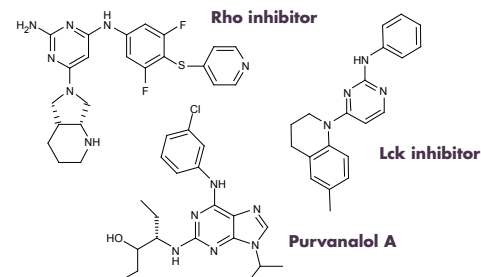
The libraries tested

- A set of 123 well-known kinase ligands described in publications and patents, including six Abl ligands, twelve EGFR ligands and eleven CDK2 ligands.
- BioPrint®, a "drug-like" database including more than 2,500 marketed drugs, reference compounds and compounds that have failed in clinical trials⁵. Around 1% of drugs included in BioPrint® presents a kinase-related mechanism of action.
- A kinase-focused library of 1,440 compounds based around 28 original pyrimidine scaffolds.

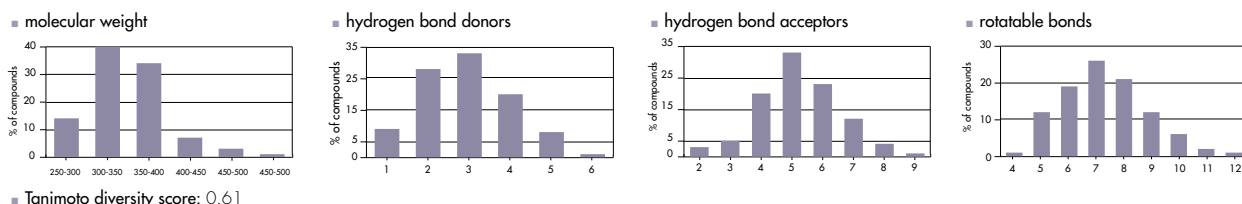
The kinase-focused library features

The kinase-focused library is centered around a diaminopyrimidine central core, which can be considered as a privileged and a rather general structure for interacting with the ATP binding site of kinases. Some examples of known diaminopyrimidines as kinase inhibitors are:

- Diaminopurine derivatives such as Purvalanol A, which are inhibitors of cyclin dependent kinases (CDKs)⁶.
- N-aryl 2,4 diaminopyrimidine derivatives have been described such as inhibitors of lck kinase⁷, FAK⁸ (focal adhesion kinase) or p38 MAP kinase⁹.
- N-aryl 4,6 diaminopyrimidine derivatives have been reported to inhibit EGFR and PDGFR kinases¹⁰, Rho kinase¹¹, src and abl kinases.



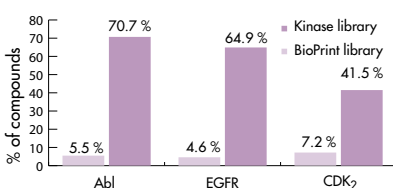
EGFR structure:
Docking of a compound from our kinase-focused library



Results: statistical validation

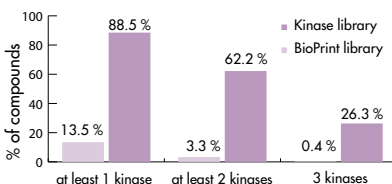
Statistics by kinase

The percentages of compounds of each library that pass the scoring cutoffs have been compared. As expected, a reduced number of compounds of BioPrint are selected as kinase ligands. In opposite, the results of the kinase-focused library show that a high number of compounds pass the scoring filters for the Abl, EGFR and CDK2 kinases separately.



Statistics for the three kinases

The percentages of compounds of each library that pass one, two or three of the scoring cutoffs have also been compared. This analysis has showed the ability of our strategy to find chemotypes that can be considered as "promiscuous".



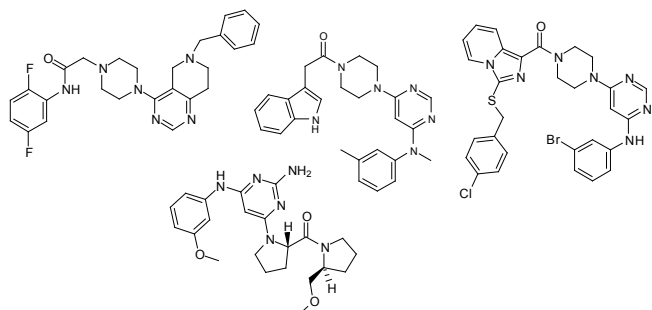
Experimental validation

We have performed a high throughput screening with a hit-seeking library of high structural diversity and the kinase-focused library on a kinase receptor structurally close to the Abl-kinase (same family). Despite the small size of the kinase library, we found more hits than in the diverse hit-seeking library.

| | inhibition > 50% at 10µmol | |
|-----------|-----------------------------|------------------------|
| | diverse hit-seeking library | kinase-focused library |
| compounds | 47256 | 1440 |
| hits | 55 | 67 |
| | 0.10% | 4.60% |

Promiscuous kinase chemotypes

Some examples of original promiscuous kinase inhibitors from Cerep focused library identified by the docking and scoring strategy.



CONCLUSIONS

- Our strategy to design a kinase-focused library has been based on medicinal chemistry knowledge and a docking and scoring strategy.
- This approach has allowed us to select promiscuous kinase oriented compounds and potential hits for a large variety of kinase targets.
- This strategy has been extensively validated in an experimental HTS assay, thus allowing us to apply it for future new libraries.

References

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