Drug Repurposing for Treatment of Cancer Through Outsourcing *In Silico* Drug Discovery

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Introduction

To speed up cancer drug development, the Drug Discovery and Development Group at University of South Australia is using drug repurposing to identify drugs with anti-cancer activity, that are clinically approved for other disease areas. Cresset Discovery has extensive experience in drug repurposing and provided support to the University of South Australia by providing *in silico* virtual screening and computational expertise through outsourced services.

Method

Virtual screening is a highly valuable computational method that enables the sampling of available chemical space from reliable suppliers. It is far more cost-effective than wet high throughput screening (HTS).

The method utilizes the XED force field model[1] to provide unique insight into the molecular characteristics behind biological activity. XED takes the 3D shape and electrostatics of molecules into account, to produce a field view of compounds. This makes it possible to compare thousands of structures and fragments on the basis of a more biological relevant 3D similarity (Figure 1).

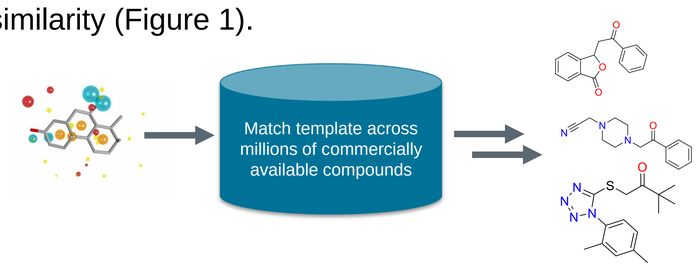

Figure 1: The 3D shape and electrostatic character of a template ligand are used to rapidly search large chemical collections for molecules with similar properties.

This approach has been successfully used to reposition drugs and extend the patents on compounds[2].

Ligand Templating

Protein kinases are major drug targets due to their critical role in all aspects of cancer biology. Ligand CDKi73 displays inhibition to multiples kinases, namely cyclin-dependent kinase 9 (CDK9) and Aurora A kinase, was used as a starting molecule[3].
CDKi73 was aligned to PDB ligand T3E (PDB:4BCI). After visual inspection, the orientation of the alkyl amino thiazole was adjusted in a manner that is consistent with the Cambridge Crystallographic Database torsion and still fits the electron density map. CDKi73 shows good electrostatic complementarity[4] to the CDK9 protein (Figure 2).

A B

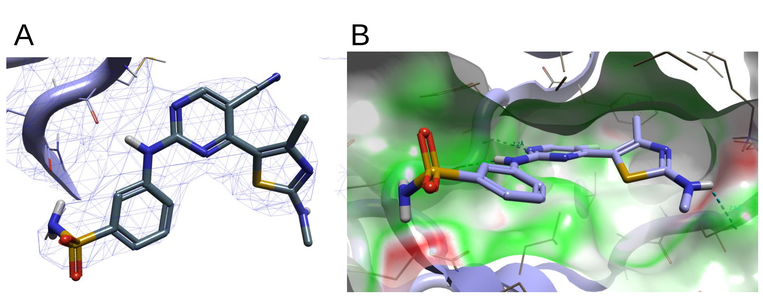

Figure 2: (A) Electron density map of PDB 4BCI and ligand T3E after adjustment of the alkyl amino thiazole bond. (B) EC map of CDKi73 to CDK9 protein (PDB:4BCI). Green, good electrostatic complementarity; red, electrostatic clash.

Virtual Screening

Ligand-based virtual screening[5] was performed against the commercial Drug bank and Drug navigator databases, which consist of approximately 9,000 drugs of different regulatory status. The top-ranking 2,000 results from this screen were filtered based on their regulatory status and target profile (Figure 3).

A B

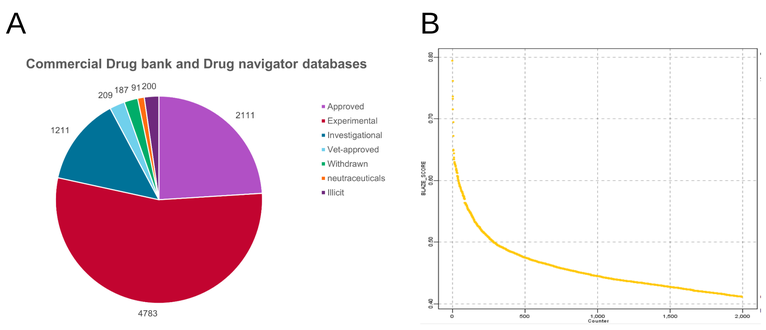

Figure 3: (A) A summary characterizing the properties of the drugs in the Drug bank and Drug navigator databases (B) illustrating a total of 2000 results selected after the Blaze™ search.

613 candidates were selected in either approved or in advanced clinical development stages. The selection was analyzed further and narrowed down to 73 candidates that displayed an appropriate fit for the binding site of the target of interest: high 3D similarity score with the known active ligand CDKi73; and no prior annotations associated with kinase activity (Figure 4). Candidates with previously reported anti-cancer activity or that are commercially unavailable were removed, and a final set of 24 drug candidates were selected for biological testing.

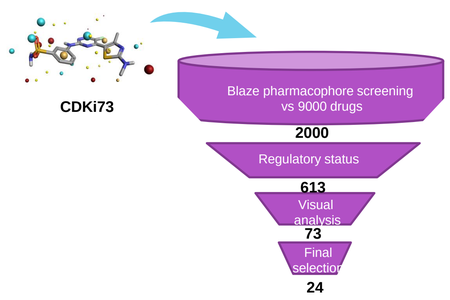

Figure 4: Cascade summary used for identifying the virtual screening drug candidates.

In Vitro Assays

The anti-proliferative properties of the 24 selected drug candidates were assessed by the University of South Australia using MTT and resazurin assays (Figure 5)[6].

A

B

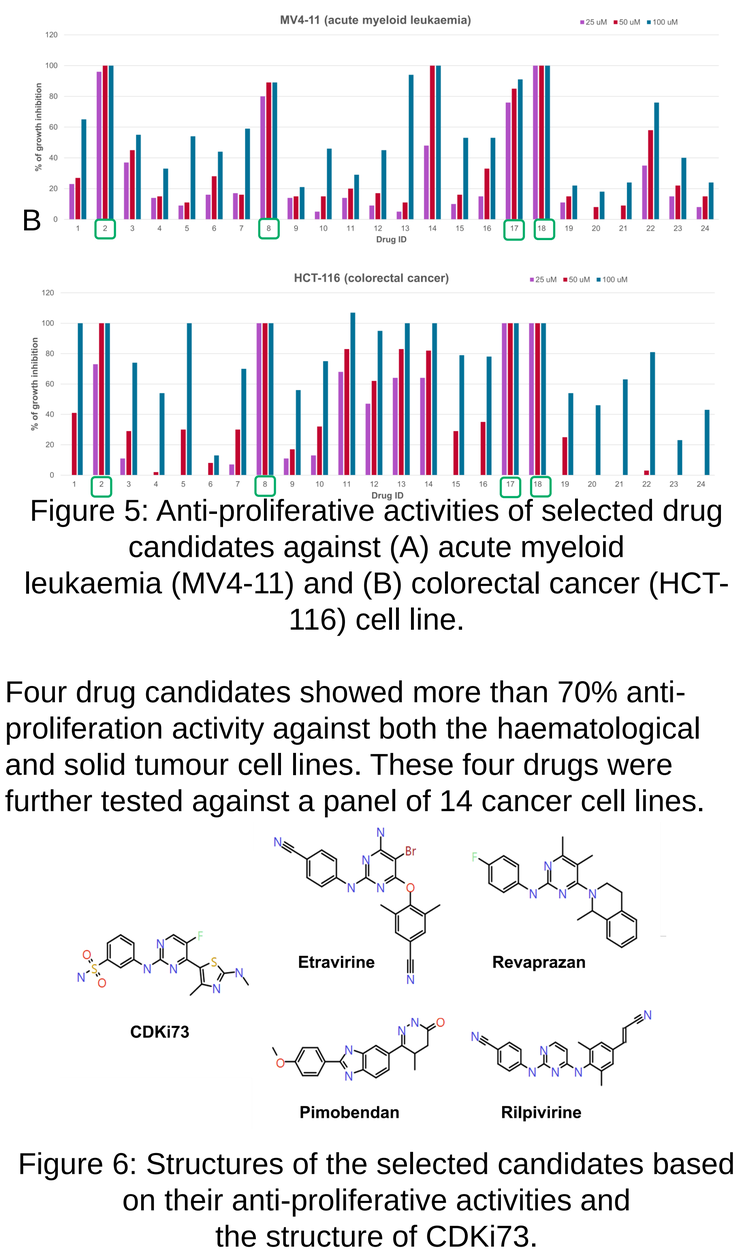

Figure 5: Anti-proliferative activities of selected drug candidates against (A) acute myeloid leukaemia (MV4-11) and (B) colorectal cancer (HCT-116) cell line.

Four drug candidates showed more than 70% anti-proliferation activity against both the haematological and solid tumour cell lines. These four drugs were further tested against a panel of 14 cancer cell lines.

Figure 6: Structures of the selected candidates based on their anti-proliferative activities and the structure of CDKi73.

Based on the cellular potency, current clinical use and existing pharmacokinetic data, rilpivirine was selected for follow-up studies. Testing against a panel of 48 different kinases revealed that the anti-viral drug rilpivirine is an Aurora A kinase inhibitor[6].

Modeling of Rilpivirine

Cresset Discovery assessed the binding characteristics of rilpivirine with Aurora A kinase and alisertib, the most clinically advanced Aurora A kinase inhibitor[7] using Flare modeling suite (Figure 7)[8]. X-ray ligands were chosen based on the closest 2D similarity to the query molecules.

A B

C D

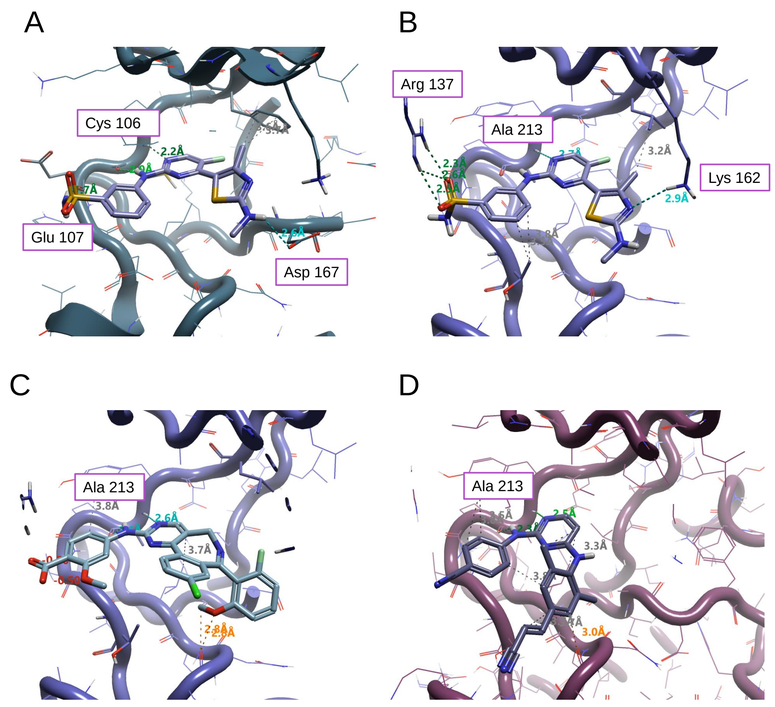

Figure 7: Proposed binding modes of (A) CDKi73 docked to CDK9 (PDB: 4BCI), (B) CDKi73 docked to Aurora A (PDB: 2X81), (C) alisertib docked to Aurora A (PDB: 2X81), and (D) rilpivirine docked to Aurora A (PDB: 3H0Y).

A common feature of the binding of CDKi73 (Ki ≤ 30nM), alisertib (Ki ≤ 2nM), and rilpivirine (Ki = 116nM), to Aurora A is the interactions involving a pair of hydrogen binding between N1 and 2C-NH of pyrimidine, a ubiquitous kinase hinge binding fragment, with the backbone amino group of Ala213 at the hinge region of Aurora A. The 2-NH-benzenesulfonamide of CDKi73 is accommodated in both Aurora A and CDK9 albeit by different recognition patterns (Figure 7 A, B). Rilpivirine was identified as an inhibitor targeting Aurora A but not CDK9, most likely due to the absence of the thiazole system at the C4-position of the pyrimidine, a key determinator for CDK9 inhibition.

Conclusion

Drug repurposing using Cresset Discovery's ligand-based virtual screening revealed Rilpivirine, originally developed as an anti-viral drug, as a promising repurposing candidate for use in cancer studies.

Cresset Discovery serves as a valued partner for computational modeling in drug discovery. Our expert team has a breadth of experience across medicinal and computational chemistry, and biology to work alongside you to deliver your project goals. To learn more, contact us or request a no-risk-free confidential discussion with our team.

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