Bespoke *In Silico* Inotropy Prediction

Morgan Morris*, Nathan Kidley*, Martin Slater*, Jeremy Billson**, Rob Scoffin*
* Cresset, Cambridgeshire, UK morgan.morris@cresset-group.com www.cresset-group.com
** InoCardia, Coventry, UK jeremy.billson@inocardia.com www.inocardia.co.uk

Introduction

Inotropy – the ability of a compound to alter the force of cardiac muscle contraction – is a source of clinical and post-clinical failure for many drug candidates. *In vivo* assays to detect inotropic agents may be supplemented with *in silico* models to conduct safety pharmacological screenings.

This poster describes *in silico* components of a bespoke analysis tool that identifies potential cardiac safety risk for candidate drug molecules. The tool incorporates, amongst other techniques, an *in silico* similarity analysis protocol to help detect potential inotropes as a pre-filter for selecting compounds to be confirmed by actual testing using InoCardia's innovative in-vitro rat myocyte assay.[1]

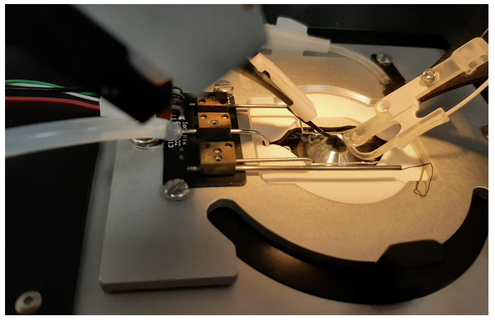

Figure 1: InoCardia's nano-scale assay device which utilizes a single isolated cardiac myocyte to test the effects of new molecules on heart muscle contractility.

An ordered workflow (Figure 2) was envisaged for pre-selection for assay testing or deselection of candidates.

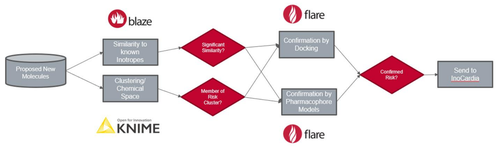

Figure 2: Workflow diagram introducing both ligand-centric and protein-centric techniques.

Method

The XED force field can be utilized to provide both accurate 3D geometries for molecules as well as an accurate assessment of their electrostatic fields, and the ways in which these fields would interact with, e.g., a biological target protein.[2] There are many applications of this technology in drug discovery, one of these being to generate a 'chemical space' for known drugs that can further be analyzed to identify regions of interest (for activity) or concern (for known side-effects, such as inotropy). The analysis was carried out by calculating a full N x N pairwise similarity matrix for a library of 2,200 known drugs, including 100 known inotropes. Up to 10 low energy conformers were generated for each molecule using the XED force field, and then the maximum similarity calculated for any pair of conformers of the two molecules.

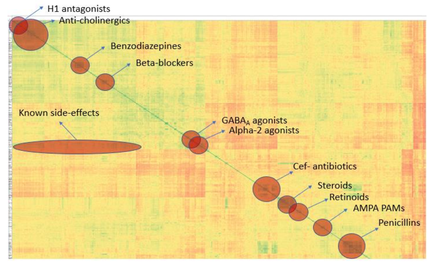

Figure 3: Analyses of similarity matrices displaying therapeutics with similar targets.

The shape and molecular field similarity between pairs of conformers were calculated, and the best alignment and similarity for any given pair of molecule conformers was kept as the score for that pair. The matrix generated a chemical space that could be used to define inotrope potential as shown in Figure 3 (matrix clustered such that groups of similar molecules appear together on the leading diagonal).

This matrix was then analyzed by principal component analysis (PCA) to reduce graph dimensionality, and RF/ SVM/ ANN machine learning techniques were used to separate molecules based on relative position (Figure 4).

Another means of analysis is to project the N-dimensional similarity matrix into a lower dimensional space, e.g., a 2-D plot. This was done using a PCA which attempts to identify new 'dimensions' such that the maximum diversity in the data set is 'explained' by the first 'principal component', followed by the second, third, etc. By then plotting compounds using the first two PC's, we retrieved a new representation of 'chemical space' where molecules (points in the space) which were highly similar appeared close to one another.

This analysis (Figure 4) highlighted that inotropy can arise from multiple mechanisms, as is shown by the diverse spread of known inotropes within the space of known drug molecules. Hierarchical and fuzzy clustering were used to identify molecule groups based on similarity, and fuzzy clustering was used to further generate probabilities for 'Inotrope group' membership for individual molecules.

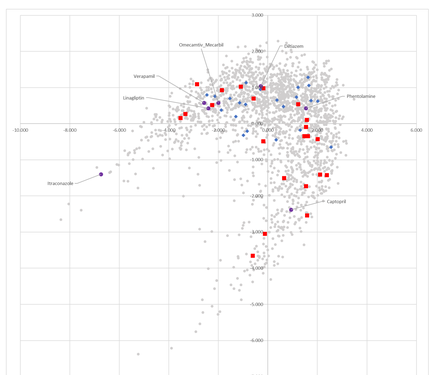

Figure 4: Drug space (PCA plot) showing known inotropes compared to 2,200 known drugs.

The diversity of structures and mechanisms leading to inotropy meant that we took a multi-faceted approach to identifying potential liability. Techniques utilized included similarity scoring, with the addition of a calculation of significance for the similarity result (using a z-score approach).

Compounds identified using the similarity screen were further assessed and confirmed/cleared using advanced techniques (Figure 5), such as docking to known target structures, or assessment using the Electrostatic Complementarity™ (EC) component of Flare™.[3]

Results and Visualization

Newly proposed structures were assessed for potential inotropy using similarity scoring to known inotropes and the comparison of these results with similarity to a broader set of known drugs (the majority of which do not show either positive or negative inotropy).

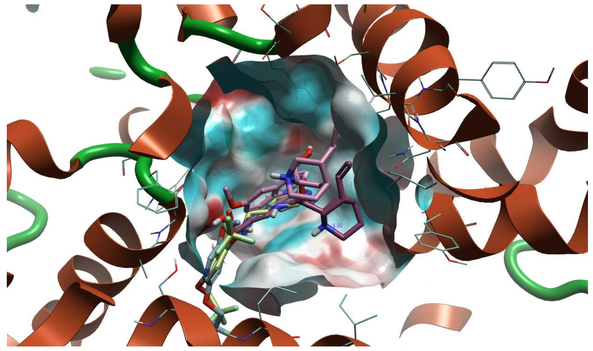

Figure 5: Proposed structure for new discovery project – similarity-based alert for potential Nav 1.5 activity confirmed by docking score.

The results are highlighted in the case of the ion channel Nav 1.5 (PDB entry 6LQA) using a proposed bioactive conformation (Figure 5).

Docking results orthogonal to similarity scoring identified known B AR antagonist atenolol and negative inotrope flecainide as structurally similar hazard molecules. This is highlighted using Torx® (Figure 6).[4]

Further screening using the InoCardia work-loop model was thus avoided through *in silico* processing of the proposed lead compound.

From the results of the field similarities, the high similarity inotropes for a given mechanism were used together to generate multi-compound pharmacophores. This was then also used with inotropic activity data to generate QSAR models.

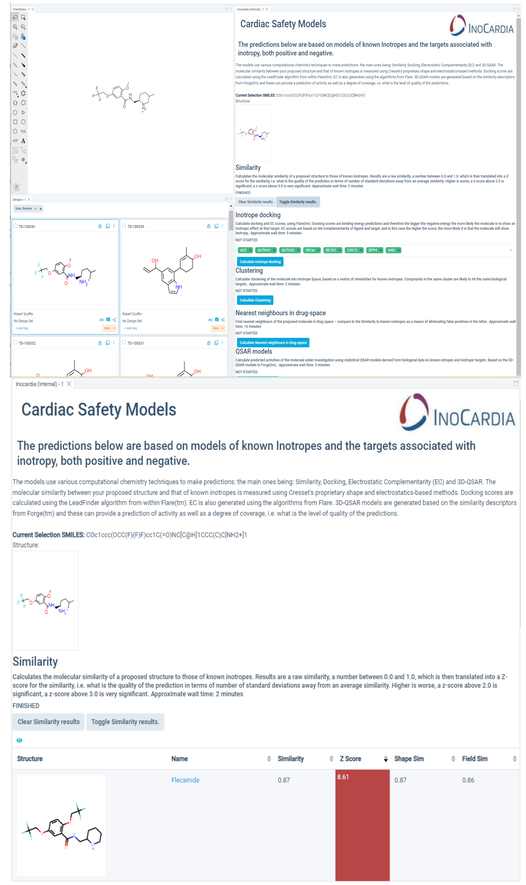

Figure 6: Torx display highlighting potential cardiac toxicity issues based on similarity and z-scores.

Conclusion

A holistic *in silico* inotropy prediction tool was developed and delivered through Torx®.[4] This included novel 3D field and shape similarity analyses, and confirmation using docking and EC methods, to provide a cardiac safety profile for candidate molecules. This was developed as a companion diagnostic and triaging pre-screen to an *in vitro* myocyte assay from InoCardia for cardiac tox screening. The similarity matrices performed well as a diagnostic tool and allowed for the development of a fail-early strategy in early-stage drug screenings.

References

1. Maddock *et al*, An in vitro platform using the primary cardiomyocyte Work-Loop assay to screen for drug-induced effects on cardiac contractility, *Journal of Pharmacological and Toxicological Methods*, Volume 105,106759, **2020**
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. Flare™, Cresset®, Litlington, Cambridgeshire, UK; https://www.cresset-group.com/flare/; 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
4. Torx®, Torx Software®, Litlington, Cambridgeshire, UK; https://www.cresset-group.com/software/torx/