Covalent Drug Discovery at Domainex: Curation of a covalent fragment library and fast follow-up via 'Direct to Biology' screening

Domainex
Enrich your Medicines Pipeline

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Introduction

Recently there has been increased interest in 'electrophile-first' covalent drug discovery, which has centred around screening of covalent libraries [1]. Fragment based screening has been a successful hit discovery approach for reversible inhibitors, providing better chemical space coverage and higher probability of binding due to lower molecular weight complexity [2]. Here, we have curated a bespoke cysteine targeted covalent fragment library for use in an 'electrophile-first' covalent drug discovery. Additionally, we present validated methods for rapid follow-up of hits through "direct-to-biology" (D2B) synthesis and mass spectrometry screening.

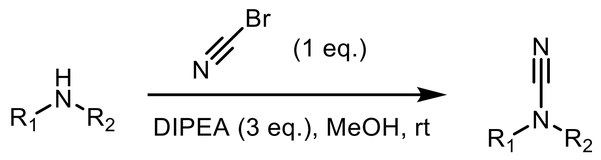
Library Curation Workflow

Cys targeted covalent fragment from commercial sources → Filtered for preferred warheads → *In silico* property prediction & filtering → Cluster per warhead and diverse selection → Visual inspection → Final selection quoted and purchased

Our approach utilised commercially available fragments. Multiple commercial supplier databases were combined and filtered for preferred warheads (based on literature precedent).
Fragment-like space was defined as HAC: 9-20, Mw: 100-300, cLogP: -2 to 3, TPSA: <100, nROT: 0-5, HBD: 0-3, HBA 0-6, nRings: 1-3, clogD -1 to 3, chiral centres ≤ 1
A set of substructure filters designed to identify and remove unsuitable screening compounds was also applied.
Compounds were clustered using fingerprints and Tanimoto similarity to yield an appropriate number of clusters compared to number of compounds and diversity found in each warhead.
Finally, each fragment was visually inspected and where necessary, replaced with structurally similar compounds using a chemically sorted 2D map.

Synthesis of cyanamides

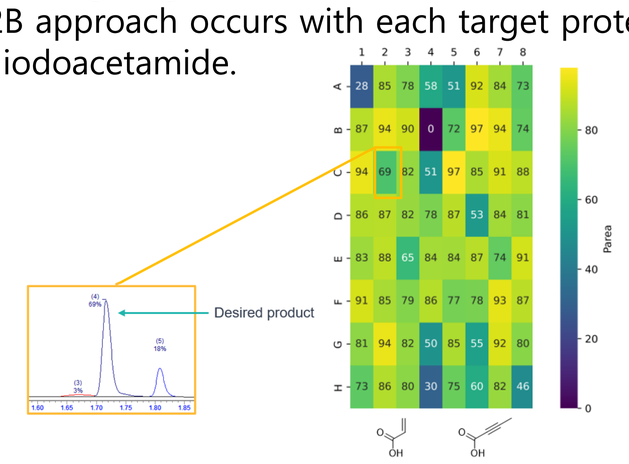
Cyanamides are a fast-developing warhead capable of reversible covalent interactions [3]. Diversity and property-space of commercial cyanamides was not considered to be ideal, therefore a synthesis campaign was conducted.



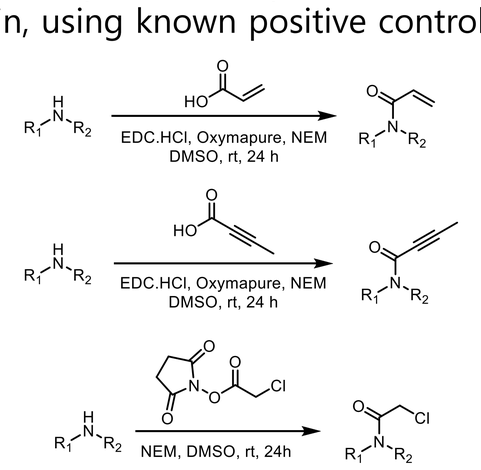
40 structurally diverse secondary amines which adhered to fragment property space were selected from our in-house collection using a Knime workflow. Fragments were synthesised and purified in an array fashion, with a success rate of 85%.

'Direct to Biology' (D2B) rapid expansion

To facilitate rapid SAR expansion, we validated acrylamide, chloroacetamide and propargyl amide for D2B synthesis and mass spec analysis. Using optimised conditions, 100s of compounds and their corresponding binding data can be obtained within a week. Synthesis can occur either at the library screening stage or after hit validation to enable rapid follow-up. Validation of D2B approach occurs with each target protein, using known positive controls or iodoacetamide.



Data from a representative experiment

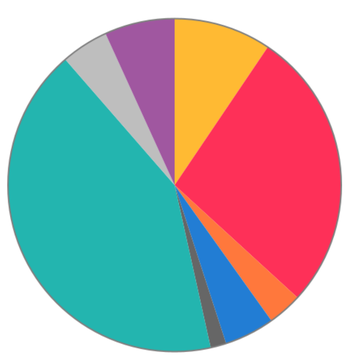


Profile of Domainex covalent fragment library

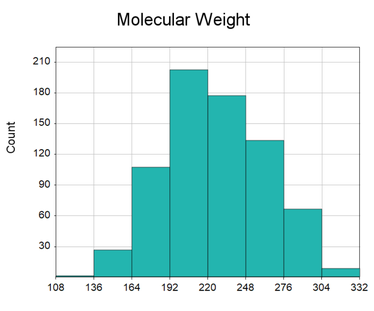
The final library contains 721 fragments, which have been profiled. Subsequent analysis demonstrates the library has good physiochemical and structural diversity, whilst maintaining excellent fragment properties.

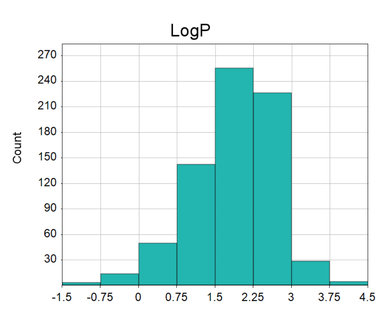
The final library contains a diverse set of warheads known to bind to cysteine. Both irreversible and reversible groups are included.

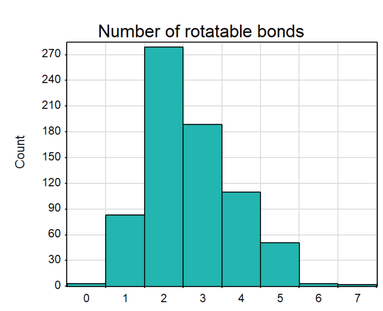
The library incorporates a bias towards acrylamides due to their presence in marketed drugs and their prevalence in commercial compounds.

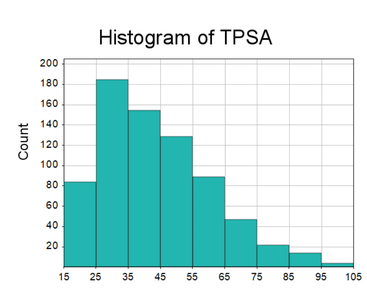


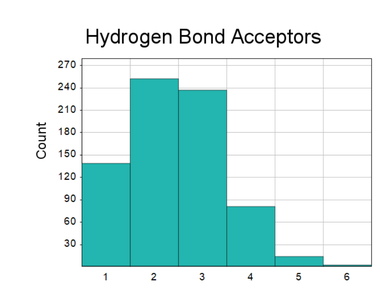
Warhead	% Library	Colour
Acrylamide	42	
2-Chloroacetamide	27	
2-Chloropropionamide	9	
Cyanoacrylamide	7	
α, β-unsaturated ketone	5	
Cyanamide	5	
Butynamide	3	
Vinyl sulphonamide or sulphone	2	

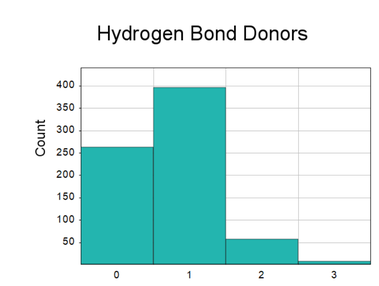












Conclusions and Next steps

- Domainex has built a high-quality covalent fragment library to facilitate hit discovery using its mass spec covalent screening platform
- Hits can be rapidly expanded using validated 'direct to biology' workflows to generate valuable SAR in the hit validation and hit-to-lead phase
- Future work will expand the library to include additional warheads (including those to target beyond cysteine), as well as further developing the collection around key warheads

References

[1] SLAS Discovery, 2024;29(3) https://doi.org/10.1016/j.slasd.2024.01.003
[2] Nat Rev Drug Discov, 2024;15:605–619 https://doi.org/10.1038/nrd.2016.109
[3] Angew. Chem. Int. Ed. 2024;63: e202318849 https://doi.org/10.1002/anie.202318849

Contact

If you would like to learn more about applying our drug-discovery platforms, please contact: enquiries@domainex.co.uk

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