Accelerated Covalent Fragment Hit Discovery for KRAS [G12C] Using Mass Spectrometry and Direct-to-Biology Screening

Joe Mason, Gary Wu, Anna Hopkins, Elisa Pileggi, Rachel Armstrong, Jason Pembroke and Andrew Ratcliffe

Domainex Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden, CB10 1XL

Introduction

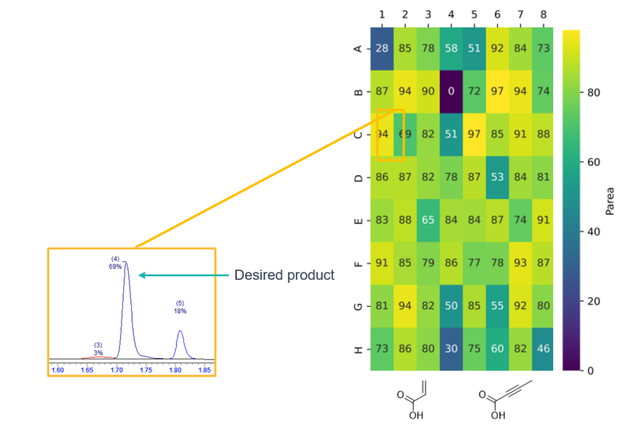
KRAS is one of the most frequently mutated oncogenes in cancer, where its mutations drive tumour growth through constitutive activation of downstream signalling pathways [1]. Once considered "undruggable," KRAS [G12C] has recently become a viable drug target with the development of covalent inhibitors like Sotorasib and Adagrasib. Recently there has been increased interest in 'electrophile-first' covalent drug discovery, which has centred around screening of covalent libraries [2]. Domainex has developed a mass-spectrometry based workflow for covalent screening, which, coupled with our Direct-to-Biology platform, enables rapid identification of fragment hits or expansion of chemical matter.

Direct to Biology and Mass Spectrometry screening workflow

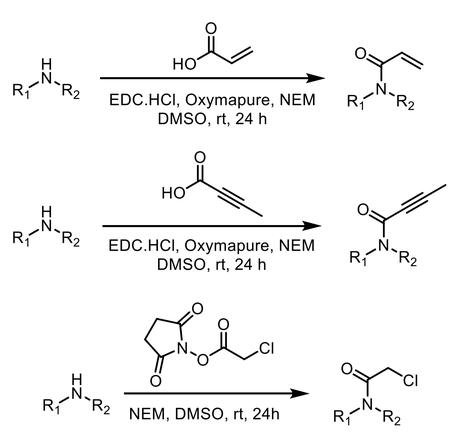
Warheads validated for D2B synthesis → Assay compatibility test → Library of Primary and Secondary amine fragments compiled → D2B synthesis → High throughput MS screening and analysis (1 µM protein + 100 µM compound) → Hit resynthesis and Testing

'Direct to Biology' (D2B) chemistry validation

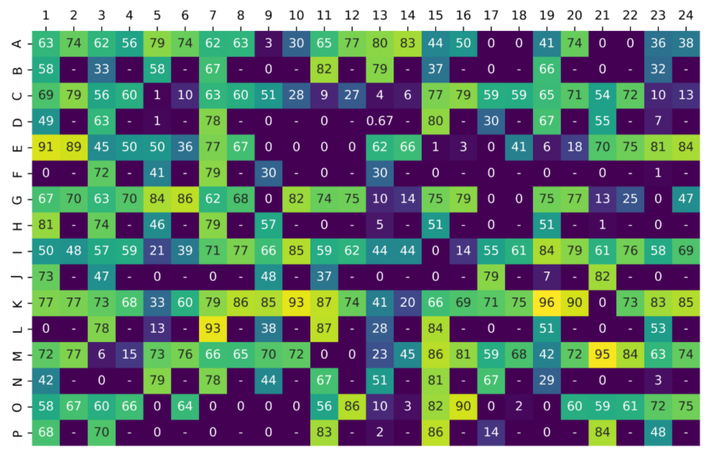
To facilitate rapid SAR expansion, we validated acrylamide, chloroacetamide and propargyl amide for D2B synthesis and mass spec analysis. Using optimised conditions, 100s of compounds and their corresponding binding data can be obtained within a week. Synthesis can occur either at the library screening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



Data from a representative experiment



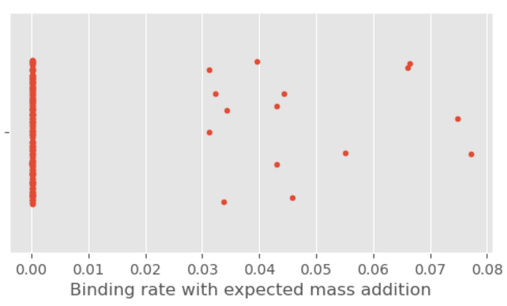
Library synthesis

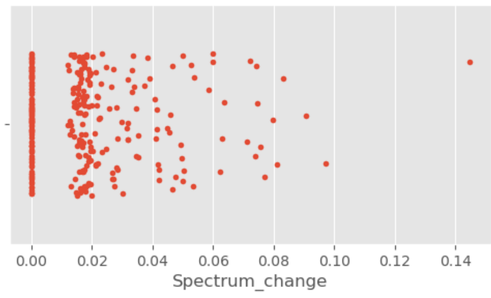


288 compounds were synthesised overnight in one plate. Automated LCMS analysis using python processing showed good conversion across all 3 warheads and all samples were submitted to assay.

High throughput Mass Spectrometry screening

LCMS data was acquired on a Waters G2-XS Q-Tof and the data analysed and visualised using an in-house data processing pipeline. Hits were observed containing all three warheads.



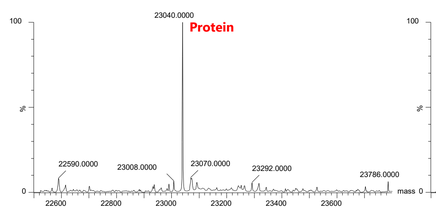


Spectrum change is used to measure bindings with unexpected mass additions (eg multiple binders)

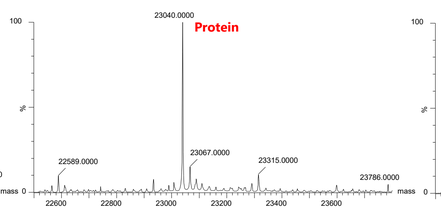
Compatibility test

Crude reaction mixtures and project specific starting material were tested in the assay, with no effect. A spiked positive control (Sotorasib) retained activity in the presence of crude samples.

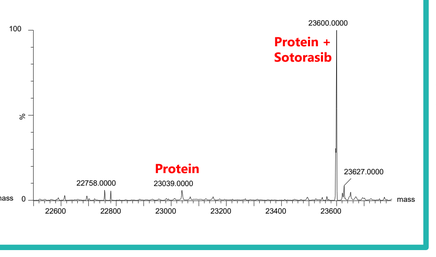
Untreated KRAS [G12C] protein



KRAS [G12C] + crude reaction mixture



KRAS [G12C]+ crude reaction mixture + Sotorasib



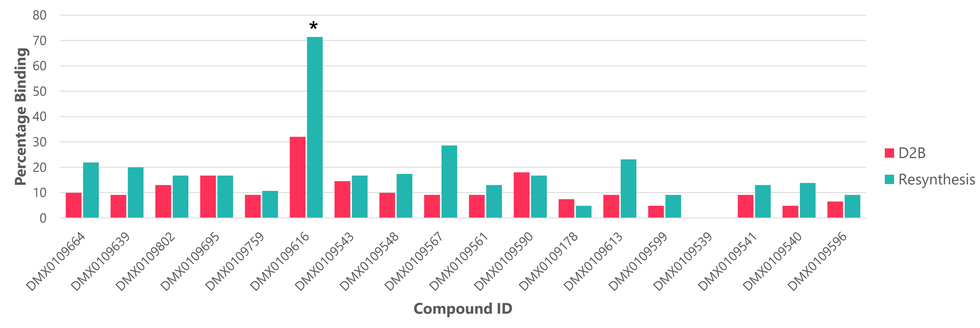
Selection of fragments monomers

Amine monomers were selected from available fragment-like in-house building blocks: Fragment-like space was defined as HAC: 8-17, Mw: 100-300, cLogP: -2 to 3, TPSA: <100 Å^2, nROT: 0-5, HBD: 0-3, HBA 0-6, nRings: 1-3, 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 and 96 compounds were chosen following visual inspection.

Hit resynthesis and confirmation

18 compounds were successfully resynthesised, purified and re-tested in the assay. All resynthesised compounds were confirmed as hits with the purified material, and a negative control remained inactive.



* Multiple bindings observed

Conclusions and Next steps

- **Domainex has utilised its Direct-to-Biology and mass spec covalent screening platform to rapidly generate and screen a covalent fragment library to obtain KRAS [G12C] binders.**
- **Hits can be rapidly expanded using validated 'Direct-to-Biology' workflows to generate valuable SAR in the hit validation and hit-to-lead phase.**
- **All synthesized compounds could be further triaged using Domainex in-house assay platform, including ChromLogD and microsomal stability (crude compounds) and GSH stability, k_{inact}/K_I and binding site identification (purified compounds).**

References

[1] Cox AD et al (2014) "Drugging the undruggable RAS: mission possible?" Nat Rev Drug Discov, 13(11), 828–851

[2] Lucas SCC et al (2024) "Covalent hits and where to find them" SLAS Discovery 29, 100142

Services/Contact

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