The Next Generation of Covalent Fragments: Sulfur(VI) Fluoride Warheads for Ligandability Assessments and Hit Identification

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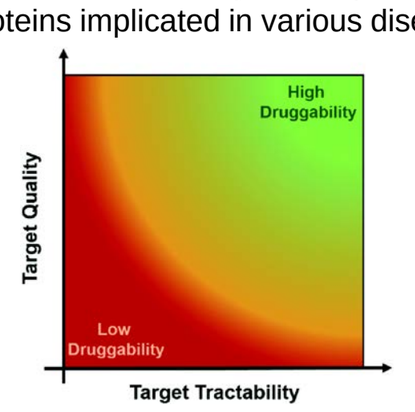
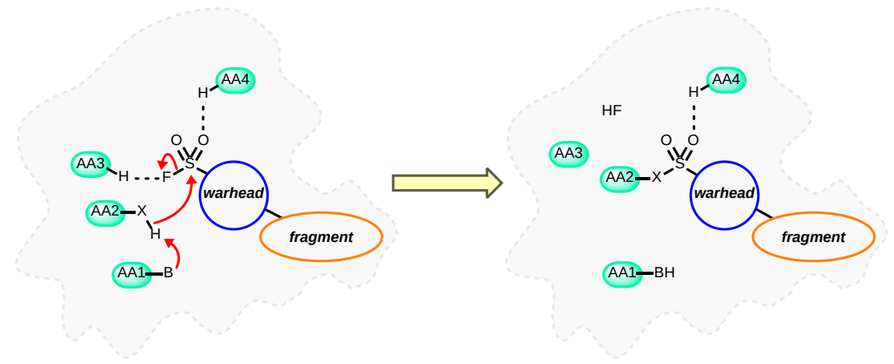
Introduction

Recent revolutionary developments in human genome sequencing technologies and functional genomic analyses present the pharmaceutical industry with many new target proteins implicated in various diseases, but for many of these proteins there has been little prior research into their structure and function.[1]

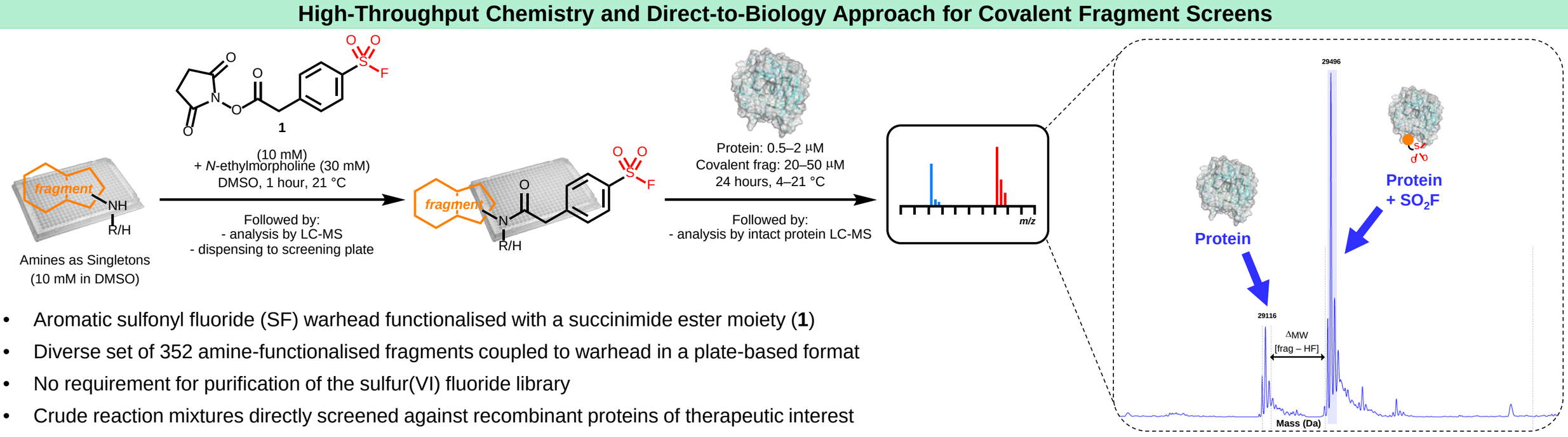


Covalent fragments represent a new strategy for hit identification through the leveraging of mass spectrometry analyses. Current covalent fragment libraries are only applicable to a sub-set of protein targets and occasionally give rise to false positives.[2,3] Sulfur(VI) fluoride fragments offer improvements on current covalent fragment strategies as they:

- are applicable to the majority of protein targets.
- allow for the robust identification of hits.[4–6]

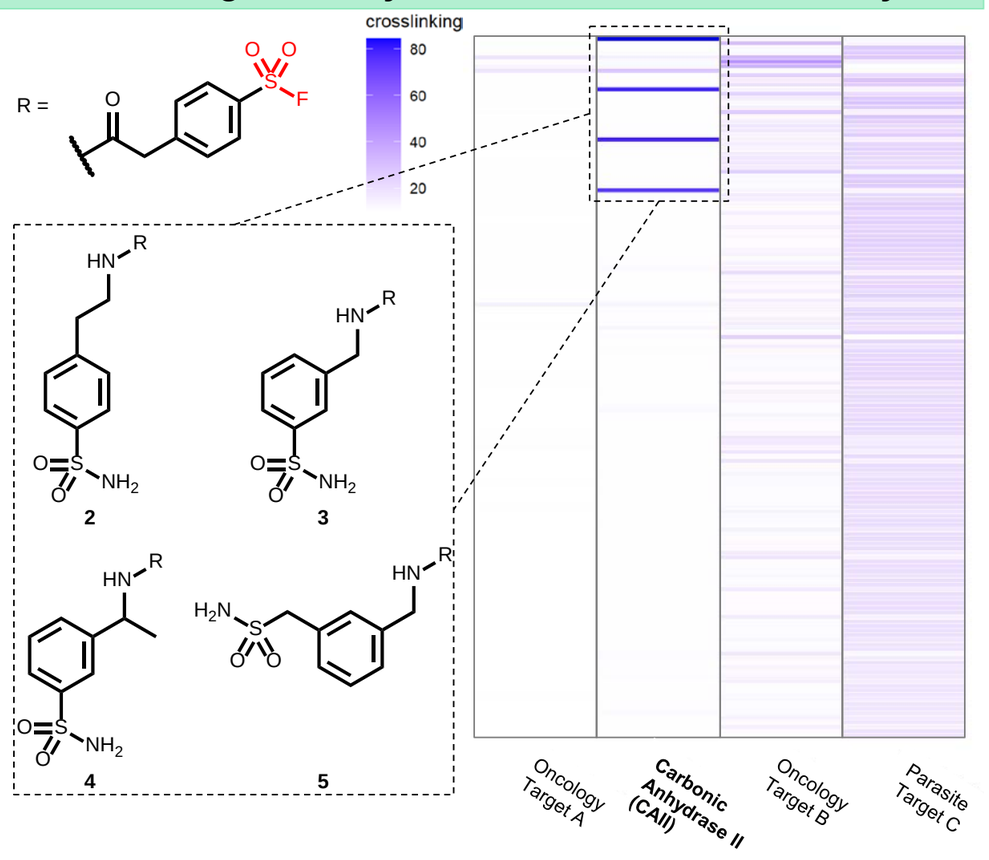


High-Throughput Chemistry and Direct-to-Biology Approach for Covalent Fragment Screens



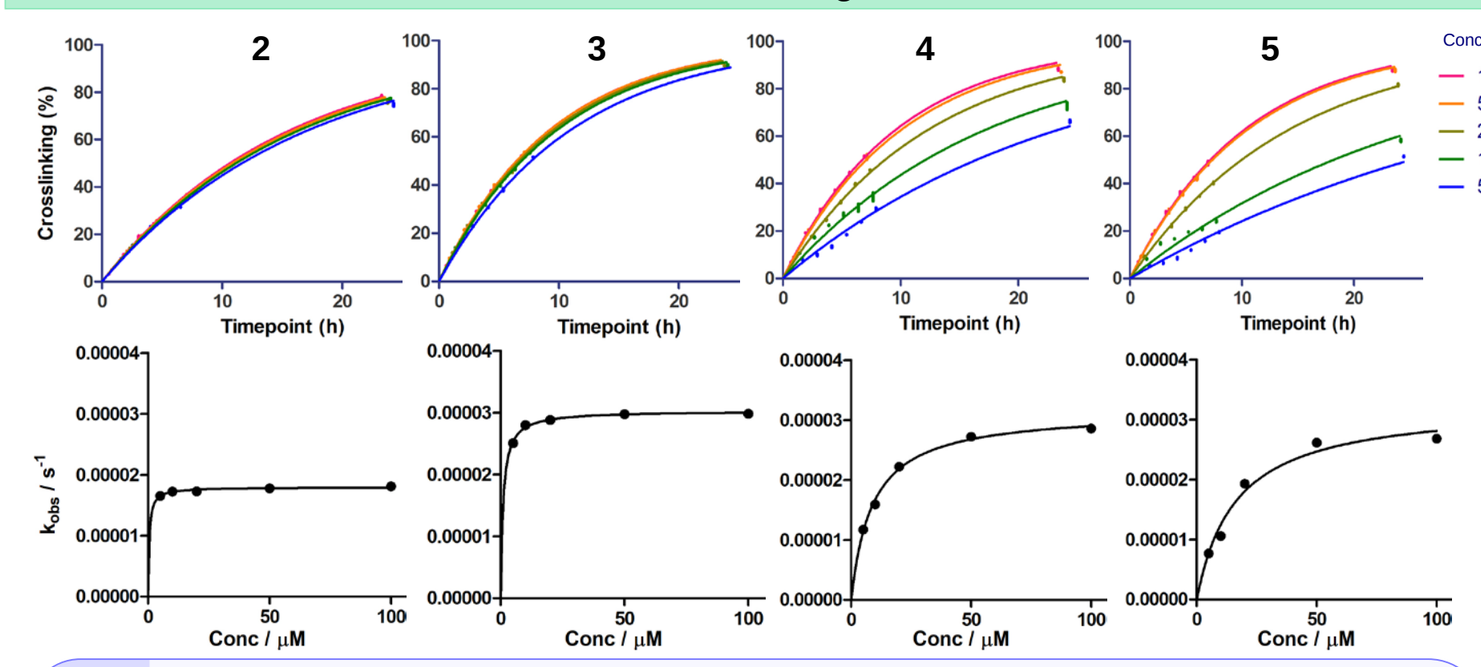
- Aromatic sulfonyl fluoride (SF) warhead functionalised with a succinimide ester moiety (**1**)
- Diverse set of 352 amine-functionalised fragments coupled to warhead in a plate-based format
- No requirement for purification of the sulfur(VI) fluoride library
- Crude reaction mixtures directly screened against recombinant proteins of therapeutic interest

Protein Ligandability Assessments with SF Library



*Screen against CAII identified four hit SFs (**2**–**5**). All contain sulfonamides – well-established pharmacophores for CAs.*[7]

Kinetics of Hit SFs against CAII



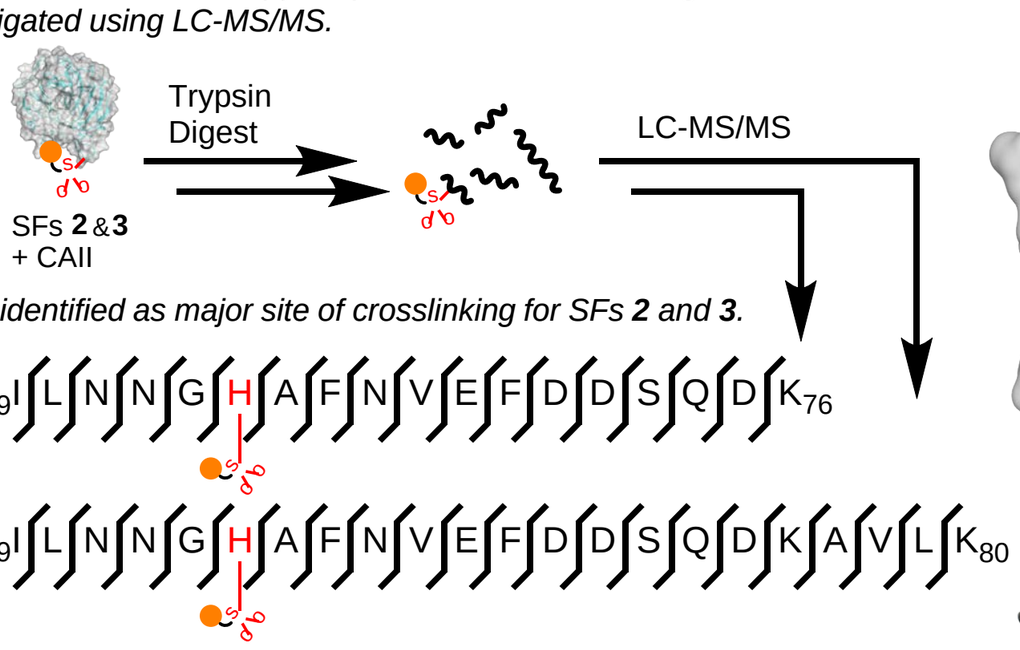
	2	**3**	**4**	**5**
k_{inact} / 10^{-5} s^{-1}	1.80	3.03**	3.16**	3.27**
K_I / μM	0.446*	0.988*	8.91	16.3

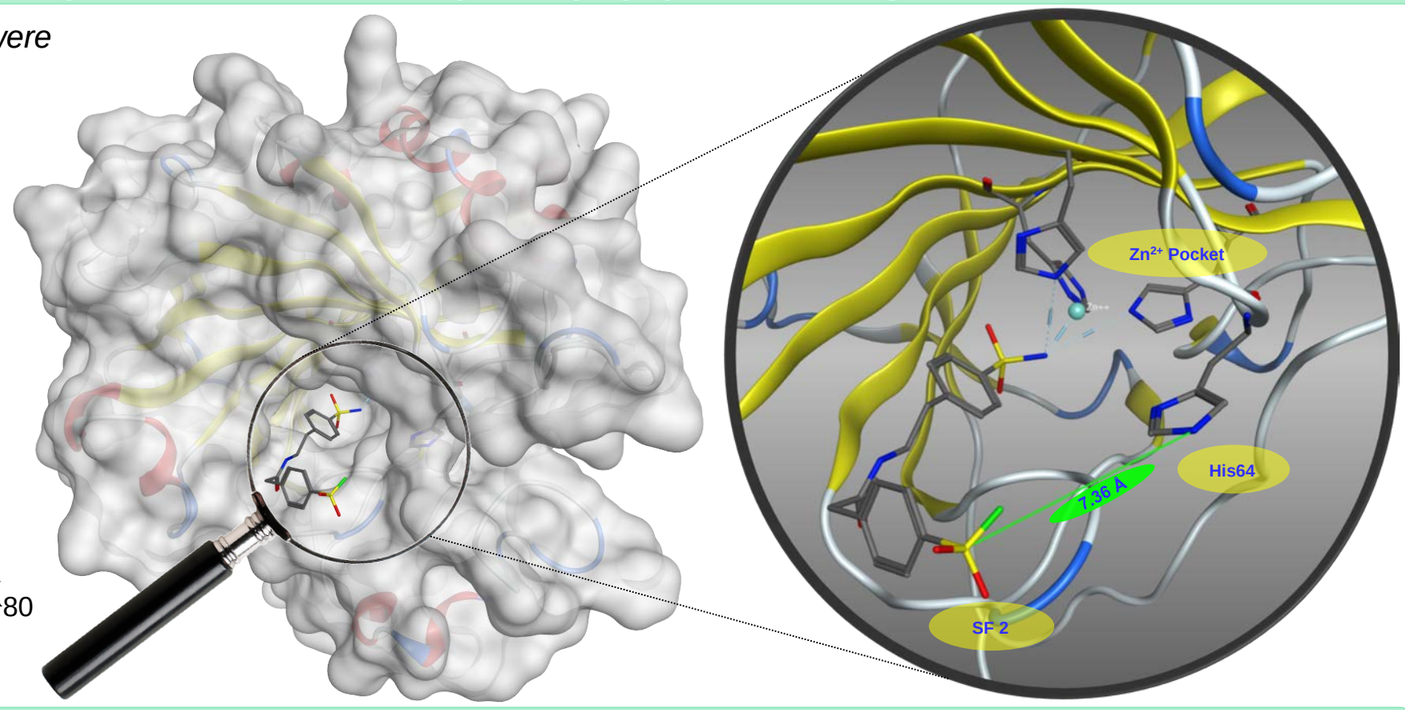
SFs **2 & **3** identified as the most potent.*

***Consistent k_{inact} observed for SFs **3**–**4**.*

Identification of the Site of Crosslinking – Rationalised with Crystallography and Docking

*The amino acid residue(s) responsible for crosslinking to SFs **2** and **3** were investigated using LC-MS/MS.*





Conclusions

These results demonstrate the applicability of covalent fragment screening with sulfur(VI) fluoride warheads for use in the early stages of drug discovery. It is anticipated that this screening platform will be used in parallel with alternative assessments of ligandability for emerging targets, such as DNA-encoded libraries, and affinity selection mass spectrometry. This will lead to the development of chemical probes for the study of diseases and provide starting points for the development of novel covalent drugs.[8,9]

References: 1) *Med. Chem. Commun.*, **2018**, *9*, 606–613. 2) *Drug Discov. Today*, **2020**, *25*, 983–996. 3) *Angew. Chem. Int. Ed.*, **2020**, *59*, 2–12. 4) *Angew. Chem. Int. Ed.*, **2014**, *53*, 9430–9448. 5) *Chem. Sci.*, **2015**, *6*, 2650–2659. 6) *ACS Med. Chem. Lett.*, **2018**, *9*, 584–586. 7) *J. Enzyme Inhib. Med. Chem.*, **2016**, *31*, 180–188. 8) *J. Am. Chem. Soc.*, **2019**, *141*, 2703–2712. 9) *J. Am. Chem. Soc.*, **2019**, *141*, 8951–8968.

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