

The Development of Next-Generation Reactive Electrophiles for Beyond Cysteine Covalent Drug Discovery

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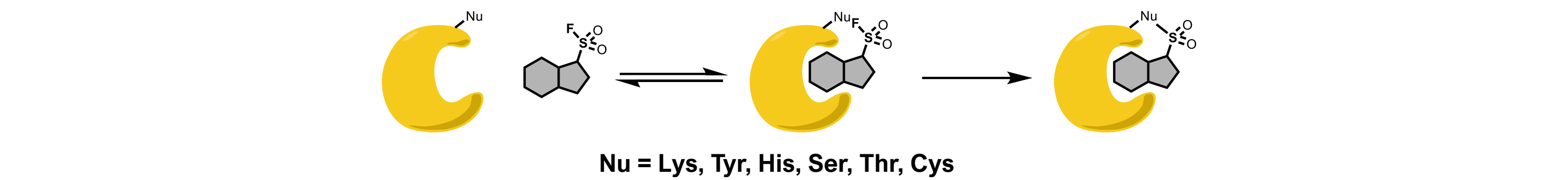
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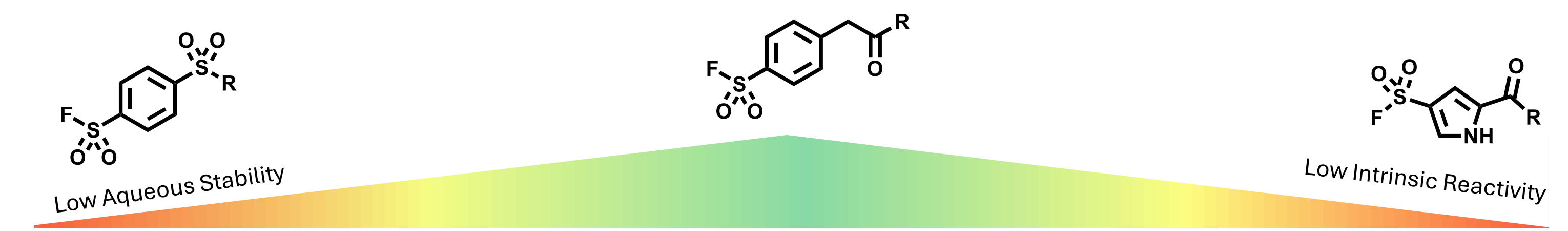
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Introduction

Covalent inhibitors have had a resurgence in drug discovery due to their benefits over reversible binders. Examples of approved drugs predominantly target cysteine, but its low proteome abundance limits applications. Targeting alternate nucleophilic amino acids would expand the scope of this approach, but the identification of suitable reactive functionalities has limited their applicability.



Sulfonyl fluorides present an opportunity to target multiple amino acids, but due to their high reactivity and low stability, they currently have limited applicability in covalent medicines.



Previous work in this field has characterised the stability and reactivity of aromatic sulfonyl fluorides.¹

Integrated SF Libraries

- ✓ Small electrophile
- ✗ Varied intrinsic reactivity
- ✗ Low synthetic tractability

Modular Phenyl SF warheads

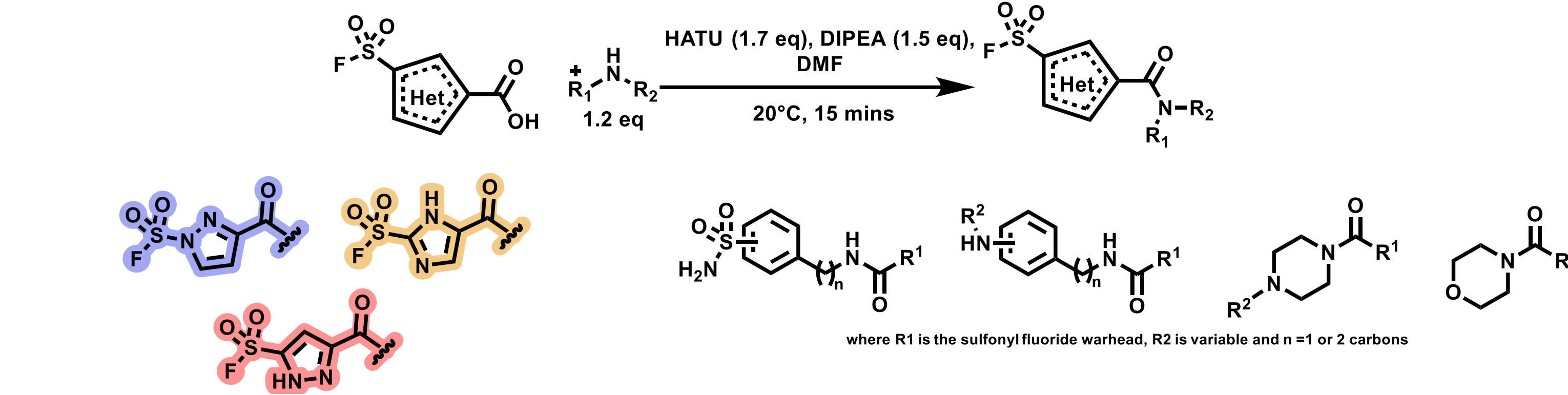
- ✓ Control of reactivity
- ✓ Synthetically tractable
- ✗ Poor physicochemical properties
- ✗ Wasteful molecular weight
- ✗ No polar interactions provided

Modular Heteroaryl SF warheads

- ✓ Controlled Reactivity
- ✓ Improved Physicochemical Properties
- ✓ Reduced Molecular Weight
- ✓ Can Form Polar Interactions

1. Library Design and Synthesis

Phenyl sulfonyl fluoride-based fragment hits (from internal screens, including CAII²) were synthesised with their respective heterocyclic sulfonyl fluoride warheads.



A library of 3 x 11 compounds were successfully synthesised for profiling the suitability of these new SO₂F warheads.

2. Electrophile Validation

Reactivity Assessment
Hydrolysis studies were performed to benchmark the stability of heterocyclic SFs with phenyl based SFs. N-heterocyclic sulfonyl fluorides were identified with good stability in aqueous buffer. SF4 had remarkably high stability likely due to the increase pK_a of the imidazole ring.

Improvement to Physicochemical Properties
Heterocyclic sulfonyl fluorides typically have improved physicochemical properties when compared with the phenyl matched pairs.

Protein Reactivity Assessment

Labelling % (4 h, 1.6 μM, 20 °C)	90%
k _{inact} / s ⁻¹	0.00014
K _i / nM	57.8
k _{inact} /K _i / M ⁻¹ s ⁻¹	2478

Selective engagement of protein targets was observed. Fragments 5-8 were known binders of CAII.

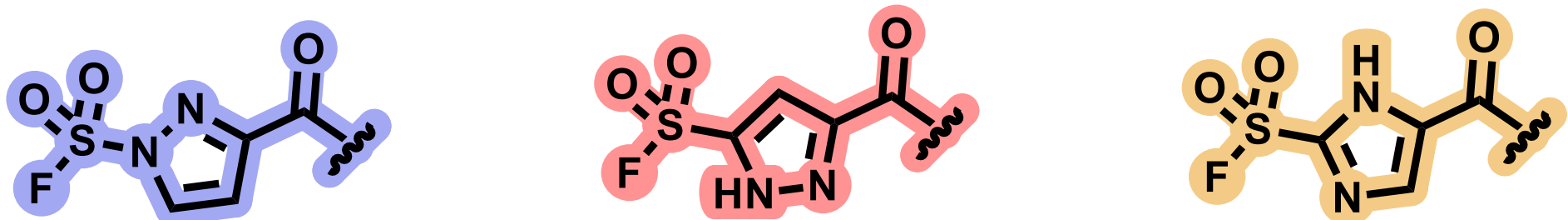
CAII	2	3	4	5	6	7	8	s1	s2	s3
SF4										
SF3										
SF2										

Proteome Wide Selectivity
Chemoproteomics can be used to assess whether we selectively engage our protein target and what other off target proteins a compound binds to.

H1 was shown to be relatively selective with only CAII coming out of the cloud of proteins.

4. Conclusions

- Heteroaromatic rings can be used to **control the reactivity of sulfonyl fluorides** whilst offering **improvements to physicochemical properties**.
- These warheads have been validated for use in **HTC-D2B screening approaches** protein targets, enabling the assessment of **target tractability** and **exploration of chemical space**.
- We have identified **novel chemical matter** that binds efficiently to **CRBN** with similar reversible affinities to known covalent CRBN binders. In addition, MS/MS analysis of these compounds have revealed that **Tyr355 can be covalently modified** – something that has not been previously reported in the literature.



3. Diverse Library Screening and HTC-D2B Validation

HTC-D2B Hit Optimisation

High-Throughput Chemistry-Direct to Biology (HTC-D2B) approaches have gained popularity as they circumvent compound purification

We have developed amide coupling conditions that result in high conversion to the desired reactive fragment that can be screened directly against recombinant protein to assess labelling efficiency.

Diverse Library Properties

The library was designed using an MGAP selection on 1000 amines to give a plate of 345 diverse amines suitable for use

HTC Synthesis of Diverse SF Reactive Fragments

HATU amide couplings were used and resulted in a high percentage purity of the desired SF reactive fragment.

HTC-D2B Screening against Protein Targets
Screening conditions: [Protein] = 1 μM, [SF] 50 μM, 24 h, 20 °C

Top CRBN Hits
*Coloured structures indicate structure expected to interact with the lenalidomide binding domain of CRBN

- 93.3%
- 89.8%
- 57.2%

5. Future Work

We are developing PROTACs to validate these novel CRBN chemotypes for targeted protein degradation applications.

Further exploration of these compounds could reveal the effect of covalently modifying different amino acid residues and the distinct differences observed in the recruitment of neosubstrates.

This could therefore be a pathway to the identification of new druggable targets that might have been deemed intractable to traditional drug discovery efforts.

