Activity-based Probes Targeting Trypsin-like Serine Proteases For Target And Biomarker Discovery[1]

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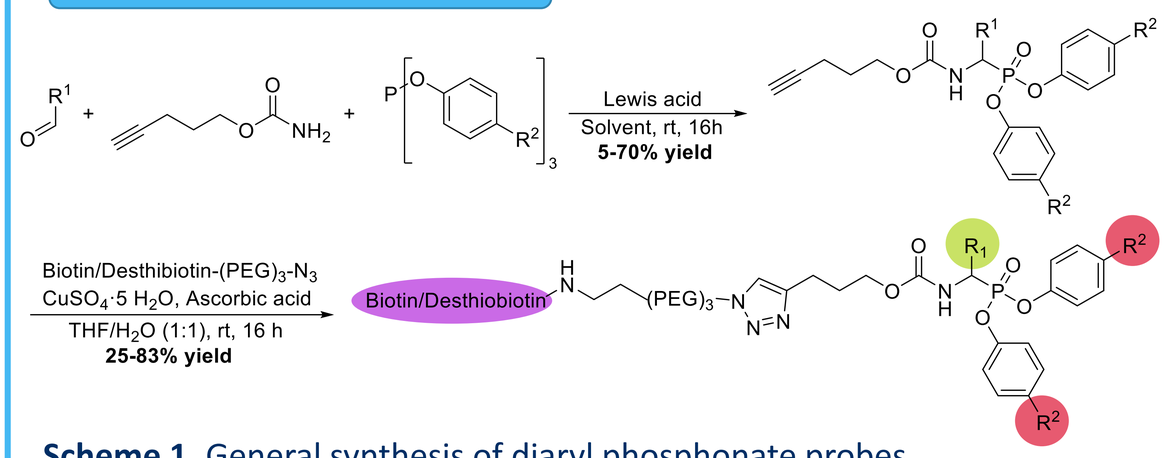
Introduction & Aim

Activity-based protein profiling (ABPP) is a proteomics technique that uses activity-based probes (ABPs) to visualise and characterise enzyme activity within a complex proteome. ABPs have the potential for target and biomarker identification in different pathologies.[2] The present study describes the synthesis and biochemical characterisation of **novel ABPs targeting trypsin-like serine proteases** with a diaryl phosphonate warhead. Even though diaryl phosphonate ABPs targeting trypsin-like serine proteases have been published,[3] the idea was to move away from mimicking the natural basic amino acids and expand the chemical diversity. This was followed by an extensive biochemical evaluation.

General chemical synthesis



Scheme 1. General synthesis of diaryl phosphonate probes

IC_{50} determination

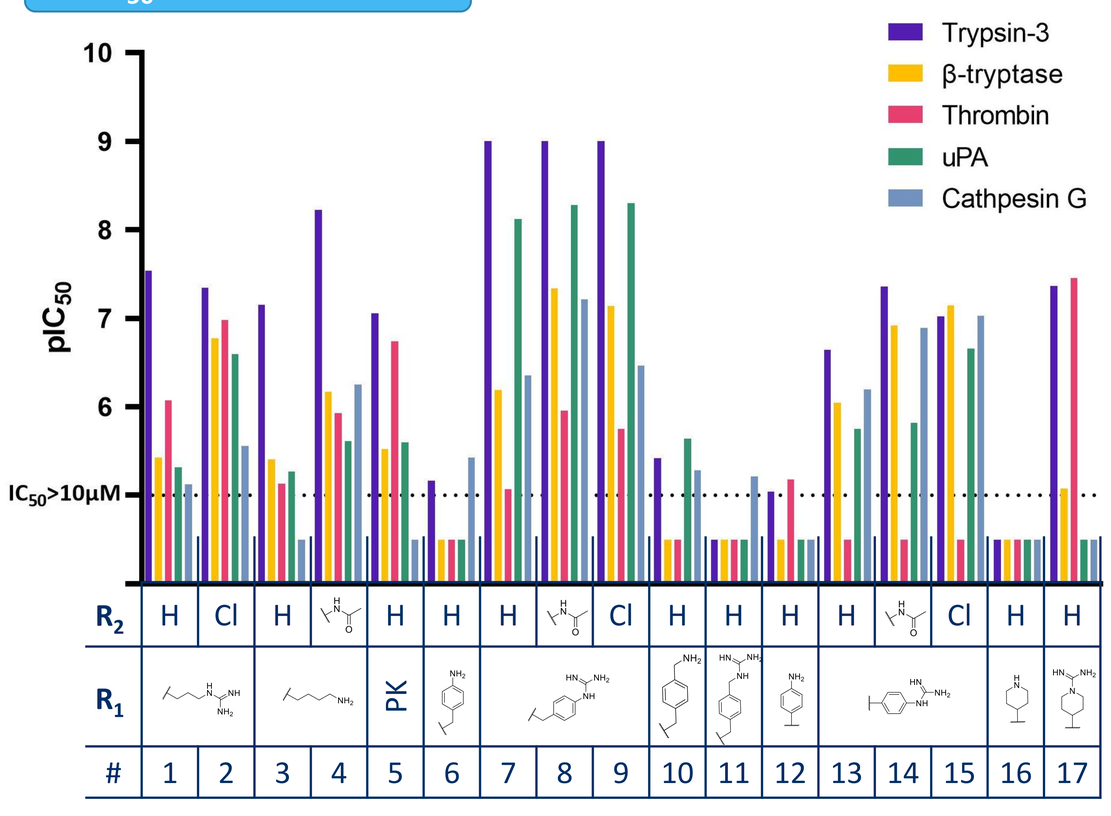


Figure 1. IC_{50} values of diaryl phosphonate alkyne probes against a panel of trypsin-like serine proteases.

Kinetic profile

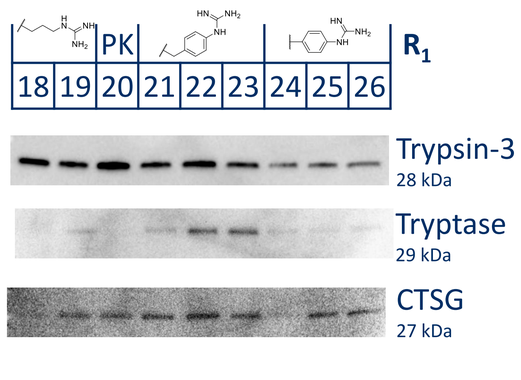
Table 1. k_{app} values of selected alkyne probes and the description of the mechanism of inhibition: slow-binding or irreversible.

	Trypsin-3	β-tryptase	Thrombin	uPA	CatG
			k_{app} ($M^{-1} s^{-1}$)		
1	$18x10^2$	41	64	41	*
2	$35x10^2$	$28x10^2$	$11x10^2$	$15x10^2$	*
5	$13x10^2$	136	$13x10^2$	188	N.D.
7	$40x10^3$	712	17	$75x10^2$	*
8	*	$70x10^2$	128	$13x10^4$	477
9	$29x10^3$	$61x10^3$	251	$32x10^4$	*
13	293	375	N.D.	124	582
14	358	$20x10^2$	N.D.	215	$14x10^2$
15	$11x10^2$	$45x10^2$	N.D.	$25x10^2$	$23x10^2$
17	$17x10^2$	27	$41x10^2$	N.D.	N.D.

The mechanism of inhibition was confirmed by jump dilution assays
N.D.: IC_{50} is greater than 10 µM, and progress curves were not performed
*: k_{app} determination is not possible by curve fitting

Labelling proteases

A. Recombinant proteases



B. Mast cell proteases

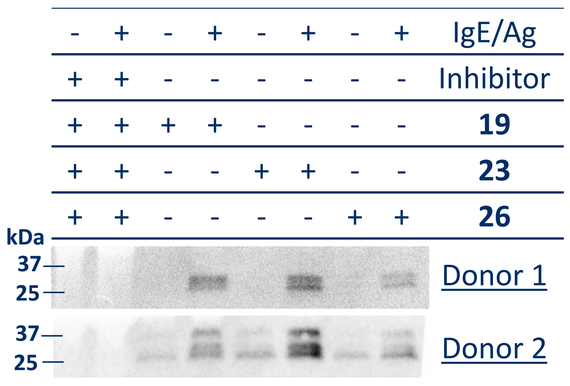


Figure 2. Labelling proteases under SDS-PAGE conditions with dichloro phenyl phosphonate desthiobiotin probes.

Conclusions

An **efficient synthetic route** has been implemented for probes bearing a diphenyl phosphonate warhead, and an **extensive chemically diverse ABP** library was achieved. Then, the newly ABPs were screened in a **detailed biochemical evaluation** in a panel of serine proteases. Surprisingly, the most potent ABPs were only irreversible in uPA, whereas they present a slow-binding mechanism for the rest. Last, we demonstrated that both irreversible and slow-binding probes **can label** recombinant **proteases** and tryptase released from mast cell degranulation.

1. A. Ramos-Llorca *et al.* **Manuscript submitted**
2. B. F. Cravatt *et al. Annual Review of Biochemistry*, **2008**, 77, 383–414.
3. Z. Pan *et al. Bioorganic & Medicinal Chemistry Letters*, **2006**, 16, 2882–2885.

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