SYNTHESIS AND CHARACTERIZATION OF ACTIVITY-BASED PROBES TO IDENTIFY NOVEL THERAPEUTIC AND DIAGNOSTIC TARGETS FOR DRY EYE DISEASE AND IRRITABLE BOWEL SYNDROME

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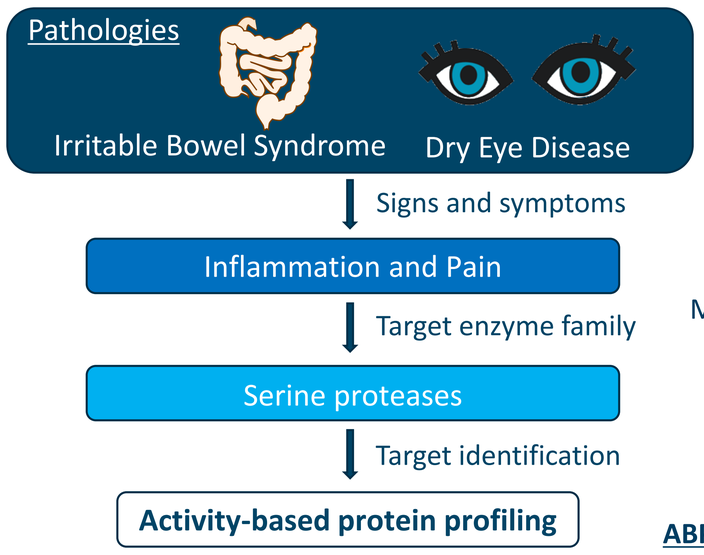
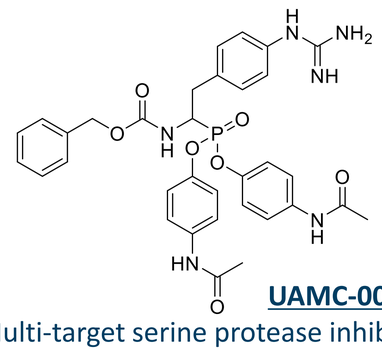
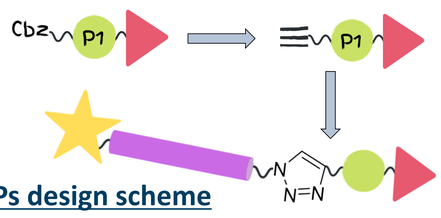
Introduction





UAMC-00050

Multi-target serine protease inhibitor

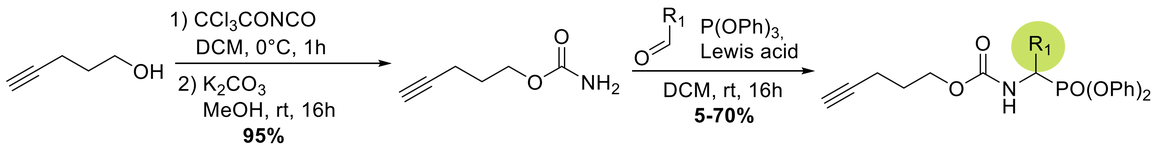


ABPs design scheme

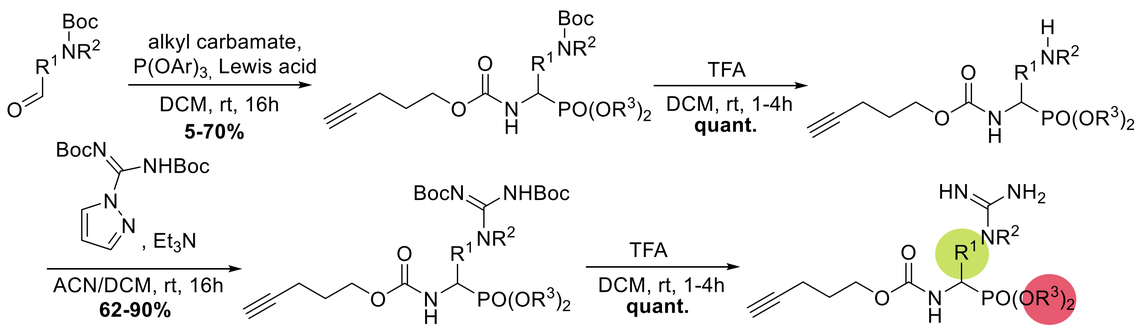
- DED is a disease of the ocular surface accompanied ocular surface **inflammation** and **tissue damage**; while IBS is a gastrointestinal disorder, characterized by abdominal **pain**.[1,2] We hypothesized that serine proteases play an important role DED and IBS.
- **Serine proteases** are involved in several physiological processes, including immune response, cell death and tissue healing.[3]
- **UAMC-00050** showed a reduction of both tissue damage and of inflammatory parameters in an eye of a dry eye rat animal model and, a decrease in visceral hypersensitivity in a rat model of post-inflammatory visceral hypersensitivity [4,5].
- **Activity-based protein profiling** is the method chosen to identify the possible upregulated serine proteases in the different pathologies.

Chemistry

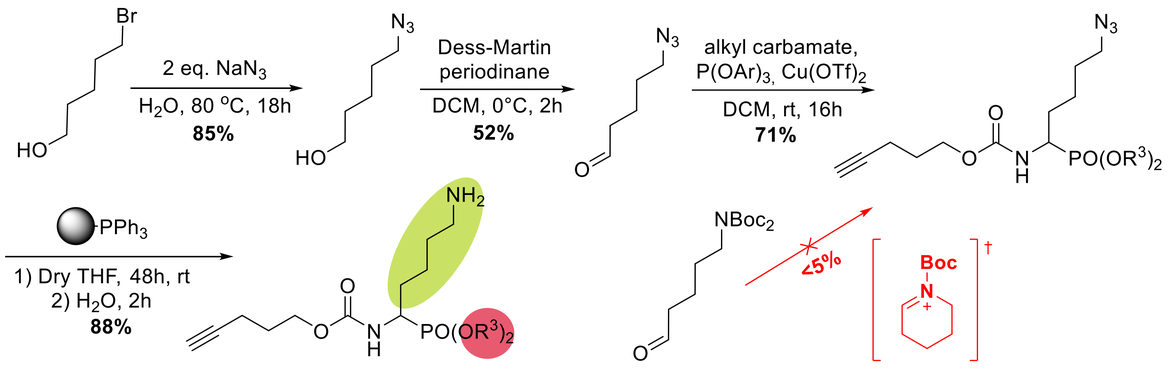
General synthesis of diaryl-phosphonates targeting serine proteases



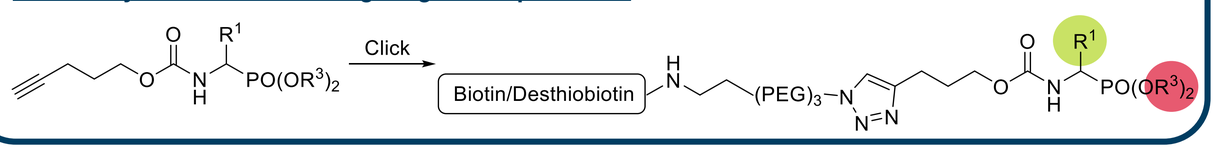
Synthesis of guanidino analogues



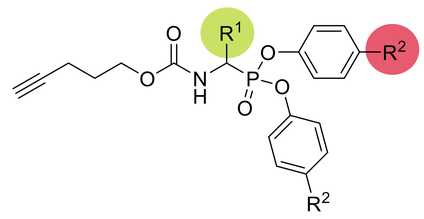
Modification for lysine analogue

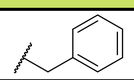
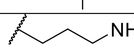
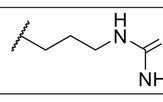
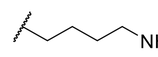
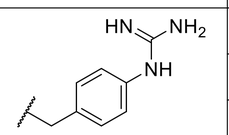
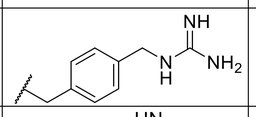
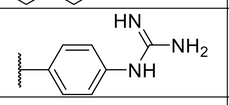
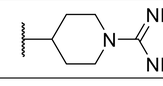


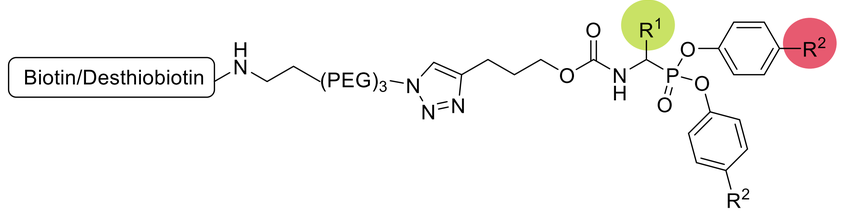
General synthesis of ABPs targeting serine proteases

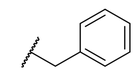
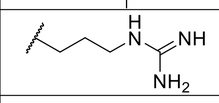
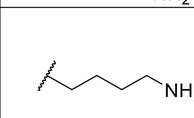
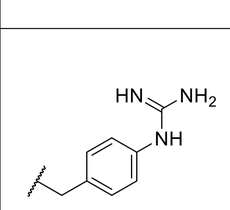


Biochemical evaluation



#	UAMC code	R^1	R^2	Tryptase	uPA
				IC_{50} (μM)	
1	3572		H	>10	>10
2	3567		H	>10	>10
3	3671		H	>10	>10
4	3672		H	3.7	4.8
5	3570		H	4.0	5.3
6	3573		NHCOMe	0.7	2.4
10	624		H	**0.6**	**0.004**
11	883		NHCOMe	**0.05**	**0.005**
12	3595		Cl	**0.07**	**0.005**
14	3569		H	>10	>10
16	3571		H	0.9	1.8
18	3576		H	8.4	>10
19	3596	Pro-Lys	H	3.0	2.5



#	UAMC code	Tag	R^1	R^2	Tryptase	uPA
					IC_{50} (μM)	
1	3461	B		H	>10	>10
2	3472	D		H	>10	>10
3	3473	D		H	>10	>10
4	3465	B		H	N.D.	N.D.
5	3495	B		H	2.1	3.5
6	3494	D		H	2.5	4.6
7	3563	D		NHCOMe	1.1	6.7
8	3464	B		H	0.9	0.04
9	3498	D		H	0.4	0.02
10	3511	D		NHCOMe	0.07	0.01
11	3510	B		NHCOMe	0.08	0.01
12	3562	D		Cl	0.1	0.006

B: Biotin; D: Desthiobiotin

Conclusions and perspectives

- A total of 19 alkyne diaryl phosphonates and 12 ABPs have been successfully synthesized and screened on uPA and tryptase. Modifications on the P1 position to target specificity for Chymotrypsin-, Elastase-, Trypsin-like serine proteases have been studied.
- The guanidine benzyl analogues are the most potent among all the available diaryl phosphonates, also more potent than the natural amino acids analogues.
- The ABPs will be used for target identification on different pathologies were serine proteases are involved.

1. Craig J. P. *et al. Ocular Surface* **2017** 15(3), 279-283.
2. Chey W.D., Kurlander J., Eswaran S. *JAMA* **2015**, *313*, 949-958.
3. Safavi F., Rostami A., *Exp. And Mol. Path*. **2012** 93: 428-433
4. Joossen C. *et al.* submitted.
5. De Winter *et al., British J. Pharmacol.* **2018**, *175*, 3516-3533

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