https://doi.org/10.17952/37EPS.2024.P2217

Synthetic strategies to prepare novel bioactive lysine and peptide conjugates with triazolium derivatives

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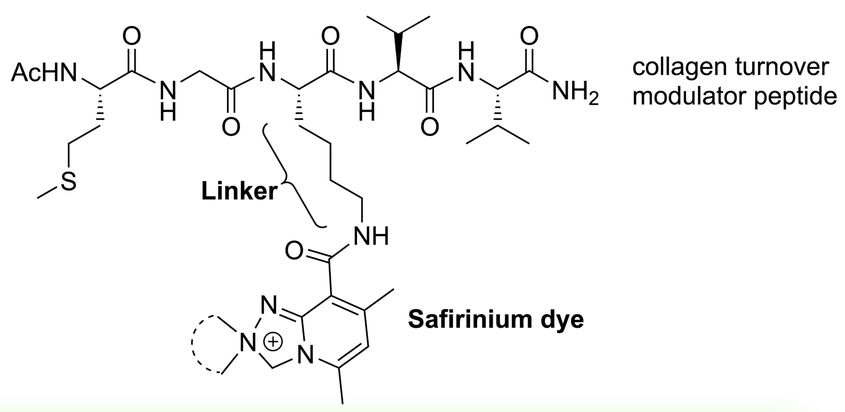
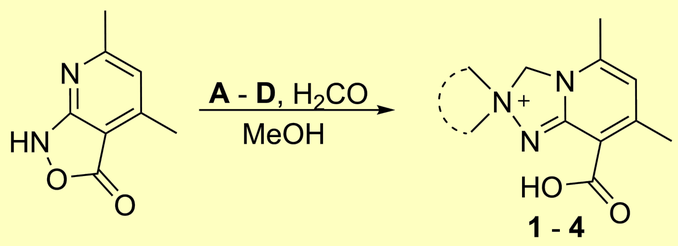
Introduction

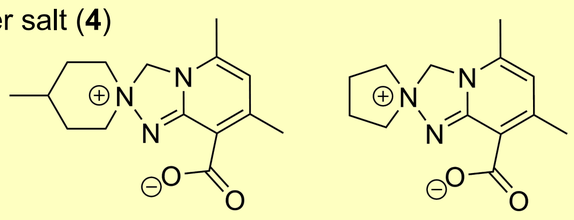
Peptide conjugates with small molecules are increasingly used, particularly in pharmaceutical applications, and can be designed as carriers, stabilizing agents, or active ingredients per se. In many conjugates a drug-like molecule is responsible for the activity, but the efficacy can be enhanced by the peptide sequence, e.g. by providing an additional binding specificity. Research for new peptide- and peptidomimetic drug conjugates is leading to the development of novel compounds with anticancer and antimicrobial activity and for diagnostic applications. Dihydro-[1,2,4]triazolo [4,3-a]pyridine-2-ium carboxylates known as *Safirinium* dyes, have been previously investigated as antibacterial and imaging agents.[1] Heterocyclic compounds, such as N-benzoylpyrazoles and N-benzoylindazoles were found to be good inhibitors of human neutrophil elastase (HNE). To the best of our knowledge, *Safirinium* derivatives and conjugates were not tested as enzyme inhibitors. Making available a straightforward synthesis strategy to prepare lysine and peptide conjugates with *Safirinium* dyes is key to enable their exploitation for pharmaceutical and cosmeceutical applications, and as building blocks for the synthesis of novel bioactive peptides.

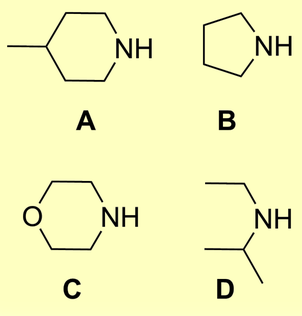


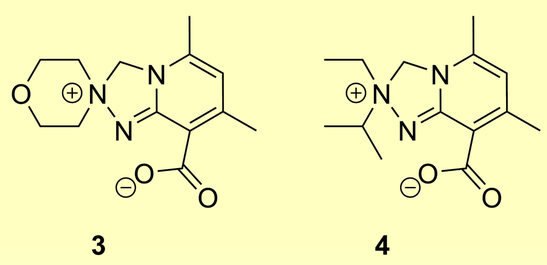
Synthesis of *Safirinium* derivatives



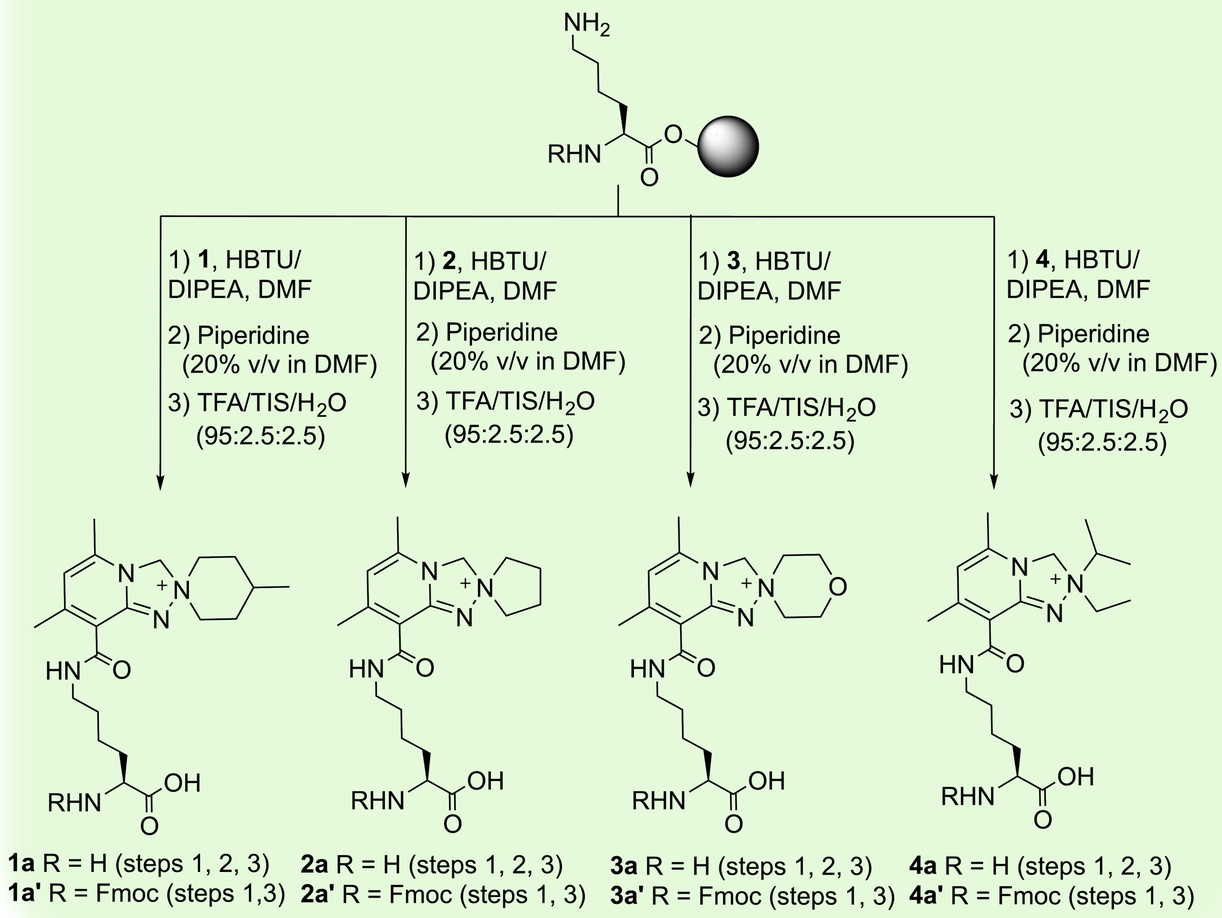
Safirinium derivatives 8'-carboxy-4,5',7'-trimethyl-3'H-spiro[piperidine-1,2'-[1,2,4] triazolo[4,3-a]pyridin]-1-ium inner salt (**1**), 8'-carboxy-5',7'-dimethyl- 3'H-spiro [pyrrolidine-1,2'-[1,2,4]triazolo[4,3-a]pyridin]-1-ium inner salt (**2**), 8'-carboxy-5',7'-dimethyl- 3'H-spiro[morpholine-4,2'-[1,2,4]triazolo[4,3-a] pyridin]-4-ium inner salt (**3**), and 8-carboxy-2-ethyl-2-(1-methylethyl)-5,7-dimethyl-2,3-dihydro-1,2,4] triazolo[4,3-a] pyridin-2-ium inner salt (**4**) were synthesised by reacting 4,6-dimethylisoxazolo [3,4-] pyridin-3(1H)-one with amine **A-D** respectively





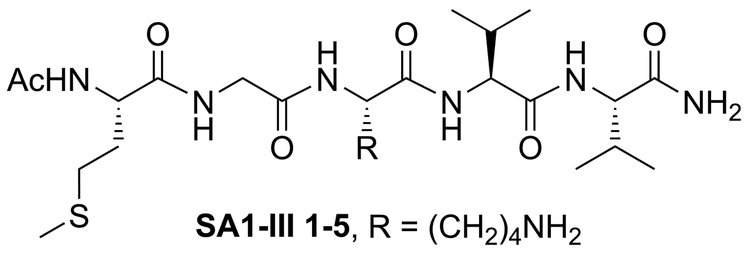


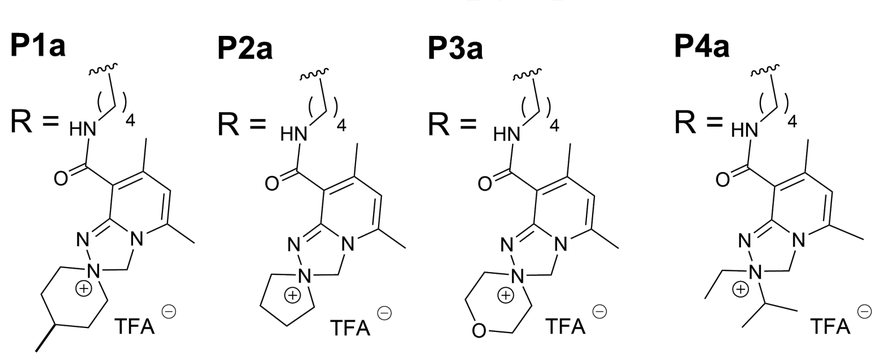
Solid phase synthesis of *Safirinium*-lysine building blocks and conjugates



1a R = H (steps 1, 2, 3) **2a** R = H (steps 1, 2, 3) **3a** R = H (steps 1, 2, 3) **4a** R = H (steps 1, 2, 3)
1a' R = Fmoc (steps 1,3) **2a'** R = Fmoc (steps 1, 3) **3a'** R = Fmoc (steps 1, 3) **4a'** R = Fmoc (steps 1, 3)

Use of building blocks 1a'-4a' for the SPPS of *Safirinium*-peptide conjugates and evaluation of elastase inhibitory activity of *Safirinium*-lysine and peptide conjugates





The building blocks were used in the synthesis of peptide conjugates (**P1a-P4a**) as Human Neutrophil Elastase (HNE) inhibitors for potential cosmeceutical applications, using the collagen turnover modulator **SA1-III 1-5**[2] as model peptide. All the new lysine and peptide conjugates were tested on Porcine Pancrease Elastase (PPE), commonly used as model of HNE. Lysine conjugates **1a-4a** showed moderate inhibitory activity on PPE, with **2a** being the most potent among the tested compounds. Peptide conjugates **P1a-P4a** are, instead, only weak inhibitors of PPE, probably because their affinity for the active site of the enzyme is reduced by steric hindrance.

C_M	1a	2a	3a	4a
10 μM	2%	8%	3%	0%
20 μM	5%	11%	10%	2%
30 μM	11%	18%	9%	10%
40 μM	12%	15%	20%	9%
50 μM	9%	25%	19%	12%

Conclusions and outlook

- *Safirinium* derivatives **1-4** as payloads for amino acid/peptide-drug conjugates acting as potential elastase inhibitors have been designed and efficiently synthesized.
- Their facile conjugation to amino acids was demonstrated by developing a straightforward solid phase synthesis of new lysine conjugates (**1a-4a**) and building blocks (**1a'-4a'**).
- Versatile conjugation to peptides has been proved by using building blocks **1a'-4a'** in the solid phase synthesis of the model peptide Ac-MGKVV-NH2 via Fmoc/tBu orthogonal strategy.
- All the *Safirinium* conjugates have been tested for their inhibitory activity toward porcine pancreas elastase. **2a** gave the best results, being a moderate elastase inhibitor.
- The described Lys-based *Safirinium* conjugates are good starting point for the development of novel elastase inhibitors which can be further used as new seedbed for the design of bioactive conjugates for pharmaceutical and cosmeceutical applications, and as building blocks for the synthesis of bioactive peptides.

Acknowledgements

The authors declare the support from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wroclaw University of Science and Technology, and from the University of Florence. PL is the participant of "BioTechNan" project– Interdisciplinary Environmental Doctoral Studies KNOW in the field of Biotechnology and Nanotechnology, co-financed by the European Union.

References

[1] J. Sączewski, K. Hinc, M. Obuchowski, M. Gdaniec, Chem. Eur. J. 2013, 19, 11531–11535
[2] F. Errante, L. Giovannelli, A. M. Papini, P. Rovero, Bioactive Peptides and Compositions Comprising Them / Peptidi Bioattivi e Composizioni Che Li Contengono, 2020, WO 2020/245772 A1
[3] P. D. Ledwoń, M. Jewginski, C. Bello, F. Nuti, P. Rovero, R. Latajka, and A. M. Papini Eur JOC 2024, e20240039

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