DEVELOPMENT OF BIOACTIVE PEPTIDES OF COSMETIC INTEREST THROUGH ENVIRONMENTALLY SUSTAINABLE PROCESSES

LUCREZIA SFORZI [a,b] ; FOSCA ERRANTE [a,b] ; JOANNA MAKOWSKA [d] ; PAOLO ROVERO [a,b] ; ANNA MARIA PAPINI [a,c] .

a) Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology, University of Florence, Sesto Fiorentino (FI), 50019, Italy;
b) Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Sesto Fiorentino (FI), 50019, Italy;
c) Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino (FI), 50019, Italy;
d) Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland.

lucrezia.sforzi@unifi.it

https://doi.org/10.17952/37EPS.2024.P2087

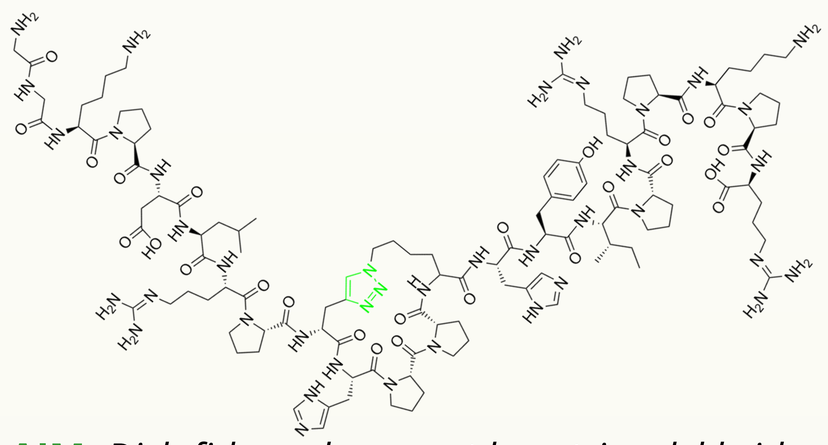
INTRODUCTION:

Peptides are more and more used in the cosmeceutical field to enhance skin beauty, health and appearance. Their synthesis is mainly performed by Solid Phase Peptide Synthesis (SPPS) that usually involves large quantities of harmful reagents and solvents like DMF, which has been recently banned by EU. This project aims to design, synthesize, and characterize metabolically stable peptides for cosmeceutical use, employing greener solvents and reagents to minimize environmental impact.

We focused our attention on **Waglerin-1**, a 22-mer peptide derived from tropical snake *Tropidolaemus wagleri*, characterized by an intramolecular disulfide bond that forms a rigid cysteine loop. The flexible N- and C- terminal parts as well as the disulphide bond contribute to the interaction of the toxin with the muscle nicotinic acetylcholine receptors (nAChR), classifying this toxin as cosmeceutical neurotransmitter-inhibiting peptide, endowed with botox-like activity.

WTX-1	**GGKPDLRPc[CHPPC]HYIPRPKPR** *Native peptide*
WTX-L	**GGKPDLRPCHPPCHYIPRPKPR** *Reduced peptide*
WTX-S	**GGKPDLRPSHPPSHYIPRPKPR** *Serine-containing peptide*

Stabilizing the structure of Waglerin-1 via CuAAC:



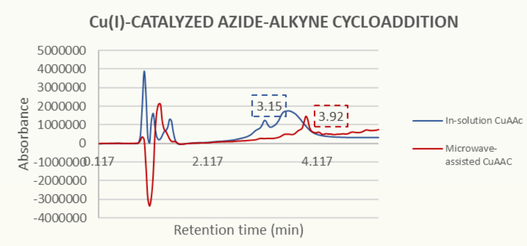
AIM: *Dislufide replacement by a triazolyl bridge*

GGKPDLRP-NHCHCO-HPP-NHCHCO-HYIPRPKPR — WTX-PN

Cu(I)-Catalyzed Azide-Alkyne cycloaddition (CuAAC) to obtain triazolyl WTX analogue

GGKPDLRP-NHCHCO-HPP-NHCHCO-HYIPRPKPR — cWTX-PN

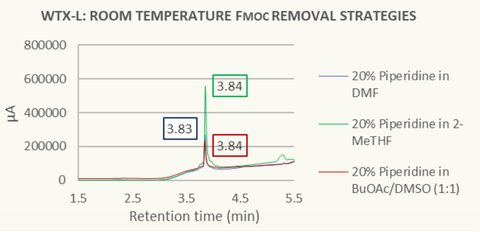
In-solution CuAAC: Peptide solution, 1 mg/mL previously cleaved and deprotected, in *tert*-BuOH:H_2O, 2:1; $CuSO_4 \cdot 5H_2O$ and ascorbic acid, 24h RT.
Microwave-assisted CuAAC: Peptide still protected and linked to the resin; CuBr and sodium ascorbate in DMSO:DMF, 3:7; DIPEA and 2,6-lutidine. Microwave cycle for 10 min at 55 °C.



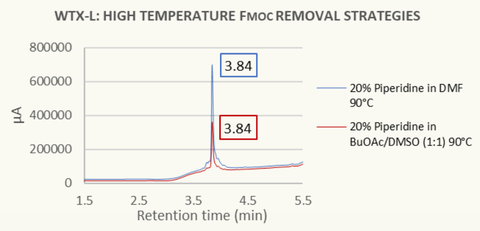
Experimental conditions: BEH C18 1.7 µm 2.1× 50 mm, flow 0.6 mL/min, T = 35 °C, solvent A: H_2O milliQ + 0.1 % (v/v) TFA, solvent B: ACN + 0.1 % (v/v) TFA, gradient 15-60 % B in 5 min.

RESULTS:
Only **In-solution CuAAC**, gave low amounts of clicked analogue.

Greening N-terminal Fmoc removal:

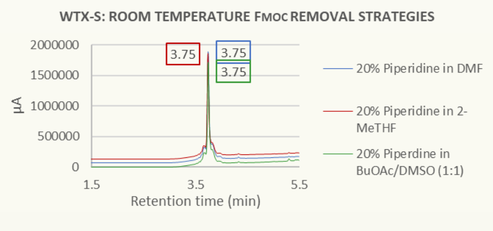


Samples:	*Rt:*	*HPLC Purity(%):*
WTX-L 20% Piperidine in DMF	3.81	16.4
	3.83	71.9
	3.9	11.7
WTX-L 20% Piperidine in 2-MeTHF	3.81	11.3
	3.84	74.2
	3.91	14.5
WTX-L 20% Piperidine in BuOAc/DMSO (1:1)	3.81	10.6
	3.84	76.1
	3.89	13.3

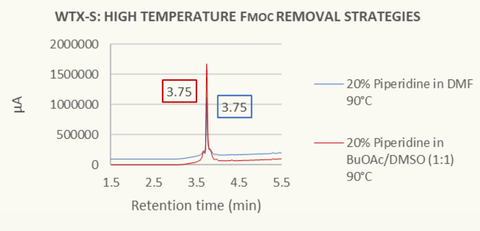


Samples:	*Rt:*	*HPLC Purity(%):*
WTX-L 20% Piperidine in DMF 90°C	3.81	4.7
	3.84	80.2
	3.89	15.1
WTX-L 20% Piperidine in BuOAc/DMSO (1:1)	3.81	4.7
	3.84	81.2
	3.88	14.0

Experimental conditions: Waters Acquity™ BEH C18 1.7µm 150mm, flow 0.5 mL/min, T = 45 °C, solvent A: H_2O milliQ + 0.1 % (v/v) Formic Acid, solvent B: ACN + 0.1 % (v/v) Formic Acid, gradient 5-75 % B in 5 min.



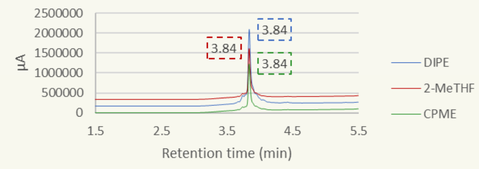
Samples:	*Rt:*	*HPLC Purity (%):*
WTX-S 20% Piperdine in DMF	3.67	7.8
	3.75	77.1
	3.83	15.1
WTX-S 20% Piperdine in 2-MeTHF	3.66	6.9
	3.75	74.1
	3.8	19.0
WTX-S 20% Piperdine in BuOAc/DMSO (1:1)	3.67	7.3
	3.75	80.0
	3.83	12.6



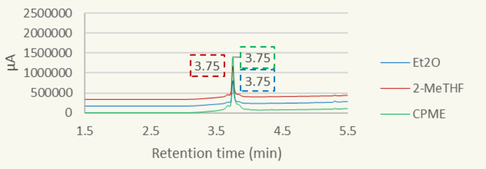
Samples:	*Rt:*	*HPLC Purity(%):*
WTX-S 20% Piperdine in DMF 90°C	3.67	4.6
	3.75	84.5
	3.82	10.9
WTX-S 20% Piperdine in BuOAc/DMSO (1:1) 90°C	3.67	7.4
	3.75	78.9
	3.83	13.7

Experimental conditions: Waters Acquity™ BEH C18 1.7µm 150mm, flow 0.5 mL/min, T = 45 °C, solvent A: H_2O milliQ + 0.1 % (v/v) Formic Acid, solvent B: ACN + 0.1 % (v/v) Formic Acid, gradient 5-75 % B in 5 min.

Greening the precipitation step:



Samples:	*Rt:*	*HPLC Purity(%):*
WTX-L DIPE	3.74	4.0
	3.78	2.5
	3.84	86.6
	3.94	6.9
WTX-L 2-MeTHF	3.74	5.5
	3.84	74.1
	3.88	20.4
WX-L CPME	3.78	14.6
	3.84	69.9
	3.95	15.5



Samples:	*Rt:*	*HPLC Purity(%):*
WTX-S Et_2O	3.72	12.5
	3.75	74.5
	3.79	13.0
WTX-S 2-MeTHF	3.72	12.1
	3.75	74.8
	3.79	13.1
WTX-S CPME	3.67	4.7
	3.75	85.6
	3.83	9.7

Experimental conditions: Waters Acquity™ BEH C18 1.7µm 150mm, flow 0.5 mL/min, T = 45 °C, solvent A: H_2O milliQ + 0.1 % (v/v) Formic Acid, solvent B: ACN + 0.1 % (v/v) Formic Acid, gradient 5-75 % B in 5 min.

RESULTS:

Greening the N-terminal Fmoc removal; for both WTX-L and WTX-S, deprotection at RT and HT using 20% Piperidine in BuOAc/DMSO (1:1) gave better results, the only exception being the deprotection of WTX-S at high temperature.

Greening the precipitation step; WTX-L and WTX-S displayed opposite behavior. In both cases 2-MeTHF could be a good compromise between efficiency and yield.

FUTURE PERSPECTIVES:

- Application of the greener Fmoc removal strategy during the full synthesis and final comparison of the results with the one obtained by classic SPPS, combined with a further investigation on greener precipitation solvents.
- Optimization of the bioactive sequence by conformational analysis and structure-activity relationship studies.
- Development of an efficient method to prepare constrained triazolyl bridged analogues.

REFERENCES:

1. Errante, F., Ledwoń, P., Latajka, R., Rovero, P. & Papini, A. M. Cosmeceutical Peptides in the Framework of Sustainable Wellness Economy. Front. Chem. 8, 572923 (2020).
2. Schmidt, J. J., Weinstein, S. A. & Smith, L. A. Molecular properties and structure-function relationships of lethal peptides from venom of Wagler's pit viper, Trimeresurus wagleri. Toxicon 30, 1027–1036 (1992).
3. Al Musaimi, O., de la Torre, B. G. & Albericio, F. Greening Fmoc/ t Bu solid-phase peptide synthesis. Green Chem. 22, 996–1018 (2020).
4. Pacini, L. et al. Optimization of peptide synthesis time and sustainability using novel eco-friendly binary solvent systems with induction heating on an automated peptide synthesizer. J. Pept. Sci. 30, e3605 (2024).
5. Roice, M., Johannsen, I. & Meldal, M. High Capacity Poly(ethylene glycol) Based Amino Polymers for Peptide and Organic Synthesis. QSAR Comb. Sci. 23, 662–673 (2004).
6. Tornøe, C. W., Christensen, C. & Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. J. Org. Chem. 67, 3057–3064 (2002).
7. D'Ercole, A. et al. On-resin microwave-assisted copper-catalyzed azide-alkyne cycloaddition of H1-relaxin B single chain 'stapled' analogues. 10.
8. Le Chevalier Isaad, A., Papini, A. M., Chorev, M. & Rovero, P. Side chain-to-side chain cyclization by click reaction: CLICKED CYCLOPEPTIDES. J. Pept. Sci. 15, 451–454 (2009).
9. Testa, C. et al. Design, synthesis, and conformational studies of [DOTA]-Octreotide analogs containing [1,2,3]triazolyl as a disulfide mimetic. Pept. Sci. 110, e24071 (2018).