Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente

Ciclo XXXVIII

The potential of marine-inspired thiol compounds as novel UV-screening agents for sun protection

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INTRODUCTION AND AIM

One of the major threats to skin aging and the risk of developing skin cancer is excessive exposure to the sun's ultraviolet radiation (UVR). The use of sunscreens containing different synthetic UVR filters is one of the most widespread defensive measures. Nowadays, consumers are increasingly aware of the origin (synthetic or natural) and eco-sustainability of their personal care products, and recent concerns have been raised on the potential eco-toxicity of some filters (benzophenone-3, octylmethoxycinnamate) to the marine environment and human health (1,2). Resorting to natural products produced in a wide range of marine species to counteract UVR-mediated damage could be an alternative strategy. In this context, photoprotective compounds derived from marine environments and extracted either from their natural sources or produced by engineering yeast or other microorganisms, represent an attractive strategy. Our attention focused on sulfur-containing histidine compounds, named ovothiols, known to be produced by several marine invertebrates (3).

The **aim of the study** was to **characterize** and **investigate** the **UV-screening properties** of marine-inspired thiol compounds **(Figure 1)** for their potential use as photoprotective molecules to obtain more biocompatible sunscreens and anti-photoaging formulations that could be both safer for human use and more environmentally friendly.

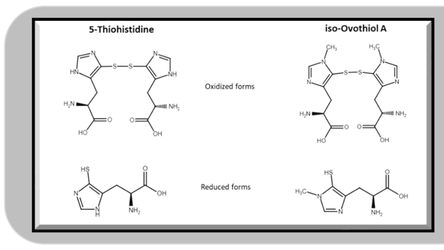
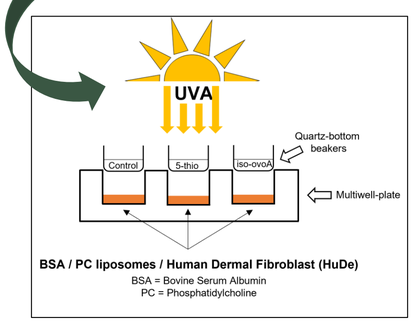


Figure 1. Chemical structure of the oxidized and reduced forms of 5-thio and iso-ovoA.

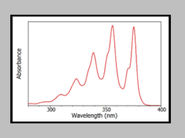
METHODS

To investigate the shielding effect of the two marine-inspired thiol compounds, we used the experimental setup here reported.



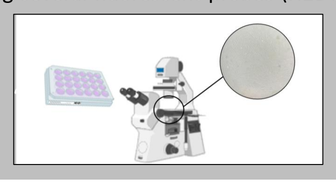
Methods - step 1

Preliminary results were obtained by spectrophotometric and fluorimetric analysis using various biological macromolecules.



Methods - step 2

Subsequently, Human Dermal Fibroblasts (HuDe) were seeded, and cell-based assays were carried out by cytofluorimetric approaches using several fluorescent probes (H2DCFDA, MitoSOX Red).



RESULTS

Comparison between oxidized and reduced forms of Marine-Inspired Thiol Compounds

Characterization before and after UVA exposure

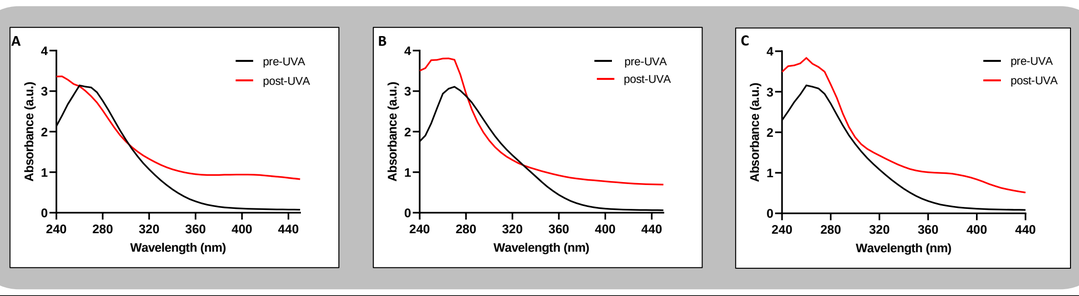


Figure 2. (**A**) Optical absorption spectra of 5-thio (1 mM), (**B**) iso-ovoA (1 mM) and of **(C)** 1-N-methyl 5-thio (1 mM) in their oxidized form, not exposed (black line) and exposed (red line) to 20 min UVA (540 kJ/m^2).

	Absorbance 412 nm	
	pre-UVA	post-UVA 20'
GSSG	-0.055 ± 0.016	-0.041 ± 0.007
GSH	0.522 ± 0.04	0.481 ± 0.020
5-Thio	0.004 ± 0.001	0.493 ± 0.016 **
iso-OvoA	-0.065 ± 0.021	0.601 ± 0.025 **

Table 1. Absorbance values of Ellman's reagent (DTNB) measured at 412 nm, after reaction with 5-thio and iso-ovoA, before and after 20 min UVA exposure. Both GSSG and GSH were used for comparison. The results are expressed as mean value ± S.D. (n = 3). ** p < 0.001 vs. pre-UVA.

Characterization before and after Sodium borohydride treatment

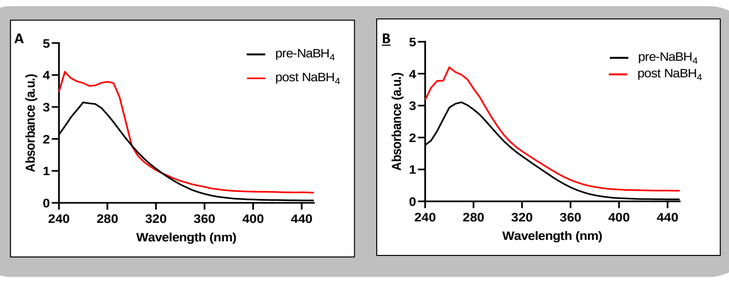


Figure 3. Optical absorption spectra of equimolar concentrations (1 mM) of 5-thio (**A**) and iso-ovoA (**B**), pre- and post-reduction with $NaBH_4$.

	Absorbance 412 nm	
	$NaBH_4$	
GSSG	-0.041 ± 0.057	-0.033 ± 0.056
5-Thio	0.003 ± 0.004	0.507 ± 0.095 *
iso-OvoA	-0.020 ± 0.028	0.449 ± 0.091 *

Table 2. Absorbance values of Ellman's reagent (DTNB) after reaction with 5-thio and iso-ovoA, pre- and post-reduction with $NaBH_4$. GSSG was used for comparison. The results are expressed as mean value ± S.D. (n = 3).* p < 0.05 vs. pre-$NaBH_4$.

Shielding effect on BSA and Liposomes

Figure 4. UVA-induced lipid peroxidation in PC liposomes in the presence or absence of marine-inspired thiol compounds. Iso-ovoA and 5-Thio (1 mM) were used as shielding agents in the oxidized **(A)** and in the reduced forms **(B)**, exposed to 20 min UVA irradiation (540 kJ/m^2). *p < 0.05, **p < 0.001 vs. control (CTR); $ p < 0.05 vs. 0 (exposed to UVA).

Figure 5. Photo-oxidative damage in BSA in the presence of 5-thio and iso-ovoA used as shielding agents. (**A**) Fluorescence intensity of Tryptophan in the presence or absence of 1 mM non-pre-irradiated compounds. (**B**) Content in carbonyl groups after 20 min UVA exposure using pre-irradiated compounds (1 mM). All exposures were performed at 20 min UVA irradiation (540 kJ/m^2). *p < 0.05; ** p < 0.001 vs. control (CTR); $$ p < 0.05, $ p < 0.001 vs. 0 (exposed to UVA).

Evaluation of the photoprotective effect on cell cultures

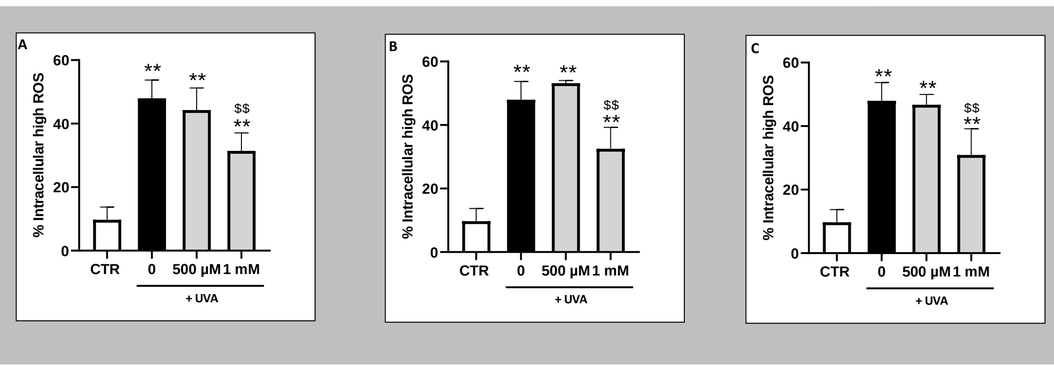


Figure 6. Production of intracellular high ROS (expressed as a percentage) in Human Dermal fibroblasts in the presence or absence of pre-irradiated compounds at different concentrations of (**A**) 5-thio, (**B**) 1-N-methyl 5-thio and (**C**) iso-ovoA, used as shielding agents. All exposures were performed at 10 min UVA irradiation (270 kJ/m^2). ** $p < 0.001$ vs. control (CTR); $$ $p < 0.001$ vs. 0 (exposed to UVA).

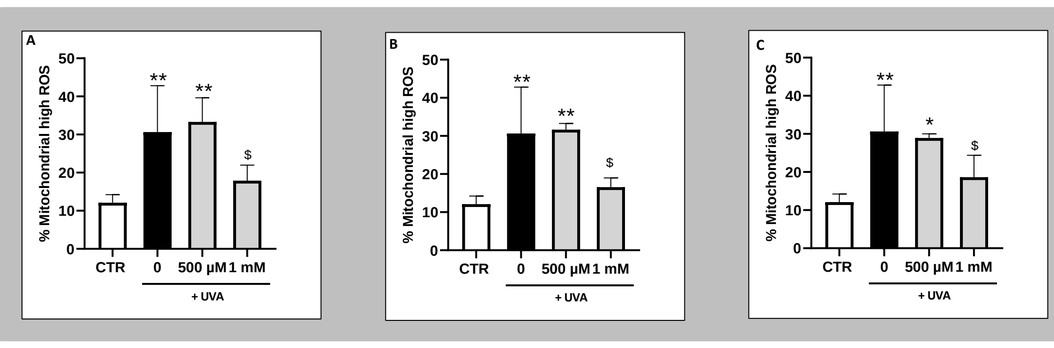


Figure 7. Production of mitochondrial high ROS (expressed as a percentage) in Human Dermal fibroblasts in the presence or absence of pre-irradiated compounds at different concentrations of (**A**) 5-thio, (**B**) 1-N-methyl 5-thio and (**C**) iso-ovoA, used as shielding agents. All exposures were performed at 10 min UVA irradiation (270 kJ/m^2). * p < 0.05 ** $p < 0.001$ vs. control (CTR); $ $p < 0.05$ vs. 0 (exposed to UVA).

CONCLUSION AND FUTURE PERSPECTIVES

Natural selection and evolution have ensured that plants and animals have developed effective protective mechanisms against the deleterious side effects of oxidative stress and ultraviolet radiation (UV). In this work, we have presented the first chemical characterization of the UVA absorption properties and UVA shielding effects of novel synthesized histidine derivatives inspired by the chemical structure of marine natural products, commonly named ovothiols. We found that the UVA properties of these compounds increase upon exposure to UVA and that their absorption activity is able to screen UVA rays. The preliminary results of this work demonstrate that these novel marine-inspired compounds could represent an alternative eco-friendly approach for UVR skin protection.

The following study demonstrates that natural compounds from marine organisms, even if at high concentrations, have significant photoprotective properties and therefore could potentially be used as sunscreens in cosmetic formulations, in order to limit the use of synthetic ones. The next aim is to investigate the possible antioxidant role of these compounds to prevent oxidative damage.

Funding: This research was funded by Next Generation EU in the framework of PRIN 2022 grant awarded by the Italian Ministry of University and Research, Grant no. 2022MJBEK9, CUP E53D23009970006.

Finanziato dall'Unione europea NextGenerationEU

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