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Review of Patrycja Ledwoń's, MSc doctoral dissertation titled Design, synthesis, and biological investigation of new peptides and peptidomimetics of cosmeceutical interest.

This evaluation was written in response to a letter dated March 6, 2023 from professor Robert Góra, chairman of the Scientific Board of the Faculty of Chemistry at Wrocław University of Technology.

The topic of the dissertation, which is a result of a collaboration between Prof. Latajka's team and Prof. P. Rovero's Italian center, is the prospective use of peptides and peptidomimetics in cosmetics. This is an important topic with a significant application potential. The search for active cosmetic ingredients with a clearly defined mechanism of action and proven efficacy in cellular models is arduous and not always effective. The undertaking of this topic by a PhD student should be highlighted for this purpose. Patrycja Ledwoń, MSc, had the opportunity to join an important field of research in reputable Polish and Italian scientific teams.

The work is 155 pages long and is divided into seven distinct chapters. As a result of an international collaboration, the work is quite lengthy and was written in English. The layout of the work is not typical of a classic doctoral dissertation, but reading such a study does not cause much difficulty. From the perspective of an Evaluator, it would have been advantageous to include an Introduction chapter in the Literature Review. This chapter (Literature Review) contains extremely valuable information on the biochemical processes and molecular targets that the PhD student investigated. She then discusses peptides and their conjugates with low-molecular-weight therapeutics, the role of collagen and the enzymes responsible for its degradation, and the current

state of knowledge concerning compounds that inhibit collagen degradation processes, using elastase as an example. In the next section, the author discusses the melanogenesis process, tyrosinase which is an essential part of this process, and inhibitors of this enzyme.

In the following chapter, titled Aim and objectives, the doctoral student describes the overall objective of the research, which is to obtain new compounds with the ability to inhibit the process of collagen degradation (potential elastase inhibitors) and the formation of skin pigmentation (potential tyrosinase inhibitors). According to the Reviewer, the objectives are correctly defined, but point 1 to conduct a literature review and point 8 to comprehend the obtained results are so apparent that their inclusion is unnecessary.

In the first twenty pages of the fourth chapter, the experimental section (consisting of 26 pages), the author describes the process of designing novel compounds, as well as their synthesis, purification, and physicochemical characterization. The methods used for the synthesis of peptides and their conjugates required outstanding abilities and a mastery of a chemist's workshop. The syntheses were designed properly, and the way they were performed leaves no doubt that the PhD candidate is a qualified synthetic experimentalist. Subsequent purification stages and characterization of the obtained compounds by means of spectroscopic techniques (NMR, CD) and mass spectrometry verified their structure. MSc. Ledwoń describes enzymatic assays of the two series of compounds obtained in the second section (6 pages). The experiments have been meticulously planned and conducted using appropriate control systems, which is not a typical activity for a young science student at their developmental stage. Enzymes (pancreatic elastase) and tyrosinase isolated from fungi as equivalents of enzymes genuinely involved in collagen degradation or melanogenesis is the only source of uncertainty. I would ask the PhD candidate to compare the structure, function, and substrate specificity of enzyme pairs (pancreatic elastase: neutrophilic elastase and human and fungal tyrosinase). In the first combination, is the substitution of a digestive enzyme for one derived from neutrophils not an oversimplification? Are any analyses of specific compounds with HNE planned?

In the following section, the author describes the theoretical methods for studying the interactions of selected compounds and the cellular studies that naturally complete her research cycle.

Chapter 5, unquestionably the most intriguing, contains 35 pages in which the doctoral student presents the obtained results and discusses them in light of contemporary scientific advancements. She discusses with great skill the synthetic issues she encountered during synthesis and the spatial structure of the resulting compounds to then proceed to enzymatic investigations in a consistent manner. All the compounds obtained by the doctoral student were either mild inhibitors of pancreatic elastase or exhibited no inhibition of this enzyme's activity. This may be due to the fact that the digestive enzyme PPE has a broad specificity range for a wide variety of substrates. HNE is an entirely distinct enzyme with a relatively limited substrate specificity; therefore, the possibility of inhibiting its activity is, with any luck, greater.

In terms of the synthesis and selection of potential tyrosinase inhibitors, the situation is quite distinct. The PhD candidate obtained a number of highly active derivatives that inhibit the enzyme several times more effectively than the commonly used inhibitor (kojic acid $IC_{50} = 20 \mu M$). The tested thiosemicarbazones (**43** and **45**) and their conjugates with peptides (**50**, **51** and **56**, **57**) had the greatest potential to inhibit the examined enzyme's activity. Notable are the derivatives of TSC43 with $IC_{50} = 5.3 \mu M$ (compound **43**) and $IC_{50} = 6.57 \mu M$ (compound **50**) and $IC_{50} = 6.66 \mu M$ (compound **51**) whereas the series where TSC45 served as the starting compound exhibited a similar inhibition potential to the control. In this context, investigations on cell lines, a near-real-world model, produced intriguing but unexpected results. Compound TSC44 demonstrated the greatest ability to inhibit melanin production (77% at 40 μM and 28% at 10 μM concentration), whereas it inhibited tyrosinase to a negligible degree (34% at 150 μM concentration). This effect may result from the relative toxicity ($IC_{50} = 71.5 \mu M$) of both the derivative and its peptide conjugates to the tested line. I have no doubt that this research represents a significant accomplishment for the PhD student, and I would recommend its continuation.

The concluding chapter of the dissertation comprises enzymatic, cellular, and molecular modeling research. Such a thorough strategy is noteworthy and outstanding.

The dissertation contains 261 articles of literature, the majority of which are from the past decade. This demonstrates the author's skill of literary navigation.

The doctoral student is the author of five publications on the topic of her dissertation, the majority of which are review papers and experimental papers thematically related to her dissertation. In addition, she has contributed to the production of four original papers, three of which are on the JCR list. MSc Ledwoń participated in a number of scientific internships (6) comprising more than a year, she is the project leader for Preludium, and her scientific conference participation is above average.

The work was undoubtedly difficult for the PhD candidate to prepare, but I have no significant editorial comments. A minor criticism pertains to the fact that "pH=8" was written erroneously (e.g., on page 102 and numerous other pages with other numerical values) in the stead of pH 8.

In conclusion, the dissertation submitted to me for review is a multidisciplinary work, and I have a special appreciation for this form of research. The author's ability to collaborate with international teams deserves a special mention. Her ability to plainly present the obtained results is an additional strength of this study. Considering the doctoral student's scientific accomplishments and her above-average activity, I request a distinction for the dissertation.

The evaluated work fulfills all formal requirements of the Act of July 20, 2018 on Higher Education and Science (Journal of Laws 2018 item 1668) for doctoral dissertations, and I request that MSc Patrycja Ledwoń be permitted to proceed to the subsequent stages of the proceedings for the award of a doctoral degree.

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