Dipartimento di
Farmacia
Università degli Studi di Napoli Federico II

Naples, April 18th, 2023

Referee report for the Ph.D. Thesis
submitted by Patrycja Ledwoń

Thesis's Title: "***DESIGN, SYNTHESIS, AND BIOLOGICAL INVESTIGATION OF NEW PEPTIDES AND PEPTIDOMIMETICS OF COSMECEUTICAL INTEREST***"

Prepared under the supervision of:
Prof. Rafał Latajka, Wroclaw University of Science and Technology, Faculty of Chemistry, Department of Bioorganic Chemistry
Prof. Paolo Rovero, Department of NEUROFARBA, Interdepartmental Research Unit of Peptide and Protein Chemistry and Biology

Introduction

Peptides are known to have diverse biological roles, most prominently as signaling/regulatory molecules in a broad variety of physiological processes including defense, immunity, stress, growth, homeostasis, and reproduction. More recently the peptides have attracted attention in dermatology and cosmetology thanks also to innovative drug delivery technology. Cosmeceutical peptides may act as signal modulators of the extracellular matrix component, as structural peptides, carrier peptides, and neurotransmitter function modulators.
The advantages of using peptides as cosmeceuticals include their involvement in many physiological functions of the skin, their selectivity, their lack of immunogenicity, and the absence of premarket regulatory requirements for their use.
This thesis is aimed at the discovery of new inhibitors of two important enzymes, elastase, and tyrosinase, involved in the breakdown of collagen fiber and the regulation of the synthesis of melanin, respectively.

General Comments

Overall, this thesis has explicit scopes and is based on original ideas, in designing and synthesizing peptides and their conjugates with small molecules. The designed molecules have been biologically investigated and rational structure-activity relationships have been rationally formulated and discussed based also using molecular modeling approaches.
The thesis consists of seven Chapters, described clearly and well-structured and I have particularly appreciated the list of discussed compounds reported before the abstract. In the first two chapters are well introduced the targets studied (elastase and tyrosinase) and the state-of-the-art concerning the latest results reported in the literature regarding the application of the peptides as cosmeceuticals.

Dipartimento di Farmacia
Via Domenico Montesano, 49 • 80131 Napoli, Italia
paolo.grieco@unina.it • + 39 081678620
www.farmacia.unina.it

Although the first two chapters include more than 40 pages, the information reported is very useful for the reader who is introduced without difficulty to better understand the aims and objectives of the research activity carried out during the doctoral activity.
Chapter 4 nicely reports the design and synthesis of new compounds starting from the previous information reported in the literature. Herein is also reported the purification, analysis, and enzymatic assays performed on the synthesized compounds.
From the point of experimental work reported in this thesis regarding the synthesis of the enzyme inhibitors (including the molecular modeling studies performed by collaboration), using classical organic and peptide synthesis, and in vitro biological testing (enzymatic assays) of these compounds. I can judge that the technical quality is excellent and certainly state-of-the-art.
Concerning the first part of the thesis related to the discovery of new elastase inhibitors, although all the presented compounds were rationally designed, most of them did not show significant inhibitory activity in experiments with Porcine Pancreas Elastase. It should be emphasized that even negative results can represent valid elements for the subsequent design of new compounds. In any case, the great work carried out for the realization of these compounds demonstrates that the Ph.D. candidate has not only a vast knowledge of the subject of research but also profound scientific skills in analyzing the data obtained and in drawing valid conclusions.
Compressively, the thesis is well structured and contains many interesting data. The design and synthetic approaches have been well described and a detailed analysis of the advantages and disadvantages of the chosen solutions has been performed. Moreover, all the methodologies sound well.
Finally, the references are up-to-date, current, and relevant to the work carried out. There are enough citations in general.
However, some minor points could be revised to improve the thesis (See **Minor typographical errors were noticed**).

Additional comments and questions to be discussed with the Defendant

In Chapter 3, the results reported in Table 14 (page 71) demonstrate the importance of the thiourea group in the inhibitory activity against tyrosinase, without modifications to R2 and R3. Therefore, the sentence “Therefore, several thiosemicarbazones having free -COOH are required for conjugation, with minor changes in the R1, R2, R3 positions” given on page 72, should be reworded.

On page 73, in Figure 26 the chirality for the compound TSC 44 should be reported, or, at least, mention the presence of chirality.

Page 75, the word “Organic” is superfluous in the summary of 4.3.2

Dipartimento di Farmacia
Via Domenico Montesano, 49 • 80131 Napoli, Italia
paolo.grieco@unina.it • + 39 081678620
www.farmacia.unina.it

Page 116, Table 25: Add the standard deviation for kojic acid. Otherwise, compound 56 (26.12 ± 9) could be less active than kajic acid. The information on page 116 regarding compound 56, when compared with kajic acid, may be speculative.

As for compounds 43-45, that is, the thiosemicarbazones used for conjugation with peptides, why these compounds have not been evaluated earlier for their inhibitory activity? Perhaps knowing in advance of their inhibitory activity towards the tyrosinase enzyme would have changed the subsequent design of the molecules.

Page 118, it is reported that "Almost all compounds (45-56) inhibited 0-3% of melanogenesis at the concentration of 10 µM and 4-13% and 40 µM." It should read "... and 2-13 % at 40 µM".
Also, the statement "However, TSC 43-45 and tripeptide conjugate 57 inhibited melanogenesis more than kojic acid at these two concentrations" should be correct.

Page 120, in the sentence "In the case of TSC 43, containing a..." Perhaps she meant the compound TSC 44.

Minor typographical errors were noticed.

- Page 37: “depsipeptide”;
- Page 87, “…used in **this** type of assay mushroom tyrosinase…”
- Page 92, “…with yields **of** around 20-30%.”
- Page 93, “…thiosemicarbazones resulted **in** pure products.”
- Page 93, “The presence of **another** hydrophobic moiety,…"
- Page 94, “…structures of **synthesized** compounds 4a-7a…”
- Page 99, “…for peptide **8** possesses a positive…”
- Page 103, “…most of them **were** found as multiplets…”
- Page 103, “…overlapping signals, **and** all assignments were…”
- Page 107, “…groups were deprotected for ~~**the purpose of**~~ the assay.”
- Page 109, “It shows **an** increasing percentage of PPE…”
- Page 111, “…after the measurements **were** performed…”
- Page 111, “…**have** been shown previously in the **Figure 25.”**
- Page 111, “…TSC 43 and 45 resulting **in** very similar inhibitory…”
- Page 111, “…The results for TSC-conjugates **shown** in **Figure 48**,”
- Page 119, “…especially <1.0, **do** not let to..”
- Page 119, “…and healthy **cell** viability.”
- Page 120, “…simulations allow **us** to better…”
- Page 120, “Isomer *E* **was** docked as default…”

Dipartimento di Farmacia
Via Domenico Montesano, 49 • 80131 Napoli, Italia
paolo.grieco@unina.it • + 39 081678620
www.farmacia.unina.it

Dipartimento di
Farmacia
Università degli Studi di Napoli Federico II

Conclusion

The quality of the work is high, and the experiments and synthetic strategies performed are at the highest level.

The planned objectives are original and clearly described and the amount of work involved in achieving these objectives is considerable. The results and conclusions confirm that Patrycja Ledwoń has achieved the main objectives and testify to the ability to carefully analyze the large amount of data obtained on the 42 synthesized compounds. Patrycja Ledwoń has never overestimated her results and has always considered all the appropriate controls in meticulously analyzing the data, providing a correct interpretation of the results obtained.

These considerations lead me to conclude that the work reported in this thesis fully satisfies the specified requirements of Art. 187 of the Act of July 20, 2018, Law on Higher Education and Science (i.e., Journal of Laws of 2018, item 1688, as amended).

Therefore, I recommend honorable mentions for this thesis of high scientific value.

Date: 18/04/2023

Signature:

Paolo Grieco

Dipartimento di Farmacia
Via Domenico Montesano, 49 • 80131 Napoli, Italia
paolo.grieco@unina.it • + 39 081678620
www.farmacia.unina.it