CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Organic and Bioorganic Chemistry

Study program: Pharmacy

Opinion of the Opponent of the Diploma Thesis

Year of the defense: 2023/2024

Student: **Seyedmohammad Khatibii**

Thesis Tutor: PharmDr. Lukáš Opálka, Ph.D.

Consultant: PharmDr. Monika Kopečná, Ph.D., RNDr. Martina Navrátilová, Ph.D.

Opponent: Dr. Georgios Paraskevopoulos, Ph.D.

Thesis title: **Factors affecting the microstructure and permeability of lipid model membranes**

Scope of work, number of 55 pages, 13 figures, 1 tables, 120 citations

Evaluation of the work:

a) Processing of the theoretical part: Excellent

b) The complexity of the methods used: Excellent

c) Preparation of the methodological part (clarity, comprehensibility): Very good

d) The quality of the experimental data obtained: Very good

e) Processing of results (clarity): Very good

f) Evaluation of results, including statistical analysis: Excellent

g) Discussion of results: Very good

h) Clarity, conciseness, and adequacy of conclusions: Very good

i) Meeting the objectives of the work: Excellent

j) Quantity and up to date of references: Excellent

k) Language level (stylistic and grammatical level): Excellent

l) Formal level of the work (text structure, graphic design): Excellent

I recommend the thesis for recognition as a rigorous thesis ☒

Comments on the evaluation:

The thesis examines the development of a protocol for the isolation of the covalently bound lipids from the tape-stripping samples of human stratum corneum. The study of the covalently bound lipids provides valuable insights for their impact on the skin lipid arrangement and helps to a better understanding of the skin's barrier function. The study was based on previously published methods and aimed to optimise the existing protocols.

The theoretical part of the thesis contains a general description of human skin structure together with the function of Ceramides (Cers) and their biosynthesis, closing with the connection between alterations of the skin lipid composition during specific skin disorders. In general, the theoretical part is written nicely and covers the described topics very well. On the other hand, I would expect to find at least a small subchapter describing previous attemps for the isolation of covalently bound lipids from the literature and I would remove the part of the Cer biosynthesis as it seems inconsistent with the aim of the study.

The results and discussion part is bult logically and it is following a nice flow through the outcomes of each experiment that were used to alter specific conditions and optimize the

isolation of covalently bound lipids. The results are systematically presented, and the discussion, including data interpretation in the context of existing literature, contributes to the scientific value of the work.

The experimental part of the thesis is also written with a nice structure. Sometimes, though, it is difficult for the reader to orient between the used tubes and the discarded supernatants. I think that a scheme would be usefull for a better orientation and I would highly recommend it during the presentation.

My general evaluation of the written text is positive and I have the following comments which are not affecting the quality of the described work:

- Some figures have not good quality eg Fig. 4

- In some cases the names used in text is not consistent with the relative figure eg 3-ketodihydrosphingosine in page 18 and 3-ketosphinganine in Fig. 5.

- Some periods at the end of setences are missing eg page 32, lines 9, 14 and 24.

- Some references are overused in the same paragraph eg ref 41 in page 20. It would be enough to add the reference number at the end of the paragraph.

- Some materials and instruments are not described eg evaporator with nitrogen stream, tabletop centrifuge, RP-18 cartridges for the desalting procedure

Questions and comments to student:

1. What is the role of the corneocyte lipid envelope (CLE)?

2. In the experimental part it is described the protein content measurement (page 40, paragraph 4.2.2) but no additonal comments are given about this in the text. What is the purpose of this measurement? What is the value 0.3? Is this measurement connected with the units showed in the Y-axis of the lipids quantification after each experiment (pmol/L)/protein. If yes, how this calculation was done?

3. What is the expected composition of the pellet after the extraction of the free lipids and the pellet purification steps? Do you have any data for the approximate mass of the remaining pellet? If yes, what was the average percentage of covalently bound lipids in the pellet?

The thesis by Seyedmohammad Khatibii meets the requirements for this type of work, and therefore, I recommend it for defense.

Evaluation of the thesis: Excellent   **For the defense:**   **Recommend**

 In Hradec Králové   12. září 2024   signature of the opponent