

О P I N I O N

by a member of the Scientific jury: Assoc. Prof. Krasimira Tasheva, PhD, Institute of Plant Physiology and Genetics – BAS, appointed as a member of the Scientific jury by Order No. 1050 of 18 December 2025 issued by the Director of Institute of Biophysics and Biomedical Engineering – BAS

For the award of the educational and scientific PhD degree

Field of Higher education 4. “Natural Sciences, Mathematics and Informatics”

Professional field: code 4.3 “Biological Sciences”

Scientific specialty “Biophysics”

Author of the Dissertation: Tihomira Tihomirova Stoyanova, MSc, Transmembrane Signalling Laboratory, Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences

Topic of dissertation: Antitumor lipids – effects on transmembrane cellular signalling

Supervisors: Prof. Dr Rumiana Tzoneva

Prof. Dr Albena Momchilova, DSc

1. BIOGRAPHICAL DATA

Tihomira Tihomirova Stoyanova was born on 28 September 1991. She obtained a Bachelor’s degree in Molecular Biology and completed two Master’s degree programs in *Cell Biology and Pathology* and *Developmental Biology* at the Faculty of Biology, Sofia University “St. Kliment Ohridski”, graduating in 2016 and 2017 with excellent academic performance. Since 2015, she has been employed at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, initially as a biologist specialist and, since 2019, as an assistant professor. In 2017, she was enrolled as a part-time PhD student at the Laboratory of *Transmembrane Signalling*.

2. RELEVANCE OF THE DISSERTATION TOPIC

The dissertation thesis is focused on investigating the effects of the antitumor lipid erufosine (EPC3) with regard to its potential application in the treatment of one of the most difficult cancer types to manage—triple-negative breast cancer. In

addition, the antitumor effects of two other lipid-based compounds, miltefosine (HePC) and D-erythro-N,N-dimethylsphingosine (DMS), are thoroughly examined in the context of developing a combination therapy for lung cancer. The research problem addressed in this dissertation is relevant and significantly important from scientific and practical perspectives in light of increasing interest in developing new therapeutic strategies effective for the treatment of oncological diseases.

3. STRUCTURE AND CONTENT OF THE DISSERTATION

The presented dissertation is organized in accordance with established scientific standards and comprises the following principal sections: Introduction, Literature Review, Aim and Objectives, Materials and Methods, Results and Discussion, and Conclusions and Contributions. The dissertation concludes with a comprehensive bibliography, which includes both recent publications from the last decade and earlier works providing essential theoretical and methodological insights relevant to the research problem.

The “*LITERATURE REVIEW*” section, comprising 29 pages, demonstrates a thorough and critical understanding of the current state of the research problem. It presents a logically structured and analytically evaluated overview of key literature data on the prevalence and etiology of oncological diseases, as well as the structural, functional and biophysical differences between normal and tumor cells. Particular attention is given to the mechanisms of action of antitumor lipids and their role within contemporary anticancer therapeutic strategies. The review is clearly organized and supplemented with sixteen figures and two tables, which enhance the clarity and interpretation of the presented data.

Overall, the literature review reflects a deep and systematic comprehension of the subject matter, offering a detailed analysis of existing challenges and future directions, while providing a coherent rationale for both the aim of the dissertation and the specific experimental tasks.

The “*MAIN AIM*” is broadly formulated and appropriately directed toward elucidating the mechanisms of action of antitumor lipids on transmembrane signaling in tumor cells. The six experimental objectives are logically interconnected, and their sequential execution has enabled the successful achievement of the overall aim of the study.

The “*MATERIALS AND METHODS*” section, spanning 14 pages, provides a detailed account of the techniques employed, ensuring reproducibility. To achieve the dissertation’s objectives, a comprehensive interdisciplinary approach was

applied, including in vitro assays for cell viability, apoptosis, migration, adhesion, morphology, and cell cycle analysis, using fluorescence and confocal microscopy, and flow cytometry. Additionally, molecular and biochemical methods such as RT-qPCR, Western blot, ELISA, chromatographic, and enzymatic assays were employed. The chosen methods are fully aligned with the experimental tasks. Data reliability was statistically validated using one-way ANOVA followed by Bonferroni post hoc testing in GraphPAD PRISM, enabling accurate interpretation. It is evident that the doctoral candidate, Tihomira T. Stoyanova, has mastered and successfully applied a wide range of classical and contemporary techniques, which will undoubtedly benefit her future research endeavors.

The “**RESULTS AND DISCUSSION**” section, spanning 54 pages, presents the experimental data alongside their interpretation, including a Summary subsection synthesizing the key findings. The section is richly illustrated with 3 tables and 45 figures, comprising graphs, histograms, photographs, and high-resolution microscopic images, which enhance comprehension of the quantitative data and facilitate interpretation. The results are described with precision and conciseness and are thoughtfully analyzed in the context of relevant literature from other research groups, demonstrating the candidate’s ability to not only generate and process experimental data but also to critically evaluate and accurately interpret them.

The dissertation concludes with **8 conclusions** and **5 contributions**, clearly categorized as either original or applied. These conclusions and contributions appropriately reflect the results obtained and their scientific significance.

4. AUTHOR’S ABSTRACT AND DISSEMINATION OF RESULTS

The presented author’s abstract comprises 71 printed pages and, in accordance with the requirements, provides a complete and accurate representation of the dissertation. In addition to the experimental work, obtained data, and main contributions, it includes information on the dissemination of results through scientific publications, participation in academic forums, and recorded citations.

The results of the research have been published in three peer-reviewed journals, indexed in established international scientific databases: *Chemico-Biological Interactions* (Q1, IF 5.4), *Biomolecules* (Q2, IF 4.6), and *Comptes rendus de l’Académie bulgare des Sciences* (Q3, IF 0.321). To date, these publications have received 17 citations. Part of the results has also been presented as reports at 2 national and 4 international scientific forums.

5. CRITICAL REMARKS AND QUESTIONS TO THE PHD CANDIDATE

I have no remarks regarding the substance of the work.

6. OVERALL ASSESSMENT OF THE DISSERTATION AND CONCLUSION

The presented dissertation is original in concept, well-planned, and executed using a diverse range of experimental techniques and approaches. The results obtained are sufficient in scope and of high quality, fully corresponding to the stated objectives and tasks, which has enabled the formulation of original scientific and applied contributions. Particularly noteworthy are the competent interpretation of the results, the clear and scientifically accurate language, and the linguistic proficiency of the PhD candidate.

All of this demonstrates that, in the course of developing the dissertation, PhD candidate Tihomira Tihomirova Stoyanova has developed into a precise, capable, and promising young scientist. The presented materials comply with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and its implementing regulations, as well as with the specific requirements of the corresponding regulations of the Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences.

For these reasons, **I am fully confident in giving a positive evaluation of the presented dissertation and recommend that the esteemed members of the Scientific Jury award the educational and scientific degree of “Doctor” in the professional field 4.3 “Biological Sciences,” scientific specialty “Biophysics” to PhD candidate Tihomira Tihomirova Stoyanova.**

February 26, 2026

Sofia

Opinion by: .


/Assoc. Prof. Dr. Krasimira Tasheva/