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STATEMENT

about the PhD thesis of PhD student **Inna Aleksandrovna Sulikovska** on the topic "**Optimization and adaptation of the method for determining phototoxicity *in vitro* 3T3 NRU phototoxicity test, to the LED - solar simulator Helios-iO**" for awarding of the educational and scientific degree "PhD" in Professional field 4.3. "Biological Sciences", Scientific Specialty "Biochemistry"

by **Prof. Dr. Romyana Mironova**, Institute of Molecular Biology "Academic Rumen Tsanev" at the BAS

With the growth of the pharmaceutical and cosmetic industries in recent decades, the number of photosensitizers used in medical and cosmetic preparations as active and/or accompanying ingredients increases. This, combined with the increasing intensity of sunbathing and excessive exposure to sunlight, leads to an increase in the incidence of skin reactions (redness, itching, swelling, etc.) as one of the most common manifestations of photosensitivity. These reactions occur both with cutaneous and systemic administration of the preparations and often lead to the need for hospital treatment of patients. To reduce the risk of such adverse reactions, it is necessary to test for phototoxicity all ingredients in the cosmetic products and medicinal preparations. This determines the relevance and significance of Inna Sulikovska's PhD thesis, which aims to investigate the possibility of using a diode simulator of sunlight to evaluate the photosafety of natural and synthetic products. The significance of the thesis is reinforced by the fact that the tested substances have potential antitumor activity.

The dissertation is written on 147 pages and includes the standard chapters as the results and the discussion are united in one chapter and ends with a concise summary. The illustrative material, 16 tables and 60 figures, is sufficient to convincingly document the presented results, and the literature reference of 322 citations testifies to an extensive and thorough acquaintance with the status and trends of research on the PhD thesis worldwide. This is even more apparent from the literature review, written in a good literary and scientific language. It addresses key research issues such as the properties of sunlight and its effect on living organisms, types of solar simulators, the phenomenon of phototoxicity and its practical application.

The aim and tasks (4 points) of the PhD thesis are precisely and clearly formulated. The specified objective is reduced to optimization and adaptation of the phototoxicity determination method "*in vitro* 3T3 NRU phototoxicity test" (recommended by the European (EMA) and

American (FDA) drug agencies) using diode solar Helios-iO simulator (LE-9ND55-H-5500K). The materials used are described in detail - parameters of the LED simulator, cell lines used (4 normal and 8 cancer), a large number of substances tested for phototoxicity – natural (4) and synthetic (8 the peptide and 6 thieno[2,3-d]pyrimidines). The applied methods (11 in number, including statistical ones) are fully adequate to the set goals and tasks and are described in sufficient detail to be able to be reproduced by an independent researcher.

Chapter ``results and discussion`` is the most voluminous one (45 pages), which makes a good impression and brings an appropriate balance to the content of the dissertation. The data obtained for the used diode light source makes it suitable for simulating natural sunlight in terms of spectrum, power and distributed power density when standard plates are used for cell cultivation. Prior to testing the target (natural and synthetic) substances for phototoxicity, appropriate model experiments were conducted. In them the applicability of the light source for research purposes was confirmed by the use of classical photosensitizers (Acridine orange, Radachlorin) and the application of three independent methods (*in vitro* phototoxicity test, light and fluorescence microscopy). The most significant result of the study is the convincingly demonstrated photosafety of the studied substances of natural and synthetic origin. Considering that these substances have a potential antitumor effect, a certain practical interest in them from the pharmaceutical and cosmetic industries can be expected, especially for three of the substances (the ethylacetate extract of *Cotinus coggygia* Scop. and two of thieno[2,3-d]pyrimidines) which showed high selectivity towards some tumor cell lines. I have no objection to the way the conclusions (5 points) and original contributions (3 points) of the study are formulated, as they very accurately and succinctly reflect the reported results.

The results of the study are reflected in two publications, one out of print in a journal (*Molecules*) referenced in Web of Science with a high JCR IF: 4.412 and quartile Q1, and one accepted for print in a journal (*International Journal of Bioautomation*) referenced Scopus with SJR: 0.198 and quartile Q3. Part of the research has been presented at three national and one international scientific meetings, and the doctoral student is the first author in the national participations. *As an insignificant critical remark, I note the fact that it is not clear whether the participations in the scientific forums are with posters or reports.* I also note the fact that the research was carried out with the financial support of the Scientific Research Fund and the Ministry of Education and Science under two projects, which means that the PhD student gained experience in teamwork and in the development of scientific research projects.

Conclusion

PhD student Inna Sulikovska presents us a thorough and complete study with scientific and scientific-applied value. Her research is interdisciplinary in nature, which has allowed her to master a variety of biophysical, biochemical and cell biological methods. The results obtained give her reason to formulate 3 original contributions, which I fully accept. The results of the PhD student's research have been published/accepted for publication in refereed international journals with IF and/or SJR, and in one of the articles she is the first author. With these scientometric indicators, the doctoral student fully meets the criteria of the BAS and the internal criteria of IEMPAM BAS for the acquisition of the educational and scientific degree "PhD". **All of this gives me reason to vote positively without hesitation for awarding the educational and scientific degree "PhD" to Inna Aleksandrovna Sulikovska in Professional direction 4.3. "Biological Sciences", Scientific Specialty "Biochemistry".**

July 8, 2022

Prepared by:



/ Prof. Dr. Romyana Mironova /