

on a dissertation for the acquisition of the educational and scientific degree "Doctor"

area of higher education 4. Natural sciences, mathematics and informatics,

professional field 4.3. Biological Sciences,

scientific specialty: "Application of the principles and methods of cybernetics in the field of structure-activity relationships of biologically active substances"

by **Prof. DSci Stefka Germanova Taneva**, Institute of Biophysics and Biomedical Engineering – BAS, member of the Scientific jury according to Order № 707 / 11.09.2023 of the Director of IBPhBME-BAS

Topic of the dissertation "In silico and in vitro studies of ADME/TOX properties and molecular interactions of flavonolignans from Silybum Marianum L. (MILK THISTLE)"

presented by **Antonia Georgieva Diukendjieva-Todorova**

Supervisors: Prof. Ivanka Tsakovska, PhD,
Corr. Member Prof. Ilza Pajeva, DSc

Description of the submitted documents

Antonia Georgieva Diukendjieva-Todorova has submitted a dissertation written in English, summaries of the PhD Thesis in Bulgarian and English, a list of publications on which the dissertation is based, a list of participations in national and international scientific forums, and all necessary documents according to the regulations of ADASRB – IBPhBME-BAS for acquiring scientific degrees and occupying academic positions at BAS.

Applicant data

Antonia Diukendjieva graduated as a Bachelor of Biotechnology at Sofia University "St. Kliment Ohridski" in 2012, and as a Master of Biochemistry, Molecular Biology after defending a thesis on the topic: "Peroxisome proliferator-activatable receptor- γ and its role on hepatic steatosis: mode of action and molecular modeling of ligand binding" in 2014. Since September 2014, she has been working on her doctoral thesis in the QSAR and Molecular Modeling Department, Institute of Biophysics and Biomedical Engineering, BAS, with scientific supervisors: Prof. Dr. Ivanka Tsakovska and corr. member Prof. DSci Ilza Pajeva.

Dissertation data

The dissertation presented by Antonia Diukendjieva is written in English and is 144 pages long, structured in the usual PhD thesis sections - Introduction, Aims and objectives, Materials and methods used in research contributions; Results and discussion; Conclusion; Contributions; 2 appendices, cited literature with 202 titles, list of publications and participation in scientific forums. The results of the conducted research are presented in 25 Figures and 16 Tables.

The dissertation aims were to evaluate the ADME/Tox properties of flavonolignans from *Silybum marianum*, the interactions of the flavonolignans with new target proteins and to elucidate the mechanisms of action associated with potential pharmacological effects.

Scientometric indicators 4 scientific papers have been published in refereed international journals (3 with an impact factor (overall IF 11.39) and 1 with SJR = 0.579; 1 of the publications is with quartile Q1, and 3 with Q2) and 2 full-text conference papers. The doctoral student has presented the results of her dissertation at 5 international and 3 national scientific forums.

It is noteworthy that Antonia Diukendjieva is the first author both in all publications and in all participations in scientific forums, which shows a significant personal contribution of the doctoral student.

Author's summary of the PhD Thesis

Two copies of the Author's summary of the PhD Thesis are presented – in English and in Bulgarian, designed according to the requirements for preparing a summary and correctly present the results and content of the dissertation.

2. Scientific and scientific-applied contributions of the dissertation work

The dissertation explored an interdisciplinary approach to evaluate the gastrointestinal absorption of the main compounds in silymarin - an active extract from the medicinal plant *Silybum marianum* (L.) Gaertn. A QSAR model for the evaluation of membrane permeation and an in vitro membrane permeation assay (PAMPA) were developed for the main components of the active extract of *Silybum marianum* and their derivatives.

The research data suggest that the majority of flavonolignans under study can pass through the gastrointestinal tract.

The mechanisms of antitumor activity of flavonolignans from *Silybum marianum* have been elucidated through a combined in silico and in vitro analysis. New antitumor target proteins were identified for flavonolignans from *Silybum marianum* based on the chemical similarity of

the studied compounds to the antitumor drugs vemurafenib and vismodegib. It was shown that the studied components of silymarin could be considered as dual inhibitors of BRAF kinase and Smoothed receptors (SMOs), which are major target proteins in modern anticancer therapies. Docking simulations show higher activity of dehydrosilybins towards BRAF kinase and the Hedgehog (HH) signaling pathway compared to silybins.

The cytotoxic properties of the compounds have been confirmed experimentally in tumor skin cell lines, and different activity has been demonstrated in different cancer cells.

The interdisciplinary approach implemented in the dissertation has the potential for preliminary assessment of gastrointestinal absorption of bioactive compounds, including natural products. The applied approach and the obtained data on flavonolignans have potential applications for the purpose of rational design of natural product derivatives with better characteristics for therapeutic application.

The model is freely available through the Database Service on Alternative Methods of the European Union Reference Laboratory for Alternatives to Animal Testing Alternatives.

3. Conclusion

In my opinion the quality of the presented dissertation work, the originality of the obtained results, reflected in 4 scientific publications, participations in scientific forums, and the prospects for future developments meet the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Application of ADASRB in BAS and the specific requirements of IBPhBME-BAS for acquisition by the candidate of the educational and scientific degree "doctor".

Based on this, I recommend to the members of the Scientific Jury to vote for the award of the educational and scientific degree "Doctor" to Antonia Georgieva Dyukendzhieva-Todorova in area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty: "Application of the principles and methods of cybernetics in the field of structure-activity relationships of biologically active substances".

21.11.2023 r.

Sofia



/Prof. DSci Stefka Germanova Taneva/