REVIEW

prof. Alexander Borisov Zlatkov, DSci,
lecturer at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University - Sofia, designated as a member of the scientific jury on the basis of art. 4, paras 1 and 2, LDASRB, Decision of the Faculty Council of the Faculty of Pharmacy at the Medical University - Varna and order No. R-109-114/09.02.2023 of the Rector of the Medical University - Varna

Abot: dissertation work for the acquisition of the ONS "Doctor" in the Higher Education Region 7. Health care and sports, professional direction: 7.3. Pharmacy and Doctoral Program: Pharmaceutical Chemistry

Topic: "Synthesis, characterization and toxicity evaluation of bexarotene esters"

Author: master of pharmacy Ivelin Rosenov Iliev, full-time PhD student in the "Pharmaceutical Chemistry" doctoral program, enrolled by order No. R-109-53/31.01.2020 at the Department of Pharmaceutical Chemistry at the Faculty of Pharmacy, Medical University - Varna.

Scientific supervisors: assoc.prof. Svetlana Georgieva, Ph.D.
assoc.prof. Yana Koleva, Ph.D.

I. General presentation of the procedure and the PhD student

The presented set of materials on paper and electronic media is in accordance with the requirements of the MU - Varna and includes the following documents:

1. Application to the Rector for the disclosure of a defense procedure;
2. Dissertation work
3. Abstract of a dissertation work
4. Autobiography signed by the doctoral student;
5. Copy of a diploma for a completed higher education educational-qualification degree EQD "Master" with its annex;
6. Enrollment order;
7. Minutes of an examination for the doctoral minimum;
8. Minutes from the SC with a positive decision on the readiness for protection;
9. Deduction order with right of defense;

10. Declaration of originality;
11. List of publications related to the topic of the dissertation with the doctoral student's signature;
12. Copy of the publications related to the topic of the dissertation work;
13. Declaration of authenticity of the presented documents;
14. Declaration for registration of profiles in scientific databases.

The PhD student has attached 4 (four) scientific publications, all related to the topic of the developed dissertation work.

I have no notes or comments on the documents.

Ivelin Rosenov Iliev, was born on 11.11.1994. He completed his higher education in the specialty "pharmacy" with a Master's degree in 2019 at the Faculty of Pharmacy at the Medical University - Varna. By Order No. R-109-53/31.01.2020, he was enrolled as a full-time doctoral student in the field of Higher Education "7. Health care and sports", professional direction: "7.3. Pharmacy", doctoral program: "Pharmaceutical Chemistry" with scientific supervisors assoc. prof. Svetlana Georgieva, Ph.D. and assoc. prof. Yana Koleva, Ph.D. to the Department of Pharmaceutical Chemistry of the Faculty of Pharmacy at the Medical University of Varna - Varna. By Order No. R-109-114/09.02.2023, he was dismissed with the right of defense for up to one year, starting from 09.02.2023.

II. Brief description of the structure of the dissertation

The presented dissertation is written on 171 pages, of which 2 pages are introduction, 64 pages literature review, 1 page goals and objectives, 15 pages experimental part, 59 pages results and discussion, 1 page conclusions, 1 page contributions, 14 pages literature. The work includes 37 tables and 63 figures, as well as 1 appendix with 5 figures, as well as 1 page of abbreviations used.

III. Relevance and dissertationability of the development

Retinoids are a class of chemical compounds derived from vitamin A used to treat a limited number of diseases, including skin conditions such as acne and psoriasis, and to treat certain types of cancer. Despite good therapeutic effects, their application is limited due to their toxicological profile. The reduction in adverse drug reactions with synthetic retinoids has led to an increase in their use as therapeutic agents and also to a better understanding of their mechanism of action.

On the other hand, one of the approaches to optimize the drug profile of drugs is the application of the so-called "pre-drug design". It aims to improve the

physico-chemical, pharmacokinetic, pharmacological, toxicological and even organoleptic properties of the molecule. Abstracting from all the difficulties researchers face in prodrug design, developing prodrugs is a faster and largely cost-effective strategy than searching for an entirely new therapeutically active agent with suitable ADMET properties. One of the main problems in this direction is the prediction of bioconversion levels and pharmacological and/or toxicological effects of prodrugs.

The present work is aimed at synthesis, characterization of synthetic retinoids from the group of ester derivatives of bexarotene, as well as studies on prediction of metabolic activity and analysis of manifested *in vivo* general toxic effects of the studied compounds. In this sense, the peer-reviewed dissertation is up-to-date with the possibility of deriving guidelines for optimizing the search for new synthetic retinoids.

IV. Critical analysis of the dissertation

The literature review (64 pages in total) is based on 213 literary sources, mainly in Latin. Of the cited literary sources, 39 are from the last ten years, and 21 - from the last five. The literature review is comprehensive, the information in it illuminates the essence of the problems and challenges discussed in the dissertation work in obtaining and researching new bexarotene derivatives and shows the good awareness of the doctoral student on the problem being developed. The review is written concisely and with understanding, but at the same time it is comprehensive and reflects in full the chemistry and pharmacology of bexarotene, as well as the possibilities of obtaining new derivatives thereof. A number of issues related to the structural characterization of retinoids, as well as some approaches for the *in silico* prediction of biological effect and theoretical assessment of the toxicity of bexarotene and derivatives, are highlighted.

The objective of the dissertation - to obtain, structurally characterize and study a group of new, not described in the literature, Bexarotene ester derivatives and to prepare a toxicological profile of the retinoid analogs, is suggested by the literature review. I believe that it would have been significantly better grounded if the literature review ended with a summary, but with clearly formulated conclusions that naturally lead to the goal. For its implementation, 6 specific **tasks** have been identified, formulated precisely and in a logical sequence.

Research methodology

In the **Experimental part** section, the PhD student has presented a detailed description of the methods used in the reviewed scientific work. The manner of their representation shows that the dissertation work was developed through appropriately and correctly selected methods, allowing the achievement of the set goal and obtaining an adequate answer to the tasks solved in the dissertation work.

Chromatographic methods are applied for analytical control of the active substances in the synthetic process. The structures of the newly obtained compounds were proved by instrumental methods of analysis.

The methodology does not give rise to doubt and is a prerequisite for obtaining the correct results discussed below.

Characterization and assessment of own research and contributions

In the "**Results and Discussion**" section, the PhD student describes in detail the obtained experimental results and, in parallel, presents their critical discussion, clearly showing the systematic approach to the implementation of the research.

In fulfillment of the main objective of the dissertation - synthesis of ester derivatives of bexarotene, proving the structure of the obtained compounds, the applicant obtained the target compounds by using acid chloride of the starting bexarotene, obtained by reaction with oxalyl chloride, and alcohols selected so that after possible hydrolysis of the product in the body to release non-toxic products.

The structures of the new compounds were confirmed by instrumental methods of analysis. The interpretation of the spectral data is adequate. It is worth noting the detailed interpretation of the data from the ATR - FTIR spectral analysis, which shows the good knowledge of the PhD student in the field. The detailed presentation of the results of the spectral studies shows that the doctoral student has mastered the use of instrumental methods for the characterization of new compounds not described in the literature. Regarding this part of the thesis, I have one remark - the recording of the melting temperature of a compound cannot be taken as structural characterization.

A modified and validated UV-VIS spectrophotometric method for the quantitative determination of Bexarotene is presented within the framework of the dissertation. The obtained results show that the method is fast and simple. It is suitable for routine analyzes in daily laboratory practice. It is characterized by good linearity and high precision. Sample preparation and analysis time are relatively short, the cost of the method is relatively low. However, the author found that the newly developed and validated method for the quantification of bexarotene was not suitable for the determination of mixtures of bexarotene and its esters.

Due attention is paid to the influence of some key factors on the chromatographic behavior of the studied compounds in order to select conditions for their chromatographic separation from the starting bexarotene, as their eventual hydrolysis product. The dissertation student applies the results of this study to the adaptation of a literature HPLC method. The study would have had much more weight if the method had been validated.

In quite detail and with understanding, the dissertation student describes the conducted research related to predicting the potential metabolic activity of Bexarotene, newly synthesized esters of Bexarotene and their metabolites using the QSAR Toolbox.

In the course of the study, metabolic models were used for the potential metabolic activity of the Bexarotene structure and its newly obtained esters. Application of such models allows identification of metabolites, characterization of physicochemical properties, and determination of properties affecting the biological activity of the parent compound and its derivatives. For this purpose, a mathematical model was applied to determine the potential metabolic activity of the studied compounds, which enables the identification and determination of the physicochemical properties of the newly obtained compounds, as well as some properties affecting the biological activity of the molecules. The results obtained by the dissertation indicate that the studied esters have a safety profile identical to that of Bexarotene, as they do not bind to DNA and proteins and therefore do not cause mutagenicity and genotoxicity. *In silico* studies performed by simulating liver and skin metabolism showed that the metabolites of the esters partially overlapped with those of bexarotene, with the E4 ester showing the highest metabolic activity. The same result was observed when simulating the so-called S9 metabolism.

Determination of pharmacokinetic parameters and prediction of possible biological activity using Molinspiration Cheminformatics software indicated that the studied compounds obeyed Lipinski's rule (1 violation) and could be expected to have oral activity, most likely acting as nuclear receptor ligands. Taking into account the latter results, as well as those from the prediction of metabolic activity, it is necessary to conclude that the studied derivatives could not be classified as bexarotene prodrugs.

In addition to these studies, the doctoral student predicted the ADME parameters and toxicity of the studied derivatives using the web-based server PredADMET. The results indicate possible high intestinal absorption and a satisfactory distribution profile accompanied by relatively low renal excretion.

As a finale of the research, an *in vivo* experiment was conducted to determine the general toxic effects of one of the studied esters - the one with ethanol. Biochemical liver markers (ALT, AST, PSA) were studied, and the observed changes in values indicate a low risk of hepatotoxicity with a single administration of the retinoid and newly synthesized ester, which in turn confirms the predictions.

The **conclusions** (7 in number) are adequate and correctly reflect the results of the conducted research. The conclusions are one more than the set tasks, moreover, they do not mention anything about the developed and validated UV-vis spectrophotometric method. On the other hand, a separate conclusion (#4) emphasizes the developed HPLC method for determining Bexarotene and its derivatives alone and in mixtures, which, however, has not been validated.

V. Assessment of the PhD student's publications and personal contributions

In relation to the dissertation work, a total of four articles were published, in two of which Iliev was the first/last author. At the time of writing this review, no citations

have been identified. The results of the dissertation were reported in two scientific forums.

With regard to these scientometric indicators, the dissertation student meets the requirements for awarding the educational and scientific degree "Doctor", laid down in the Regulations for the development of the academic staff at the MU - Varna.

VI. Abstract

The abstract (total volume 108 pages) is made according to the requirements and accurately and sufficiently reflects the content of the dissertation work.

Recommendations, questions and comments:

The dissertarion is written in good scientific language, there are almost no typographical and grammatical errors in the text.

CONCLUSION

In general, the dissertation concerns a current topic from a theoretical point of view. The set objectives and tasks were successfully completed, and the doctoral student mastered and used a number of modern synthetic and analytical methods.

The dissertation mainly contains theoretical and applied results, which represent an original contribution regarding the preparation and research of synthetic retinoids and their derivatives and meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Rules for the Development of the Academic Staff in MU-Varna.

The dissertation shows that the master pharmacist Ivelin Rosenov Iliev possesses the necessary theoretical knowledge and professional skills and demonstrates qualities and skills for independent conduct of scientific research.

Given the above, I give my ***positive assessment*** of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I ***propose to the honorable scientific jury to award the educational and scientific degree "doctor"*** to mag.-pharm. Ivelin Rosenov Iliev in a PhD program in Pharmaceutical Chemistry.

Sofia.

31 March 2023

Reviewer:

(prof. A. Zlatkov, DSci)