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Review

on the doctoral dissertation of **Kamila Buzun "Molecular mechanism of anticancer activity of novel 4-thiazolidinone derivatives"**

(Dissertation is based on a series of scientific publications)

Special field: Medical and health sciences in pharmaceutical sciences

Institution: Faculty of Pharmacy, Division of Laboratory Medicine, Medical University of Bialystok

Supervisors:

Prof. Anna Bielawska, Medical University of Bialystok, Poland

Prof. Roman Lesyk, Danylo Halytsky Lviv National Medical University, Ukraine

This is to recommend doctoral dissertation of MSc Kamila Buzun for approval by Pharmaceutical Sciences Discipline Council at Medical University of Bialystok.

MSc Kamila Buzun obtained 13 novel 4-thiazolidinone derivatives using the organic synthesis. She examined their anticancer properties and selected a derivative, Les-3331, which demonstrated the highest anticancer activity in vitro. The thiazolidinone core was confirmed as a molecular fragment with most promising biological activity, in particular, the anticancer one. It was demonstrated that apoptosis is one of the main mechanisms responsible for the antiproliferative effects of the thiazolidinones. Since detailed mechanisms of anticancer action of thiazolidinone derivatives are poorly studied, doctoral dissertation of MSc Kamila Buzun is a novel and actual investigation in the field of pharmaceutical sciences.

MSc Kamila Buzun successfully designed a new group of Ciminalum-thiazolidinone hybrid molecules with selective and pronounced effect towards cells

of human gastric cancer (AGS), colon cancer (DLD-1) and breast cancer (MCF-7 and MDA-MB-231 lines). A lead compound Les-3331 was selected among studied heterocyclic compounds. It was established that Les-3331 induced both intrinsic and extrinsic pathways of apoptosis. In molecular scales, it caused a decrease in concentration of LC3A, LC3B, and Beclin-1 and a reduction in concentration of Topoisomerase II in treated human breast cancer cell lines. A significant inhibitory effect of Les-3331 towards Topoisomerase II was found with using two different methods – ELISA measurement (Fig. 11) and FACS analysis (Fig. 12). A possibility of direct interaction of the molecule of Topoisomerase II with the Les-3331 molecule was confirmed with the help of the Molecular Docking Simulation (Fig. 13).

There are not so many anticancer medicines which realize their antineoplastic action through induction of autophagy. Thus, the author of dissertation should take a principal decision if she would like to continue studying this process of the programmed cell death under the action of the applied 4-thiazolidinone derivatives.

The most probable metabolic pathways for Les-3331 targeting were found in the in vitro model.

Chemical structure of new synthesized compounds was confirmed using modern methods of physico-chemical and physical studies, namely, 1H and 13C NMR, LC-MS, and X-ray analyses.

Thus, the obtained experimental data showed that Les-3331 is a promising compound that represents multi-targeted potential in chemotherapy of breast cancer.

Summarizing, MSc Kamila Buzun demonstrated in her dissertation high scientific level that resulted in four original papers and three review papers published in journals with the total Impact Factor of 33.688. It should be stressed that in all four articles presented in the dissertation, MSc Kamila Buzun is the 1st co-author that indicates her important role in the accomplishment of dissertation work.

Major and minor (editorial) remarks:

The aim of the work was to synthesize novel 4-thiazolidinone derivatives. However, the "synthetic" part in the "Introduction" section is described only briefly (1 page) without chemical details. Most attention in the Introduction section is paid to biological problems and targets: 2.2. Autophagy, 2.3. Apoptosis, and 2.4. Topoisomerases (altogether, 6 pages). Most "chemical data" were put in the Supplementary materials.

The Aim of the work is presented in a too broad form. Instead of that, the main Research tasks should have been formulated for explanation of the structure and logistics of the work.

Conclusion 1 should have more distinct "chemical character", like the design and synthesis of .....

Conclusion 2 "…. the correlation between the anticancer activity of the synthesized compounds and the nature of substituents at the N3 position of the thiazolidinone's ring" should be more related to the biological activities.

In the results presented in manuscript in Molecules, 2021 (Vol. 26, No. 10, 15 pp., Article ID: 3057), "positive control" (ex. doxorubicin) is absent in Tables 2, 3, 4.

In Fig. 5 of this article (Molecules, 2021), compounds 2i, 2f, 2j at 1 and 10 uM doses were more toxic towards activated normal human lymphocytes, comparing to compound 2h that was the most toxic for human tumor cell lines. Why?

In the Conclusions, it is stated about low toxicity of studied compounds towards normal lymphocytes, while one can see more than 50% inhibition of survival of these cells under their treatment with the studied compounds.

Two lines of breast cancer cells – MCF-7 and MDA-MB-231 - were treated with studied compounds. Why these cell lines were selected? Did the author observe any difference in their response to the action of these compounds? If yes, which and why?

On page 122 of the Dissertation, the issue about early and late apoptosis is discussed. However, neither in the Results section of the corresponding article (page 6 of the article), nor in the Materials and Methods section (page 18 of the article, Item 4.7 of that section), no distinct criteria are provided for discrimination between early and late apoptotic cells (Fig. 4). To do such discrimination with FACS analysis, measurements of Annexin V+ and Annexin V^- cells should be conducted in parallel with measurements of Propidium Iodide+ and Propidium $Iodide^-$ cells. According to such analysis, only Annexin V+ / Propidium $Iodide^-$ cells are considered as early apoptotic, while other combinations of these biomarkers suggest late apoptosis and/or necrosis.

There are no time dependence experiments for apoptosis induction in human breast cancer cells – only one time point (24 h) was used in the study.

Same remark concerns measuring mitochondrial membrane potential. At apoptosis induction with specific alkaloids, we observed a release of cytochrome C from mitochondria as early as in 2 min (Kaminskyy V. et al. Toxicology Letters, 2008, Vol. 177, N 3. P. 168-181). Measuring mitochondrial membrane potential in 24 h after the start of cell treatment with Les-3331 is too late for determination of early apoptosis (see Fig. 4 and 5 in the corresponding article).

ELISA measurement of caspases applied in the dissertation (Caspase-9 (Fig. 6) and Caspase-8 (Fig. 7) revealed their relatively high level in control (untreated) cells and very small increase of that level in a positive control (Etoposide). Probably, here, the traditional Western-blot analysis would be better method.

Summarizing, the dissertation of Kamila Buzun "Molecular mechanism of anticancer activity of novel 4-thiazolidinone derivatives" is of high scientific value

since it is addressed to an actual goal of the design and synthesis of novel anticancer compounds, as well as substantiation of their application in cancer chemotherapy. To reach these research goals, the author used modern methods, conducted the appropriate experiments, and provided perfect discussion of the existing problems

Thus, my estimation mark of the dissertation work of Kamila Buzun is "**positive with recognition**".

The dissertation work of MSc Kamila Buzun was prepared in the accordance with the proceedings for the award of a doctoral degree commenced after 01.10.2019 with the following legal basis: article 187 of the Act of 20 July 2018 and the law on Higher Education and Science (Journal of Laws of 2022, item 574).

With respect, **Rostyslav Stoika**, Doctor of Biol. Sciences, Professor, Foreign Member of Polish Academy of Science and Arts (Academija Umetnosti), Corresponding Member in Biochemistry, National Academy of Sciences of Ukraine

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Signature of prof. Rostyslav Stoika is certified by Dr. K. Dmytruk,

Deputy Director of the Institute of Cell Biology, NAS of Ukraine

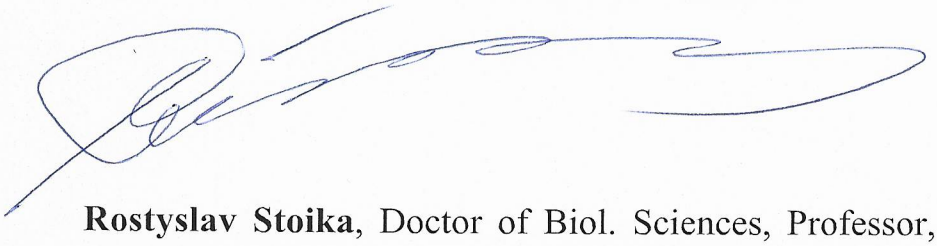
Application

on the recognition of the doctoral dissertation of M.Sc. Kamila Buzun "Molecular mechanism of anticancer activity of novel 4-thiazolidinone derivatives"

I would like to recommend doctoral dissertation of MSc Kamila Buzun for distinction by the Pharmaceutical Sciences Discipline Council of the Medical University of Bialystok.

In her work, she focused at the thiazolidinone core that is known as a molecular fragment providing a variety of biological activities, among which anticancer activity is one of the best studied. She conducted investigations which demonstrated that apoptosis is one of the main mechanisms related of antiproliferative effects of the thiazolidinones. Since detailed mechanisms of anticancer action of thiazolidinone derivatives were poorly studied, MSc Kamila Buzun successfully designed a new group of *Ciminalum*-thiazolidinone hybrid molecules with selective and pronounced effect towards cells of human gastric cancer (AGS), colon cancer (DLD-1) and breast cancer (MCF-7 and MDA-MB-231). Among the studied heterocyclic compounds, a lead compound Les-3331 was selected. MSc Kamila Buzun established that Les-3331 induced the intrinsic and extrinsic apoptotic pathway, caused a decrease in LC3A, LC3B, and Beclin-1 concentration and a reduction of Top II concentration in tested human breast cancer cell lines. Furthermore, the most probable metabolic pathways for Les-3331 were found in the *in vitro* model. The obtained experimental data proved that Les-3331 is a promising compound, representing multi-targeted potential in breast cancer therapy.

Summarizing, doctoral dissertation of MSc Kamila Buzun demonstrated high scientific level and has resulted in four original papers and three review papers published in journals with the total Impact Factor of 33.688. Thus, with full conviction, I recommend doctoral dissertation of MSc Kamila Buzun **for recognition**.



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