OPINION

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on the dissertation of Prof. Dr. Iva Ugrinova Zlatkova

"A Common Target, Different Agents: Fundamental Vulnerabilities of the Tumour Cell as a Basis for Selective Antitumour Activity"

for the award of the scientific degree of Doctor of Sciences
in professional field 4.3. Biological Sciences,
scientific specialty Molecular Biology

1. General overview of the procedure and the candidate

The dissertation submitted for assessment was prepared at the Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences. I have reviewed the dissertation, the dissertation summary, the included scientific publications, the curriculum vitae and the accompanying reports on scientific activity and compliance with the criteria. I hold the scientific content of the dissertation in high regard and set out the grounds for this assessment below.

Prof. Iva Ugrinova graduated in Biotechnology from Sofia University "St. Kliment Ohridski" and in 2002 obtained the educational and scientific degree of Doctor (PhD) with a study on the post-synthetic acetylation and DNA-binding properties of HMGB1. She has held the academic position of Professor since 2019. Her scientific career includes extensive work on chromatin structure and function, research training abroad, and leadership of a research unit and research projects. This experience explains the consistent focus of the studies presented here on the molecular mechanisms of the cellular response to antitumour agents.

The present dissertation addresses a subject distinct from that of the dissertation for the PhD degree. In the submitted lists, the publications for the two degrees are presented separately and do not overlap bibliographically. The research submitted for the award of the scientific degree of Doctor of Sciences encompasses new synthetic compounds, natural products and delivery systems, brought together through an analysis of tumour selectivity and mechanisms of cellular stress.

2. Relevance of the research topic

The challenge of selectively damaging tumour cells remains central to the development of antitumour agents. High cytotoxicity alone is insufficient unless accompanied by a distinction between effects on tumour and non-tumour cells and an understanding of the processes that determine their sensitivity. In this respect, I consider the choice of topic well justified: to investigate structurally different agents through shared functional characteristics of the cellular response.

The scientific relevance derives from the relationship between redox homeostasis, replication stress, the DNA damage response and mechanisms of cell survival. The applied relevance lies in the potential of these relationships to support the selection of lead compounds and the optimisation of their delivery. The dissertation addresses both biological activity and some of the limitations arising from the physicochemical properties of the agents studied.

3. Knowledge of the research problem

The literature review provides the necessary foundation for defining the experimental objectives. It examines cell cycle regulation, p53 and NF-κB signalling, apoptosis, autophagy and the DNA damage response. These mechanisms are linked to the characteristics of the chemical classes and natural products under investigation, as well as to the potential use of nanocarriers.

I view favourably the discussion of the results of the individual publications within a common biological context. Prof. Ugrinova demonstrates an in-depth understanding of the research problem and an ability to compare diverse experimental observations. The literature analysis is linked to the interpretation of her own results and supports the formulation of the scientific generalisations.

4. Research methodology

The methodological approach is appropriate for the objectives and combines the assessment of viability and proliferation with analyses of the cell cycle, cell death and molecular markers of the stress response. Of particular significance are the comparison of tumour and non-tumour cell models and the investigation of the lung carcinoma cell lines A549 and H1299, which differ in TP53 status. The calculation of selectivity indices allows activity to be assessed relative to the corresponding non-tumour model.

Combining metabolic assays with clonogenic analysis extends the assessment beyond the short-term suppression of cell viability. Analyses of EdU incorporation, γH2AX, cell cycle phase distribution and apoptotic markers allow the relationship between inhibited DNA synthesis, the accumulation of damage and the subsequent cellular response to be traced. Annexin V/PI assays and measurements of caspase activity contribute to a more robust characterisation of cell death.

For the natural products, the biological studies are complemented by fractionation and chemical characterisation, while the delivery systems are also examined by physicochemical analyses. This combination allows the observed effects to be considered in relation to the composition or formulation of the material tested. The methodological breadth and the combination of complementary approaches are substantial strengths of the dissertation.

5. Characteristics and assessment of the dissertation and its contributions

The dissertation brings together three interrelated areas: the investigation of synthetic antitumour agents, the biological characterisation of natural products and the development of delivery systems for active substances. The overarching scientific problem is to elucidate the relationship between antitumour activity, selectivity and the cellular processes that determine sensitivity to these treatments. The publications are connected through a literature review and a general discussion that trace the development of this line of research.

The studies of ferrocene-containing camphorsulfonamides investigate how structural differences between compounds affect activity, selectivity and the nature of cell death. The comparison between DK-164 and CC-78 reveals different cellular response profiles. DK-164 stands out for its selective activity in the models studied, accompanied by oxidative stress, changes in p53 and NF-κB signalling and predominantly apoptotic cell death. CC-78 exhibits a different selectivity profile and predominantly necrotic features. These results support the selection of DK-164 as a lead compound for further development.

For the heterocycle-annulated naphthalimides, the central issue is the relationship between the inhibition of DNA synthesis, disturbances in genomic stability and selective antitumour activity. The benzodioxin and benzofuran derivatives exhibit activity in the low nanomolar range. For the lead benzofuran derivative, the reported IC50 values are 61 nM in A549 and 11 nM in H1299, compared with 352 nM in MRC-5, with a selectivity index of up to 32 in the corresponding comparison. Reduced EdU incorporation, an increased γH2AX signal, cell cycle changes and apoptotic markers link this activity to replication stress and the DNA damage response. Differences in the cellular response between models with different TP53 status, together with retained activity in p53-deficient cells, have been established.

The studies of *Rapana venosa* consider marine bioresources as a source of components with selective antitumour activity and the potential to enhance the effects of established antitumour drugs. Haemolymph fractions active in breast cancer cell models have been characterised, and potentiation of the effect of cisplatin has been demonstrated. For the active HRv 50–100 kDa fraction, evidence has been obtained for oxidative stress, p53 activation and changes in the autophagic response. The work combines biological testing with characterisation of the composition of the fractions studied.

For the plant and fungal products, the question addressed is how the composition of the material tested and the characteristics of the cell model determine activity and the nature of the cellular response. The cannabidiol studies show differences in the manifestations of cell death in A549 and H1299. Extracts from Bulgarian mushrooms, including *Amanita muscaria*, exhibit cell model-dependent antiproliferative effects. The separate study of *Amanita muscaria* combines cytotoxicity assessment with analytical determination of ibotenic acid, muscimol and ergosterol content, complementing the biological observations with specific data on extract composition. For the ethanol extract of *Croton lechleri*, evidence is presented for selective cytotoxicity and an apoptotic response in the A375 melanoma model relative to the non-tumour HaCaT control used. The comparison of conventional and modern extraction approaches for *Croton lechleri*, complemented by chemical characterisation, allows biological activity to be considered in relation to the preparation methods and composition of the extracts. Natural products thus extend the comparative investigation to agents of different origins and compositions.

The research on delivery systems addresses the physicochemical limitations of biologically active substances. The low aqueous solubility of DK-164 has been improved through incorporation into polymeric micelles while retaining its biological activity. pH-sensitive niosomes have also been developed and characterised for the delivery and controlled release of active substances. The niosomal formulation of curcumin has shown improved cytotoxic and proapoptotic activity compared with the free substance in the tumour cell models studied. These results link the selection of promising compounds to the optimisation of their formulations and their subsequent preclinical development.

Taken together, these research areas form a coherent approach: selecting and characterising active agents, comparing their effects on tumour and non-tumour cell models, examining the processes associated with sensitivity and cell death, and optimising delivery. Their added scientific value lies in linking activity to the biological context in which it is expressed. This enables the results of the individual studies to inform the selection of candidates and the directions for their development.

In the general discussion, Prof. Ugrinova relates the effects of the agents studied to three interacting groups of processes: redox homeostasis, maintenance of genomic stability, and

regulation of cell survival and death. The experimental results support functional commonalities among some of the cellular responses to structurally different agents. The comparative data highlight the importance of cellular context for selectivity and the manner in which the antitumour effect is exerted. The new findings on the involvement of p53, together with retained activity in p53-deficient models, contribute to the understanding of the response to the specific compounds studied. The nanoformulation results demonstrate how improved physicochemical characteristics can be combined with the preservation of biological activity.

In assessing the contributions, the principal emphasis may be placed on the scientific synthesis supported by the comparative experimental studies. In this respect, I consider the following generalisations to be substantial contributions of the dissertation.

The results for synthetic and natural agents are integrated into a scientific framework linking antitumour activity and selectivity to changes in redox homeostasis, genomic stability and the regulation of cell death. This synthesis gives coherence to the studies and provides a basis for the comparative assessment of promising antitumour candidates.

The characterisation of ferrocene-containing camphorsulfonamides establishes relationships between structural differences, selectivity profiles and cellular responses, supporting the selection of DK-164 for further development.

The investigation of benzodioxin and benzofuran naphthalimides establishes a combination of high activity and selectivity in the models used and links their effects to the inhibition of DNA synthesis, DNA damage markers, cell cycle changes and apoptosis.

The comparative studies provide new data on the involvement of p53 in the response to the specific agents and on differences between cell models with different TP53 status. Retained activity in p53-deficient cell lines broadens the experimental basis for investigating sensitivity to the lead compounds.

Comparative data have been obtained on cellular responses associated with changes in autophagy following exposure to ferrocene derivatives, the micellar formulation of DK-164, the benzofuran naphthalimide derivative 5d and a haemolymph fraction from *Rapana venosa*. The analysis of LC3-positive structures, complemented for 5d by Western blot analysis of LC3-I/LC3-II, is compared with measures of viability and cell death, including the apoptotic response. The differences observed in relation to the cell model and agent concentration extend the characterisation of changes in autophagy in the context of cellular stress and mechanisms of cell death.

The characterisation of haemolymph fractions from *Rapana venosa* establishes their antitumour activity and their potential to potentiate the effect of cisplatin in breast cancer cell models. The results support the development of these fractions as a source of biologically active components.

The studies of cannabidiol and plant and fungal extracts extend the evidence on how biological activity and cell death depend on the composition of the products tested and the characteristics of the cell model.

The development and characterisation of micellar and niosomal systems demonstrate opportunities to improve solubility and control the release of active substances. The preservation of the biological activity of micellar DK-164 links this contribution to applied research with the central theme of the studies.

6. Assessment of the publications and the candidate's personal contribution

The separate list includes 10 publications from 2021–2026, submitted as the basis of the dissertation. According to the scientometric report provided, eight are classified as Q1, one as Q3 and one as Q4. The reported totals are 227 points under indicator group Г and 87 citations, corresponding to 174 points under indicator group Д, of the criteria for awarding the scientific degree of Doctor of Sciences. These reported values exceed the thresholds of 100 points for each of these groups in the accompanying regulations. The final assessment of these totals should be based on harmonised lists and verified allocation of the publications and citations to the relevant indicators.

The publications have been disseminated and accepted by the specialist scientific community, and the accompanying list also includes 20 conference contributions presenting results on the topic. The candidate's personal contribution is clearly documented by the information in the publications, which shows that Prof. Ugrinova participated in defining the concepts and methodology, providing scientific supervision, interpreting the results and preparing the manuscripts, as well as in project administration and funding acquisition for a substantial proportion of the projects. This information supports her substantial personal contribution and leading role in the development of the research.

7. Dissertation summary

The dissertation summary presents the aim and objectives, the main results, the synthesis, conclusions and contributions of the dissertation. It allows the logic of the research and the relationships between its different areas to be followed. I consider that it reflects the essential scientific content of the dissertation. The recommendation to harmonise the bibliographic information also applies to its final version.

Conclusion

The dissertation contains original experimental results and scientific generalisations addressing a significant problem in the molecular biology of the tumour cell. The decisive factor in my positive assessment is the integration of studies on different agents through the relationship between selectivity, cellular stress and the regulation of cell death. This synthesis is supported by the characterisation of promising antitumour candidates, the new data on the involvement of p53 in the response to these agents, and the results of delivery optimisation. Taken together, these contributions constitute a significant original scientific contribution and, in their scientific content, meet the requirement for theoretical generalisations and solutions to major scientific or applied research problems for the degree of Doctor of Sciences.

On the basis of the above, I give a positive assessment of the dissertation and recommend that the esteemed academic jury award Prof. Dr. Iva Ugrinova Zlatkova the scientific degree of Doctor of Sciences in professional field 4.3. Biological Sciences, scientific specialty Molecular Biology.

Date: 13 September 2026
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