REVIEW

of a dissertation submitted for the award of the scientific degree
„Doctor of Sciences“ in the professional field 4.3 Biological Sciences (Molecular Biology)

Dissertation title: „One target, different agents: fundamental vulnerabilities of the tumor cell as a basis for selective antitumor action“

Author: Prof. Iva Ugrinova Zlatkova, PhD

Reviewer: Acad. Prof. Rumen Georgiev Pankov, DSc, member of the Scientific Jury appointed by order of the Director of the Institute of Molecular Biology „Academician Roumen Tsanev“ – Bulgarian Academy of Sciences, No. 150-OB/13.07.2026

Brief biographical data on the candidate

Professor Iva Ugrinova, PhD, was born in 1968 in Sofia. She completed her higher education at Sofia University „St. Kliment Ohridski“, obtaining a bachelor's degree in Biotechnology and a master's specialization in Genetic and Cell Engineering in 1992. Her professional career has developed almost entirely at the Institute of Molecular Biology „Academician Roumen Tsanev“, where in 2002 she successfully defended her PhD thesis and subsequently held the positions of senior assistant, associate professor, and, since 2019, professor. Since 2013 she has headed the „Chromatin Structure and Function“ section; from 2014 to 2022 she was elected Director of the Institute of Molecular Biology, and since 2022 she has been Deputy Director of the same institute.

General information on the documentation related to the dissertation

The dissertation, the author's abstract, and the accompanying defense documents have been prepared in full compliance with the Bulgarian Law on the Development of the Academic Staff and its Implementing Regulations as adopted by the Institute of Molecular Biology. For the format of her dissertation, Prof. Ugrinova has used the form „a collection of scientific publications accompanied by a comprehensive literature review and a connecting discussion“, as indicated in point 2.2 of the Institute's Regulations for the Implementation of the Law. The submitted scientific output fully corresponds to the profile of the announced competition.

The dissertation comprises 281 pages and consists of an Introduction, a Literature Review, Aims and Objectives, Results – presented in the form of 10 full-text scientific articles, a General Discussion, a Conclusion, Scientific Contributions, Conclusions, Perspectives and Future Development of the Research, and a Cited Literature section comprising 158 sources.

Relevance of the dissertation topic

The dissertation of Prof. Ugrinova puts forward a clear unifying idea: antitumor agents of different natures act on the same fundamental vulnerabilities of the tumor cell. This is not merely a description of individual compounds; it is an attempt at generalization – all the agents studied, regardless of their origin, affect a similar range of processes: oxidative stress, DNA damage, and disturbances in cell survival.

This idea is close to a concept well established in contemporary oncology. Since the early 2000s, the field has adopted the notion that all types of cancer, for all their diversity, share a common, limited set of functional characteristics. In order to survive and proliferate uncontrollably, a tumor cell must “achieve” several things: secure a constant proliferative signal, circumvent the mechanisms that halt division, evade programmed cell death, secure a blood supply, and acquire the ability to spread through the body. Later, further hallmarks were added to this list – genomic instability, chronic inflammatory response, and altered energy

metabolism. Each of these characteristics represents a kind of “weak point” – a process on which the tumor cell has become dependent for survival, and precisely for that reason a convenient target for treatment. This framework has changed the way new drugs are sought: instead of looking for an action on a single gene or protein alone, the search increasingly targets the process on which the tumor cell has become dependent.
The topic of the dissertation fits exactly into this logic – the agents studied are chemically different, but their action converges on similar, well-known vulnerabilities of the tumor cell. The topic is also relevant because contemporary pharmacology increasingly seeks not a single, narrowly targeted action, but selectivity achieved through several affected processes simultaneously – a direction into which the research in the dissertation fits as well.

Structure and aims of the dissertation
The literature review, supported by 158 references, is balanced in volume and content and, though concise, provides a highly informative analysis of the existing data. It is built in four logically connected parts that gradually lead the reader from the general cell biology of the tumor response to the specific agents studied in the dissertation. The first part examines the cellular response to antitumor action – the role of p53 as a central tumor suppressor, apoptosis as the main mechanism of cell death, and NF-κB as a factor associated with both cell survival and therapeutic resistance. The second part goes deeper into genomic stability – the cell cycle and its checkpoints, the types of DNA damage and the mechanisms for their repair, as well as replication stress as a specific type of cellular vulnerability. The third, shorter part examines autophagy as an adaptive mechanism with a dual role – protective or leading to cell death, depending on context. The fourth part presents the agents studied themselves – ferrocene-containing compounds, annulated naphthalimide derivatives, bioactive substances from marine molluscs, natural products of plant and fungal origin, and polymeric nanosystems for delivery. The inclusion, within this part, of an „Own Contribution“ section outlining the main results of the research conducted makes a particularly good impression. This, together with the indication of the methods used to establish the status of key molecules – used as markers of the activity of the signaling pathway under study – gives a clear picture of the experimental approaches and outlines well the parameters of the studies performed. A good addition to the review is also the 18 original or adapted schematic figures, which are highly informative and visually well executed.
The aim of the dissertation is formulated clearly and has two clearly distinguishable components: on one hand, to show that structurally different synthetic and natural agents can act through a common set of fundamental vulnerabilities of the tumor cell; on the other, to develop approaches for improving their pharmaceutical applicability through polymeric nanosystems. This duality of the aim is logical given the structure of the work itself. The five formulated objectives (although the text lists them as six) provide a solid basis for achieving the stated aims.

Analysis of the experimental results, conclusions, and contributions
The presented scientific results comprise a coherent and thematically related group of studies aimed at the discovery, characterization, and mechanistic investigation of new biologically active compounds with potential antitumor action, as well as at developing approaches for their more effective delivery to tumor cells.
The experimental data obtained are presented and discussed in an order well chosen by the author, which I will also follow, summarizing the main scientific achievements.
The first group includes two publications devoted to the ferrocene-camphorsulfonamide derivatives DK-164 and CC-78, covering their synthesis, the study of their antitumor activity, the elucidation of their molecular mechanism of action, and the development of a suitable

nanoformulation for their application. The two articles form a clear two-stage line within a single research direction. The first article introduces and characterizes a new ferrocene camphorsulfonamide, designated CC-78, comparing it directly with the previously synthesized compound DK-164. The second article takes this further and solves a specific practical problem of DK-164, namely its low water solubility.

The genuine scientific contribution of the first article lies in the structure–function comparison between the two compounds, which share a similar skeleton. Changing only a single structural element – replacing the tert-butyl group of DK-164 with a piperazine group in CC-78 – leads to a set of biological consequences. The change results in increased overall cytotoxicity but also a complete loss of tumor selectivity (CC-78 proves more toxic to normal MRC5 cells than to the tumor cell lines). A shift in the predominant mechanism of cell death from apoptosis (for DK-164) to necrosis (for CC-78) was also established, and, most tellingly, a complete loss of the ability to induce nuclear translocation of NF-κB, while the ability to activate p53 remained unchanged for both compounds. It is precisely this combination of accompanying changes – rather than an isolated observation – that makes the comparison valuable and provides good support for the dissertation's claim that the loss of a specific molecular mechanism coincides with the loss of selectivity.

The second article makes a contribution of a different nature, related not so much to a biological mechanism as to solving a specific pharmaceutical-technological barrier. Encapsulation of DK-164 in micelles based on the triblock copolymer PEO-b-P(CyCL-co-CL)-b-PEO achieves a high encapsulation efficiency (about 98%), nanoparticles with a small hydrodynamic diameter, and, most importantly, delayed release of the active substance. The biological activity of the encapsulated form retains the characteristics of the free substance. Its selectivity toward tumor cells and its ability to activate NF-κB and p53 are preserved, albeit with a slightly attenuated effect, explainable by the slower release.

Methodologically, both articles employ a wide range of methods (cytotoxicity assays, flow cytometry, immunofluorescence for LC3/p53/NF-κB, ROS, and mitochondrial membrane potential), which makes the comparison between the free and encapsulated substance correct and directly comparable.

As a limitation of these results, I would note the absence of in vivo data, but this is entirely logical given the stage the research is currently at.

The second group is likewise represented by two articles, published during the current year, devoted to the study of new annulated naphthalimide derivatives with antitumor activity. In both works an approach is used in which structural modification of the naphthalimide core through annulation of a benzodioxin or benzofuran fragment, combined with changes in electronic properties and substituent position, is used to investigate the relationship between chemical structure, cellular selectivity, and biological effect. In this way, the studies go beyond simple cytotoxicity screening and form a complete structure–activity–mechanism framework.

A significant contribution of these studies is the identification of highly effective compounds with nanomolar cytotoxicity and good selectivity toward tumor cells. The structure–activity analysis shows that the type of the annulated heterocyclic fragment and the nature and position of the substituents, especially the nitro group, are decisive for the antitumor activity and selectivity. Particularly significant is also the mechanistic finding that the active compounds suppress DNA synthesis and induce replication stress, accompanied by γH2AX accumulation, cell-cycle disturbances, and subsequent caspase-dependent apoptosis. This mechanistic aspect is especially important, as it places the developed compounds within the context of contemporary strategies for selectively targeting the increased replication vulnerability of tumor cells. It is also worth highlighting the established dependence of the cellular response on the functional status of p53. The comparison between p53-competent and p53-deficient cell models shows that the antitumor effect is not exhausted by a classical p53-dependent

mechanism. The data support the simultaneous involvement of p53-dependent and p53-independent pathways, which has potential significance for the applicability of this class of compounds in tumors with impaired p53 function. In this context, the differences in cell-cycle behavior are particularly interesting, as they show that the cellular response to induced replication stress can be modified by the genetic context of the cell. The data from the second publication complement the mechanistic characterization by establishing an LC3-related cellular response, pointing to the involvement of autophagy-related processes as well.

In these studies, too, a complex of mutually complementary approaches was again applied, once more demonstrating high professionalism. Chemical synthesis and structural characterization of the compounds, quantitative determination of cell viability, clonogenic potential analysis, flow cytometry for studying the cell cycle and apoptosis, immunofluorescence tracking of DNA synthesis and damage, and analysis of key protein markers were all employed. It is precisely the combination of these independent methodological levels that allows a more reliable connection between the chemical structure, the observed cellular phenotype, and the proposed mechanism of action.

The scientific value of the two works is determined by the combination of original chemical design, structure–activity relationship analysis, and comprehensive cellular-mechanistic characterization. In this way, they contribute to the development of new naphthalimide derivatives targeting the increased vulnerability of tumor cells to replication stress. It should be noted that the primary molecular event triggering replication stress has not been established directly. Nevertheless, the totality of the results obtained provides a convincing basis for the proposed mechanistic sequence and qualifies the two publications as original and of substantial scientific contribution to the development of new strategies for pharmacological targeting of the replication vulnerability of tumor cells.

The last, third group, combining six publications, is a thematically related complex of studies aimed primarily at identifying and characterizing the antitumor potential of natural bioactive products of various origins – marine organisms, fungi, and plants – complemented by the development of pH-sensitive nanosystems for the delivery of antitumor agents. The common feature of these studies is the drive to discover poorly studied natural sources and to transform them from objects of primary cytotoxic screening into more thoroughly characterized potential sources of bioactive molecules.

Among the more significant contributions in this group is the identification of antitumor activity of a low-molecular-weight protein fraction from the hemolymph of the Black Sea snail Rapana venosa, including a pronounced synergistic effect with cisplatin. The identification, via MALDI-MS/MS, of specific protein components in this fraction extends the knowledge of the species' bioactive molecules beyond the well-studied hemocyanin. Another aspect of originality is the study of Bulgarian wild fungal species as a potential source of cytotoxic substances. The subsequent in-depth study of Amanita muscaria is particularly valuable in that, through the use of independent analytical approaches, it shows that the observed cytotoxic effect is not due to the main psychoactive components, ibotenic acid and muscimol, thereby directing the search toward other, as yet unidentified, bioactive constituents.

A significant contribution is also made by the study of Croton lechleri, in which a comprehensive approach was implemented, combining various extraction technologies, chemical characterization of the fractions obtained, and a multifaceted assessment of their biological activity. Particularly significant are the identification of previously unstudied derivatives and the established selectivity of certain extracts toward melanoma cells compared with normal keratinocytes. In this way, the work contributes both to the search for new natural antitumor agents and to the utilization of plant material that constitutes a byproduct.

The study of cannabidiol in lung tumor cell models complements this line of research with an analysis of the dependence of the cellular response on p53 status. The established difference

between predominant apoptosis in p53-positive cells and a more pronounced necrotic response in p53-deficient cells represents an interesting result, although the contribution of this work lies more in detailing already-known biological effects of cannabidiol than in discovering a fundamentally new mechanism.
A separate but logically complementary contribution to the thematic block is the development of pH-sensitive niosomal systems for the delivery of curcumin. The creation of new hexadecyl-poly(acrylic acid) copolymers for the modification of niosomal carriers represents an original approach in the field of drug delivery and extends the research program beyond the identification of natural bioactive substances toward the development of systems for their targeted delivery.
Methodologically, the six publications show a progression from initial cytotoxic screening toward an increasingly complex characterization of the biological effect. Alongside the assessment of cell viability, clonogenic assays, flow cytometry, Annexin V/PI staining, cell-cycle analysis, EdU labeling, immunofluorescence, and molecular-analytical methods were used. The use of independent methods to confirm key conclusions makes a particularly positive impression – for example, two different approaches for assessing drug synergy in Rapana venosa, and several independent analytical platforms for identifying and quantifying the components of Amanita muscaria. In the study of Croton lechleri, the combination of cell-cycle analysis with EdU and a wound-healing assay allows a more accurate distinction between the antiproliferative and purely migratory effects.
I consider that the overall value of the publications in this group lies not so much in establishing fundamentally new mechanisms of cell death as in the systematic application of contemporary cellular, analytical, and technological approaches to insufficiently studied natural resources, and in the gradual progression from phenotypic screening toward mechanistic and structural characterization. In this sense, the six articles form a coherent and scientifically productive complex that contributes to expanding the base of potential natural antitumor agents and to the development of approaches for their pharmaceutical application.
The presented „General Discussion“ can be described as a strength of the dissertation. It goes beyond a mechanical summary of the results from the individual publications and integrates them into a unified conceptual framework based on the common biological vulnerabilities of the tumor cell – disrupted redox homeostasis, dependence on the maintenance of genomic stability, and disrupted control over cell death. Particularly successful is the treatment of p53 as an integrator of various stress signals and the analysis of tumor selectivity as a common outcome of action on these vulnerabilities. The inclusion of polymeric nanosystems as a logical continuation of the research on the bioactive molecules, aimed at optimizing their potential application, is also convincing. The discussion demonstrates the ability to achieve a comprehensive scientific synthesis and to derive general patterns from results obtained with chemically and biologically different agents.
From the studies conducted, 15 general conclusions have been drawn, and four contributions of a fundamental nature and four of an applied nature have been formulated. The formulated scientific contributions and conclusions are logically connected to the stated aims and the experimental results obtained, and convincingly reflect the scientific value of the dissertation.
The author's abstract has been prepared in accordance with the requirements and reflects all the main results and contributions of the dissertation.

Compliance of the submitted documentation with the Law on the Development of the Academic Staff and with the scientometric indicators

The dissertation includes ten scientific articles published over a five-year period (2021–2026), with Prof. Ugrinova as corresponding author in 9 of them. Eight of the articles are published in Q1 (first-quartile) journals, and the total impact factor of the articles is 48.9. Although

published quite recently, the results of these studies have been very well received by the scientific community and have already been cited 87 times.
The reference on the fulfillment of the minimum national requirements under Article 2b of the Law on the Development of the Academic Staff for scientific field 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences, submitted by Prof. Ugrinova, includes the following indicators: group A – 50 points; group B – 100 points; for indicator D – 227 points against a required 100 points; and for indicator E (citations) – 174 points against a minimum of 100 points. Thus, against a required minimum of 350 points for the scientific degree „Doctor of Sciences“ under the Regulations for the Implementation of the Law, Prof. Ugrinova accumulates 551 points, thereby not only meeting but substantially exceeding the minimum national requirements for this scientific degree.

Conclusion:
The dissertation submitted by Prof. Ugrinova, entitled „One target, different agents: fundamental vulnerabilities of the tumor cell as a basis for selective antitumor action“, represents an in-depth monographic study of high scientific value that fully meets the criteria for the award of the scientific degree „Doctor of Sciences“. The work is the result of consistent and purposeful research and addresses key problems related to identifying the main vulnerabilities of tumor cells, discovering new antitumor agents, and developing approaches for their delivery. Within the framework of the study, a number of innovative investigations have been carried out, the results of which have received considerable recognition both within the Bulgarian and the international scientific community – a fact that establishes the author as a highly qualified specialist in the field of molecular biology.
On the basis of all of the above, I give a positive assessment and confidently recommend to the esteemed members of the Scientific Jury that they award Prof. Iva Ugrinova Zlatkova, PhD, the scientific degree „Doctor of Sciences“.

10.09.2026

Reviewer:
/Acad. Prof. Rumen Pankov, DSc/