International Institute of Molecular
and Cell Biology in Warsaw
4 Księcia Trojdena St., 02-109 Warsaw, Poland

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Dr. hab. Elżbieta Nowak
Preclinical Drug Development Facility
IN-MOL-CELL

**Review of the PhD Thesis of Kavya Kondaka entitled**
**"Yeast DNA Topoisomerase II as a model molecular target for novel antifungal compounds"**

The reviewed PhD thesis was prepared at the Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, under the supervision of Dr. hab. Iwona Gabriel.

The doctoral dissertation of Ms. Kavya Kondaka addresses a highly relevant and timely topic at the intersection of molecular biology, microbiology, medicinal chemistry, and drug discovery. The increasing incidence of invasive fungal infections, together with the growing prevalence of resistance to currently available antifungal agents and the limited number of validated molecular targets, creates an urgent need for novel therapeutic strategies. Within this context, the Candidate investigates DNA topoisomerase II (TopoII) as a potential molecular target for antifungal therapy and explores approaches for the identification and development of compounds acting through this mechanism. The Author translated this broad scientific objective into several complementary research goals encompassing literature analysis, target validation, structural characterization, inhibitor identification, and mechanistic investigations. Together, these objectives formed a coherent research strategy that effectively addressed the central hypothesis and enabled a comprehensive evaluation of fungal TopoII as a target for antifungal drug development.

The thesis is based on four thematically coherent scientific publications, comprising three original research articles and one review paper. Ms. Kondaka is the first author of two experimental studies published in *Scientific Reports* and the *European Journal of Medicinal Chemistry*, and the second author of a third experimental paper published in *Scientific Reports*. The publication portfolio is complemented by a comprehensive review article published in *Molecules*, for which she is the first author. Overall, this represents a strong and well-balanced body of work, demonstrating both scientific productivity and a substantial intellectual contribution to the field. It is worth emphasizing that all publications forming the basis of the dissertation appeared in well-recognized international journals and underwent rigorous peer review, providing confirmation of the originality, scientific quality, and relevance of the reported findings.

The review article *Targeting DNA Topoisomerase II in Antifungal Chemotherapy* should not be regarded merely as a literature survey. Rather, it establishes the conceptual framework for

the entire doctoral project by critically discussing the biological role of fungal TopoII, known mechanisms of enzyme inhibition, possibilities for drug repurposing, and structural determinants of selective targeting. Importantly, the review identifies key knowledge gaps and outlines research directions that subsequently became the focus of the experimental studies presented in the dissertation.

Especially valuable is the experimental validation of fungal DNA topoisomerase II as a pharmacologically relevant target. In the publication *Anticancer Drugs Targeting Topoisomerase II for Antifungal Treatment*, the Author demonstrated that several clinically used TopoII inhibitors, including daunorubicin, doxorubicin, bisantrene, epirubicin, amsacrine, idarubicin, mitoxantrone, and pixantrone, also inhibit fungal TopoII; however, antifungal activity was observed only for the last three compounds. Notably, idarubicin displayed activity against various fungal species, including fluconazole-resistant *Candida glabrata* isolates. Although these compounds are unsuitable as antifungal agents in their current form due to their anticipated toxicity toward human cells, the findings provide important proof-of-concept evidence supporting fungal DNA topoisomerase II as a therapeutically exploitable target.

Equally noteworthy are the molecular dynamics studies comparing human TopoII with enzymes derived from various fungal species. These results were described in the article entitled *Structural Insights into Fungal and Human Topoisomerase II with Implications for In Silico Antifungal Drug Design*, published in *Scientific Reports*. By identifying structurally divergent regions, including the HLR-HLR and K-loop-HLR interfaces, the Author established a rational foundation for the development of selective antifungal inhibitors. Importantly, these findings have practical implications for structure-based drug design and virtual screening approaches aimed at identifying compounds with increased selectivity toward fungal TopoII while minimizing potential interactions with the human enzyme. The identification of fungal-specific structural features significantly advances our understanding of the molecular determinants governing target selectivity.

The final publication, printed in the *European Journal of Medicinal Chemistry*, represents the culmination of the research program. Building upon knowledge generated in the preceding studies, the Candidate participated in the design and biological evaluation of novel bisacridine derivatives targeting fungal TopoII. The compounds were investigated using a combination of biochemical assays, microbiological analyses, cytotoxicity studies, and molecular modeling approaches. Particularly noteworthy is the identification of compound IKE16, which demonstrated preferential inhibition of yeast TopoII over its human counterpart while retaining antifungal activity against *Saccharomyces cerevisiae*. However, clinically relevant fungal strains, including drug-resistant isolates, remained resistant to this compound, which also exhibited substantial cytotoxicity toward human cells. Nevertheless, comparative studies described in this article identified several other bisacridine derivatives as potent fungal TopoII inhibitors with reduced cytotoxicity and significant activity against fluconazole-resistant clinical strains. In particular, compounds IKE18, IKE19, and IKE21, which – in contrast to

IKE16 – contained linker without piperazine, provide compelling evidence that the development of selective fungal TopoII inhibitors is feasible and further support the therapeutic potential of this molecular target.

One of the most impressive aspects of the dissertation is the coherence of the overall research strategy and the consistency with which the successive stages of the project were executed. The work evolved through a logical sequence of studies, beginning with a critical review of the literature on fungal DNA topoisomerase II as a therapeutic target, followed by experimental validation of this hypothesis, and culminating in the identification and evaluation of novel inhibitory compounds based on comparative structural characterization of fungal and human enzymes. This progression reflects not only careful project planning but also the Candidate's ability to formulate and refine research questions on the basis of previously obtained results. Moreover, the presented experimental work is distinctly interdisciplinary, integrating approaches from molecular biology, enzymology, microbiology, medicinal chemistry, and computational biology.

In my opinion, the most important achievements of Ms. Kondaka's PhD project are:

• Establishment of fungal DNA topoisomerase II as a valuable molecular target for antifungal drug development.
• Identification of previously unrecognized structural differences between fungal and human TopoII enzymes.
• Discovery of fungal-specific ligand-binding hotspots suitable for selective inhibitor design.
• Demonstration that clinically used TopoII inhibitors possess antifungal activity, thereby providing experimental validation of the target.
• Identification of compounds active against fluconazole-resistant fungal isolates.
• Discovery and characterization of novel bisacridine derivatives capable of inhibiting fungal TopoII.
• Development of a coherent scientific framework linking target identification, target validation, structural characterization, and inhibitor development.

With regard to editorial aspects, the dissertation is generally very well written, logical, and scientifically rigorous. The publication-based format is appropriate and effectively documents the progression of the project. Nevertheless, despite the high quality of the individual publications, I would have appreciated a more substantial integrative discussion summarizing how the findings collectively advance the validation of fungal DNA topoisomerase II as an antifungal target. Such a section would have further strengthened the dissertation by placing the individual studies within a broader scientific context and discussing their implications for future antifungal drug development.

I also noted several minor editorial issues, including the absence of page numbering in the electronic version of the dissertation and a small number of formatting inconsistencies.

Although these shortcomings somewhat affect the ease of navigation through the document, they do not detract from its scientific merit or overall quality.

Given the high quality of the published work, most methodological and scientific aspects of the research have already undergone thorough peer review. Nevertheless, the dissertation raised several scientific questions that I would like to discussed by Ms. Kondaka during the defense. At the same time, these issues should be viewed primarily as future research directions rather than shortcomings of the presented work. They do not diminish the scientific value of the dissertation but rather illustrate the substantial research potential generated by the obtained findings.

1. Which of the fungal-specific hotspots identified in the structural study do you consider the most promising for future structure-based drug design and why?
2. How would you experimentally validate the biological role of the fungal-specific K-loop and helix-supported loop regions identified through molecular dynamics simulations?
3. What factors may explain the discrepancy between enzymatic selectivity and antifungal activity observed for certain investigated compounds?
4. The presented studies demonstrate selective inhibition of fungal TopoII by several compounds. In your opinion, what are the key molecular determinants governing the relationship between enzyme inhibition, intracellular accumulation, and overall antifungal efficacy?
5. Which strategy do you consider to have greater long-term potential: repurposing existing anticancer TopoII inhibitors or developing entirely new TopoII-targeting antifungal compounds?
6. Most biological studies presented in the dissertation were performed *in vitro*. Which experimental infection model would you consider most appropriate for validating the therapeutic potential of the identified compounds *in vivo*?
7. Although several compounds demonstrated promising selectivity profiles, what do you consider the major challenges in further optimizing their pharmacological properties and toxicity profiles before progression toward preclinical development?

In summary, the presented dissertation constitutes an original, rational, and scientifically mature body of work. The Candidate successfully progressed from the identification of an important biomedical problem, through comprehensive target characterization and validation, to the development and evaluation of novel inhibitory compounds. By integrating approaches from molecular biology, computational modeling, medicinal chemistry, microbiology, and pharmacology, Ms. Kondaka has made a valuable contribution to the field of antifungal drug discovery. The results presented in the dissertation significantly advance our understanding of fungal DNA topoisomerase II and provide a solid framework for the future development of TopoII-directed antifungal therapies.

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The dissertation clearly demonstrates the Candidate's ability to conduct independent, high-quality scientific research and to address complex interdisciplinary problems.

I therefore conclude that the dissertation fulfills the requirements set forth in the Act of 20 July 2018, *Law on Higher Education and Science* (Journal of Laws 2018, item 1668, as amended). Accordingly, I recommend that the Scientific Council of the Faculty of Chemistry, Gdańsk University of Technology, admit Ms. Kavya Kondaka to the subsequent stages of the doctoral degree procedure.

Sincerely,

Elżbieta Nowak

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