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Prof. dr. hab. Joanna Kruszewska
Laboratory of Fungal Biology
Institute of Biochemistry and Biophysics
Polish Academy of Sciences

Review

of the doctoral dissertation of Kavya Kondaka, MSc, Eng., entitled "Yeast DNA Topoisomerase II as a model molecular target for novel antifungal compounds," prepared under the supervision of Iwona Gabriel, PhD, DSc, Eng.
The doctoral thesis was carried out at the Gdańsk University of Technology.
The formal basis for this review is the Resolution of the Disciplinary Council for Chemical Sciences of the Gdańsk University of Technology of June 9, 2026, appointing me as the reviewer of the above-mentioned doctoral dissertation of Kavya Kondaka, M.Sc. Eng.
The legal basis is Article 13, Section 1 of the Act of March 14, 2003, on Academic Degrees and Academic Titles and on Degrees and Titles in the Field of Art (Journal of Laws of 2003, No. 65, item 595, as amended).

Selection and importance of the research topic

Candida albicans is a common component of the human microbiota and the leading cause of opportunistic fungal infections in immunocompromised patients, with reported mortality rates ranging from 30% to 50%. Its remarkable ability to colonize and infect diverse host niches results from a broad repertoire of virulence factors, exceptional adaptability to changing environmental conditions, efficient nutrient acquisition systems, and robust stress response mechanisms.
The treatment of fungal infections remains challenging because the number of available antifungal drug classes is limited. Their clinical effectiveness is further compromised by the emergence of drug resistance, host toxicity, and clinically significant drug–drug interactions. Moreover, most newly introduced antifungal agents are based on existing drug classes and target well-established mechanisms, primarily inhibition of ergosterol biosynthesis or direct interaction with ergosterol in the fungal cell membrane.
In the Introduction, the doctoral candidate presents a comprehensive overview of the current limitations of antifungal therapy, emphasizing the growing problem of antifungal

resistance in *Candida* species and the urgent need for the development of novel therapeutic strategies. She correctly points out that the search for new antifungal agents has largely focused on already established molecular targets, which inherently restricts the scope of innovation. To overcome these limitations, she advocates an interdisciplinary approach that integrates advances in molecular biology, genomics, proteomics, medicinal chemistry, and pharmacology.

The candidate thoroughly reviews contemporary strategies for antifungal drug discovery, including the screening of plant-derived natural products, genomic and proteomic identification of essential fungal genes and metabolic pathways absent in humans, optimization of drug formulations to improve bioavailability and reduce toxicity, and the development of combination therapies aimed at achieving synergistic effects, lowering effective drug doses, and overcoming antifungal resistance. She also discusses the growing interest in drug repurposing, highlighting that several compounds originally developed for non-fungal diseases exhibit antifungal activity and may expand the therapeutic arsenal when used alone or in combination with established antifungal agents.

The doctoral candidate further notes that antifungal drug discovery is particularly challenging because fungi and humans are both eukaryotic organisms and therefore share many fundamental metabolic pathways, limiting opportunities for selective targeting. Against this background, she identifies fungal DNA topoisomerase II as an especially promising molecular target. Although this enzyme has a human homolog, structural differences between the fungal and human proteins may enable the development of selective inhibitors with reduced host toxicity.

The Introduction then provides a detailed description of the structure and biological function of DNA topoisomerase II, including its ATPase domain, DNA-binding and cleavage core, and C-terminal regulatory domain. Given its indispensable role in DNA replication, transcription, chromosome segregation, and overall genome maintenance, DNA topoisomerase II has long been recognized as an important target of anticancer therapy. The candidate discusses studies demonstrating that human and fungal topoisomerase II, when expressed in yeast, display differential sensitivity to several anticancer compounds. Some of these compounds exhibit antifungal activity by simultaneously targeting fungal DNA topoisomerase II and compromising membrane integrity.

The Introduction concludes with a well-structured comparison of fungal and human DNA topoisomerase II, clearly outlining both their similarities and their structural differences, thereby providing a strong scientific rationale for the objectives of the doctoral research.

Evaluation of the scientific publications

The first review article, "**Targeting DNA Topoisomerase II in Antifungal Chemotherapy,"** presents DNA topoisomerase II as a well-established therapeutic target in antibacterial and anticancer chemotherapy and discusses its potential as a novel target

for antifungal drug development. Since considerably less is known about exploiting this enzyme in antifungal therapy, the review provides a comprehensive and timely overview of the field.

The doctoral candidate first outlines the currently available antifungal therapies and emphasizes the urgent need to identify new molecular targets. She introduces DNA topoisomerase II as a particularly attractive candidate. Human topoisomerase II inhibitors are widely used in anticancer therapy, while bacterial topoisomerases are established targets of antibacterial agents. These compounds generally act by stabilizing the cleaved DNA–topoisomerase II complex, thereby preventing DNA religation and inducing lethal DNA damage. Catalytic inhibitors, in contrast, interfere with ATP hydrolysis and trap DNA within the enzyme without stabilizing the cleavage complex. The review further summarizes the structure and biological function of fungal topoisomerase II from *Saccharomyces cerevisiae*. Importantly, the candidate highlights structural and functional differences between fungal and human enzymes, providing a strong rationale for exploiting topoisomerase II as a selective target for antifungal therapy.

The second publication, "**Anticancer Drugs Targeting Topoisomerase II for Antifungal Treatment**," investigates whether structural differences between fungal and human topoisomerase II can be utilized to identify compounds that selectively inhibit the fungal enzyme. The authors evaluated several clinically used anticancer drugs that function as topoisomerase II poisons and assessed both their inhibitory activity against yeast topoisomerase II and their antifungal properties.

Interestingly, although most of the tested compounds strongly inhibited yeast topoisomerase II in vitro, only a limited number exhibited significant antifungal activity. The study demonstrated that even subtle structural modifications, such as the absence of a single methoxy group, could markedly influence antifungal efficacy. Particularly noteworthy was the observation that several *Candida* strains resistant to fluconazole remained susceptible to idarubicin and selected other topoisomerase II inhibitors. Microscopic analyses of *S. cerevisiae* cells treated with idarubicin revealed profound alterations in both nuclear and mitochondrial DNA, including DNA fusion events accompanied by increased accumulation of reactive oxygen species (ROS), suggesting that apoptosis contributes to the antifungal mechanism of action. The authors also investigated the contribution of fungal efflux pumps to idarubicin susceptibility. Collectively, these findings demonstrate that topoisomerase II inhibitors constitute promising candidates for antifungal drug development.

The third publication, "**Structural Insights into Fungal and Human Topoisomerase II with Implications for In Silico Antifungal Drug Design**," provides a detailed structural comparison between human Topoisomerase IIα and *S. cerevisiae* Topoisomerase II. The analysis was based on the crystal structure of the yeast Topoisomerase II homodimer in complex with DNA and an ATP analogue together with available structures of the human enzyme.

The comparative analysis identified several structural differences that could be exploited for the rational design of selective antifungal inhibitors. The most significant variations were found within the transducer (TDL) domain, particularly in the K-loop, the α-helix (or helix-like region), and the helix-supporting loop. These observations provide an important structural framework for structure-based drug design aimed at selectively targeting fungal Topoisomerase II while minimizing inhibition of the human enzyme.

The fourth publication, "**Bisacridine Derivatives as Effective Eukaryotic Topoisomerase II Inhibitors**," focuses on the synthesis and biological characterization of novel bisacridine derivatives as inhibitors of fungal and human Topoisomerase II. Although bisacridines are well known for their anticancer activity, their antifungal potential has remained largely unexplored.

Building upon their previous findings, the authors designed and synthesized a series of new bisacridine derivatives and evaluated their effects on Topoisomerase II activity in vitro. The study clearly demonstrated that even minor structural modifications profoundly affected biological activity. For example, the selective inhibition of yeast Topoisomerase II observed for compound IKE16 was completely lost in the closely related analogues IKE17 and IKE17a, which differ only by the presence of additional methyl or methoxy substituents on the acridine rings.

The compounds also differed in their mechanisms of enzyme inhibition. While several derivatives effectively inhibited Topoisomerase II-mediated DNA unwinding, IKE16 exhibited little activity through this mechanism, suggesting an alternative mode of enzyme inhibition. The strongest antifungal activity was observed for a compound containing two acridine moieties connected by an ethylamino linker, whereas compounds containing a piperazine linker, including IKE16, displayed considerably weaker antifungal activity. Importantly, the most active compounds retained activity against fluconazole-resistant fungal strains.

The authors further demonstrated that several bisacridines stimulated intracellular ROS production, indicating that oxidative stress may contribute to their antifungal mechanism of action. They concluded that antifungal efficacy depends not only on selective inhibition of fungal Topoisomerase II but also on fungal strain-specific properties and the activity of drug efflux pumps. Surprisingly, the most selective Topoisomerase II inhibitor, IKE16, also exhibited considerable cytotoxicity toward mammalian cells. The authors propose that the fungal cell wall constitutes a major barrier limiting intracellular drug accumulation, thereby necessitating higher extracellular drug concentrations and increasing host toxicity. They therefore identify optimization of drug transport across the fungal cell wall as an important direction for future research.

Summary

The series of publications included in Ms. Kavya Kondaka's doctoral dissertation presents a coherent and logically structured research program aimed at developing novel

antifungal agents targeting fungal DNA Topoisomerase II. The work convincingly demonstrates that successful antifungal drug design requires a multidisciplinary approach, beginning with the identification and characterization of an appropriate molecular target, followed by rational inhibitor design, structural and biochemical validation, and comprehensive biological evaluation in fungal cells.
The individual publications represent successive stages of this research strategy. The first article establishes fungal Topoisomerase II as a promising therapeutic target. The second investigates the antifungal potential of clinically used Topoisomerase II inhibitors and elucidates their mechanisms of action. The third provides a detailed structural comparison between fungal and human enzymes, laying the foundation for the rational design of selective inhibitors. Finally, the fourth publication describes the synthesis and biological evaluation of novel bisacridine derivatives optimized to exploit these structural differences.
Taken together, these publications form a coherent and scientifically well-justified body of work that successfully progresses from the identification of a novel antifungal target to the rational design and biological characterization of promising lead compounds.

Final Conclusions

The doctoral dissertation entitled "Yeast DNA Topoisomerase II as a Model Molecular Target for Novel Antifungal Compounds" fulfills the requirements set forth in Article 13, Section 1 of the Act of 14 March 2003 on Academic Degrees and Academic Title and on Degrees and Title in the Arts (Journal of Laws No. 65, item 595, as amended).
In view of the scientific quality of the dissertation, the originality of the research, and the significance of the obtained results, I conclude that the dissertation satisfies all the statutory requirements for the award of the doctoral degree.
Accordingly, I recommend that the Council of the Discipline of Chemical Sciences at the Gdańsk University of Technology admit Ms. Kavya Kondaka, M.Sc., to the subsequent stages of the doctoral proceedings leading to the award of the degree of Doctor of Natural Sciences in the discipline of Chemical Sciences.

Kind regards,

Prof. Joanna Kruszewska PhD, DSc

Questions to Ms Kavya Kondaka

1. One of the main arguments for selecting fungal DNA topoisomerase II as a therapeutic target is the structural difference between the fungal and human enzymes. Which of these structural differences do you consider the most promising for the design of selective inhibitors, and why?
2. Your studies demonstrated that strong inhibition of Topoisomerase II activity in vitro does not always correlate with potent antifungal activity. In your opinion, which factors, apart from enzyme inhibition itself, are primarily responsible for this discrepancy?
3. You showed that several Topoisomerase II inhibitors remain active against fluconazole-resistant *Candida* strains. Do you believe that fungal Topoisomerase II represents a therapeutic target that is independent of the mechanisms responsible for azole resistance, or could resistance mechanisms eventually compromise the efficacy of this class of compounds as well?
4. Several of the investigated compounds induced the production of reactive oxygen species (ROS). In your opinion, is ROS generation a primary mechanism underlying their antifungal activity, or is it more likely a secondary consequence of DNA damage and mitochondrial dysfunction?
5. Your work suggests that the fungal cell wall represents a significant barrier limiting intracellular drug accumulation. What strategies would you propose to improve the penetration of Topoisomerase II inhibitors into fungal cells while minimizing their toxicity toward human cells?
6. Among the compounds investigated in your dissertation, which do you consider the most promising candidate for further preclinical development, and what would be the next key experiments required to advance its development?
7. Do you envisage fungal Topoisomerase II inhibitors being developed primarily as standalone antifungal agents or as components of combination therapy with existing antifungal drugs? What do you consider the major advantages and potential limitations of each approach?
8. Most of the experimental work was performed using *Saccharomyces cerevisiae* as a model organism. What are the main limitations of this model when translating your findings to clinically relevant pathogens, particularly *Candida albicans*?