Yeast DNA Topoisomerase II as a model molecular target for novel antifungal compounds

PhD thesis

Kavya Kondaka

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DESCRIPTION OF DOCTORAL DISSERTATION

The author of the doctoral dissertation: Kavya Kondaka

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Abstract of doctoral dissertation in English:

Fungal infections, particularly those caused by *Candida* species, represent a growing health concern due to rising multidrug resistance and the limited repertoire of antifungal agents. The significant similarity between fungal and mammalian cells at a biochemical level, combined with the development of resistance to existing chemotherapeutic drugs, highlights the need to explore new molecular targets. DNA topoisomerase II (TopoII), a key enzyme in DNA replication, transcription, and chromosome segregation, has emerged as a promising antifungal target because of its reported vital role in the survival of certain fungal strains. Moreover, some enzyme structural divergence from human homologs were described.

The aim of the research presented in this PhD thesis was to evaluate fungal TopoII as a therapeutic target with the use of biochemical, molecular, antifungal, and computational approaches.

Firstly, a high-throughput screening tests of antifungal and yeast topoisomerase II inhibitory effect of known, clinically used human topoisomerases inhibitors were performed to finally verify the hypothesis that inhibition of fungal enzyme may result in an antifungal activity and to characterize the effect of enzyme inhibition on cellular level. Screening of known TopoII inhibitors identified idarubicin as the most effective compound against yeast TopoII. Mechanistic studies revealed that its antifungal action involves mitochondrial disintegration, fusion of mitochondria into nuclear DNA and progressive reactive oxygen species (ROS) accumulation. Our results also indicated that in the presence of clinically used idarubicin some candidal virulence factors might be strongly affected. Although idarubicin therapeutic plasma levels are lower than minimal inhibitory concentrations determined as antifungal, reported liposomal encapsulation improves its bioavailability to achieve concentrations sufficient for killing fungal cells.

Studies based on previously reported results on bisacridine derivatives inhibitory potential against yeast TopoII were then carried out. A series of novel derivatives were evaluated for their ability to inhibit yeast topoisomerase II, their antifungal activity, and their selectivity. Selected compounds demonstrated strong yeast TopoII inhibitory activity and antifungal effect against both fluconazole-resistant and sensitive *Candida albicans* and *Candida glabrata* strains. Among them, IKE16 was most interesting; unlike idarubicin or m-AMSA, it is not a classical TopoII poison but functions through a distinct inhibitory mechanism. Moreover, our results also demonstrate that IKE-16 is a selective inhibitor of the fungal enzyme. Molecular docking simulations performed in cooperation supported these findings, showing that IKE-16 interacts with key amino acid residues in yeast TopoII (GLU831 and ARG906). These residues are crucial for DNA binding and their disruption may inhibit the enzyme's catalytic function.

To investigate structural distinctions between yeast and human topoisomerase II, aiming to identify if fungal enzyme can be a selective target for antifungal drug development a comprehensive *in-silico* analysis was performed. A sequence analysis, extending to various fungal strains and evolutionary ancient organisms, revealed dissimilarities in the transducer and transducer linker domains of these proteins, as well as in the lysine rich K-loop region. Molecular dynamics simulations emphasized structural differences in the K-loop, α-helix (or helix-like region), and helix supporting loop region, as well as showed unique patterns in hydrophilic and hydrophobic intramolecular interactions in yeast TopoII. Thus, three potentially selective inhibitor binding areas ("hotspots") were identified.

Complementing these findings, an *in-silico* screen of approximately seven million compounds across three predicted fungal-specific TopoII "hotspots" yielded promising scaffolds. Biochemical validation identified two derivatives as potent selective yeast TopoII inhibitors. However, neither compound demonstrated measurable MICs in fungal cells, suggesting limited penetration or intracellular stability. To address this, chemical modifications of fluoroquinolone derivative will be carried out, generating novel derivatives with the potential to improve cellular uptake and antifungal efficacy.

Overall, this work provides mechanistic insights into fungal TopoII inhibition, highlights novel scaffolds with selective antifungal activity, and establishes an integrated framework for antifungal drug discovery.

Abstract of doctoral dissertation in Polish:

Zakażenia grzybicze, zwłaszcza te wywoływane przez gatunki Candida, stanowią coraz poważniejszy problem zdrowotny ze względu na rosnącą oporność wielolekową i ograniczony repertuar leków przeciwgrzybiczych. Istotne podobieństwo między komórkami grzybów i ssaków na poziomie biochemicznym, w połączeniu z rozwojem oporności na istniejące leki chemioterapeutyczne, podkreśla potrzebę poszukiwania nowych celów molekularnych. Topoizomeraza DNA II (TopoII), kluczowy enzym w replikacji, transkrypcji i segregacji chromosomów DNA, stała się obiecującym celem terapeutycznym ze względu na jej istotną rolę w przeżywalności niektórych szczepów grzybowych. Ponadto, opisano pewne różnice strukturalne enzymu w stosunku do homologów ludzkich.

Celem badań przedstawionych w niniejszej rozprawie doktorskiej była ocena grzybiczej TopoII jako celu terapeutycznego z wykorzystaniem metod biochemicznych, molekularnych, mikrobiologicznych i obliczeniowych.

W pierwszej kolejności przeprowadzono wysokoprzepustowe testy przesiewowe działania przeciwgrzybowego i inhibicyjnego wobec drożdżowej topoizomerazy II puli znanych, klinicznie stosowanych ludzkich inhibitorów topoizomerazy. Ostatecznie zweryfikowano hipotezę, że hamowanie enzymu grzybowego może prowadzić do efektu przeciwgrzybowego. Scharakteryzowano również wpływ hamowania enzymu na poziomie komórkowym. Badania przesiewowe znanych inhibitorów TopoII wskazały idarubicynę jako najskuteczniejszy związek wobec drożdżowej TopoII. Badania mechanistyczne wykazały, że jej działanie przeciwgrzybicze obejmuje rozpad mitochondriów, fuzję DNA mitochondrialnego z jądrowym oraz progresywną akumulację reaktywnych form tlenu (ROS). Wyniki wskazują również, że obecność klinicznie stosowanej idarubicyny może silnie wpływać na niektóre czynniki wirulencji drożdżaków. Mimo, że terapeutyczne stężenia idarubicyny w osoczu są niższe niż minimalne stężenia hamujące wzrost, określane jako przeciwgrzybicze, opisane liposomalne kapsułkowanie poprawia jej biodostępność, umożliwiając osiągnięcie stężeń wystarczających do zabicia komórek grzybowych.

Następnie przeprowadzono badania oparte na wcześniej opublikowanych wynikach dotyczących potencjału hamującego pochodnych bisakrydyny wobec drożdżowej TopoII. Oceniono szereg nowych pochodnych pod kątem ich zdolności inhibicyjnej, aktywności przeciwgrzybiczej oraz selektywności. Wybrane związki wykazały silne działanie hamujące aktywność drożdżowej TopoII oraz działanie przeciwgrzybicze wobec szczepów *Candida albicans* i *Candida glabrata*, zarówno opornych, jak i wrażliwych na flukonazol. Spośród nich najbardziej interesujący okazał się IKE16; w przeciwieństwie do idarubicyny lub m-AMSA, nie jest on klasyczną trucizną dla TopoII, lecz działa poprzez odrębny mechanizm hamujący. Co więcej, nasze wyniki wskazują również, że IKE-16 jest selektywnym inhibitorem enzymu grzybowego. Symulacje dokowania molekularnego, przeprowadzone we współpracy, potwierdziły te ustalenia, pokazując, że IKE-16 oddziałuje z kluczowymi resztami aminokwasowymi drożdżowej TopoII (GLU831 i ARG906). Reszty te są istotne dla wiązania DNA, a zaburzenie możliwości oddziaływania z DNA może hamować katalityczną funkcję enzymu.

Aby zbadać różnice strukturalne między drożdżową a ludzką topoizomerazą II, dążąc do ustalenia, czy enzym grzybowy może być selektywnym celem dla rozwoju leków przeciwgrzybiczych, przeprowadzono kompleksową analizę in silico. Analiza sekwencyjna, obejmująca różne szczepy grzybów i badania ewolucyjne, ujawniła różnice w obrębie tzw. domeny, „transduktora" i łącznika „transduktora", a także w bogatym w lizynę regionie pętli K. Symulacje dynamiki molekularnej podkreśliły różnice strukturalne w pętli K, α-helisie (lub regionie helisopodobnym) oraz regionie pętli podtrzymującej helisę, a także wykazały unikalne wzorce hydrofilowych i hydrofobowych oddziaływań wewnątrzcząsteczkowych w drożdżowej topoizomerazie II. W ten sposób zidentyfikowano trzy potencjalnie selektywne obszary wiązania inhibitora (tzw „gorące punkty").

Uzupełniając te odkrycia, badania przesiewowe in silico około siedmiu milionów związków wobec trzech przewidywanych obszarów strukturalnych, specyficznych dla grzybowej TopoII (tzw. „gorących punktów") pozwoliło na zaproponowanie obiecujących struktur. Walidacja biochemiczna zidentyfikowała dwie pochodne, jako silne, selektywne inhibitory drożdżowej TopoII. Jednak żaden ze związków nie wykazał aktywności przeciwgrzybowej. Wyniki wstępnych badań sugerują ograniczoną penetrację lub

stabilność wewnątrzkomórkową związków. Aby rozwiązać ten problem, zaplanowano badania nad chemicznymi modyfikacjami pochodnej fluorochinolonu, które mają doprowadzić do otrzymania nowych pochodnych o wyższym potencjale wnikania do komórki i skuteczności przeciwgrzybiczej.

Podsumowując, niniejsza praca pozwoliła na pozytywną weryfikację możliwości celowania terapeutycznego wobec grzybowej TopoII. Wskazuje również na struktury o możliwym selektywnym działaniu inhibicyjnym i przeciwgrzybiczym.

I wish to express my deepest gratitude to my supervisor, Dr. Iwona Gabriel, for her invaluable guidance, encouragement, and support throughout my doctoral journey. Her expertise and insightful feedback have been instrumental in shaping this work.

I would also like to extend my sincere thanks to Dr. Kamila Rzad, who dedicated efforts greatly enriched my knowledge and skills. Working alongside her has been an inspiring and rewarding experience.

My heartfelt appreciation goes to my dearest husband, Dr. Subrahmanyam Sappati, whose unwavering support, motivation, and encouragement have been a constant source of strength. His words, actions, and expertise became an integral part of my research, directly contributing to the progress of my project. I feel truly blessed to have him as my life partner, always motivating me with immense care and joy.

This journey holds a special place in my heart as it coincided with the birth of my beloved daughter, whose very first breaths began during this period. She, too, has been a part of this work in her own beautiful way.

I am deeply grateful to my mother, my sister and my in-laws for their unconditional love, encouragement, and support, without which this journey would have been impossible.

Finally, I am thankful to my friends for their constant encouragement and for standing by me during the challenges and triumphs of this journey.

TABLE OF THE CONTENT OF DOCTORAL DISSERTATION

1. ARTICLES PRESENTED IN THE DISSERTATION WITH AUTHOR'S CONTRIBUTION

1.1 ARTICLE 1 (Review)

Targeting DNA topoisomerase II in antifungal chemotherapy

Kavya Kondaka, and Iwona Gabriel

Molecules, 2022, 27(22), 7768; doi: 10.3390/molecules27227768

IF_{2022}=4.6, Q2, Ministry of Science and Higher Education Points (MHES): 140

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Kavya Kondaka's contributions to this manuscript included participation in developing the manuscript concept, and conducting an in-depth literature review on fungal topoisomerase II and its therapeutic relevance. The author performed sequence alignments, homology-based modeling of yeast TopoII with the program MODELLER based on the sequence alignment to support literature data, data interpretation and table organization. The author synthesized biochemical and structural data into a coherent framework, drafted the full manuscript, integrated references, and actively contributed to the revision and refinement of the final version. For the supplementary file, the author was responsible for designing and creating all figures and alignments, validating analyses, preparing the analytical content.

1.2 ARTICLE 2

Anticancer drugs targeting topoisomerase II for antifungal treatment

Kavya Kondaka, Kamila Rząd, Natalia Maciejewska, and Iwona Gabriel

Scientific Reports, 2025, 15, 9311; doi: 10.1038/s41598-025-93863-z

IF_{2025}=4.379, Q1, Ministry of Science and Higher Education Points (MHES): 140

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Kavya Kondaka's contribution to this manuscript included active participation in conducting the majority of the experimental work. This involved in vitro enzyme inhibition assays, antifungal susceptibility testing, MIC and MFC determination and drug accumulation studies. The author also contributed to the assessment of drug effects on *Candida albicans* morphological transformation and biofilm formation, as well as ROS detection and flow cytometry-based oxidative stress analysis. Additionally, the author participated in interpreting the results and contributed to writing parts of the manuscript draft.

1.3 ARTICLE 3

Structural insights into fungal and human topoisomerase II with implications for in silico antifungal drug design

Subrahmanyam Sappati, **Kavya Kondaka**, Iwona Gabriel, and Maciej Bagiński

Scientific Reports, 2025, 15, 9467; doi: 10.1038/s41598-025-93122-1

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Kavya Kondaka participated in interpreting the results of detailed comparative sequence and structural analyses of fungal and human topoisomerase II, with a focus on identifying key differences relevant to antifungal drug targeting. Based on these findings, the author wrote the corresponding section of the manuscript and prepared the related figures (Figure 1A and 1B, Figure S1 and S2), and edited several other images included in the manuscript to enhance visual clarity and consistency. The author supported evolutionary based differences analysis.

1.4 ARTICLE 4

Bisacridine derivatives as effective eukaryotic topoisomerase II inhibitors

Kavya Kondaka, Subrahmanyam Sappati, Kamila Rząd, Ewa Paluszkiewicz, Natalia Maciejewska, Maciej Bagiński, and Iwona Gabriel

European Journal of Medicinal Chemistry, 2026, 301, 118174;

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Kavya Kondaka made substantial contributions to this manuscript by conducting all experiments studies related to the antifungal evaluation of the tested compounds, including minimum inhibitory concentration (MIC) assays, time dependent ROS development, and mechanism-of-action investigations. She was responsible for the preparation and organization of all tables and figures, ensuring accurate and effective presentation of the experimental data. The author also drafted the initial version of the manuscript, synthesizing the experimental findings into a scientifically coherent and well-structures narrative.

1.5 SUMMARY OF IMPACT FACTOR (IF)

The summary of IF of the articles presented in the dissertation is 19.258

2. INTRODUCTION

2.1 Background on fungal infections

2.1.1 Epidemiology and clinical significance

Fungal pathogens are increasingly recognized as major contributors to global morbidity and mortality, particularly in immunocompromised individuals (1,2). Opportunistic yeasts such as *Candida albicans*, *Candida glabrata* as well as molds like *Aspergillus fumigatus*, are primary agents of systemic mycoses, often resulting in severe illness and high mortality rates (3). Among these, *Candida albicans* is notable as the fourth most common cause of nosocomial bloodstream infections worldwide (4,5).

The clinical management of fungal infections has been further complicated by the alarming rise of Multi-Drug Resistant (MDR) species, particularly *Candida auris*, which exhibits resistance to multiple antifungal drug classes (4,6–8). As shown in Figure 1, the cumulative number of reported resistance cases has risen sharply over the past four decades, with a notable acceleration after 2015, and resistance has now been reported across multiple continents (9).

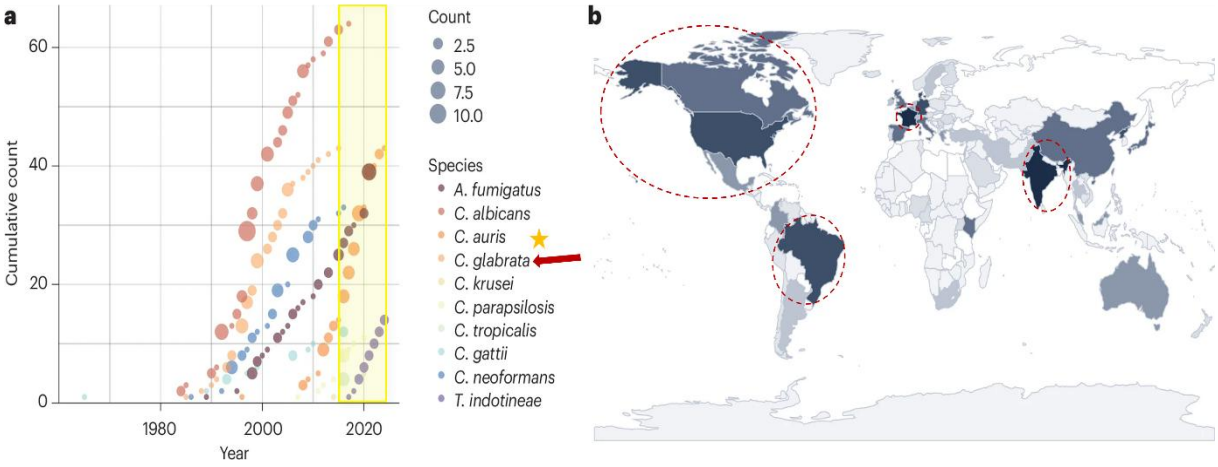

Figure 1: Global distribution and temporal trends of antifungal resistance among clinically significant fungal pathogens (a) Cumulative resistance reports of antifungal resistance from 1980 to 2020 across ten fungal species. Bubble size corresponds to the number of resistance reported annually. The yellow box highlights the period 2015-2020, showing a sharp rise in resistance cases. Red arrow indicates the increasing trend in resistance-associated reports for *Candida glabrata.* (b) World map depicting countries that have reported resistance cases. Color intensity increases with the number of resistant species reported per country (lighter = fewer species, dark = more species; gray indicates no reported cases for these species. Red circle indicates geographic hotspots with high resistance burden. Yellow star indicates *C. auris* as an emerging multidrug-resistant (MDR) threat. Adapted with permission from "Evolution of antifungal resistance in the environment" by Rhijn N.V. and Rhodes J., 2025, Nature Microbiology, Aug; 10(8): 1804-1815. Copyright 2025 by Springer Nature Limited.

Several key observations from Figure 1 are relevant to the present study. First, the sharp increase in resistance cases after 2015 coincides with the global emergence of multidrug-resistance *C. auris* and the spread of fluconazole-resistant *C. glabrata*. Second, the geographic distribution of resistance (Figure 1b) shows that high burden regions include India, United States, Brazil and France.

Notably, all four high-burden regions identified in Figure 1b have reported cases of fluconazole-resistant *C. glabrata* and emerging *C. auris* – the very strains against which our lead compound Idarubicin and bisacridine derivatives show potent activity.

Resistance in *Candida* species is frequently attributed to target mutations and the overexpression of efflux pump proteins, which lower intracellular antifungal concentrations to sub-lethal levels (4,10). These efflux mechanisms diminish the effectiveness of key antifungal classes such as polyenes, azoles, and pyrimidine analogues (11). The need to overcome such resistance has sparked interest in efflux pump inhibitors as therapeutic adjuvants and in the identification of novel drug targets like G-quadruplex structures, which have shown promise in combating resistant fungal strains (4,12,13).

Delayed diagnosis exacerbates disease progression and increases the risk of co-infections with bacteria and other pathogens (4,8). Hafeez et al., proposed the uses of PCR-based diagnostics targeting topoisomerase II (TopoII) and efflux-related genes such as phospholipase B gene (PLB) and, *Candida* drug resistance gene (CDR) for early detection of resistant *Candida* strains (14). The urgency is compounded by the increasing incidence of *Candida* and non-*Candida* fungal infections (6,7) and their association with worsened outcomes in COVID-19 patients, particularly in intensive care units (15,16).

The limited pipeline of new antifungal drugs has not kept pace with the rising incidence of fungal infections. This gap, combined with escalating resistance, compounds the challenges of antifungal therapy. Existing antifungal therapies are often hampered by poor pharmacokinetics, toxicity, and a narrow spectrum of activity (10,11). This highlights the critical need for novel, fungus-specific molecular targets to enable the selective and effective therapy while minimizing toxicity to human cells. Among these, fungal topoisomerase II (TopoII) has emerged as a particularly attractive target for antifungal drug development, offering the potential for improved efficacy in overcoming multidrug resistance.

2.1.2 Limitations of current antifungal drugs

Despite prophylactic use of antifungals, infections like invasive candidiasis continue to afflict high-risk surgical patients, largely due to pharmacokinetic variability and complex pathogen-host interactions (17). Antifungal resistance, driven in part by efflux-mediated drug clearance as mentioned above, remains a major clinical challenge. Efflux pump inhibitors are being investigated as adjuvants to restore antifungal efficacy (18).

Existing antifungal agents are constrained by poor oral bioavailability, nephrotoxicity, genotoxicity, reduced specificity, susceptibility to proteolytic degradation, and off-target toxicity (19,20). Amphotericin B, though effective, causes severe side effects and contributes to resistance upon overuse (19). Furthermore, fungal cells share significant biochemical similarities with mammalian cells, limiting the potential for selective drug targeting.

Clinically employed antifungal agents are classified according to their molecular targets and mechanisms of action. Azoles inhibit lanosterol 14α-demethylase (Erg11), a cytochrome P450 enzyme essential for ergosterol biosynthesis, thereby altering membrane structure and function (e.g., fluconazole, itraconazole, voriconazole). Polyenes bind ergosterol with high affinity, creating

transmembrane pores that facilitate ion leakage and cell death (e.g., amphotericin B, nystatin). Echinocandins block β-1,3-glucan synthase, impairing cell wall biosynthesis and leading to osmotic instability (e.g., caspofungin, micafungin, anidulafungin). Pyrimidine analogues such as flucytosine are converted intracellularly to 5-fluouracil, thereby inhibiting RNA and DNA synthesis. Allylamines inhibit squalene epoxidase, leading to ergosterol depletion and toxic squalene accumulation (e.g., terbinafine, naftifine) (21). This mechanistic classification highlights established drug targets and provides a framework for guiding novel drug discovery.

Therapeutic drug monitoring (TDM) remains essential to maintain optimal drug levels, particularly for agents with narrow therapeutic windows such as voriconazole (22). Although advancements in drug delivery systems such as cyclodextrins, cochleates, nanoparticles, long-circulating liposomes have improved pharmacokinetics these do not compensate for the lack of fundamentally novel drug classes (23).

Current antifungal therapies are limited in efficacy and spectrum. The slow pace of novel antifungal drug development, coupled with escalating prevalence of resistant fungal pathogens, underscore the urgent need for innovative strategies (24,25). These include identification of novel molecular targets, the development of hybrid therapeutic modalities, and utilization of model systems like *Saccharomyces cerevisiae* for high-throughput screening. Future antifungal development must integrate computational modelling, SAR-guided design, and systems biology along with model organism study approaches to overcome current limitations, which is the main focus of this study.

2.2 Ongoing antifungal therapies

2.2.1 Natural Products in Antifungal Treatment

Natural compounds offer a rich scaffold resource for antifungal drug development. Barhouchi et al., identified 1.8-cineole as a major antifungal component of *Myrtus communis* essential oil via molecular docking studies targeting fungal TopoII, SARS-CoV-2 protease, and angiotensin-converting enzyme 2 (ACE2) (26). Zhang et al., also compiled over eighty antifungal natural products, performing SAR analysis that identified multiple mechanisms of action, including oxidative stress induction, membrane disruption, transporter inhibition, and interference with signal transduction (27,28). Studies by Barhouchi et al., and Zhang et al., have shown that essential oils and secondary metabolites from Myrtus communis and *Aframomum atewae* exhibit antifungal activity, particularly against *C. albicans* and *S. cerevisiae*, supported by in-silico docking to TopoII targets (26,27,29).

Natural compounds such as flavonoids, terpenoids, and alkaloids have been shown to bind DNA-binding or catalytic domains of TopoII, modulating the cleavage-religation cycle and mimicking the effects of synthetic topoisomerase poisons like doxorubicin and etoposide, sometimes even enhancing their efficacy (30,31). Essential oils and plant derived secondary metabolites from pomegranate and lemon extracts have emerged as a rich source of bioactive antifungal agents (32).

From a pharmacological perspective, natural compounds like punicalagin, punicalin, and ellagic acid exhibit potent inhibition of *C. albicans* TopoI and TopoII with IC_{50} values as low as 4.6 μM (33,34), while benzoquinoline derivatives surpass the antifungal activity of polyene nystatin.

2.2.2 Genomics-Guided Antifungal Drug discovery

The decline of classical screening methods has ushered in a new era of genomics and proteomics driven approaches to target identification. Significant advances in fungal genomics, including the full genome sequencing of model and pathogenic fungi such as *S. cerevisiae*, *C. albicans*, *C. glabrata*, *A. fumigatus*, *Cryptococcus neoformans*, *Debaryomyces hansenii*, *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Yarrowia lipolytica*, *Neurospora crassa* and *Eremothecium gossypii*, have enabled high resolution functional and comparative genomic studies. These genomic resources facilitated the identification of essential genes open reading frames (ORFs), and species-specific pathways, significantly broadening the scope for antifungal target prediction and validation (14,16,35,36).

Tools like ResFungi platform use Hidden Markov Models to systematically analyze genomic data and detect conserved motifs associated with antifungal resistance (37). By scanning fungal genomes, these tools not only identify known resistance determinants but also reveal novel, uncharacterized genes that may serve as future therapeutic targets (37). These platforms play a vital role in functional annotation and enable the prioritization of essential genes for experimental validation. Integration with transcriptomic and proteomic datasets enhances the predictive accuracy, facilitating the development of highly selective and effective antifungal agents (14,38).

2.2.3 Nanotechnology in antifungals delivery

Building on the challenges outlined in section 2.1.2, nanotechnology offers strategies to overcome poor solubility, systemic toxicity, and non-specific drug distribution. Nanocarriers such as lipid-based nanoparticles, nanosponges, and self-assembling antimicrobial peptides (AMPs) have shown significant potential (20,39–42). These systems provide a protective environment for drug molecules, minimizing premature degradation and facilitating controlled release at the site of infection.

Advanced nanomaterials are engineered to respond to specific physiological cues, including pH gradients and electrostatic interactions or specific targeting of the fungal microenvironments for e g., by targeting cell wall and cell membrane. This site-specific delivery like smart release of drugs in the specific environment enhances antifungal efficacy while reducing systemic toxicity and off-target effects (43).

Collectively, nanotechnology enables a new generation of antifungal agents with improved pharmacological profiles, enhances targeting precision, and greater therapeutic safety, contributing meaningfully to the advancement of antifungal drug design.

2.2.4 Repurposing strategy in antifungal treatment

Drug repurposing, or repositioning, represents a pragmatic approach to bypass resistance mechanisms described in section 2.1.1, either through intrinsic antifungal properties or synergistic action with existing drugs. By leveraging existing pharmacokinetic, safety and toxicology data, this approach offers an accelerated route to clinical application compared with de novo drug discovery. Importantly, repurposed agents may exhibit intrinsic antifungal properties or act synergistically with established antifungals, potentially overcoming resistance and expanding therapeutic options. Systematic screening

of repurposing libraries encompassing approved drugs, discontinued clinical candidates, and bioactive compounds has identified several promising antifungal leads.

Beyond antifungals, diverse therapeutic domains are being repurposed for antifungal indications. Auranofin, an antirheumatic agent, demonstrates antifungal activity against *Candida*, *Cryptococcus*, and *Aspergillus* spp. particularly when combined with the antiparasitic pentamidine (44,45). Similarly, drospirenone (a synthetic hormone), perhexiline (anti-anginal), and mefloquine (antimalarial) has shown inhibitory activity against *Candida* and *Cryptococcus* species (44,46,47). Other agents such as bifonazole, econazole, alexidine, cetylpyridinium chloride, otilonium bromide, benzethonium chloride, niclosamide, disulfiram, temsirolimus have demonstrated activity against *Cryptococcus neoformans* (46). Furthermore, miltefosine, originally used in leishmaniasis, is being investigated for its antifungal activity against various *Candida* species and other fungi (46,48,49).

Preclinical exploration has further broadened this landscape, revealing antifungal activity in unexpected therapeutic classes. Examples include the antidepressant sertraline, the estrogen receptor antagonist tamoxifen, the lincosamide antibiotic clindamycin, the anti-inflammatory derivatives AR-12, and the PPAR-γ agonist pioglitazone. Their mechanisms of action range from interference with ergosterol biosynthesis and disruption of fungal membrane integrity to inhibition of mitochondrial function and alteration of lipid metabolism (Table 1) (45,49–51).

Together, these examples underscore the breadth of repurposing as a therapeutic strategy, highlighting both the diversity of pharmacological origins and the potential to rapidly expand the antifungal arsenal. By exploiting existing drugs and exploring synergistic combinations, repurposing provides a realistic and cost-effective pathway to address the urgent need for new antifungal therapies.

Table 1: Representative repurposed drugs with antifungal potential

Drug/Compound	Original or clinical indication	Proposed antifungal target/ Mechanism of action	Tested pathogens	Current evaluation
Sertraline (49)	Depression, anxiety (SSRI)	Inhibits lanosterol 14α-demethylase disrupts ergosterol biosynthesis	*Candida* spp., *Cryptococcus* spp., *Sporothrix* spp., dermatophytes	Preclinical, animal models
Auranofin (45)	Rheumatoid arthritis	Disrupts thiol-redox homeostasis; synergistic with pentamidine	*Candida* spp., (including fluconazole-resistant isolates)	Preclinical
Pentamidine (45)	Antiparasitic (trypanosomiasis, leishmaniasis)	DNA/RNA synthesis interface; synergistic with auranofin	*Candida* spp.,	Preclinical
Tamoxifen (51)	Breast cancer (ER antagonist)	Disrupts calmodulin-dependent pathways; membrane destabilizing	Various pathogenic fungi	Preclinical
Mefloquine derivatives (51)	Malaria prophylaxis and treatment	Membrane disruption, inhibition of protein synthesis	*Candida* spp., *Cryptococcus* spp.,	Preclinical

AR-12 (51)	Anti-inflammatory (celecoxib derivative)	Inhibits mitochondrial function, protein folding, and autophagy pathways	Broad-spectrum fungi	Preclinical
Clindamycin (50)	Bacterial infections (lincosamide)	Inhibits protein synthesis; species-specific antifungal effect	Select fungal pathogens	Preclinical
Pioglitazone (49,50)	Type 2 diabetes (PPAR-γ agonist)	Alters fungal lipid metabolism; potential indirect immunomodulatory effects	Select fungal pathogens	Preclinical

Reformulation of existing antifungals remain an integral component of this strategy. Notably, itraconazole, posaconazole, voriconazole, while long established as frontline azoles, are under evaluation for improved delivery systems such as SUBA-itraconazole, which enhances oral bioavailability (52–54). Amphotericin B cochleates have been designed to mitigate nephrotoxicity and enhance delivery, addressing the limitations previously described in section 2.1.2 (52,53,55). Next-generation echinocandins, exemplified by rezafungin currently in Phase III evaluation, represent another platform where repurposing converges with structural innovation (53). Similarly, the topical re-exploration of terbinafine reflects the drive to extend the utility of wee-established antifungals in recalcitrant conditions such as onychomycosis (52,53,55,56).

2.3 Need for novel molecular targets

Current antifungals target fungal-specific pathways like cell wall synthesis (e.g., β-glucans, chitin) and ergosterol biosynthesis in the plasma membrane (e.g., azoles, polyenes) (5,57–61). Despite their therapeutic efficacy, currently available antifungal treatments are often limited by adverse side effects, significant drug-drug interactions, and the requirement for prolonged treatment regimens. Despite reported advances, antifungal drug development has lagged far behind antibacterial research. The eukaryotic nature of fungi demands the identification of targets with structural and functional divergence from human homologs to enhance specificity. Enzymes involved in DNA processing, protein biosynthesis, and amino acid metabolism are promising candidates (62–64). Of particular interest is fungal TopoII, which shares conserved mechanisms with its human counterpart but also harbors unique structural features exploitable for selective antifungal design. DNA topoisomerases are a ubiquitous family of enzymes essential for DNA topology maintenance and are already validated as drug targets in cancer therapy.

2.4 DNA Topoisomerases

The discovery of DNA topoisomerases began with Wang's identification of the *E. coli* omega protein in 1969, capable of relaxing supercoiled DNA (65–67), followed by the characterization of eukaryotic TopoII isoforms (68–70). These are ubiquitous enzymes essential for maintaining DNA topology during vital cellular processes such as replication, transcription, recombination, and chromosome segregation (71–81). These enzymes alleviate topological strain by introducing transient

breaks into DNA strands and are classified into two major types based on their mechanism of action: a) Type I that cleave a single DNA strand and alter linking number by ±1; b) Type II that cleave both strands of DNA simultaneously, altering linking number by ±2 (82–84).

2.4.1 Classification

Type I topoisomerases are ATP-independent and relive supercoiling by transiently nicking one strand of DNA. Subtypes IA and IB are defined by their structural folds and reaction mechanisms. Type IA enzymes such as *E. coli* TopoI, TopoIII form a covalent 5'-phosphotyrosine intermediate and require divalent metal ions. Type IB enzymes such as human TopoI, poxvirus TopoI form a 3'-phosphotyrosine bond and unwind DNA via controlled rotation (85–88).

TopoII catalyzes ATP-dependent double stranded DNA cleavage, enabling strand passage and religation to resolve topological stress (89). Type IIA enzymes include eukaryotic TopoII isoforms like human TopoIIα, TopoIIβ, yeast TopoII, and bacterial DNA gyrase. Type IIB enzymes, such as TopoVI, are predominantly found in archea and plants and are structurally distinct due to the absence of a conserved C-gate (90).

2.4.2 Structure and Function of Topoisomerase II

Eukaryotic TopoII is a homodimeric enzyme composed of three core functional domains. The N-terminal ATPase domain (N-gate) binds and hydrolyzes ATP, driving conformational changes required for DNA strand passage (82). The central DNA-binding and cleavage domain (DNA-gate) transiently cleaves both DNA strands and facilitates the passage of another DNA segment, while the C-terminal dimerization and gate control domain (C-gate) enables exit of the transported DNA and resets the enzyme for subsequent catalytic cycles (Figure 2) (91,92).

The catalytic mechanism is highly conserved across eukaryotes. TopoII binds a gate-DNA (G-DNA) segment, cleaves both strands via covalent phosphotyrosyl intermediates, and passes a transported DNA (T-DNA) segment through the break. ATP binding and hydrolysis are essential for strand passage, whereas the cleavage and religation steps occur independently of ATP. This cycle relieves DNA supercoiling, untangles interwined chromosomes, and ensures proper replication, transcription, and chromosome segregation. Catalytic tyrosines in the Winged Helical Domain (WHD) form covalent 5'-phosphotyrosyl intermediates to prevent accidental genome fragmentation, and the enzyme's strand passage and religation steps are tightly coordinated to maintain genomic integrity (93). The conserved mechanism across yeast, fungi, and mammals provides a foundational framework for TopoII inhibition design.

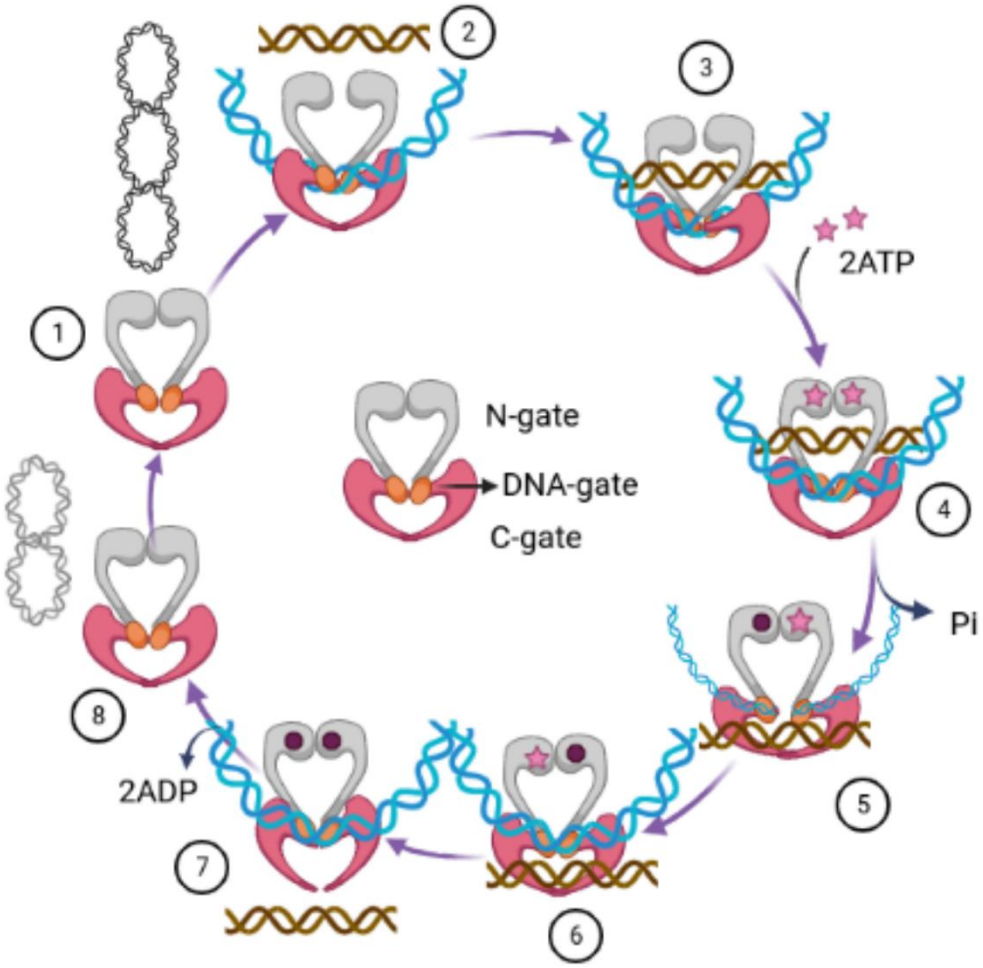

Figure 2: Catalytic cycle of yeast Topoisomerase II (1) Association of the enzyme with relaxed duplex DNA (linking number n+1). Dimerization of TopoII subunits and binding of G-segment. (3) ATP binding and closure of the N-gate. (4) ATP hydrolysis and capture of the T-segment. (5) Formation of a transient DSB formation in G-segment and passage of the T-segment through DNA-gate. (6) Re-ligation of the cleaved G-segment. (7) Opening of C-gate and passage of T-segment with release of 2 ADP molecules. (8) Restoration of the enzyme to its initial state, yielding duplex DNA with linking number n. Created in https://BioRender.com

Structural modeling and crystallographic studies (e.g., PDB ID 4GFH) have elucidated key steps of the TopoII catalytic cycle, including ATP hydrolysis, DNA cleavage, strand passage, and religation (94). The enzyme bends DNA at ~150°, with active site residues facilitating cleavage and religation. The ATPase domain features a unique K-loop structure, critical for catalysis (94). Additional modeling (80,89,95) has illuminated transitions within the TOPRIM and tower domains that guide catalysis.

Wojtaszek and Williams highlighted the role of DNA-protein crosslinks (TOP-DPCs) and discussed strategies for addressing such lesions, including targeting the protein component of the crosslink for degradation (96). Collins combined single-molecule FRET and crystallography to study DNA gate dynamics, clarifying how topoisomerase poisons exert cytotoxicity (89). Collectively, these

insights underscore the indispensable roles of TopoII in maintaining DNA topology during fundamental cellular processes.

2.5 Role of Topoisomerase II in DNA metabolism

2.5.1 Biological roles in Eukaryotic cells

Distinct mammalian TopoII isoforms were identified as TopoIIα (p170) and TopoIIβ (p180). They are encoded by separate genes located on chromosomes 17q21-22 and 3p24, respectively, and perform both overlapping and unique cellular functions (97–103). TopoIIα is primarily expressed in proliferating cells and is closely associated with DNA replication, chromosome segregation, and the cell cycle progression (104,105). In contrast, TopoIIβ is expressed throughout the cell cycle and plays essential roles in transcriptional regulation, chromatin remodeling, and the spatial organization of the genome (106,107). Their distinct expression patterns and non-redundant functions highlight the importance of isoform-specific regulation of DNA topology in maintaining genomic stability and supporting diverse cellular processes (Table 2) (108,109).

Table 2: Distinct cellular processes regulated by Topoisomerase II in eukaryotic systems

Cellular processes	**The distinct role**
DNA Replication	TopoII alleviates torsional stress ahead of replication forks and decatenates daughter chromatids post-replication. Loss of TopoII activity leads to accumulation of catenated DNA and defective segregation, as observed in yeast mutants (104,105).
Transcription regulation	TopoII collaborates with TopoI to relieve supercoils generated by RNA polymerase, supporting elongation and expression of long genes. It also modulates transcription by generating transient breaks that facilitate chromatin remodeling at promoters (106,107).
Recombination and DNA repair	TopoII resolves DNA entanglements that rise during homologous recombination and repair. Its cleavage intermediates can serve as recombinogenic sites, aiding in recombinase recruitment and strand exchange (110,111).
Chromosome condensation and segregation	Yeast TopoII is essential for proper chromosome segregation during mitosis and meiosis. Mutants lacking TopoII show mitotic arrest and cell death due to unresolved catenation (112,113). TopoII is also a component of the mitotic chromosome scaffold (76).
Chromatin architecture and nuclear organization	TopoII localizes to scaffold-associated regions and interacts with condensing and cohesion complexes to organize chromatin into topologically associated domains, influencing gene regulation and 3D genome structure (108,109).

2.5.2 Therapeutic significance

DNA topoisomerases are vital for genomic maintenance and dynamic DNA metabolism. Type II is especially critical in untangling DNA during replication and mitosis, making it a validated target for anticancer therapies (72–79). TopoII inhibitors function by stabilizing the cleavage complex, causing replication fork collapse and accumulation of cytotoxic double-stranded breaks (DSBs) in the DNA duplex (82,114) or act as catalytic ones (115). This mechanism underlies their potent therapeutic potential. Although TopoII is well established as an anticancer target, its potential as a selective antifungal target remains underexplored, providing the central rationale for this study.

2.6 Eukaryotic Topoisomerase II as a drug target

2.6.1 As an antitumor target

TopoII is a well-established therapeutic target in cancer chemotherapy due to its crucial role in DNA replication and chromosome segregation. TopoII targeting agents are broadly classified into two pharmacological categories based on their mechanism of action. The first group, known as TopoII poisons, includes drugs such as anthracyclines (etoposide, aclarubicin, idarubicin, doxorubicin) and m-AMSA. These compounds function by stabilizing the transient TopoII-DNA cleavage complex, thereby preventing religation and resulting in the accumulation of lethal DSBs (116).The second group comprises catalytic inhibitors, such as ICRF-187 (dexrazoxane), which act by interfering with ATP binding or the catalytic cycle of the enzyme without inducing DNA strand breaks. These inhibitors suppress TopoII activity in a more subtle manner and are typically associated with reduced off-target toxicity (117).

2.6.2 Dual antifungal and antitumor potential

Recent advances in medicinal chemistry have facilitated the development of dual acting inhibitors capable of targeting both TopoI and TopoII (118,119). Classical anticancer agents such as etoposide and doxorubicin exemplify this strategy by stabilizing the TopoII-DNA cleavage complex, inducing cytotoxic DSBs (120,121). Comparative studies in yeast systems engineered to express either human or fungal TopoII revealed isoform-specific sensitivities, highlighting exploitable differences between human and fungal enzymes (122–124). For example, ICRF-193 preferentially inhibits the human enzyme, whereas mitoxantrone displays stronger activity against yeast TopoII in these assays, although clinically mitoxantrone is a potent antineoplastic agent used to target human topoisomerase II in the treatment of various cancers (125–127).

Within this context, acridine derivatives constitute a particularly versatile class of agents, displaying activity across both therapeutic domains due to their DNA intercalation ability and TopoII inhibition (128–139). While originally explored for their antitumor potential, many derivatives demonstrate significant antifungal activity. They impair DNA relaxation, suppress *Candida* filamentation, and disrupt biofilm formation. For instance, quinacrine blocks yeast-to-mycelia transition and interferes with biofilm formation in *Candida* species (140). Further chemical innovations include imidazoacridinones (e.g., C-1330, C-1415 and C-1558) and acridine-octaarginine conjugates demonstrate enhanced fungal cell penetration and inhibition of TopoII relaxation activity (131,132,135,141–144). These acridine-peptide conjugates exert dual mechanism of action by targeting both fungal DNA and membrane integrity, offering novel antifungal strategies.

Of particular relevance are the imidazoacridinone C-1311 and the triazoloacridinone C-1305, which act as potent human TopoII poisons in cancer, inducing lethal DNA breaks in cancer cells. In contrast, derivatives of these compounds, notably benzamide and isoquinoline classes, have been systematically optimized to achieve fungal TopoII selectivity, thereby retaining potent antifungal activity while reducing inhibition of the human enzyme (121,144).

Together, these findings represent the therapeutic duality of TopoII, oncology-validated scaffolds such as C-1311 and C-1305 serve as potent anticancer agents, while their structural derivatives provide a promising foundation for the development of fungal selective inhibitors with reduced host toxicity (132). This conceptual framework supports the rational repurposing and design of TopoII directed scaffolds towards fungal selective therapeutics, exploiting structural divergences between human and fungal enzymes.

2.6.3 Computational approaches in eukaryotic topoisomerase II inhibitor design

These approaches provide a rapid and cost-effective framework for designing selective eukaryotic TopoII inhibitors. These tools allow prediction of enzyme-ligand interactions, assessment of binding stability, and rational scaffold optimization before experimental validation.

2.6.3.1 Structure-based modeling and molecular simulations

In silico techniques such as molecular docking, molecular dynamics (MD) and AMDET profiling are central to modern antifungal drug discovery. These computational methods are used for virtual screening, optimization, and prediction of pharmacological properties. Crystal structures of the ATPase region of yeast TopoII bound to ICRF-187 (PDB 1QZR) (145) and structure of *S. aureus* gyrase complex with an antibiotic GSK299423 and DNA (PDB 2XCS) (146) were used to model enzyme-ligand interactions. These simulations demonstrated stable binding of triazoles, pyrazoles, and monoterpene hydrocarbons to the fungal TopoII active site (147,148). MD simulations using PDB structure model 2XCS further confirmed stable interactions and suggested that these compounds inhibit haoDNA replication by stabilizing the enzyme-DNA complex (147). Docking and MD simulations were integral to our strategy for identifying lead compound that selectively target fungal TopoII.

2.6.3.2 Structure- Activity-Relationship (SAR) guided optimization and Scaffold diversification

SAR analysis constitutes a fundamental strategy for rational drug design, enabling systematic correlation between chemical modifications and biological activity, selectivity, and pharmacokinetic properties. SAR studies revealed critical insights into the antifungal potential and anti-cancer potential of nitrogen-containing heterocycles, including triazoles, quinolines, pyridines, and thiazoles (38,149,150). Broad scaffold screening revealed diverse TopoII-inhibiting classes: pyrimidines, pyridines, quinolines, carbazoles, β-carbolines, naphthalimides, acridones, benzoxazoles, and piperazine-based hybrids. These heterocyclic scaffolds have been optimized through chemical diversification to enhance their biological activity and specificity. Schiff base metal complexes, indazole derivatives, and chrysin based hybrids have also shown potent inhibitory effects on TopoII, particularly

when these cores are fused with polycyclic or aromatic moieties, resulting in enhanced antifungal efficacy (151–153).

Pyrazole and azole analogues have been optimized for improved binding and efficacy (151,154). Macrocyclic Schiff base complexes effectively inhibit TopoIIβ, while α-aminophosphonate-pyrazole hybrids and triazine derivatives show antibacterial potential (155,156).

In silico methods are central to designing selective fungal TopoII inhibitors. Docking, MD simulations, and SAR-guided scaffold diversification collectively enable rational design of potent, selective, and stable antifungal candidates, directly supporting the goal of next-generation antifungal therapeutics. While human TopoII has been extensively exploited as an anticancer target, structural and functional divergences in fungal TopoII present opportunities for selective antifungal drug design.

2.7 Fungal Topoisomerase II as a therapeutic target

2.7.1 Evolutionary Conservation

TopoII enzymes are conserved across species and maintain genomic integrity through management of DNA topology during replication, transcription, and segregation.

Phylogenetic investigations of topoisomerases encoding genes from twenty-nine fungal species revealed a strong evolutionary relationship among these organisms (157). Analysis of TopoII enzyme sequences in different *Candida* species showed that *C. albicans, C. dubliniensis, C. parapsilosis,* and *C. tropicalis* are closely associated with the ADPT cluster, whereas *S. cerevisiae*, *C. glabrata* and *C. kefyr* are grouped within the SCGK cluster. In addition, the amino acid sequences of human TopoIβ and Topo IIα enzymes were compared with those of fungal species. Two phylogenetic trees generated for medically important fungi indicated that fungal TopoI sequences share great similarity with human Topoβ than fungal TopoII sequences do with its human counterpart (Figure 3) (119).

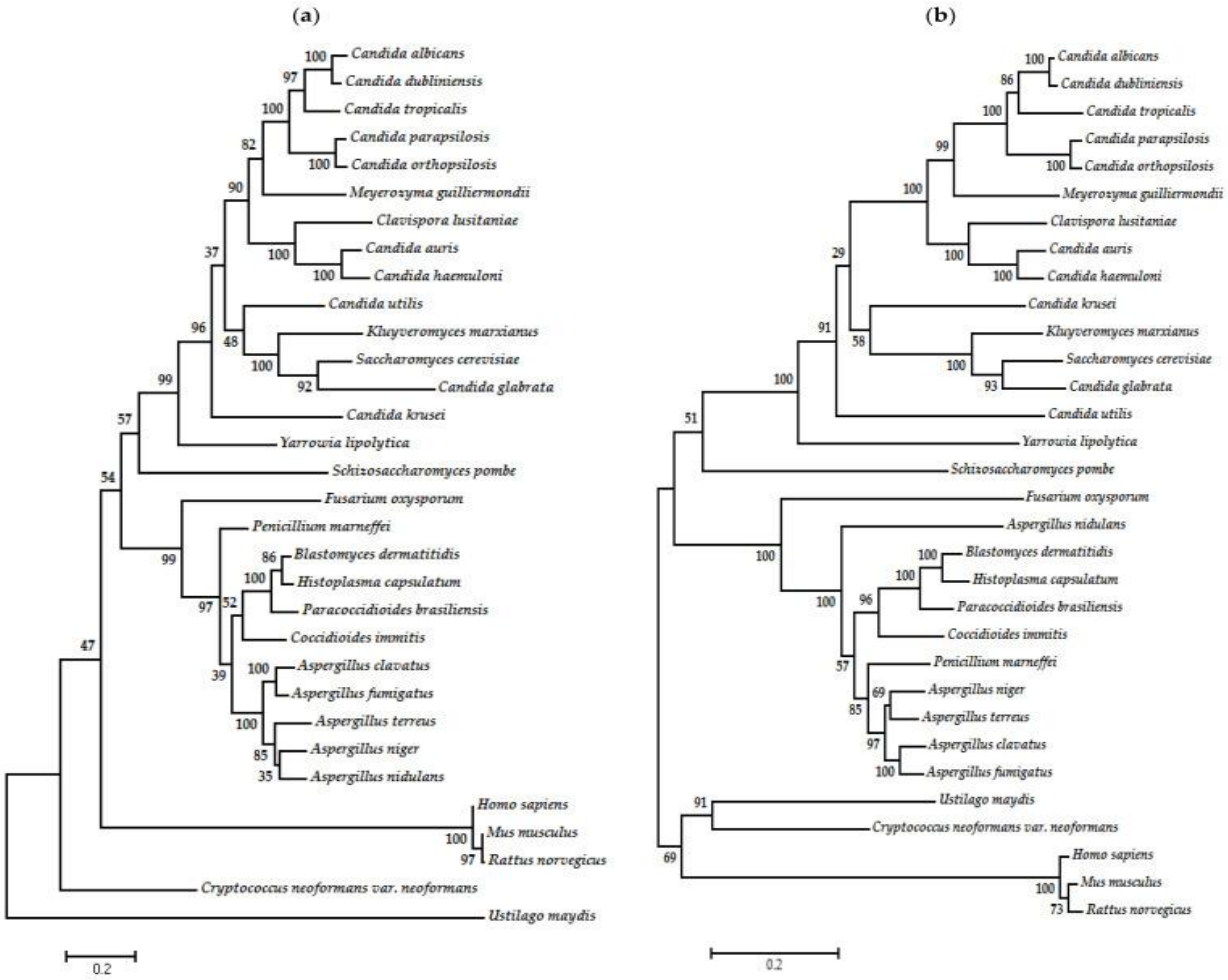

Figure 3: Phylogenetic relationships among 32 representative mammalian and fungal DNA topoisomerases of type I (a) and type II (b) were inferred using the maximum-likelihood method implemented in MEGA 6 with the WAG + G evolutionary model. Bootstrap values obtained from 100 runs are displayed at the branch nodes. (*Source:* [Andrade-Pavón D, Gómez-García O, Villa-Tanaca L.], [Review and Current Perspectives on DNA Topoisomerase I and II Enzymes of Fungi as Study Models for the Development of New Antifungal Drugs], [2024, 10, *Journal of Fungi*], reproduced under the terms and conditions of the Creative Common Attribution CC BY 4.0 license.

2.7.2 Differences between fungal and human Topoisomerase II: A therapeutic opportunity

2.7.2.1 Structural deviations and mechanistic insights

While the general catalytic cycle of TopoII is conserved, fungal TopoII exhibits distinctive structural and mechanistic features that can be exploited for selective antifungal therapy. Notably, the fungal TopoII possesses distinctive structural motifs absent in human TopoII, including a six-lysine K-loop compared to three in other eukaryotic TopoIIs, that is indispensable for DNA-stimulated ATP hydrolysis and strand passage (94). Mutational replacement of this lysine-rich region (KKKK to AAAA or KKKK to AEEA) drastically diminished decatenation and supercoil relaxation activities, highlighting its central mechanistic role (94).

High resolution crystallographic studies of *S. cerevisiae* TopoII have provided structural insights into its catalytic cycle. PDB IDs: 4GFH, 1PVG, 1QZR, 2RGR, 3L4J, 3L4K, 1BJT, 1BGW reveal extensive conformational transitions during strand passage and DNA cleavage/religation (94,158–162). Structural differences are also evident in the TOPRIM domain and C-gate, where the absence of the α11 helix and β-hairpin offer sites for selective antifungal targeting (92). Key catalytic residues, including Tyr782. Arg690, Asp697, Lys700, Arg704, and Arg781, are critical for yeast TopoII enzymatic activity, and mutations at these sites disrupt TopoII function (163). Functionally, yeast TopoII is tightly coupled to replication and chromosome segregation, whereas human TopoIIβ additionally regulates transcription of long genes, reflecting divergent roles shaped by chromatin organization and non-catalytic domain variations. These fungal specific motifs and structural deviations provide “hotspots” for the rational design of selective inhibitors, enabling potent antifungal activity while minimizing host toxicity.

2.7.2.2 Functional and evolutionary divergence

Fungal TopoII differs significantly from its human homolog in several non-catalytic domains, including the K-loop, transducer domain, and helix-supporting loop. These regions are less conserved and serve as pharmacophore “hotspots” for selective inhibitor design (14,164). Such divergence is thought to reflect evolutionary adaptation to distinct chromatin architectures in fungi versus mammals. In higher eukaryotes, human TopoII isoforms, particularly TopoIIα, also participate in transcriptional regulation of long, activity-induced genes by introducing transient DNA DSBs that facilitate chromatin remodeling, a process less prominent in yeast (165). In contrast, yeast TopoII remains tightly coupled to replication and chromosome segregation, highlighting functional specialization across species. Moreover, differences in higher order chromatin organization, including topologically associating domains (TADs) in metazoans, may have further shaped isoform-specific regulatory roles and drug sensitivities (76,166).

These insights, coupled with structural divergence, led to the classification of Topo enzymes and the discovery of therapeutic agents that exploit the reversible cleavage-religation process (167). Comparative analyses across species reveal striking differences in DNA cleavage patterns mediated by TopoII, which are essential for the rational design of selective inhibitors. Specific mutations such as Ser740Trp in yeast and Ser763Trp in humans modulate both drug sensitivity and site-specific cleavage activity (168). Strumberg et al., further demonstrated sequence selectivity variations in TopoII from human, yeast, and bacterial enzymes when challenged with intercalators like amsacrine and non-intercalators such as fluoroquinolones (164).

Importantly, functional divergence extends beyond replication into morphogenesis. In *C. albicans*, the yeast-to-hypha transition, a key determinant of virulence can be suppressed by distinct TopoII targeting chemotypes. 2-Alkylaminoquinoline derivatives attenuate filamentation without imparing growth, acting through cAMP-PKA and MAPK signaling and downregulating regulators (PDE2, CDC35, TEC1, HST7, CPH1) and hyphal-sepcific genes (ALS3, HWP1) (169). Similarly, styryl ketones such as NC1184 abolish germ tube formation via thiol-dependent redox interference, implicating cysteine-rich regulatory proteins (170).

Collectively, fungal TopoII exhibits structural and functional divergence that underlies species-specific genome regulation and morphogenesis, providing a foundation for selective inhibitors that impair both fungal viability and pathogenicity.

2.7.2.3 Differences in sensitivity to Topoisomerase II inhibitors

Fungal TopoII displays reduced sensitivity to several classical anticancer TopoII poisons such as m-AMSA and etoposide (73,130). Podophyllotoxins and bisdioxopiperazines such as ICRF-187 show differential activity, with greater selectivity for human TopoII isoforms (Table 3) (132,171,172).

The quinolone derivatives A-80198 and A-75272 exhibit species-specific inhibition, with A-80198 more potent against mammalian TopoII, whereas A-75272 is more effective against fungal TopoII (171). Similarly, natural products such as resveratrol and eupolauridine have also demonstrated species-specific inhibitory profiles (33,34,171–173).

Preliminary investigations by our research group suggest that acridine derivatives e.g., C-1305, C-1311 known to target human TopoII effectively are less effective against fungal enzyme. C-1311 as well as C-1305 totally inhibited the yeast topoisomerase II mediated relaxation at a concentration 50 μM, much higher than previously reported for human enzyme (10 μM for C-1305 and IC_{50} 6.5 μM for C-1311 (174). While human TopoII appears more sensitive to several of these compounds, structural divergence raises the possibility of identifying analogs that preferentially inhibit fungal TopoII while sparing the host enzyme.

Table 3: Comparative Sensitivity of TopoII inhibitors – yeast Vs. human

Compound	**Target & Assay**	**Species/Enzyme**	**Potency Metric**
Capridine β	yTopoII relaxation inhibition	*C. albicans* TopoII	Inhibits yTopoII at <200 μM (metabolite IE1 only)
m-AMSA	yTopoII catalytic activity assay	Yeast TopoII	Moderate inhibitor
ICRF-187	Catalytic inhibition	Human TopoII	Low μM inhibitor potency
	Sensitivity comparison	Yeast vs. human	Greater inhibition in human enzyme
Etoposide (Mechanistic)	DNA loop compaction & cleavage (optical tweezer assays)	Yeast Vs. human	Yeast TopoII exhibits stronger DNA compaction and higher breakpoint sensitivity

2.7.2.4 Molecular basis of differential sensitivity

Mutational studies in *S. cerevisiae* reveal amino acid residues responsible for altered drug sensitivity. Mutants such as S741W display hypersensitivity to etoposide, while substitutions like H507Y, M887I, R886I, H1012Y confer resistance to multiple drugs (168,175,176).

ATP binding site mutants e.g., G437S causes hypersensitivity to anticancer agents such as CP-115,953 and m-AMSA in the absence of ATP, but restores wild-type sensitivity in its presence, implicating conformational shifts (177,178). Similarly, the T744P mutation in the CAP homology domain increases cleavage activity independent of ATP, reflecting an alternative inhibition mechanism (179). Collectively, these studies help map the drug interaction landscape and identify residues essential for TopoII-targeting antifungals.

These structural differences guide the rational design of novel antifungal scaffolds and the repurposing of existing TopoII inhibitors for fungal selectivity.

2.7.3 Antifungal activity of TopoII inhibitors

2.7.3.1 Established TopoII inhibitors exhibiting antifungal activity

Evidence supporting fungal topoisomerase II as an antifungal target initially emerged from studies on established antibacterial and anticancer TopoII inhibitors. While bacterial gyrase inhibitors show minimal antifungal activity, several mammalian TopoII poisons, including etoposide, aclarubicin, and idarubicin, were reported to inhibit fungal growth, indicating exploitable functional divergence between fungal and human TopoII isoforms (114,120,122,164).

Among these agents, idarubicin consistently exhibited the strongest antifungal activity against *Candida* species, including fluconazole-resistant *Candida glabrata*. Biochemical and cellular studies demonstrated preferential inhibition of yeast TopoII, efficient fungal uptake, induction of DNA damage, mitochondrial dysfunction, and oxidative stress, resulting in fungicidal effects and suppression of virulence traits such as hyphal formation and biofilm development (Section 6). Additional support for TopoII as an antifungal target comes from studies on acridine-based TopoII poisons and quinacrine, which further linked TopoII interference with impaired fungal morphogenesis and pathogenicity (128–136,139,180).

Recent studies have highlighted the antifungal potential of acridine-based scaffolds. Rzad et al., demonstrated that imidazoacridinone and triazoloacridinone derivatives (IKE1-IKE14), designed as TopoII poisons, effectively inhibited yeast TopoII activity and showed fungicidal activity against *Candida* species. Notably, IKE7 displayed MIC_{90} values of 32-64 μg mL^{-1} against fluconazole-resistant *Candida glabrata* strains, while exhibiting a direct correlation between enzyme inhibition and antifungal efficacy, supporting fungal TopoII as a valid drug target (144).

Collectively, these findings provided critical proof-of-concept for fungal TopoII targeting and motivated the development of fungal-selective synthetic inhibitors with improved efficacy and safety profiles.

2.7.3.2 Acridine and Triazole based synthetic derivatives

Rzad et al., recently reported the synthesis of triazoloacridinone (IKE1-8) and imidazoacridinone (IKE9-14) derivatives, structurally derived from the prototypical TopoII poisons C-1305, C-1311. These agents demonstrated a multimodal antifungal profile. They stabilize the TopoII-DNA cleavage complex, induce reactive oxygen species (ROS), perturb fungal morphology, and disrupt biofilm formation. In

quantitative terms, the most active compounds exhibited MICs of 0.5-4 μg mL^{-1} against *Candida* species with IKE7 displaying notable potency against fluconazole-resistant *Candida glabrata* while retaining low mammalian cytotoxicity. Biochemical assays further established selective TopoII inhibition, with IC_{50} values ranging from 4-6 μM for yeast TopoII, contrasting with limited activity against the human isoform. Moreover, at concentrations approximating the MIC, these derivatives reduced *Candida albicans* biofilm biomass by 40-65%, thereby coupling antifungal activity with pronounced antibiofilm efficacy. Mechanistic studies corroborated through decatenation and DNA unwinding assays provided direct validation of their TopoII targeting mode of action (144).

Complementary studies by Kritika et al., further reviewed the multi-faceted potential of 1,2,4-triazole derivatives, noting their antifungal, anticancer, and antiviral activities (18). Docking and enzymatic assays confirmed their ability to simultaneously engage TopoI and TopoII, reinforcing the utility of these heterocycles in multi-target drug development strategies (181).

2.7.3.3 Polycyclic and heterocyclic compounds

Merzouki et al., developed heterocyclic scaffolds with triazole rings that enabled dual TopoII inhibition (182). Their selective cytotoxicity toward pathogenic cells, combined with low mammalian toxicity, underscores their promise. Further, Sinicropi et al. developed hybrid trimethoxybenzene, thiazolidinone and thiazole motifs that selectively targeted MCF-7 breast cancer cells while sparing non-cancerous MCF-10A cells. Docking and enzymatic studies confirmed TopoI/II inhibition, and scaffold optimization suggested preferential interaction with fungal isoforms (181).

2.7.3.4 Structure Activity Relationship (SAR) Insights

Extending the general SAR framework, fungal-specific studies have focused on exploiting structural divergences in fungal TopoII to achieve selective inhibition while minimizing off-target activity against human isoforms. In acridine derivatives, substitution at the ninth position (C9) with alkylamino or arylamino moieties significantly enhanced fungal cell permeability and improved inhibitory potency against *Candida* species, as reflected by reduced MIC values (183). Triazole analogs exhibited increased antifungal activity upon incorporation of electron-donating substituents, optimizing interactions with the fungal-specific K-loop and TOPRIM domains of TopoII (144).

Hybrid heterocyclic scaffolds, including imidazoacridinones and triazoloacridinones, demonstrated that strategic functionalization of polycyclic cores could simultaneously augment enzyme binding affinity and disrupt biofilm formation. Biochemical characterization revealed selective inhibition of yeast TopoII, with IC_{50} values in the 4-6 μM range, while exhibiting minimal activity against fluconazole-resistant *C. glabrata* strains, highlighting the translational potential of these compounds (144).

Nitrogen and sulphur containing heteroaryl or fused ring systems show activity against *Candida* species and filamentous fungi, including biofilms, while hybrid TopoII inhibitors may bypass conventional resistance mechanisms (119,184). The clinical urgency of this approach arises from the limited repertoire of antifungal classes polyenes, azoles, echinocandins, and antimetabolites whose efficacy is

eroded by target modification, efflux mediated resistance, and biofilm tolerance, particularly in azole-resistant *A. fumigatus* and multidrug- resistance *C. auris* (185).

Recent evidence also highlights thiophene-thiazole derivatives with docking supported interactions at fungal targets as well as indole-thiazole hybrids where halogen substitution enhances antifungal activity (186). Mechanistic insights suggest dual inhibition of fungal TopoII and interference with ergosterol biosynthesis (144). These modifications exploit subtle structural divergences between fungal and human TopoII, facilitating selective antifungal efficacy while minimizing host toxicity.

Collectively, such SAR-driven approaches exemplify how rational scaffold design can contribute to the development of next-generation antifungal agents and broaden the therapeutic landscape against resistant mycoses (Section 5).

3. Yeast Topoisomerase II as a model organism

3.1 Essential role in yeast viability

yTopoII is vital for *Saccharomyces cerevisiae* viability, with no functional redundancy from TopoI (79,112,187,188). yTopoII functions as a homodimer and mediates ATP and Mg^{+2} dependent supercoiling, decatenation, and knot resolution (79,82,112,188,189). Additionally, it participates in transcriptional regulation of long genes (>3kb) and chromatin remodeling, particularly during replication and mitosis (72,82,189).

3.2 Role in gene regulation, chromatin dynamics and morphogenesis

TopoII regulates transcriptional subsets in *S. cerevisiae* during stress or drug treatment, thus resulting in repression of essential gene pathways and arrest in nuclear division. Genes upregulated upon TopoII inactivation are often TATA-box containing and non-essential, linked to membrane transport. In contrast, downregulated genes are essential and involved in chromatin remodeling and transcription regulation (190,191). Characterization of the yTopoII gene showed the ORF encoding a 1429-amino acid polypeptide, providing a model system to study the structure-function relationship and drug responses (192).

In pathogenic fungi, TopoII additionally functions as a nuclear checkpoint in morphogenesis. In *C. albicans*, stabilization of DNA-TopoII cleavage complexes disrupts chromatin remodeling and transcriptional reprogramming essential for yeast-to-mycelium transition (193) . Both acridinone-based inhibitors and repurposed anticancer poisons (doxorubicin, etoposide, mitoxantrone) impair filamentation, directly linking TopoII activity to morphogenetic plasticity (144). Triazoloacridinones such as IKE5 and IKE7 completely blocked hyphal development, retained efficacy against fluconazole-resistant strains, and showed enhanced uptake with reduced cytotoxicity after lysine conjugation (144).

Together, these findings establish TopoII as a pivotal regulator at the intersection of nuclear processes and morphogenesis, linking genomic stability with virulence control.

3.3 Validation as a fungal-specific drug target

TopoII is an established therapeutic target in antibacterial and anticancer therapy (120,194–203). Drugs such as novobiocin, ciprofloxacin, and coumermycin inhibit bacterial gyrase, while etoposide, irinotecan, aclarubicin, and idarubicin target mammalian TopoII. Notably, fungal TopoII exhibits key structural distinctions from human homologs, supporting selective inhibition (94).

Importantly, inhibition of fungal TopoII yields fungicidal effects and concurrently attenuates fungal virulence by preventing yeast-to-mycelium transition, a key determinant of pathogenicity (193). Together, these findings highlight functional specificity and regulation of long-gene transcription and mitotic progression, reinforcing its candidacy as a selective and mechanistically validated antifungal drug target (204).

3.4 Structural divergence and selectivity hotspots

Comparative sequence and structure analyses reveal that while ATP-binding and catalytic residues (e.g., Tyr782 in yeast Vs. Tyr805 in humans) are conserved, domains such as the K-loop, TOPRIM, and DNA interaction motifs differ significantly (91,92,160,161,205). These regions constitute pharmacophoric “selectivity hotspots” that can be exploited for designing antifungal TopoII inhibitors (122).

These findings support the strategic design of antifungal agents targeting fungal TopoII, exploiting both conserved enzymatic function and structural divergence from human isoforms. This study specifically focuses on DNA TopoII as a strategic fungal-specific target. A set of known TopoII inhibitors and a set of newly synthesized acridine and acridone derivatives from our department are used to identify the selectivity among the fungal and human isoforms of the TopoII enzyme. Furthermore, in silico screening of chemical databases was performed to identify candidate molecules with high affinity for fungal TopoII. The top hits were subsequently validated through biochemical assays and microbiological evaluations, confirming their antifungal activity and target specificity.

Based on the previous studies we came to a hypothesis to improve antifungal drug discovery approaches that support my PhD objectives by demonstrating the feasibility of using computational and synthetic chemistry approaches to design selective antifungal agents targeting TopoII. This integrated workflow lays the groundwork for in vitro and in vivo evaluations, advancing promising candidates toward translational antifungal development.

4. AIM AND OBJECTIVES

4.1 AIM

Verification of the hypothesis of the possibility of using fungal topoisomerase II as a molecular target for antifungal chemotherapy.

4.2 OBJECTIVES

1. A high-throughput screening tests of antifungal and yeast topoisomerase II inhibitory effect of known, clinically used human topoisomerase inhibitors.

2. A high-throughput screening tests of antifungal and yeast topoisomerase II inhibitory effect of acridine and acridone derivatives (from the collection of the Department of Pharmaceutical Technology and Biochemistry)

3. The design of new inhibitors of fungal topoisomerase II - potentially promising compounds will be selected by Structure Activity Relationship (SAR) Analysis as well as by a high-throughput screening procedure (using molecular docking methods) of available chemical databases, followed by modification and further selection of the docking results.

4. The leading compounds inhibition studies against the above-mentioned yeast topoisomerase II enzyme and in vitro antifungal activity.

5. The mechanism of yeast topoisomerase II inhibition determination.

4.3 Significance of the study:

This research contributes to the broader field of antifungal chemotherapy by identifying novel chemical scaffolds with antifungal activity, offering a model for selective antifungal drug development based on molecular differences in conserved targets. It also provides insights into the structure-function relationships of fungal TopoII. This research aims to bridge the gap in antifungal drug discovery by identifying and validating fungal TopoII as a druggable target. By leveraging structural and biochemical differences between fungal and mammalian enzymes, it seeks to uncover novel antifungal compounds with high specificity, potentially expanding the therapeutic arsenal against life-threatening fungal infections.

5. TARGETING DNA TOPOISOMERASE II IN ANTIFUNGAL CHEMOTHERAPY

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The first article (Article 1) published in *Molecules (MDPI)* focuses on the potential of fungal DNA topoisomerase II as a novel molecular target for antifungal drug discovery. Topoisomerase II is a crucial enzyme responsible for regulating DNA topology during replication, transcription, chromosome segregation, and repair. Inhibition of this enzyme leads to DNA damage, including double-strand breaks, ultimately resulting in cell death.

This review highlights structural and functional aspects of fungal TopoII, particularly from *Saccharomyces cerevisiae*, and compares it with human TopoII isoforms. Although both enzymes share overall structural similarity, important differences exist in regions such as the TOPRIM domain, K-loop, and DNA-binding interfaces. These variations provide opportunities for designing selective inhibitors that target fungal enzymes without affecting human counterparts.

The mechanism of TopoII involves a multi-step strand passage process requiring ATP hydrolysis and transient DNA cleavage. Based on their mode of action, inhibitors are classifies into two groups: catalytic inhibitors, which reduce enzyme activity, and Topo II poisons, which stabilize the enzyme-DNA cleavage complex. Several known anticancer drugs, including etoposide, doxorubicin, and m-AMSA, act as Topo II poisons and have been investigated for antifungal potential.

The article also discusses the differential sensitivity of fungal and human Topo II enzymes to various inhibitors. For example, compounds like ICRF-187 and resveratrol show higher activity against human enzymes, whereas certain quinolone derivatives and natural compounds demonstrate better selectivity toward fungal Topo II. Additionally, mutation studies in yeast TopoII have identified key amino acid residues responsible for drug sensitivity or resistance, providing valuable insights for drug design.

Importantly, acridine and its derivatives are highlighted as promising antifungal agents due to their ability to intercalate into DNA and inhibit TopoII activity. Some acridine-based compounds have demonstrated strong antifungal activity, including against drug-resistant strains, often correlating with inhibition of TopoII-mediated DNA relaxation.

Overall, this study emphasizes that fungal topoisomerase II is an essential and underexplored target for antifungal chemotherapy. Structural differences between fungal and human enzymes, along with differential drug sensitivity, support the fungal and human enzymes, along with differential drug sensitivity, support the possibility of developing selective antifungal agents. Future research focusing on these differences and incorporating medicinal chemistry approaches may lead to the discovery of novel and effective antifungal drugs.

Article 1

Targeting DNA topoisomerase II in antifungal chemotherapy

Molecules, 2022, 27(22), 7768; doi: 10.3390/molecules27227768

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IF_{2022}=4.6, Q2, Ministry of Science and Higher Education Points (MHES): 140

6. ANTICANCER DRUGS TARGETING TOPOISOMERASE II FOR ANTIFUNGAL TREATMENT

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The second article (Article 2) published in *Scientific Reports (Nature Publishing group)* focuses on evaluating anticancer drugs as potential antifungal agents through their interaction with fungal TopoII. Since TopoII is highly conserved between humans and fungi, but possesses subtle structural differences, these variations may be exploited to identify compounds with improved specificity towards the fungal enzyme.

The study explores several well-known TopoII-targeting anticancer drugs, including etoposide, doxorubicin, amsacrine, and mitoxantrone, for their antifungal potential. These compounds are classified as TopoII poisons, which act by stabilizing the transient DNA-TopoII cleavage complex, leading to accumulation of double-strand DNA breaks and ultimately cell death. While these drugs are primarily used in cancer therapy, their mechanism of action makes them suitable candidates for antifungal applications.

The research highlights that some of these compounds exhibit inhibitory activity against fungal TopoII, particularly from *Saccharomyces cerevisiae,* suggesting their potential as antifungal agents. However, one of the major challenges is the lack of selectivity, as these drugs can also affect human TopoII, leading to toxicity. Therefore, understanding the structural differences between fungal and human TopoII is critical for designing selective inhibitors.

Additionally, the study also discusses the possibility of modifying existing anticancer drugs to enhance their antifungal properties while reducing cytotoxic effects on human cells.

Overall, this study demonstrates that anticancer drugs targeting TopoII represent a promising starting point for antifungal drug development. Although challenges related to selectivity and toxicity remain, combining biochemical studies with computational modeling and medicinal chemistry approaches may lead to the development of novel antifungal agents with improved efficacy and safety profiles.

Article 2

Anticancer drugs targeting topoisomerase II for antifungal treatment

Scientific Reports, 2025, 15, 9311; doi: 10.1038/s41598-025-93863-z

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IF_{2025}=4.379, Q1, Ministry of Science and Higher Education Points (MHES): 140

7. STRUCTURAL INSIGHTS INTO FUNGAL AND HUMAN TOPOISOMERASE II WITH IMPLICATIONS FOR IN SILICO ANTIFUNGAL DRUG DESIGN

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The third article (Article 3) published in *Scientific Reports (Nature Publishing group)* focuses on comparative structural analysis of fungal and human TopoII enzymes to support structure-based antifungal drug design. Using computational approaches, including homology modeling, molecular docking, and molecular dynamics simulations, the study examines key structural features of TopoII from *Saccharomyces cerevisiae* and compares them with human TopoII.

Despite overall structural similarity, important differences were identified in functional domains such as ATPase region, TOPRIM domain, and DNA-binding interface. These variations influence ligand binding and provide opportunities for designing selective inhibitors that preferentially target fungal TopoII. The study also evaluates the binding interactions of known TopoII inhibitors, revealing differences in biding affinity and stability between fungal and human enzymes.

The findings highlight that specific amino acid residues and structural motifs unique to fungal TopoII can be exploited for selective drug design. In silico analysis further supports the potential of repurposing and optimizing existing TopoII-targeting compounds for antifungal applications.

Overall, this study demonstrates that integrating structural biology with computational modeling is a powerful strategy for identifying and designing novel antifungal agents targeting TopoII, with improved selectivity and reduced toxicity toward human cells.

Article 3

Structural insights into fungal and human topoisomerase II with implications for in silico antifungal drug design

Scientific Reports, 2025, 15, 9467; doi: 10.1038/s41598-025-93122-1

Accepted: 04th March, 2025; Published: 19th March, 2025

IF_{2025}=4.379, Q1, Ministry of Science and Higher Education Points (MHES): 140

8. BISACRIDINE DERIVATIVES AS EFFECTIVE EUKARYOTIC TOPOISOMERASE II INHIBITORS

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The fourth article (Article 4), published in European Journal of Medicinal Chemistry, focused on design, synthesis, and biological evaluation of novel bisacridine derivatives as potential antifungal agents targeting eukaryotic topoisomerase II, with particular emphasis on selective inhibition of the fungal enzyme.

The rationale for this study arises from the increasing incidence of fungal infections, especially those caused by *Candida* species, and the growing resistance to conventional antifungal drugs. Although bisacridines are well known for their antitumor activity, their antifungal potential and mechanism of action remain insufficiently explored. Therefore, the objective of this study as to design structurally modified bisacridine derivatives and evaluate their inhibitory activity against yeast TopoII, antifungal efficacy, selectivity over the human enzyme, and underlying molecular mechanisms.

The research involved a series of symmetrical and unsymmetrical bisacridine derivatives, as well as corresponding monoacridines, differing in linker structure and substituents within the acridine moieties. The obtained compounds were characterized using spectroscopic methods, including NMR and mass spectrometry, and their purity as confirmed by chromatographic techniques. Subsequently, the biological activity of the synthesized compounds was evaluated using in vitro enzymatic assays to determine their inhibitory effects on yeast and human TopoII. The relaxation and decatenation assays were employed to assess enzyme inhibition, while during unwinding assays were conducted to investigate potential intercalation mechanisms.

Among the analyzed derivatives, compound IKE16 demonstrated the most potent and selective inhibition of yeast TopoII, with significantly lower activity against the human counterpart. Importantly, its mechanism of action differed from classical topoisomerase II poisons, as it did not induce DNA double-strand breaks and exhibited limited DNA intercalation, suggesting a non-intercalating mode of inhibition. In contrast, several other derivatives, such as IKE19 and IKE21, showed the ability to intercalate into DNA, indicating a dual mechanism involving both enzyme inhibition and DNA interaction.

To further elucidate the molecular basis of selectivity, in silico studies, including molecular docking simulations, were performed using models of yeast and human TopoII-DNA complexes. The results revealed that bisacridine derivatives, particularly IKE16, exhibit stronger and more specific interactions within the yeast enzyme binding pocket, especially involving key amino acid residues such as GLU831. In contrast, interactions with the human enzyme were weaker and more dispersed, which may explain the observed selectivity. These findings suggest that structural differences between fungal and human TopoII can be exploited for the development of selective antifungal agents.

The antifungal activity of the synthesized compounds was evaluated against a panel of reference and clinical fungal strains, including fluconazole-resistant isolates. The most active derivatives, namely IKE18, IKE19, and IKE21, exhibited significant antifungal effects and, importantly, were able to overcome resistance to fluconazole. In contrast, although IKE16 showed strong enzymatic inhibition, its antifungal activity was more limited, likely due to reduced cellular uptake or other biological factors. Additionally, selected compounds demonstrated the ability to induce oxidative stress in fungal cells, suggesting that reactive oxygen species generation may contribute to their antifungal mechanism.

Cytotoxicity studies performed on human cell lines indicated that some derivatives, particularly IKE18 and IKE19, exhibited a more favorable balance between antifungal activity and toxicity, as reflected by higher selectivity indices. Conversely, IKE16, despite its high enzymatic selectivity, showed increased cytotoxicity, indicating the need for further structural optimization.

In summary, this study demonstrated that bisacridine derivatives represent a promising class of antifungal agents targeting yeast Topoisomerase II. The result highlight the importance of structural modifications in determining enzyme selectivity, mechanism of action, and biological activity. Moreover, the identification of compounds capable of overcoming antifungal resistance underscores their potential therapeutic relevance. Further optimization and detailed mechanistic studies are required to improve their selectivity and reduce toxicity, paving the way for the development of novel antifungal drugs.

Article 4

Bisacridine derivatives as effective eukaryotic topoisomerase II inhibitors

European Journal of Medicinal Chemistry, 2026, 301, 118174; doi.org/10.1016/j.ejmech.2025.118174

IF_{2025}=5.9, Q1, Ministry of Science and Higher Education Points (MHES): 140

Accepted: 12th September, 2025; Published: 5th January, 2026.

9. LIST OF ABBREVIATIONS

ACE2: Angiotensin-Converting Enzyme 2

ADPT cluster: Alanine-Aspartic Acid-Proline-Threonine

AMPs: antimicrobial peptides

BE: Binding Energy

CAP: Catabolite Activator Protein

CDR: *Candida* Drug Resistance

DSB: Double Stranded Breaks

ER: Estrogen Receptor

Erg11: Lanosterol 14α-demethylase

FRET: Fluorescence Resonance Energy Transfer

G-DNA: Gate-segment DNA

hTopoII: Human Topoisomerase II

MD: Molecular Dynamics

MDR: Multi-Drug Resistant

MFC: Minimum Fungicidal Concentration

MIC: Minimum Inhibitory Concentration

mtDNA: Mitochondrial DNA

ORF: Open Reading Frames

PCR: Polymerase Chain Reaction

PLB: Phospholipase B gene

PPAr-γ: Peroxisome Proliferator-Activated Receptor Gamma

ROS: Reactive Oxygen Species

SAR: Structure-Activity-Relationship

Sc: *Saccharomyces cerevisiae*

SCGK cluster: Serine-Cysteine-Glycine-Lysine

ScTopoII: *Saccharomyces cerevisiae* Topoisomerase II

SSRI: Selective Serotonin Reuptake Inhibitor

TAD: Topologically Associating Domains

TDM: Therapeutic Drug Monitoring

T-DNA: Transported-segment DNA

TOP-DPCS: Topoisomerase DNA-Protein Crosslinks

TOPRIM: Topoisomerase-Primase

TopoII: Topoisomerase II

WHD: Winged Helical Domain

10. SUMMARY

This study demonstrates that yeast DNA topoisomerase II (ScTopoII) is a promising molecular target for antifungal drug development. Although many compounds were strong ScTopoII inhibitors, only a select few, such as idarubicin, and certain acridine and bis-acridine derivatives, showed effective antifungal activity, primarily due to their inability to penetrate fungal cells and evade efflux pumps. Notable, TopoII inhibition had little impact on fungal virulence factors like morphological switching and biofilm formation. Structural differences between fungal and human TopoII- particularly in the HLR region and K-loop interactions-provide a basis for designing selective inhibitors. Integrated workflow for antifungal drug discovery targeting yeast Topoisomerase II demonstrated in this study is presented in Figure 4.

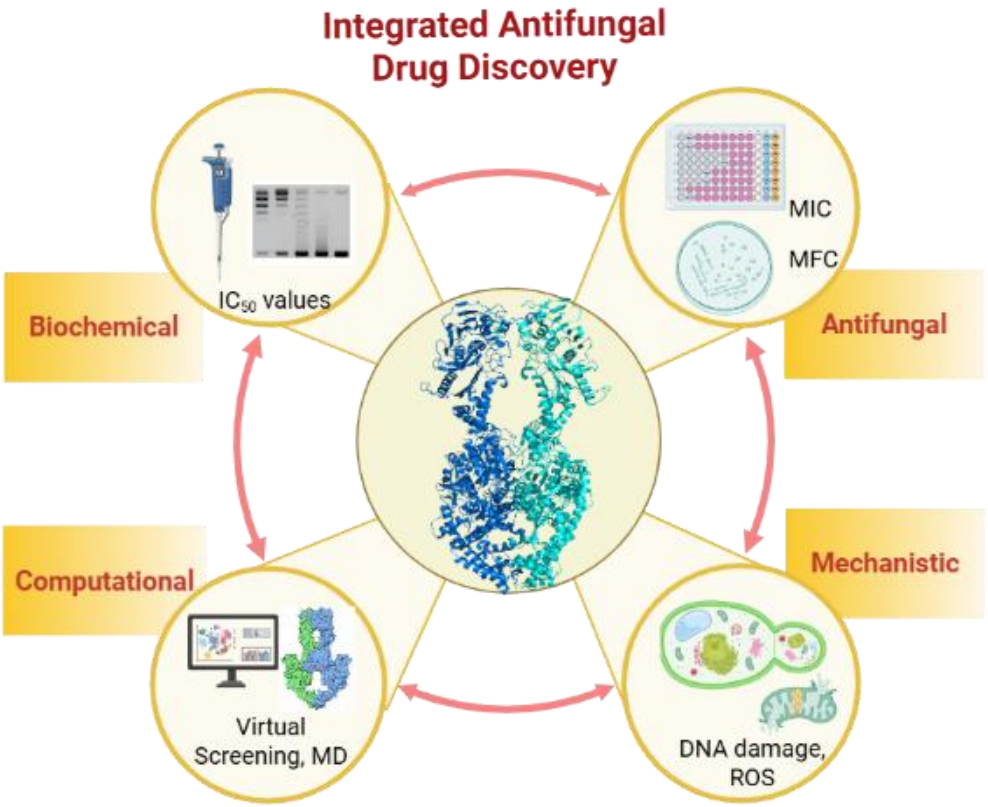

Figure 4: Integrated workflow for antifungal drug discovery targeting yeast Topoisomerase II. Created in http://Biorender.com

Article 1, which presents a comprehensive review of fungal TopoII as a drug target, lays the conceptual foundation of this thesis. It highlights the urgent need for novel antifungal strategies due to rising drug resistance and emphasizes the potential of fungal TopoII given its essential role in DNA metabolism and its structural differences from the human homolog. The paper categorizes existing TopoII inhibitors, differentiating between catalytic inhibitors and TopoII poisons, and provides a framework for identifying fungal-specific inhibitors that can be selectivity toxic to fungal cells.

Article 2 translates this conceptual groundwork into experimental validation by assessing the antifungal potential of eleven known anticancer drugs. Despite many of them being potent inhibitors of

yeast TopoII in vitro, only a few demonstrated effective antifungal activity, suggesting that cellular penetration and efflux pump evasion are critical for in vivo efficacy. Idarubicin was identified as the most effective compound, exhibiting fungicidal activity through mechanisms such as DNA fragmentation, mitochondrial damage, oxidative stress induction, and vacuole formation. These effects were observed even in fluconazole-resistant strains, positioning idarubicin as a strong candidate for antifungal repurposing.

Article 3 builds upon these results by providing a detailed structural comparison of fungal and human TopoII enzymes. Through homology modelling and docking studies, the research identifies unique structural features-particularly in the HLR and K-loop regions of fungal TopoII that are absent or differently configures in the human enzyme. These findings pave the way for rational, structure-guided drug design using in silico high-throughput screening.

Article 4 demonstrates work that bisacridines can be chemically optimized to exploit subtle structural divergences between fungal and human TopoII, achieving selective inhibition while maintaining antifungal potency. The integration of enzymatic profiling, antifungal susceptibility testing, and docking-based interaction mapping establishes a clear framework for rational lead optimization, positioning these compounds as promising scaffolds for next-generation antifungal drug development.

11. ACKNOWLEDGEMENTS

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OPUS grant NZ project “Yeast DNA topoisomerase II as a model molecular target for novel antifungal compounds with grant number UMO-2022/45/B/NZ6/01149 from October 2025 to September 2026.

I would like to thank Springer Nature for granting permission to reproduce the figure used in Chapter 1 of this thesis, originally published in Nature Microbiology.

I am grateful to the authors of the original works in the Journal of Fungi for making their research available via open access, which allowed for the reproduction of Figure 3 in Chapter 1 in this thesis under the CC BY 4.0 license.

12. SCIENTIFIC ACHIEVEMENTS

A. PUBLISHED ARTICLES IN SCIENTIFIC JOURNALS

1. **Kondaka, K.**, & Gabriel, I.* (2022) Targeting DNA topoisomerase II in antifungal chemotherapy. *Molecules.*, 27(22), 7768.

2. **Kondaka, K.**, Rząd, K., Maciejewska, N., & Gabriel, I.* (2025) Anticancer drugs targeting topoisomerase II for antifungal treatment. *Sci. Rep.*, 15, 9311.

3. Sappati, S., **Kondaka, K.**, Gabriel, I.*, & Baginski, M.* (2025) Structural insights into fungal and human topoisomerase II with implications for in silico antifungal drug design. *Sci. Rep.*, 15, 9467.

4. **Kondaka, K.**, Sappati, S., Rząd, K., Paluszkiewicz, E., Maciejewska, N., Baginski, M., & Gabriel, I. (2026) Bisacridine derivatives as effective eukaryotic topoisomerase II inhibitors. *Eur. J. Med. Chem.,* 301, 118174.

5. Ezzemani, W., Kettani, A., Sappati, S., **Kondaka, K.**, El Ossmani, H., Tsukiyama-Kohara, K., Altawalah, H., Saile, R., Kohara, M., Benjelloun, S., & Ezzikouri, S. (2023). Reverse vaccinology-based prediction of a multi-epitope SARS-CoV-2 vaccine and its tailoring to new coronavirus variants. *J. Biomol. Struct. Dyn.*, *41*(11), 4917–4938.

B. SCIENTIFIC CONFERENCES

1. 06/09/2022-09/09/2022 - 4th international symposium on frontiers in molecular science, Florence, Italy. Poster presentation entitled “Acridine as antifungal agents- Topoisomerase II targeting
Authors: Rząd, K.; **Kondaka, K.**; Olszewski, M.; Paluszkiewicz, E.; Kozlowska-Tylingo, K.; Gabriel, I.

2. 13/09/2023 -16/09/2023 - 5th Congress of Polish Biosciences BIO2023 – “Different faces of biosciences” and 52nd Meeting of the Polish Biochemical Society, Szczecin, Poland.
Presentation of the poster entitled “Yeast DNA topoisomerase II targeting: the search for an antifungal compound with a novel mechanism of action.”
Authors: Rząd, K.; **Kondaka, K.**; Jaroszewska, W.; Paluszkiewicz, E.; Kozłowska- Tylingo, K.; Gabriel, I.,

3. 07/04/2025-09/04/2025 - 1st International Conference on Advancements of Microbiology: The relevance of microbes in tackling threats to health and environment, Warsaw, Poland.

Poster presentation entitled “Development and evaluation of bis-acridine derivatives as novel antifungal agents targeting yeast topoisomerase II”.

Authors: Rząd, K.; **Kondaka, K.**; Paluszkiewicz, E.; Olszewski, M.; Kozłowska-Tylingo, K.; Gabriel, I.

4. 14/07/2025-17/07/2025 - FEMS Micro-Milan Congress & Exhibition, Milan, Italy.

Poster presentation entitled “Symmetrical and Unsymmetrical Bisacridines as Potential Antifungal Agents with Ability to Overcome Fluconazole Resistance”

Authors: **Kondaka, K.**; Rząd, K.; Paluszkiewicz, E.; Olszewski, M.; Gabriel, I.

5. 15/09/2025-19/09/2025 - EMBO Workshop: DNA Topology and topoisomerases in genome dynamics, Szklarska Poreba, Poland

Poster presentation entitled “Breaking Barriers in Antifungal Therapy: A Computational and Experimental Journey with Topoisomerase II”

Authors: **Kondaka, K.**; Sappati, S.; Rząd, K.; Bagiński, M; Gabriel, I.

C. SCIENTIFIC INTERNSHIPS

ENHANCE Research Internship Programme 2025-2026 entitled “Application of peptide-centric local stability assay (PELSA) based chemo-proteomics coupled with DIA mass spectrometry in *Saccharomyces cerevisiae*” under the guidance of Prof. Markus Künzler, Institut für Mikrobiologie, ETH Zürich, Switzerland from 1st March to 31st May, 2026.

D. PARTICIPATION IN PROJECTS

OPUS grant NZ project “Yeast DNA topoisomerase II as a model molecular target for novel antifungal compounds with grant number UMO-2022/45/B/NZ6/01149 from October 2025 to September 2026.

E. SCHOLARSHIPS

11/2021-09/2022	POLONIUM International doctoral fellowship under the IDUB programme
10/2022-11/2022, and 04/2023-09-2023	Francium Supporting outstanding doctoral candidates – scholarship of the IDUB programme

10/2025-01/2026	Francium Supporting Outstanding doctoral candidates – scholarship of the IDUB programme
03/2026-05/2026	ENHANCE Research Internship 2025 financed under the programme Support for European University Alliances, no. FERS.01.05-IP.08-0219/23 – call 2024, project titled "Strengthening the Potential of Gdańsk University of Technology within the ENHANCE Alliance", no. BPI/WUE/2024/1/00020 financed by Polish National Agency for Academic Exchange (NAWA).

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2. Odds FC. Antifungal Agents: Resistance and Rational Use.
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14. APPENDIX

14.1 LIST OF FIGURES

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14.2 List of Tables

INTRODUCTION

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