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Bioorg. Med. Chem. Rep. 8:2 (2025) 28-53

Bioorganic & Medicinal Chemistry Reports

Novel multi-axis therapeutic targeting approaches in *Candida albicans*: emerging dual-target strategies to overcome antifungal resistance

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(Received December 01, 2025; Revised December 22, 2025; Accepted December 23, 2025)

Abstract: Global climate change, the increasing prevalence of immunosuppressed patient populations, and mounting environmental selection pressures have collectively enhanced the adaptive capacity of pathogenic fungi (most notably *Candida albicans*) transforming invasive fungal infections into a major global clinical threat. The limited chemical diversity and narrow target spectrum of current antifungal agents, particularly those acting through a unidirectional mechanism centered on CYP51 inhibition, facilitate the rapid emergence of drug resistance. *C. albicans* simultaneously activates a network of interconnected resistance mechanisms to survive antifungal exposure. CYP51 point mutations and gene amplification, overexpression of efflux pumps, HSP90–calcineurin–dependent stress adaptation, and epigenetic reprogramming mediated by HDAC/HAT imbalance constitute the core components of this defensive architecture. Moreover, the central regulatory role of the EGFR–MAPK axis in epithelial invasion underscores that *C. albicans* pathobiology extends far beyond metabolic pathways, relying instead on the coordination of a sophisticated signaling network. This multilayered defense system inherently restricts the efficacy of single-target antifungal agents. Consequently, combination therapies that simultaneously engage distinct biological pathways hold the potential to enhance antifungal efficacy through pharmacodynamic synergy. However, substantial variability in clinical responses, drug–drug interactions and limited translational evidence have hindered the routine implementation of combination regimens in clinical practice. These constraints have positioned dual-target antifungal design as a more rational and feasible alternative. Notably, clinical evidence supports multi-axis antifungal engagement, with late-stage pipelines advancing toward novel resistance-relevant targets such as the Phase 3 Gwt1 inhibitor fosmanogepix. Recent studies on dual active architecture, including CYP51 and HDAC, HSP90 and HDAC, CYP51 and SE, as well as CYP51 and HSP90, identify four core axes governing *C. albicans* fitness: ergosterol biosynthesis, epigenetic regulation, proteostatic stress response, and epithelial invasion. Coordinated modulation of these axes results in synergistic suppression of antifungal resistance and virulence. In this review, we provide an integrated evaluation of the molecular foundations of antifungal resistance in *C. albicans*, the pharmacodynamic advantages of combination therapies, and the therapeutic promise of dual-target design strategies. Collectively, the evidence supports multi-target antifungal strategies as a transformative paradigm capable of achieving durable, resistance-agnostic efficacy.

Keywords: Invasive fungal infection; histone deacetylase; ergosterol; lanosterol 14α-demethylase; dual inhibitors.

1. Introduction

Fungal infections have emerged as increasingly prominent global health threats. Recent meta-analyses estimate approximately 6.5 million invasive fungal infections (IFIs) annually, with 3.8 million deaths of which nearly 2.5 million are attributed directly to fungal pathogens [1]. These infections pose

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The article was published by ACG Publications
http://www.acgpubs.org/journal/bioorganic-and-medicinal-chemistry-reports © July-December 2025 EISSN: 2667-6028
DOI: http://doi.org/10.25135/bmcr.36.2512.3745
Available online: December 30, 2025

a particularly severe risk to individuals with impaired immunity or immune dysregulation, such as patients with HIV/AIDS, in whom fungal diseases can become rapidly fatal [2]. The emergence of drug-resistant fungal species (such as *Candida* strains shaped by climate-driven selective pressures) [3] alongside the increased incidence of COVID-19 associated fungal infections has further complicated the already limited therapeutic landscape [4]. Diagnostic limitations (including low-sensitivity culture methods, delayed detection, and substantial underreporting) further obscure the true burden of disease [5]. Major risk factors include immunosuppression (e.g., chemotherapy, organ transplantation, corticosteroid therapy), prolonged hospitalization in high-risk units, catheterization, and sustained antimicrobial or antifungal drug pressure. In parallel, global warming, extreme climate events, geographic expansion of fungal habitats, adaptive fungal evolution, and intensified fungicide exposure have collectively accelerated the spread of pathogenic fungi and the development of antifungal resistance, thereby magnifying the global IFI burden. Recognizing these alarming trends, the World Health Organization (WHO) has designated critical species (including *Candida*, *Aspergillus*, and *Cryptococcus*) as highest-priority threats in its 2022 Fungal Priority Pathogens List [6,7]. Climate change has emerged as a major ecological driver shaping both the expanding burden and the geographical redistribution of fungal infections. Rising global temperatures, extreme weather events, and the expansion of fungal ecological niches facilitate pathogen proliferation, geographic spread, and the selection of adaptive traits such as thermotolerance and enhanced virulence. These climate-driven pressures, together with intensified agricultural fungicide use, accelerate the emergence of antifungal resistance and further limit the efficacy of existing therapies. Collectively, this evolving landscape underscores the urgent need to move beyond classical single-target antifungal strategies toward resistance-modulating and mechanistically innovative therapeutic approaches (Figure 1) [8].

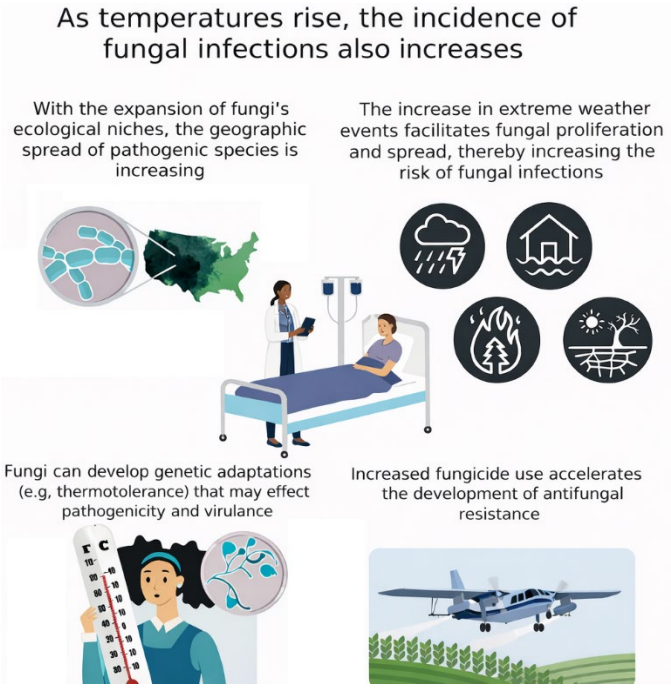


Figure 1. Potential impacts of climate change on pathogenic fungi and fungal infections.

Currently available antifungal agents used in the treatment of fungal infections display considerable chemical diversity and are generally classified into four major groups: azole derivatives (e.g., fluconazole, voriconazole, itraconazole, and posaconazole), polyenes (notably amphotericin B), echinocandins (e.g., micafungin and caspofungin), and pyrimidine analogues (flucytosine). Among these, azole-based compounds (including imidazole, triazole, and tetrazole derivatives) remain the most widely utilized systemic antifungal agents. Azoles exert their pharmacological activity by targeting ergosterol biosynthesis, a fundamental process required for fungal cell membrane integrity. Specifically, they inhibit lanosterol 14α-demethylase (CYP51), the enzyme that catalyzes the 14α-demethylation step

of lanosterol. Inhibition of this step blocks ergosterol production, disrupts membrane fluidity and permeability, and ultimately leads to cell death. Although this mechanism renders azoles indispensable in both clinical and research settings, it also imposes a significant selective pressure that promotes resistance development [9].

Despite their therapeutic advantages, the increasing prevalence of azole resistance has become a major global concern [10,11]. Pathogenic fungi have evolved multiple mechanisms to evade the effects of azole drugs. Mutations within lanosterol 14α-demethylase, the primary target enzyme, can reduce azole-binding affinity, thereby diminishing inhibitory potency [12]. Alternatively, overexpression of efflux pumps facilitates active drug extrusion from fungal cells, lowering intracellular azole concentrations and reducing efficacy [13,14]. Surveillance studies further indicate an alarming upward trend in azole resistance among fungal pathogens. Among *Candida* species, antifungal resistance has been extensively documented, particularly in *C. albicans* and *Nakaseomyces glabratus* (*Candida glabrata*) [15,16]. This challenge is further exacerbated by the emergence of *Candidozyma auris* (*Candida auris*), a multidrug-resistant pathogen associated with persistent nosocomial outbreaks [17].

Similarly, resistance in *Aspergillus* species, notably against itraconazole and voriconazole, has critical implications for the management of aspergillosis a life-threatening condition particularly in immunocompromised individuals [18,19]. Importantly, azoles may lead to therapeutic failure even in *Candida* isolates that appear susceptible *in vitro*; this phenomenon is largely attributed to the fungus's intrinsic capacity to tolerate and neutralize azole compounds at the cellular level. Such intrinsic tolerance reduces the effective drug accumulation at the target site, weakening the inhibitory impact on CYP51 and compromising clinical efficacy [20].

Intrinsic tolerance is inherently linked to the fungistatic mode of action of azoles. Although tolerant *Candida* isolates exhibit minimum inhibitory concentration (MIC) values comparable to susceptible strains, their ability to sustain growth even at supra-MIC concentrations markedly differentiates them. This capacity to proliferate despite pharmacologically inhibitory doses represent a key biological basis for the incomplete fungistatic effect of azoles and contributes substantially to treatment failure [21]. Ultimately, azole tolerance in *Candida* species undermines the therapeutic performance of azole drugs in candidiasis, facilitates the stepwise evolution of acquired resistance, and plays a major role in persistent candidemia cases [22].

2. *Candida albicans*: New Therapeutic Paradigms Beyond Drug Resistance

Candida species rank among the leading causes of nosocomial bloodstream infections and are associated with higher acute mortality rates compared with major bacterial pathogens [23]. Moreover, the prevalence of drug-resistant *Candida* isolates in clinical settings is alarmingly high. Surveillance data collected by the Centers for Disease Control and Prevention (CDC) indicate that a substantial proportion of *Candida* strains isolated from hospitalized patients with bloodstream infections exhibit resistance to commonly used antifungal agents. Approximately 90% of *Candida* isolates show resistance to at least one commercially available antifungal drug, while nearly 30% demonstrate reduced susceptibility to multiple antifungal agents [24].

Candidemia and invasive *Candida* infections represent a major global clinical threat, with reported mortality rates ranging from 36% to 60% across the United States, Europe, Asia, and Latin America. These infections disproportionately affect high-risk populations, including patients with hematological or solid organ malignancies, individuals receiving immunosuppressive therapies, transplant recipients, recent surgical patients, and those undergoing hemodialysis. In these settings, the use of central venous catheters, parenteral nutrition, and intravascular medical devices promotes biofilm formation, which substantially undermines antifungal efficacy and contributes to persistent infection and poor clinical outcomes. Although *Candida* species constitute a natural component of the human microbiota, they can cause endogenous infections when host defense mechanisms are compromised. Under conditions of immune dysfunction, these opportunistic fungi can disseminate and invade deep-seated tissues, including visceral organs, the vascular system, bone and joints, the eyes, and the central nervous system [25].

Candidemia is a severe systemic infection resulting from the proliferation of *Candida* species in the bloodstream and is associated with high mortality. Among the primary causative agents, *C.*

albicans (normally a commensal organism residing on human mucosal surfaces) possesses the capacity to rapidly transition to a pathogenic phenotype when host immunity is impaired. Clinical evidence identifies *C. albicans* as one of the most frequent etiological agents of invasive candidiasis. Globally, nearly 1.6 million cases of *Candida* bloodstream infections are reported annually, with an estimated mortality approaching 64% [1,26].

C. albicans is one of the most clinically significant opportunistic yeast pathogens in humans, and the integrity of its cell membrane is a critical determinant of susceptibility to antifungal agents. Ergosterol is the principal sterol component of the fungal membrane, and its biosynthesis directly influences membrane fluidity and permeability. The rate-limiting enzyme of this pathway, squalene epoxidase (SE), catalyzes the first oxidative step in sterol biosynthesis by converting squalene into 2,3-oxidosqualene. This reaction ensures the continuous production of ergosterol and maintains membrane stability. Pharmacological inhibition of SE leads to intracellular squalene accumulation and ergosterol depletion, resulting in disrupted lipid composition, impaired ion homeostasis, and ultimately loss of membrane integrity. These structural and functional defects trigger an irreversible cascade culminating in cell death. Thus, SE is not only a central regulator of ergosterol metabolism but also a strategically important therapeutic target due to its essential role in survival, virulence, and antifungal resistance mechanisms of *C.* albicans [27].

As a pathogen, *C. albicans* exhibit remarkable adaptability, rapidly transitioning from a commensal to a pathogenic phenotype under conditions of immune suppression or microbiota imbalance. This transition is supported by multilayered virulence strategies that enable immune evasion and resistance to antifungal therapy. Key contributors to its pathogenicity include biofilm formation, yeast-to-hyphal morphological switching, overexpression of adhesion molecules such as the ALS gene family and HWP1, and activation of efflux pump systems including CDR1 and MDR1. During the shift to a pathogenic state, surface invasin proteins (particularly Als3, Ssa1, and Hyr1) interact directly with host epithelial receptors such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and c-Met, thereby triggering receptor-mediated endocytosis. This interaction compromises epithelial barrier integrity and constitutes a primary molecular mechanism of invasion and tissue penetration. In parallel, the peptide toxin candidalysin, secreted by *C. albicans*, induces EGFR phosphorylation and activates the MAPK signaling cascade. This signaling axis amplifies epithelial cell damage, inflammatory responses, and invasion depth, facilitating the progression of infection from local mucosal surfaces to systemic dissemination. Collectively, the dynamic molecular dialogue established by *C. albicans* with its host (mediated through both invasin-driven receptor activation and toxin-induced signaling) constitutes a bidirectional regulatory network central to fungal pathogenesis. Deciphering this network, particularly through pharmacological blockade of receptor–invasin interactions, holds substantial promise for the development of next-generation host-targeted antifungal therapeutic strategies [28,29].

Resistance mechanisms in *C. albicans* clearly demonstrate that adaptive responses to antifungal therapy are tightly integrated with epigenetic regulation and stress-signaling pathways. In particular, histone deacetylases (HDACs) play a pivotal role in shaping the molecular basis of antifungal resistance by dynamically modulating chromatin architecture and thereby controlling gene expression at the epigenetic level. Through transcriptional repression or activation, HDACs regulate key resistance-associated targets such as the ergosterol biosynthesis gene CYP51 (ERG11) and efflux pump genes including CDR1, CDR2, and MDR1. Enhanced HDAC activity promotes transcriptional tolerance to antifungal agents, reduces gene silencing, and facilitates the epigenetic reprogramming of resistance-related gene clusters. Consequently, HDAC inhibitors, when co-administered with azole antifungals, induce transcriptional rebalancing, downregulate CYP51 expression, limit efflux pump activity, and substantially enhance cellular susceptibility. This epigenetic intervention functions as a complementary mechanism to classical inhibition of ergosterol biosynthesis and significantly potentiates antifungal efficacy. Therefore, HDAC-mediated regulation represents not only an epigenetic checkpoint in resistance but also a clinically actionable therapeutic node that can be targeted to achieve azole synergy [30].

Chaperone-mediated resistance constitutes another critical regulatory network supporting the molecular persistence of antifungal tolerance in *C. albicans*. Heat shock protein 90 (HSP90), a central molecular chaperone responsible for protein folding under stress conditions, stabilizes the calcineurin

and MAPK signaling cascades, thereby maintaining cellular resilience against antifungal agents. This protective role of HSP90 supports both the persistence of tolerance in azole-resistant strains and the profound drug resistance characteristic of biofilm-associated cells. Pharmacological or genetic inhibition of HSP90 disrupts the integrity of these stress adaptation networks, leading to destabilization of the calcineurin and MAPK pathways. This disruption yields strong synergistic fungicidal activity when combined with azoles and markedly enhances *in vivo* antifungal efficacy when used in combination with echinocandins. In addition, HSP90 inhibition (exemplified by agents such as ganetespib or related inhibitors) downregulates the expression of CYP51 and efflux pump genes (CDR1, MDR1). Accordingly, HSP90 functions not only as a protein-stabilizing chaperone but also as a determinant of transcriptional regulation within resistance-associated gene networks. These properties position HSP90-targeted combination strategies as an innovative therapeutic approach that strengthens conventional antifungal agents and enables eradication of drug-resistant *C. albicans* strains [31-34].

3. Fungal Targets

Beyond sterol biosynthesis, fungal survival and pathogenicity are sustained by multiple druggable nodes spanning cell wall construction, membrane anchoring, mitochondrial energetics, nucleotide and protein biosynthesis, and proteostasis. Clinically validated cell-wall targets such as β-(1,3)-D-glucan synthase remain central because perturbation of glucan assembly triggers lethal wall stress and can retain activity against azole-resistant isolates; notably, the first-in-class oral triterpenoid ibrexafungerp inhibits β-(1,3)-D-glucan synthase and has documented translational/clinical relevance in *Candida* disease [35,36]. Long-acting echinocandin pharmacology further illustrates how optimizing exposure at a wall target can improve real-world utility; rezafungin exemplifies this by enabling extended-interval dosing while maintaining potent glucan-synthase–directed activity [37,38]. A second, increasingly emphasized axis is cell-surface and secretory pathway biology, exemplified by GPI-anchor maturation (Gwt1), where the investigational prodrug fosmanogepix has advanced into Phase III development for candidemia and/or invasive candidiasis, underscoring clinical confidence in non-sterol fungal targets [39]. Metabolic essentiality beyond sterols is also being exploited through de novo pyrimidine biosynthesis, where the orotomide olorofim selectively inhibits fungal dihydroorotate dehydrogenase (DHODH) and has progressed in late-stage evaluation for invasive mold disease, highlighting the broader antifungal target landscape across fungal taxa [40]. Mitochondrial homeostasis offers another vulnerability: the investigational agent T-2307 collapses fungal mitochondrial membrane potential with reported selectivity over mammalian mitochondria, supporting energetics as a feasible antifungal axis [41]. Protein biosynthesis can be targeted at the level of aminoacylation fidelity; tavaborole inhibits fungal leucyl-tRNA synthetase (LeuRS) by engaging the editing site, demonstrating that translation-linked enzymes can be clinically actionable. In addition to sterol-centered pathways, several fungus-specific biochemical processes (absent from or fundamentally divergent in mammalian cells) constitute highly attractive antifungal targets. One prominent example is sphingolipid biosynthesis, particularly inositol phosphorylceramide (IPC) synthase encoded by *AUR1* (a membrane-associated enzyme essential for fungal viability and lacking a mammalian counterpart), which has been mechanistically validated through recent structural and biochemical analyses of the cyclic peptide aureobasidin A [42]. Another fungus-exclusive pathway is trehalose biosynthesis (a stress-protective carbohydrate system entirely absents in mammals but critical for fungal survival, virulence, and stress adaptation), in which trehalose-6-phosphate synthase (Tps1) has emerged as a druggable node supported by recent target-engagement and structural studies [43]. Chitin biosynthesis represents an additional classical fungus-specific axis (as chitin is a structural polysaccharide that is not present in mammalian cells), and renewed interest in this pathway has been driven by advances in the pharmacological characterization and clinical tolerability of chitin synthase inhibitors such as nikkomycin Z [40]. Beyond cell surface and cell wall architecture, fungal metabolic plasticity is further supported by the glyoxylate cycle (a metabolic shunt absent in mammals that enables fungal persistence in nutrient-limited host niches), with isocitrate lyase (ICL) playing a central role in virulence and intracellular survival and therefore representing a rational fungus-specific metabolic target [44].

Despite this broad target landscape, lanosterol CYP51 and SE were selected for detailed coverage because, although they are the direct targets of widely used antifungal classes such as azoles

and allylamines, respectively, they are also associated with prevalent and multilayered resistance mechanisms. For CYP51, target-site mutations, CaUpc2-mediated transcriptional overexpression, and enhanced efflux pump activity collectively undermine azole efficacy, whereas resistance linked to SE involves enzyme mutations and metabolic bypass adaptations. This converging body of clinical and molecular evidence positions CYP51 and SE not only as the most thoroughly validated nodes of sterol homeostasis but also as central hubs of antifungal resistance biology, thereby providing a rational, tractable, and therapeutically meaningful foundation for dual-fungal targeting strategies.

3.1. Lanosterol 14α-Demethylase (CYP51) Axis: The Core of Ergosterol Biosynthesis and Antifungal Resistance

The sterol dynamics of the fungal cell membrane represent one of the most fundamental biological determinants of *C. albicans* structural integrity, permeability, and adaptive capacity under environmental stress. This membrane constitutes a complex lipid matrix composed of phospholipids, sphingolipids, and sterols, providing a dynamic platform for numerous transmembrane proteins and transport enzymes. Among fungi, ergosterol serves as the primary sterol component (functionally distinct from cholesterol in mammalian cell membranes) and plays a central role in maintaining membrane fluidity and functional stability [45]. One of the most critical enzymes within this biosynthetic system is CYP51 (Erg11), which catalyzes the obligatory oxidative demethylation step in the ergosterol biosynthesis pathway (Figure 2).

Azole-derived antifungal agents have long served as first-line pharmacotherapeutic options for the treatment of *C. albicans* infections, exerting their mechanism of action directly through inhibition of this enzyme. Azoles bind to both the prosthetic heme group and the substrate-binding pocket of CYP51, thereby blocking the catalytic cycle. This inhibition halts ergosterol synthesis, leads to the accumulation of toxic sterol intermediates, and irreversibly disrupts membrane permeability [46].
Recent studies indicate that CYP51 functions not only in sterol biosynthesis but also in secondary biological processes such as cell morphogenesis and stress response. Deletion of the CYP51 gene in *C. albicans* results in impaired hyphal extension and reduced invasive growth, as well as defects in reactive oxygen species detoxification, collectively leading to attenuated *in vivo* virulence. Furthermore, mutant strains lacking ERG11 are more readily phagocytosed by macrophages, suggesting that CYP51 is a key regulatory component not only of sterol metabolism but also of immune evasion mechanisms [47].

Transcriptional and genetic adaptation constitutes a central component of antifungal resistance in *C. albicans*, operating through a multilayered network of target modification, transcriptional rewiring, and drug efflux. Resistance-associated mutations in sterol biosynthesis enzymes, particularly CYP51/ERG11 (e.g., Y132F and K143R), induce conformational changes that weaken azole binding and reduce drug susceptibility. In parallel, transcriptional regulators such as CaUpc2 modulate the coordinated upregulation of sterol biosynthetic genes, thereby sustaining intracellular ergosterol levels under antifungal pressure. Importantly, CaUpc2-mediated transcriptional activation functions in concert with additional resistance mechanisms, including the overexpression of efflux transporters (CDR1 and MDR1) and broader metabolic reprogramming, rather than acting as an isolated determinant. Together, these interconnected processes preserve membrane sterol homeostasis, limit intracellular antifungal accumulation, and enable fungal survival during prolonged pharmacological stress [48-50].

This complexity reveals that antifungal tolerance arises not only from weakened drug–target interactions but also from an adaptive resistance network integrated with epigenetic regulators and stress-response pathways. Therefore, future antifungal strategies must extend beyond classical blockade of ergosterol biosynthesis to target multiple regulatory checkpoints such as transcriptional regulators (e.g., CaUPC2), epigenetic modulators (e.g., HDACs), chaperone proteins (e.g., HSP90), and key components of host pathogen receptor interaction networks (e.g., EGFR). Such a multidimensional therapeutic approach offers substantial potential not only for the eradication of drug-resistant *C. albicans* strains but also for weakening host–pathogen interactions and preventing systemic dissemination of invasive infections.

Figure 2. Ergosterol biosynthesis pathway and clinically relevant antifungal drug targets [50].

3.2. Squalene Epoxidase (SE): A Central Regulator of Fungal Cellular Functions

The SE axis occupies a central position in preserving structural and functional integrity within the fungal sterol biosynthesis pathway. Squalene epoxidase (SE; ERG1) catalyzes the oxidative conversion of squalene to 2,3-oxidosqualene, a critical step required for maintaining membrane integrity, permeability, and fluidity in fungal cells (Figure 2). Inhibition of this enzymatic step leads to intracellular accumulation of squalene and a reduction in ergosterol synthesis, ultimately compromising membrane stability; depending on the extent of disruption, this results in fungistatic or fungicidal activity. For this reason, SE has been recognized as a key antifungal target in multiple pathogens, including *Candida* and *Aspergillus* species [51].

Although a similar enzymatic step exists in human cholesterol biosynthesis, fungal SE exhibits pharmacologically significant differences in its binding pocket architecture and inhibitor sensitivity compared with its mammalian counterpart. These structural distinctions enable allylamine derivatives (e.g., terbinafine, naftifine) to act with high fungal specificity at low concentrations, providing these agents with a broad therapeutic safety margin [45]. Recent studies have shown that SE functions not only in sterol biosynthesis but also in essential biological processes including fungal morphogenesis, cellular stress responses, and drug susceptibility. This multifunctionality positions SE as a biologically robust synergistic node for combination therapy with azoles and other sterol-targeting antifungals [52]. One of the most well-documented examples of clinical resistance involves terbinafine resistance in dermatophytes, in which missense mutations within the SE gene (particularly F397L, L393F, and A448T) exhibit strong correlations with resistant phenotypes. These associations have been validated

through multicenter clinical studies and meta-analyses [53,54]. Structural and bioinformatic modeling indicates that these mutations alter the conformation of the enzyme's binding pocket, thereby reducing terbinafine binding affinity [55]. Importantly, the therapeutic relevance of SE is not confined to dermatophytes. Opportunistic pathogens such as *Exophiala dermatitidis* have also demonstrated strong *in vitro* susceptibility to terbinafine, confirming the evolutionary conservation of SE as well as its sustained viability as a therapeutic target across fungal species [56].

Recent investigations aimed at pharmacologically revisiting this target have further revealed that certain phenothiazine-derived antipsychotics may act as competitive SE inhibitors, exhibiting preferential binding to fungal SE over human homolog.

Taken together, these findings indicate that SE represents the "critical nexus" of fungus-specific sterol metabolism, where surveillance of resistance-associated mutations can inform clinical diagnostic and therapeutic strategies. Moreover, SE provides a strong conceptual and biochemical foundation for the development of new chemical scaffolds that enhance human–fungus selectivity in next-generation antifungal agents. In this regard, SE emerges as a redefined therapeutic node—operating beyond classical targets at both biochemical and pharmacodynamic levels with significant potential for innovative antifungal drug discovery.

4. The Bridge Between Host Defense and Fungal Virulence

4.1. Epidermal Growth Factor Receptor (EGFR) Axis: The Gateway Through Which C. albicans Accesses Host Cells

Host receptor signaling constitutes an active biosignaling interface that enables *C. albicans* to traverse the epithelial barrier by exploiting host cellular communication systems rather than relying solely on passive adhesion or cell wall–mediated interactions. Central to this process is the epidermal growth factor receptor (EGFR)-centered signaling axis, which functions as a critical molecular gateway for fungal entry. Surface-expressed invasin proteins, including Als3 and Hyr1, engage EGFR and human epidermal growth factor receptor 2 (HER2) on the host cell membrane, initiating receptor autophosphorylation and downstream signaling cascades. Activation of these pathways promotes receptor-driven endocytosis coupled with actin cytoskeleton reorganization, thereby facilitating fungal internalization. Collectively, these events underscore that epithelial invasion by *C. albicans* is an actively orchestrated process driven by host receptor reprogramming rather than a consequence of nonspecific surface attachment (Figure 3) [57].

Recent molecular analyses indicate that epithelial barrier penetration by *C. albicans* is not mediated by an isolated receptor–ligand event but instead emerges from a coordinated, multi-receptor signaling architecture operating in parallel. Beyond the previously described EGFR–HER2 signaling route, invasion is increasingly recognized as being governed by an integrated receptor platform that synchronizes multiple host pathways. Using both human oral epithelial cell models and *in vivo* oropharyngeal candidiasis systems, Phan *et al.* (2023) demonstrated that the fungal invasins Als3 and Hyr1 simultaneously engage EGFR and c-Met on the host cell surface, assembling an E-cadherin–dependent receptor complex. Formation of this platform amplifies receptor tyrosine kinase activation, driving actin cytoskeleton reorganization and coordinated receptor-mediated endocytosis. Importantly, disruption of Als3 or Hyr1 markedly attenuates EGFR and c-Met phosphorylation, resulting in defective internalization and reduced invasive capacity. Collectively, these findings define the Als3–EGFR–c-Met–E-cadherin axis as a higher-order invasion hub that integrates signaling and endocytic machinery to enable regulated epithelial entry by *C. albicans* [58].

In a complementary investigation, Ponde and colleagues delineated the EGFR–MAPK signaling cascade as a central integrative pathway that links epithelial invasion with inflammatory amplification during *C. albicans* infection. In line with earlier receptor-network models, deletion of Als3 or Hyr1 impaired the assembly of the EGFR–c-Met signaling platform, leading to attenuated receptor phosphorylation and a pronounced reduction in fungal internalization. Rather than reiterating passive-versus-active invasion paradigms, these findings refine the concept by positioning epithelial penetration as a signaling-driven event embedded within broader host response circuits. Beyond receptor

engagement, the study demonstrated that the pore-forming virulence factor candidalysin is locally released at invasion foci, where it reinforces EGFR activation and propagates MAPK signaling. Subsequent phosphorylation of key adaptor proteins (including Grb2, Gab1, Shc, Shp2, and c-Cbl) serves to amplify and sustain the EGFR–MAPK axis, thereby coupling invasive signaling with downstream inflammatory outputs. This adaptor-mediated signal propagation drives robust production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, exacerbating epithelial damage and inflammatory severity. Importantly, genetic silencing or pharmacological inhibition of these adaptor nodes markedly suppressed EGFR–MAPK activation and reduced epithelial invasion, establishing the EGFR–MAPK–adaptor protein network as a bidirectional regulatory module that synchronizes fungal persistence with host tissue injury during *C. albicans* infection [59].

Recent studies focusing on the EphA2–EGFR signaling axis reveal that epithelial barrier traversal by *C. albicans* is governed by a multilayered and temporally coordinated receptor network rather than by isolated invasin–receptor interactions. Extending the EGFR–MAPK adaptor framework described previously, these findings position EphA2 as an upstream fungal-sensing module that operates in functional synergy with EGFR. Using both human oral epithelial cell systems and *in vivo* oropharyngeal candidiasis models, investigators demonstrated that the fungal invasin Als3 and the cytolytic toxin candidalysin activate the host EphA2 receptor, thereby initiating a signaling cascade that culminates in EGFR tyrosine phosphorylation. Mechanistically, EphA2 functions as an early pattern-recognition sensor by detecting fungus-specific cell wall components, such as β-glucans, and triggering the initial wave of host cell activation. In contrast, EGFR engagement predominates at later stages of infection, where it sustains MAPK and NF-κB signaling to reinforce epithelial invasion and amplify pro-inflammatory cytokine production, including IL-1β, IL-6, and CXCL8. Cross-talk between EphA2 and EGFR thus establishes a temporally stratified signaling program in which early pathogen detection is coupled to prolonged inflammatory and invasive responses. Consistent with this hierarchical model, pharmacological inhibition of EGFR or genetic silencing of EphA2 markedly attenuates *C. albicans* adhesion, endocytosis, and epithelial invasion, underscoring the cooperative role of this dual-receptor system in coordinating host invasion and immune activation [60].

During epithelial interaction with *C. albicans*, the EGFR–MAPK axis extends beyond its role in facilitating intracellular entry to function as a central regulator of host transcriptional reprogramming. Building on the EphA2–EGFR synergy that initiates early host activation, EGFR signaling here is positioned as a downstream amplifier that shapes the molecular trajectory of infection through coordinated signal propagation and gene regulation. Exposure of human oral epithelial cells to *C. albicans* rapidly induces EGFR tyrosine phosphorylation, followed by robust activation of the Raf/MEK/ERK cascade. This signaling sequence drives the induction of the immediate-early transcription factor Early Growth Response 1 (EGR1), thereby linking receptor activation to transcriptional control. Concurrently, EGFR–MAPK signaling operates in concert with NF-κB pathways to potentiate the expression of pro-inflammatory mediators, including IL-6, IL-8, and CXCL1, reinforcing epithelial defense programs. Pharmacological disruption of this axis via EGFR inhibition (gefitinib) or MEK blockade (U0126) markedly attenuates EGR1 induction and cytokine output. Collectively, these data define the EGFR–MAPK–EGR1 module as a regulatory interface that integrates early stress signaling with sustained inflammatory gene expression during *C. albicans* infection [61].

Candidalysin, a major virulence determinant of *C. albicans*, functions as a pivotal regulator of epithelial damage and inflammatory signaling by engaging EGFR-dependent host pathways. Extending the EGFR–MAPK–EGR1 transcriptional framework described above, accumulating evidence indicates that candidalysin acts not solely as a membrane-disruptive toxin but as a fungal agonist capable of actively reprogramming host signaling networks. During hyphal invasion, candidalysin is locally released within epithelial invasion pockets, where it directly induces EGFR tyrosine phosphorylation. This activation propagates downstream signaling through ERK1/2, p38 MAPK, and JNK cascades, culminating in the activation of NF-κB and AP-1 transcription factors and robust induction of pro-inflammatory mediators, including IL-1α, IL-1β, IL-6, CXCL8 (IL-8), and GM-CSF. Mechanistically, candidalysin engages EGFR in a ligand-like manner, thereby sustaining receptor signaling independently of canonical host growth factors. Consistent with this model, pharmacological inhibition or genetic silencing of EGFR markedly suppresses ERK phosphorylation and cytokine production. Together, these findings establish candidalysin as a specialized fungal signal modulator that exploits

EGFR to couple epithelial injury with amplified inflammatory responses during *C. albicans* infection [62].

Collectively, converging molecular evidence indicates that epithelial barrier traversal by *C. albicans* is driven not by passive invasin–receptor engagement but by a hierarchically organized, EGFR-centered signaling architecture. Through coordinated actions of fungal invasins (Als3, Hyr1) and the peptide toxin candidalysin, host receptor tyrosine kinases (including EGFR, HER2, c-Met, and EphA2) are dynamically rewired into functional signaling networks that integrate cytoskeletal remodeling, receptor-mediated endocytosis, and inflammatory gene regulation. Within this framework, EGFR emerges not simply as a portal for fungal entry but as a master regulator that, via the MAPK–EGR1 axis, governs early stress adaptation, transcriptional reprogramming, and epithelial damage responses. These findings support a *signaling invasion* paradigm in *C. albicans* pathogenesis, wherein successful epithelial invasion is contingent upon active manipulation of host receptor networks rather than direct physical penetration alone. Importantly, this model reframes antifungal intervention strategies by highlighting host receptor axes (such as EGFR, EphA2, and c-Met) as actionable therapeutic nodes, thereby opening new avenues for host-targeted or dual-target antifungal approaches aimed at disrupting invasion-associated signaling programs [58,61-63].

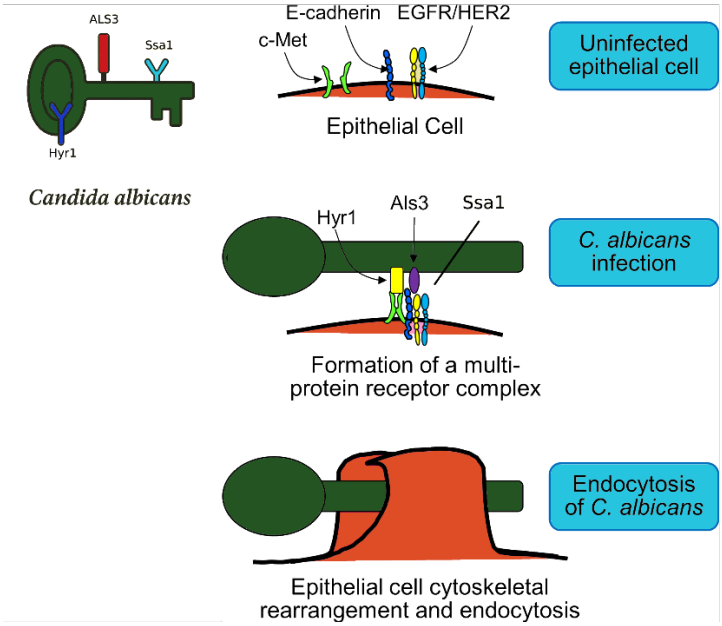


Figure 3. *C. albicans* possess the capacity to transition between yeast and filamentous hyphal forms.

This morphological plasticity plays a critical role in mucosal infections, as hyphae enables the fungus to breach host epithelial barriers. Strong adhesion of hyphae to host cells is mediated by several adhesins, including Hwp1p, Als3p, and Hyr1p. Epithelial invasion occurs through two distinct mechanisms: (i) active penetration facilitated by fungal hydrolytic enzymes, and (ii) induced endocytosis triggered by fungal invasins Als3p and Ssa1p interacting with host cell receptors EGFR/HER2 and E-cadherin. This interaction generates an “invasion pocket” around the fungal cell. The cytolytic peptide toxin candidalysin is secreted into this pocket, where it accumulates. By integrating into host cell membranes, candidalysin induces epithelial damage, which in turn activates epithelial-derived innate immune responses [64].

4.2. Histone Deacetylase (HDAC) Axis: Its Role in C. albicans Virulence

The HDAC axis of epigenetic regulation represents a multilayered mechanism through which gene expression is dynamically controlled via modulation of chromatin architecture. Histone acetylation and deacetylation constitute the most fundamental forms of reversible epigenetic modification,

governing chromatin compaction and thus the transcriptional accessibility of genes. This regulatory cycle is orchestrated by two opposing enzyme families—histone acetyltransferases (HATs) and histone deacetylases (HDACs). HAT enzymes use acetyl-CoA as a cofactor to transfer acetyl groups to the ε-amino groups of lysine side chains on histones; this relaxes chromatin structure, increases transcriptional permissiveness, and facilitates gene expression [65,66]. Conversely, HDACs remove these acetyl groups, leading to chromatin recondensation and transcriptional silencing [67,68]. For nearly two decades, this reversible acetylation cycle on histone lysine residues has been recognized as a principal determinant of epigenetic gene regulation.

Within this regulatory landscape, bromodomain-containing proteins act as the "readers" of acetylated lysine, recognizing these marks and contributing to the spatial and temporal coordination of gene expression. HDACs, in particular, influence not only chromatin architecture but also cellular differentiation, proliferation, metabolic reprogramming, and stress adaptation. Owing to this multifaceted regulatory capacity, HDACs have emerged in recent years as highly valuable therapeutic targets for both cancer and infectious diseases [69].

Recent studies demonstrate that HDAC inhibitors can function as epigenetic modulators not only in human cells but also in microbial systems. For instance, in co-cultures of *Streptomyces* sp. 13F051 with certain fungal species, bacterial-derived HDAC inhibitors markedly enhanced fungal secondary metabolite production [70]. This finding offers an innovative approach for discovering novel bioactive natural products by triggering the activation of silent biosynthetic gene clusters and highlights that epigenetic intervention can serve not only as a tool for gene repression but also as a driver of metabolic reprogramming.

Functionally, Class I HDACs exhibit strong similarity to the Rpd3 homologs described in fungi and display potent deacetylase activity localized to the nucleus. These enzymes typically serve as the catalytic subunits of repressor complexes regulated by inositol phosphates and play a central role in the silencing of target genes [71]. In contrast, Class II HDACs share structural homology with fungal HDAC I and contain conserved C-terminal deacetylase domains. Notably, members of the Class IIa subgroup (HDAC4, 5, 7, 9) possess an N-terminal adaptor region that binds the transcription factor MEF2, as well as phosphorylation-dependent interaction sites for 14-3-3 proteins. This structural configuration enables phosphorylation-mediated nucleo–cytoplasmic shuttling [72].

Recent findings strongly demonstrate that fungal HDACs, particularly through the Rpd3 complex, play a central role in the epigenetic regulation of virulence. Numerous studies have shown that the Rpd3 HDAC is a key determinant of pathogenicity-associated processes in *C. albicans*, including hyphal development, protease secretion, and cell wall remodeling [73-75]. Pho23, a component of the Rpd3 complex, exerts multifaceted regulatory functions in fungal physiology. Experiments on the *C. albicans* Pho23Δ/Δ mutant revealed striking phenotypic alterations such as upregulation of ATG genes, increased autophagosome formation, suppression of the cell wall integrity (CWI) pathway, and reduced protease secretion. Pho23 deficiency was also shown to impair filamentous growth and attenuate virulence. Collectively, these findings position Pho23 as a critical regulatory node not only in autophagy and cell wall biogenesis but also in the transcriptional control of fungal virulence [76].

Similarly, Sir2 (a Class III deacetylase in *C. albicans*) promotes systemic infection by enhancing immune evasion and adhesion through cell wall remodeling [77]. Moreover, targeting Hst3p results in genome-wide elevation of H3K56 acetylation, driving the reprogramming of gene networks linked to morphogenesis and pathogenicity [78]. These mechanisms are not restricted to yeast-form pathogens but are conserved among filamentous fungi as well. For example, deletion of Hda1 leads to impaired oxidative and osmotic stress tolerance, altered secondary metabolite biosynthesis, and disruption of mycoparasitic mechanisms [79].

In *C. albicans*, HDACs serve as key regulatory elements governing the epigenetic control of morphological transitions and virulence. Hda1 and Rpd31 promote invasive growth by supporting the expression of hypha-specific genes such as *HWP1* and *ECE1*, whereas the Set3C complex (composed of Set3 and Hos2) counteracts this process and maintains morphological equilibrium. Rpd31 also strengthens hyphal differentiation by conditionally activating the transcription factor *UME6*. Conversely, loss of Set3C function leads to transient dysregulation of *EFG1* and *NRG1*, resulting in exaggerated filamentous growth and reduced virulence. Members of the sirtuin family, such as Sir2 and Hst3, contribute to the epigenetic repression of the white–opaque transition. Together, this network

orchestrates gene expression associated with yeast–hypha switching, biofilm formation, and antifungal resistance through master transcriptional regulators including Nrg1, Brg1, and Tec1. Although HDAC inhibitors disrupt this regulatory balance and enhance hyphal formation, they generally lead to reduced virulence and increased antifungal susceptibility. Thus, the opposing activities of the Hda1–Rpd31 axis and the Set3–Hos2 complex establish a critical epigenetic equilibrium that determines *C. albicans* adaptability to environmental stress and host niches (Figure 4) [80].

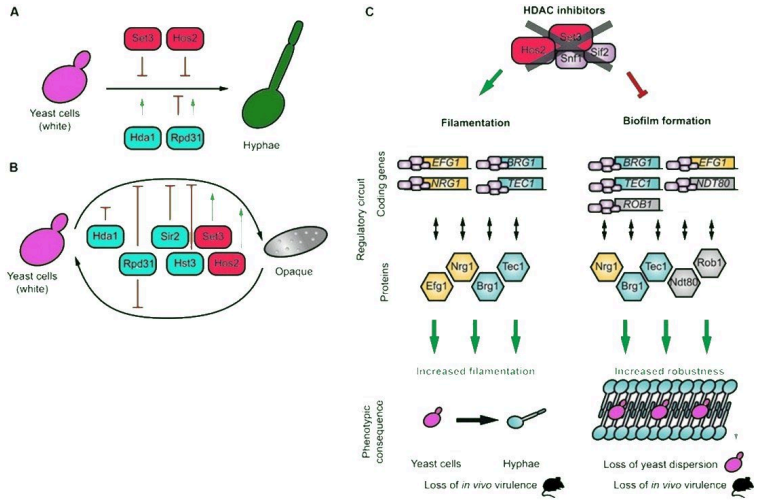


Figure 4. HDACs and morphogenesis in *C. albicans*. (A) Phenotypic effects of distinct HDACs during filamentation. (B–C) Regulation of the white–opaque transition. HDACs modulate the expression of core transcription factors that govern regulatory circuits controlling filamentation, biofilm development, and morphogenesis. Inhibition of HDAC activity disrupts these transcriptional networks, resulting in a hyperfilamentous phenotype accompanied by a marked reduction in *in vivo* virulence. Similarly, treatment with HDAC inhibitors produces structurally denser biofilms but reduces yeast dispersal, ultimately leading to diminished pathogenicity *in vivo*. Adapted from [80]

In *C. albicans*, the Rpd3 complex and its component Pho23 play decisive roles in coordinating pathogenicity-associated networks, including hyphal development, autophagy, cell wall integrity, and protease secretion. Other HDAC family members, such as Sir2 and Hst3p, contribute to adaptive advantages in morphogenesis and host interaction by controlling histone acetylation homeostasis and transcriptional remodeling. Parallel epigenetic control mechanisms observed through Hda1 in filamentous fungi further support the evolutionary conservation of this regulatory architecture across species.

Thus, the HDAC axis represents the central backbone of chromatin-dependent virulence regulation in fungal pathogenesis. Targeting these enzymes provides a strong scientific foundation for the development of epigenetic-based next-generation antifungal therapies that move beyond the limitations of classical antifungal strategies.

4.3. Heat Shock Protein 90 (HSP90) Axis: The Master Regulator of Fungal Stress Responses and Drug Resistance

Heat shock proteins (HSPs) in fungal pathogens such as *C. albicans* and *Aspergillus fumigatus* are essential regulators that sustain virulence and drive the development of antifungal drug resistance. Among them, HSP90 is an evolutionarily conserved molecular chaperone that operates at the core of cellular stress responses, protein folding, and signal transduction through its extensive interaction network with multiple co-chaperones [32,81,82]. In *C. albicans*, HSP21 functions in concert with HSP90 and is directly associated with trehalose biosynthesis, enhancing the organism's capacity to adapt to environmental stresses. Indeed, HSP21 has been shown to provide protection under thermal and

oxidative stress by maintaining trehalose homeostasis and modulating Cek1 kinase activity [9]. In this context, trehalose (a non-reducing disaccharide of glucose) acts not only as a carbon and energy reserve but also as a metabolic mediator of fungal stress tolerance [83].

HSP90 functions not merely as a protein-folding chaperone but as a central signaling regulator that integrates stress responses, cell wall integrity, and drug tolerance in fungal cells. This protein coordinates stress-induced phosphatases such as calcineurin and Pkc1, as well as mitogen-activated protein kinase (MAPK) pathways, thereby supporting virulence and preserving antifungal resistance [32,84]. At the molecular level, HSP90 stabilizes a wide array of signaling components, including the Ca^{2+}–calmodulin–activated phosphatase calcineurin and members of the PKC–MAPK cell wall integrity pathway such as Pkc1, Bck1, Mkk2, and Mkc1 (Figure 5). Stabilization of these effectors enhances the fungal cell's resilience to environmental stresses. Conversely, inhibition of HSP90 disrupts calcineurin-dependent stress responses and the PKC signaling network, thereby abolishing tolerance and resistance mechanisms to azoles and echinocandins [31,85-87].

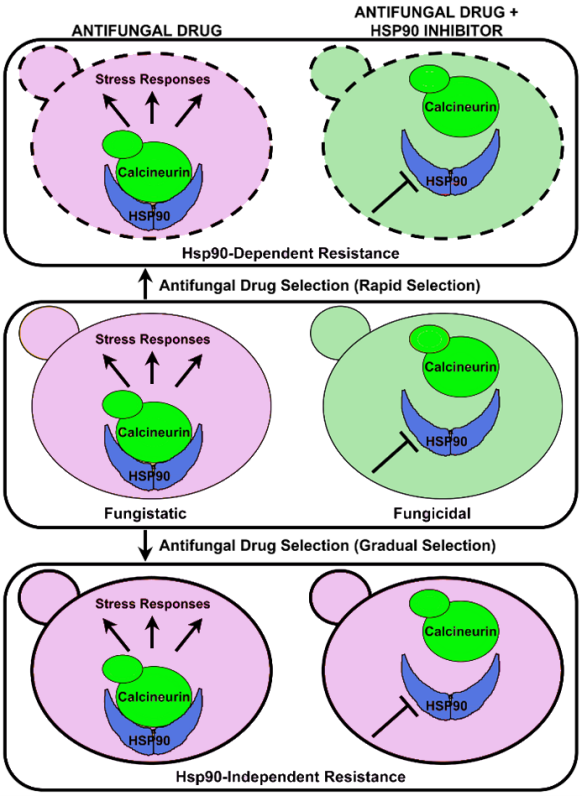


Figure 5. Disruption of HSP90 function markedly enhances the activity of fungistatic antifungal agents, converting them into fungicidal combinations and constraining the evolutionary emergence of drug resistance. In wild-type fungal cells (shown here with revised coloration), HSP90 stabilizes the calcineurin phosphatase, thereby sustaining the calcineurin-dependent stress responses required for survival in the presence of azoles and echinocandins in *C. albicans*, and echinocandins in *A. fumigatus*. When HSP90 activity is compromised, these stress response pathways collapse, causing fungistatic agents to become fungicidal, as reflected by the nonviable grey fungal cell. Under rapid selection pressure in *Saccharomyces cerevisiae*, azole resistance arises via an HSP90-dependent mechanism (illustrated by the orange cell with a dashed outline), whereas gradual selection favors the evolution of HSP90-independent resistance phenotypes (represented by the orange cell with a continuous outline). Adapted from [84].

In *Candida* species, HSP90 also plays a critical regulatory role in resistance to polyene antifungals, underscoring its evolutionarily conserved function across diverse antifungal drug classes

and positioning it as a universally targetable molecular stabilizer [88]. Consequently, therapeutic targeting of HSP90 has emerged as a promising strategy for both improving treatment outcomes in fungal infections and mitigating the development of drug resistance [89-91]. Clinical serum samples from patients with invasive *C. albicans* infections, as well as data from animal models, have identified immunodominant antigens in the 45–52 kDa range. Among these, a 48-kDa antigen corresponds to enolase, while a 47-kDa antigen represents the C-terminal fragment of *C. albicans* HSP90, which has gained recognition as a valuable diagnostic biomarker [92].

Recent research has highlighted that this chaperone is not only a cornerstone of tumor biology but also a fundamental regulator of fungal life cycles and antifungal drug resistance. Because HSP90 is highly conserved across eukaryotes, achieving therapeutic selectivity requires inhibitors that can discriminate between fungal and human HSP90 orthologs. A recent structural study focusing on the nucleotide-binding domain (NBD) of *C. albicans* HSP90 revealed that this region possesses unique conformational flexibility, enabling the rational design of the first fungus-selective HSP90 inhibitors based on semi-synthetic oxime derivatives of the resorcylic macrolactone radicicol. Whitesell and colleagues conducted detailed residue-by-residue comparisons of the fungal HSP90 NBD with its human counterpart and demonstrated that fungal-specific residues (particularly Leu130 and Phe131) play decisive roles in shaping inhibitor-binding selectivity [93].

In a complementary study, Huang and coworkers aimed to design the first fungal-selective inhibitors with strong affinity for *C. albicans* HSP90. Semi-synthetic oxime derivatives of the macrolactones radicicol and monocillin I were generated, among which monocillin analog CMLD013075 exhibited approximately 25-fold higher affinity for the fungal HSP90 NBD compared to the human ortholog. This compound significantly impaired fungal growth in cell-based assays while displaying markedly lower toxicity toward human cells relative to the nonselective radicicol scaffold [94].

In summary, the HSP90 axis represents an evolutionarily conserved chaperone system that lies at the center of fungal pathogenesis and antifungal drug resistance. By stabilizing the calcineurin–PKC–MAPK signaling network, HSP90 forms a molecular bridge between stress adaptation, cell wall integrity, and drug tolerance, thereby sustaining virulence and facilitating multidrug resistance in pathogens such as *C. albicans* and *A. fumigatus*. Structural and biochemical analyses conducted in recent years have revealed that the unique conformational dynamics of the HSP90 nucleotide-binding domain (particularly those governed by fungal-specific residues Leu130 and Phe131) constitute a critical molecular determinant for designing fungus-selective inhibitors. As a result, next-generation radicicol- and monocillin-based derivatives with enhanced fungal selectivity and reduced human cytotoxicity have positioned HSP90 not only as a master regulator of virulence and resistance but also as a promising molecular foundation for the development of targeted, host-sparing antifungal therapies.

5. Dual-Target Approaches in Antifungal Therapy

Dual-target antifungal drug design has emerged as one of the most compelling examples of rational multi-targeting strategies that are reshaping the pharmacotherapy of fungal infections. This approach aims to enhance therapeutic efficacy, suppress the emergence of resistance, and minimize host toxicity by simultaneously inhibiting multiple biological pathways within the fungal cell. Whereas resistance to classical single-target antifungals often arises rapidly through mechanisms such as target-enzyme mutations or efflux pump activation, dual inhibitors circumvent these singular resistance routes by exerting coordinated pressure on interconnected metabolic networks.

The scientific validity of this strategy has been strongly supported by numerous molecular and pharmacodynamic studies in recent years. For instance, dual-target combinations such as CYP51–HDAC, HSP90–CYP51, HSP90-HDAC and SE–CYP51 can concurrently inhibit ergosterol biosynthesis, epigenetic regulation, and signaling pathways, thereby disrupting fungal morphogenesis, stress adaptation, and drug resistance (Figure 6). Targeting such interaction networks enables the restoration of synergistic antifungal activity even against azole-resistant *Candida* isolates, where classical antifungal agents typically fail to achieve therapeutic efficacy [34,95-98].

In conclusion, dual-target antifungal strategies represent a significant therapeutic advancement by effectively suppressing resistance development and simultaneously modulating multiple virulence-

related pathways. Nevertheless, the capacity of fungal pathogens to activate compensatory signaling networks or alternative biological routes under prolonged selective pressure should be carefully considered. This consideration should not be viewed as a limitation of dual target approaches per se, but rather as a critical design principle emphasizing the importance of rational target pairing, minimization of functional pathway redundancy, and anticipatory evaluation of adaptive escape potential. Within this framework, dual-target antifungal designs should be assessed through a balanced lens that integrates both their resistance-suppressing efficacy and the long-term risks associated with biological adaptation.

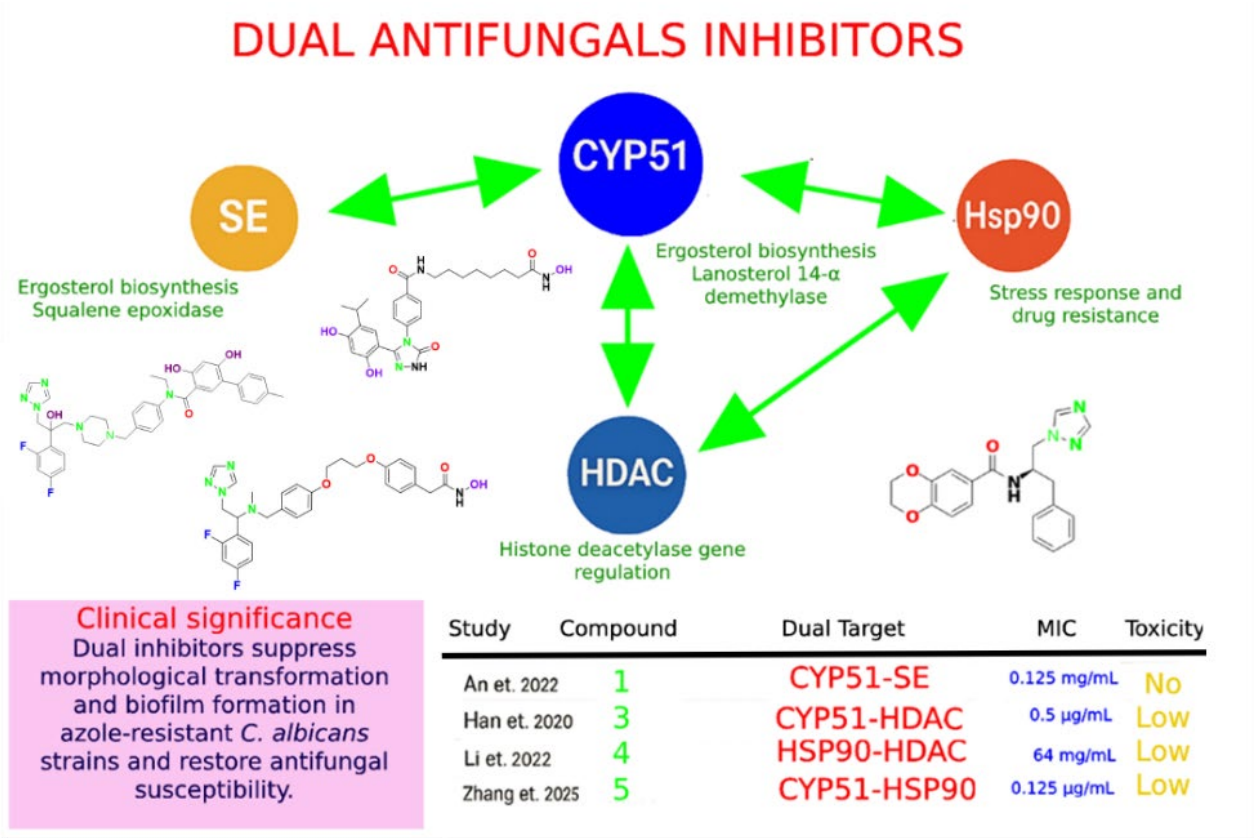


Figure 6. The interaction network, representative chemical scaffolds, and clinical relevance of dual antifungal inhibitors that simultaneously target the CYP51, SE, HDAC, and HSP90 axes. These multi-target agents disrupt ergosterol biosynthesis, epigenetic regulation, and stress-adaptation circuitry in parallel, thereby suppressing morphological transitions and biofilm formation in azole-resistant *C. albicans* strains [34,95-98].

Looking forward, dual-inhibitor design is evolving into an integrated drug-discovery paradigm that encompasses not only the development of novel chemical scaffolds but also AI-driven pharmacophore optimization and simultaneous modeling of protein–protein interaction interfaces. In this respect, the dual-target antifungal approach represents a strategically innovative framework, strongly supported by the current literature, with the potential to drive a transformative shift in the clinical management of fungal drug resistance.

5.1. Lanosterol 14α-Demethylase (CYP51) – Squalene Epoxidase (SE)

In a study conducted by An and colleagues (2022), the authors reported the design and biological evaluation of novel benzodioxane-derived dual inhibitors capable of simultaneously targeting SE and CYP51, with potent activity against *C. albicans* and other resistant fungal species. Among the synthesized compounds, compound 1 displayed the most favorable profile, exerting dual inhibition of two sequential steps in ergosterol biosynthesis, as evidenced by squalene and eburicol accumulation in

both *in vitro* and *in vivo* settings. In line with this mechanism, representative benzodioxane derivatives showed strong antifungal activity against *C. albicans*, including azole-resistant clinical isolates (MIC_{50} = 0.125–2.0 μg/mL), and achieved therapeutic efficacy in murine infection models, as reflected by reduced fungal burden and improved tissue pathology, consistent with concurrent targeting of CYP51 and SE (Figure 7). This metabolic disruption compromised membrane integrity and ultimately induced fungal cell death. Compared with classical antifungals, these dual inhibitors demonstrated markedly lower MIC values and produced strong synergistic antifungal effects against resistant isolates. Moreover, their low toxicity and favorable biocompatibility in murine models highlight their potential as powerful therapeutic candidates for the treatment of invasive candidiasis [95].

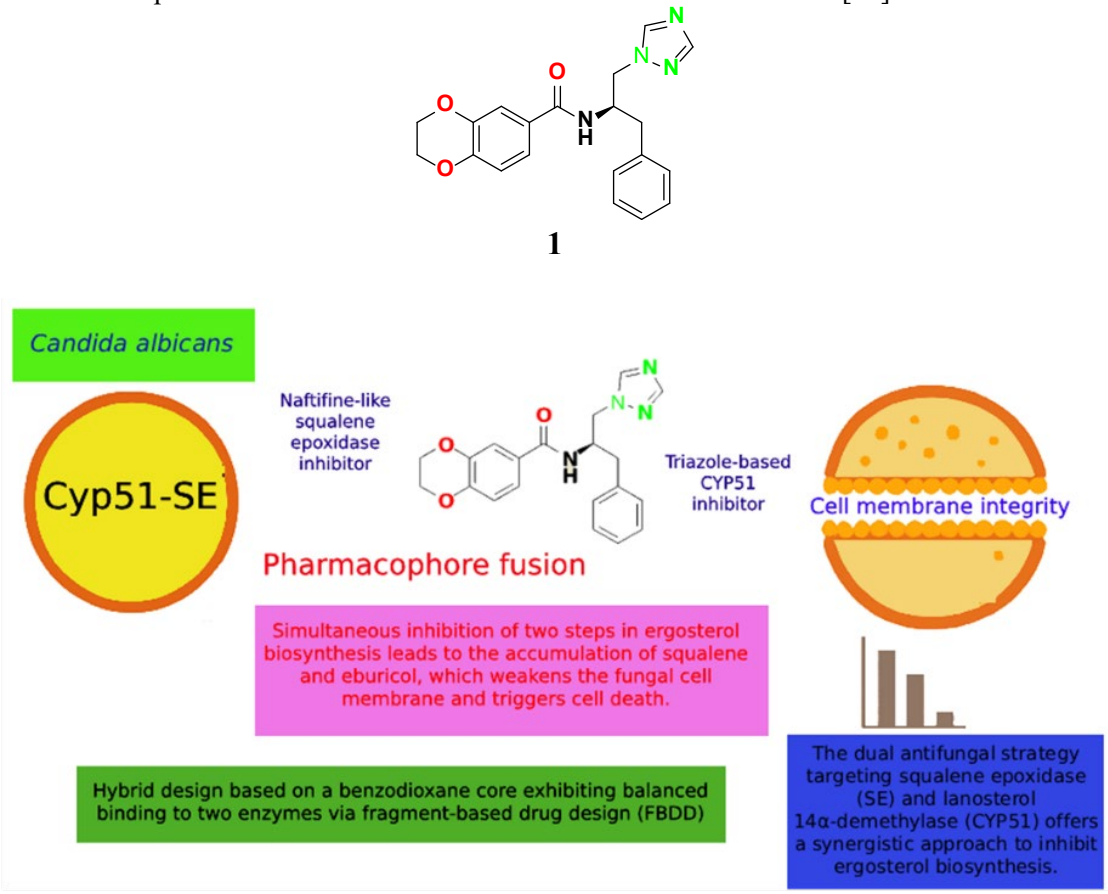
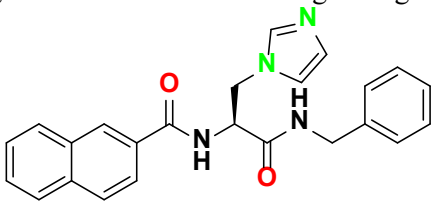


Figure 7. Lanosterol 14α-Demethylase (CYP51)–Squalene Epoxidase (SE) Dual Targeting Strategy: Sequential Blockade of Ergosterol Biosynthesis in *C. albicans.*

In a 2019 study, Dong and colleagues proposed a dual antifungal design strategy targeting both squalene epoxidase (SE) and lanosterol 14α-demethylase (CYP51), two essential enzymes in the ergosterol biosynthetic pathway. Using Discovery Studio-based pharmacophore workflows, Dong et al. constructed a HipHop ligand-based model for SE and a receptor/structure-informed pharmacophore for CaCYP51 (selected from available CaCYP51 crystal structures), then designed fragment-linked candidates guided by feature overlap and validated binding by CDOCKER docking. Motivated by the limitations of classical azole and allylamine derivatives (such as resistance, poor bioavailability, and narrow-spectrum activity), the authors employed a pharmacophore-based rational drug-design approach. Ligand-based pharmacophore models were constructed for SE, whereas receptor-based models were developed for CYP51, enabling the identification of shared structural features suitable for dual inhibition. Guided by these models, three novel compounds were synthesized. Among them, Compound 2 demonstrated strong binding affinity toward both targets, broad-spectrum antifungal activity, and significant potency even against fluconazole-resistant *Candida* isolates. Mechanistic analyses showed that the compound inhibited both SE and CYP51, leading to the accumulation of

squalene and lanosterol and a consequent reduction in ergosterol levels. Additionally, *in silico* ADMET profiling revealed favorable pharmacokinetic characteristics, including high predicted absorption, low toxicity, and an appropriate lipophilic balance. Collectively, this study highlights pharmacophore-guided dual targeting as a powerful strategy to overcome resistance and improve clinical efficacy, providing a valuable methodological framework for antifungal drug development [96].



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5.2. Lanosterol 14α-Demethylase (CYP51) – Histone Deacetylase (HDAC)

In a study conducted by Han and colleagues (2020), the authors developed the first generation of dual inhibitors designed to simultaneously target CYP51 and HDAC as an innovative strategy against azole-resistant *C. albicans* infections (Figure 8). Among the synthesized compound 3, exhibited potent antifungal activity against azole-resistant isolates in both *in vitro* and *in vivo* models. Mechanistic analyses revealed that these compounds suppress ergosterol biosynthesis by inhibiting CYP51 activity while concurrently blocking HDAC catalytic function, leading to reduced expression of efflux pump genes (*CDR1, CDR2, MDR1*) as well as *ERG11*. This dual inhibition effectively disrupts both sterol metabolism and epigenetic resistance circuitry within fungal cells.

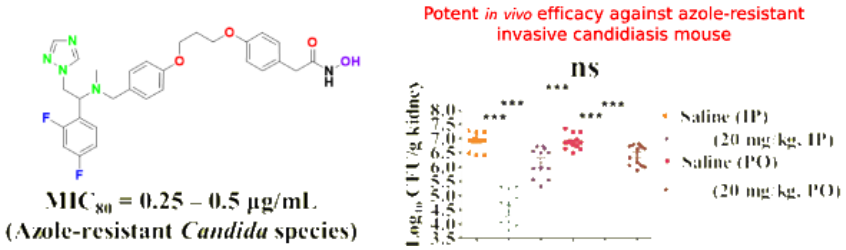
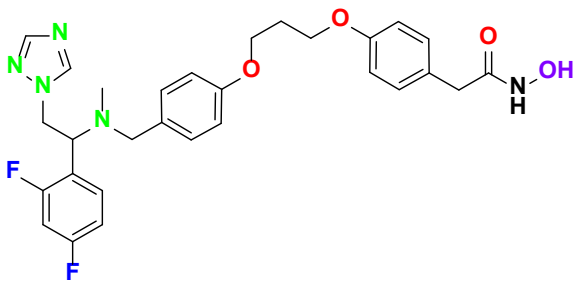


Figure 8. Lanosterol 14α-Demethylase (CYP51)–Histone Deacetylase (HDAC) Dual Targeting Strategy: Integrated Disruption of Ergosterol Biosynthesis and Epigenetic Regulation in *C. albicans*.



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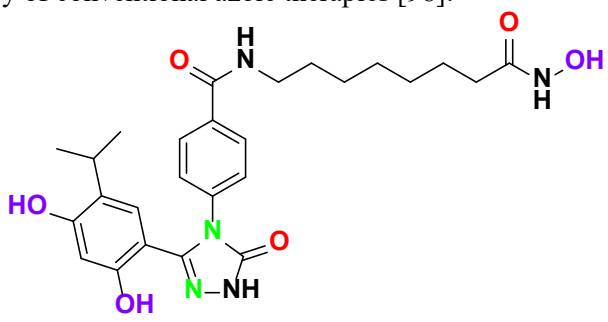
This dual mode of action disrupts membrane lipid integrity by blocking ergosterol synthesis while simultaneously suppressing resistance-associated genes at the epigenetic level, thereby reversing acquired azole resistance. Dual inhibition of CYP51 and HDAC disrupts ergosterol-dependent

membrane integrity while simultaneously suppressing biofilm maturation through epigenetic impairment of hypha-associated gene expression programs. This coordinated metabolic and chromatin-level interference effectively limits the yeast–hypha transition and prevents the establishment of structurally stable, drug-tolerant biofilm communities, leading to a marked attenuation of fungal virulence. Consistent with these *in vitro* effects, both compounds significantly reduce fungal burden in the kidneys and prolonged survival in murine infection models, underscoring the translational therapeutic potential of this dual target strategy [97].

5.3. Heat Shock Protein 90 (HSP90) – Histone Deacetylase (HDAC)

A novel therapeutic strategy targeting azole-resistant *C. albicans* infections was proposed by Li et al. (2022), who focused on the simultaneous inhibition of HSP90 and HDAC enzymes (Figure 9). In their study, newly developed carbazole-based dual inhibitors were shown to exert synergistic antifungal activity against resistant *C. albicans* strains by concurrently disrupting cellular stress-response pathways and epigenetic regulatory circuits. Among these, compound 4 demonstrated potent dual inhibition of HSP90 and HDAC, leading to marked downregulation of resistance-associated genes such as *ERG11* and *CDR1* and significantly enhancing the fungicidal activity of fluconazole. Targeting the HSP90–HDAC axis attenuates biofilm-associated virulence by destabilizing stress-response signaling pathways that sustain hyphal maintenance and biofilm persistence under antifungal pressure. This dual inhibition disrupts adaptive tolerance mechanisms required for biofilm maintenance and dispersal, thereby effectively suppressing the yeast-to-hypha transition and biofilm development while reducing both virulence and drug tolerance. Collectively, these findings position HSP90–HDAC dual targeting as a mechanistically robust antifungal strategy capable of dismantling multidimensional resistance networks and enhancing the efficacy of conventional azole therapies [98].



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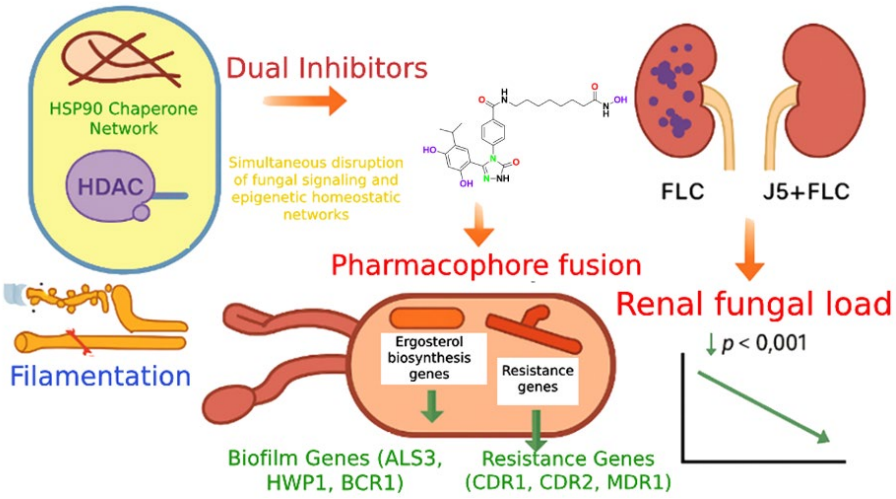


Figure 9. Heat Shock Protein 90 (HSP90)–Histone Deacetylase (HDAC) Dual Targeting Strategy: Disruption of Stress Adaptation and Epigenetic Control in *C. albicans.*

5.4. Heat Shock Protein 90 (HSP90) – Lanosterol 14α-Demethylase (CYP51)

A novel therapeutic paradigm targeting antifungal drug resistance was established by Zhang *et al.* (2025), who introduced a dual-target strategy based on the simultaneous inhibition of CYP51 and HSP90 (Figure 10). Their study demonstrated that the classical single-target action of azole antifungals on CYP51 becomes insufficient over time due to resistance-associated ERG11 mutations, compensatory upregulation of efflux pumps, and dose-limiting toxicity. Importantly, the authors showed that HSP90 functions as a "molecular buffer" that stabilizes stress-response pathways governing fungal survival, morphogenetic switching, and biofilm maturation, thereby supporting the persistence of azole resistance. Consequently, pharmacological disruption of HSP90 was identified as a critical intervention point to dismantle compensatory mechanisms that protect fungal cells from CYP51 inhibition.
Guided by this rationale, Zhang *et al.* designed a series of dual CYP51/HSP90 inhibitors capable of suppressing both ergosterol biosynthesis and HSP90-dependent virulence networks within a single molecular framework. Among these, compound 5 emerged as the most potent candidate, exhibiting markedly superior antifungal activity compared with conventional azoles while maintaining low cytotoxicity. Mechanistic assays revealed that compound 5 simultaneously impaired ergosterol production, inhibited hyphal morphogenesis, and abolished biofilm development. Furthermore, *in vivo* studies demonstrated a significant reduction in renal fungal burden, confirming the therapeutic promise of dual CYP51–HSP90 inhibition for treating drug-resistant *C. albicans* infections.

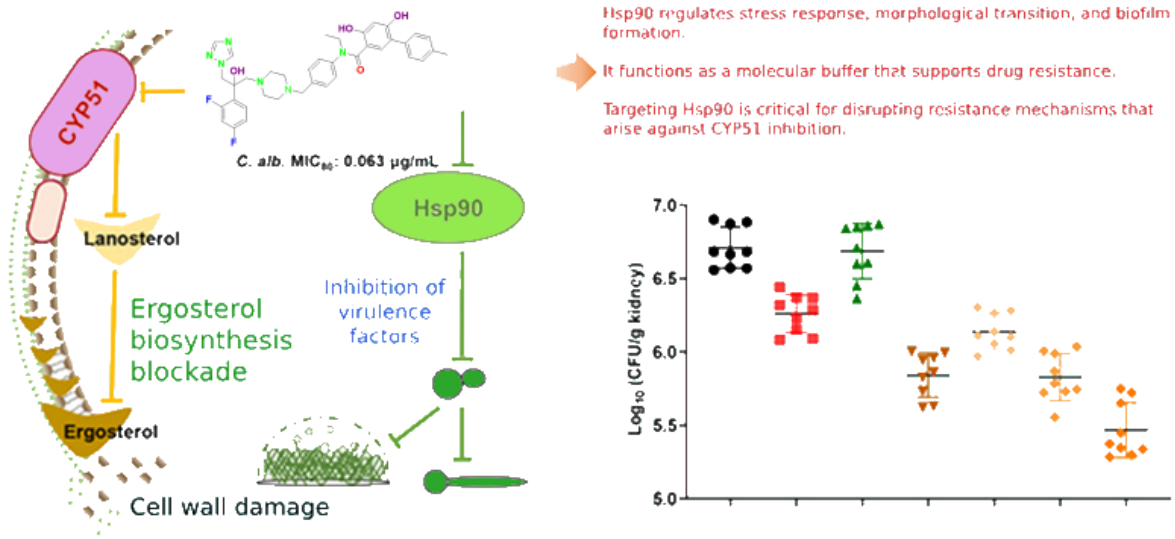
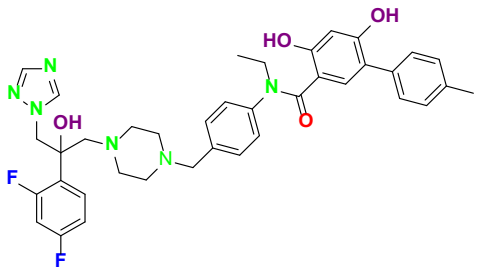


Figure 10. Heat Shock Protein 90 (HSP90)–Lanosterol 14α-Demethylase (CYP51) Dual Targeting Strategy: Coupled Disruption of Stress Adaptation and Ergosterol Biosynthesis in *C. albicans.*Adapted from [34].



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These findings demonstrate that dual-target antifungal strategies can enhance therapeutic outcomes in difficult-to-treat infections such as azole-resistant candidiasis by enabling multi-pathway inhibition through a single molecule. By simultaneously interfering with ergosterol biosynthesis and stress-response signaling, such approaches offer a mechanistically grounded means of attenuating virulence, limiting adaptive tolerance, and improving antifungal efficacy in settings where monotherapies often fail [34].

Taken together, these findings underscore not only the central role of HSP90 in fungal pathogenesis and adaptive stress tolerance but also highlight the CYP51–HSP90 axis as a particularly compelling platform for next-generation antifungal drug discovery. By coupling disruption of membrane homeostasis with destabilization of stress-response networks, this dual-target paradigm provides a rational strategy to overcome resistance while enhancing the durability and translational potential of antifungal therapies.

6. The Era of Multi-Target Antifungal Therapeutics in the Treatment of *Candida* Infections

The therapeutic paradigm for antifungal treatment has undergone a profound transition as invasive *Candida* infections rise globally and multidrug resistance becomes increasingly pervasive. Traditional monospesific antifungals, which primarily inhibit a single biological target (such as CYP51 or SE in ergosterol biosynthesis, β-(1,3)-D-glucan synthase in cell wall formation, or DNA/RNA polymerases in nucleic acid synthesis) are often insufficient to suppress the compensatory stress responses and epigenetic reprogramming that enable fungal survival under drug pressure. This limitation has catalyzed the emergence of multi-target antifungal strategies designed to overcome the adaptive robustness of fungal pathogens [99,100].

Next-generation multi-target approaches aim to enhance pharmacological efficacy and suppress resistance by simultaneously modulating multiple essential biological networks within fungal cells. Mechanistically, this encompasses concurrent interference with ergosterol biosynthesis (CYP51 and SE), epigenetic regulation (HDACs, HATs, and bromodomain-containing proteins), protein folding and stress signaling (the HSP90 chaperone complex), and host–pathogen communication (EGFR signaling). Coordinated inhibition of these nodes disrupts membrane fluidity, genomic stability, metabolic adaptability, and host–cell interaction thereby incapacitating the pathogen at multiple cellular and molecular levels.

Dual inhibition of the CYP51–SE axis exemplifies the power of this paradigm: blocking both early and late steps of ergosterol synthesis not only prevents azole-induced resistance but also triggers the toxic accumulation of sterol intermediates, resulting in irreversible membrane destabilization. In parallel, inhibition of stress-response and epigenetic regulators such as HDACs and HSP90 suppresses the transcriptional programs that drive drug tolerance, particularly the upregulation of ERG11 and efflux pump genes (CDR1, CDR2, MDR1). The synergistic blockade of HSP90–HDAC disrupts both stresses signaling coherence and epigenetic compensation, restoring fungicidal activity of azoles, echinocandins, and polyenes even against highly resistant isolates [34,95-98].

On the host side, the EGFR–HSP90–HDAC axis introduces an additional therapeutic dimension by targeting the molecular interface of host invasion. *C. albicans* invasin proteins (Als3, Hyr1, Ssa1) manipulate host EGFR signaling to facilitate epithelial penetration, while HSP90 stabilization and HDAC-driven chromatin remodeling maintain the fungal virulence and stress-response state needed for persistence. Thus, pharmacological inhibition of EGFR not only impairs epithelial invasion but also attenuates the fungus's ability to sustain its signal-dependent adaptive programs. This multi-layered perspective reframes antifungal therapy as a bidirectional intervention aimed at both the pathogen and the host–pathogen signaling interface [28,29,57,98].

The therapeutic superiority of multi-target strategies derives from their network-synergy principle: the simultaneous blockade of multiple pathways increases antifungal potency in a multiplicative manner while reducing the required drug dose, thereby lowering both host toxicity and pharmacokinetic burden. Clinically, this approach yields robust activity against *C. albicans*, *C. glabrata, C. krusei*, and particularly the highly resistant *C. auris*. Importantly, the multi-target paradigm not only

accelerates the discovery of new chemical entities but also expands the potential for drug repurposing. HDAC and HSP90 inhibitors originally developed for oncology are now being re-evaluated for antifungal activity, while azoles are being combined with epigenetic modulators or host-receptor antagonists to generate potent synergistic effects [30,45,50,101].

The dual-target antifungal paradigm offers a systems-level strategy for destabilizing fungal pathogenicity by selectively co-modulating key regulatory nodes rather than isolated molecular targets. Coordinated interference with signaling and metabolic hubs such as EGFR, HDAC, HSP90, CYP51, and SE disrupts the integration of stress adaptation, epigenetic responsiveness, and sterol homeostasis that underpins fungal persistence. By dismantling the crosstalk between these axes, dual-target interventions restrict compensatory network rewiring and attenuate the post-transcriptional flexibility required for long-term survival under antifungal pressure. Importantly, this approach reframes antifungal intervention as a targeted perturbation of host–pathogen signaling interfaces and regulatory plasticity, highlighting the value of network-guided design principles for next-generation antifungal development [34,95-98].

7. Conclusion

The increasing ecological adaptability, thermal tolerance, and multilayered resistance strategies of fungal pathogens have exposed the fundamental limitations of current antifungal paradigms. Most conventional agents (including azoles, echinocandins, polyenes, and pyrimidine analogues) target a narrow spectrum of molecular pathways, making rapid and stable resistance development inevitable, particularly under CYP51-centered selective pressure. As a result, invasive fungal infections have become a critical global health challenge with escalating clinical and economic burden.

The evidence summarized in this review demonstrates that multi-target antifungal strategies (especially dual-target approaches) hold significant therapeutic potential by simultaneously modulating the interconnected biological networks that define fungal fitness. The HSP90–calcineurin stress response, HDAC-mediated epigenetic reprogramming, sterol metabolism governed by the CYP51–SE axis, and EGFR–MAPK-driven host invasion signaling collectively constitute the four major pillars sustaining fungal pathogenicity. Concurrent perturbation of these axes reinforces pharmacodynamic synergy while substantially weakening the evolutionary stability of adaptive resistance programs.

Unlike conventional combination therapy, dual-target inhibitors consolidate multi-pathway suppression within a single chemical framework, offering distinct advantages in terms of reduced toxicity, improved pharmacokinetic coherence, and minimized resistance emergence. Encouraging data from dual inhibitors targeting CYP51/HDAC, HSP90/HDAC, CYP51/SE, and CYP51/HSP90 axes underscore the translational value of this strategy and validate its potential for clinical advancement.

Overall, multi-target antifungal strategies that comprehensively disrupt the complex signaling, metabolic, and epigenetic networks of fungal pathogens signal a transformative paradigm shift in future antifungal therapy. These approaches promise to decelerate resistance evolution, deliver more selective and durable antifungal efficacy, and significantly enhance clinical treatment outcomes. Future research must focus on optimizing the chemical scaffolds of dual-target agents, integrating systems-biology–based target mapping, and expanding clinical adaptability assessments to translate these advances into effective therapeutic interventions.

Given the central role of fungal invasins such as Als3, Hyr1, and Ssa1 in coordinating epithelial penetration and endocytic signaling through the host EGFR/HER2 axis, the invasin–receptor interface emerges as an attractive and underexplored target space for advanced antifungal intervention. The development of dual-target architectures capable of simultaneously modulating fungal invasion machinery and host receptor–driven signaling represents a rational extension of antifungal strategies beyond classical cell wall or sterol-centered pathways into the domain of host–pathogen signal integration. In this context, ongoing and future efforts aimed at designing dual inhibitors that integrate suppression of invasin-mediated signaling with regulation of receptor tyrosine kinase activation may offer a promising framework for next-generation antifungal therapeutics grounded in infection biology. Such approaches have the potential to expand the therapeutic landscape by targeting invasion-associated signaling programs that are critical for fungal pathogenicity and persistence.

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