Exploring Quinoxaline Derivatives as Potential Antifungal Agents: A Comprehensive Study on Candida and Aspergillus Species Susceptibility

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ABSTRACT

The escalating prevalence of fungal diseases presents a significant global health concern, impacting millions of individuals annually. Fungal infections encompass a spectrum of conditions ranging from superficial to life-threatening systemic diseases, with Candida, Aspergillus, and Cryptococcus species posing substantial threats. Particularly, Candida albicans is a leading cause of invasive fungal infections, including candidiasis and candidemia, with significant mortality rates and economic burdens. Addressing the challenges posed by fungal infections requires innovative therapeutic approaches, especially in the face of emerging drug-resistant strains such as Candida auris. In this context, the antifungal potential of quinoxaline derivatives, exemplified by 2,3-dimethylquinoxaline (DMQ), has garnered attention for its efficacy against fungal infections and wound healing properties. Building on previous research, our study aims to explore the antifungal activity of various quinoxaline compounds against *Candida* and *Aspergillus* species. Notably, compounds such as 2-Methyldibenzo[f,h] quinoxaline and 4-Chloro-7-fluoropyrrolo[1,2-a]quinoxaline demonstrated significant activity against specific *Candida* strains, while 2-Chloro-6-(trifluoromethyl)quinoxaline and 2-Chloro-7-(trifluoromethyl)quinoxaline exhibited efficacy against multiple *Candida* species. However, none of the tested compounds showed activity against certain *Candida* and *Aspergillus* strains, highlighting the diverse responses of different fungal species to these compounds. The observed variations in antifungal activity can be attributed to differences in chemical structure, mode of action, and target specificity among the compounds. While some compounds effectively target fungal vulnerabilities, others may lack the necessary biochemical properties for interaction with fungal cells. In conclusion, our study underscores the need for continued research to develop effective antifungal therapies capable of combating a broad spectrum of fungal infections. By elucidating the molecular mechanisms underlying compound efficacy and exploring novel therapeutic strategies, we can address the evolving challenges posed by fungal diseases and improve patient outcomes.

Keywords: *Candida*, *Aspergillus,* Quinoxaline derivative, drug resistance

INTRODUCTION

The growing worldwide health issue in the realm of public health revolves around the burgeoning impact of fungal diseases on human well-being. Statistical estimations reveal that close to a billion individuals contend with fungal infections, impacting their skin, nails, and hair, with tens of millions confronting challenges posed by mucosal candidiasis. Furthermore, a staggering 150 million people grapple with severe fungal diseases, which have the potential to significantly alter or even terminate lives. Fungal infections, despite being frequently underestimated and overlooked, exert a profound and far-reaching influence on the overall quality of life for individuals on a global scale [1,2]. These afflictions span a spectrum ranging from superficial conditions affecting the skin and nails to serious systemic fungal infections that pose a significant threat to life [3]. The ramifications of fungal infections are particularly significant within the demographic of immunocompromised individuals, including those undergoing chemotherapy for cancer treatment or recipients of organ transplants. In this vulnerable population, fungal infections can escalate the frequency of illnesses and elevate mortality rates [4,5]. Fungi possess the capacity to exert a far-reaching impact on millions of individuals globally each year, contributing to an estimated annual mortality rate of around 1,350,000 [6].

Candida, *Aspergillus*, and *Cryptococcus*, among various genera, are primary culprits for invasive infections, notably *Candida* causing the most prevalent invasive fungal disease, candidiasis, in developed

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nations. Candidiasis is estimated to affect two to 14 individuals per 100,000, and candidemia, a Candida bloodstream infection, impacts over 250,000 people annually, resulting in 50,000 deaths globally. The economic toll of candidemia is considerable, involving an average hospitalization period of three to 13 days in the US, with associated costs ranging from $6,000 to $29,000 [6–9]. The rise and dissemination of resistant *Candida* isolates are becoming more prevalent. Notably, the emergence of the extensively drug-resistant *Candida auris* in recent years poses a substantial global threat to human health. This pathogen is accountable for infections resistant to all major antifungal drug classes, significantly affecting individuals with weakened immune systems [6,10].

Currently, addressing candidiasis poses dual challenges, the swift and accurate identification of the invading pathogen and the limited availability of treatment options. This necessitates comprehensive strategies to enhance diagnostic efficiency and broaden therapeutic alternatives [6]. Beyond concerns surrounding drug resistance, polyenes are recognized for their elevated toxicity and possess a narrow therapeutic window. The administration of both polyenes and echinocandins is constrained by limited oral bioavailability, confining their delivery to intravenous routes and compelling prolonged hospitalization. Additionally, extended-spectrum triazoles, like posaconazole, encounter obstacles associated with fluctuating bioavailability, immediate adverse effects, and the emergence of resistance, collectively constraining their efficacy [4,11,12].

In a precedent investigation led by Elfadil and collaborators, they established the antifungal properties of quinoxaline derivatives, particularly highlighting the effectiveness of 2,3-dimethylquinoxaline (DMQ) in combating fungal infections and promoting wound healing [13]. Inspired by these findings, our research aims to extend this exploration to other quinoxaline compounds. Specifically, we will evaluate the antifungal potential of compounds including 2-Methyldibenzo[f,h] quinoxaline, 4-Chloro-7-fluoropyrrolo[1,2-a]quinoxaline, 2-(Thiophen-2-yl)quinoxaline, Quinoxaline-2-carboxamide, Methyl quinoxaline-2-carboxylate, Quinoxaline, Quinoxaline-2,3-diamine, 2-Chloro-3-(trifluoromethyl)quinoxaline, and 2-Chloro-6-(trifluoromethyl)quinoxaline. These compounds will undergo rigorous *in vitro* assessments to determine their efficacy against various *Candida* and *Aspergillus* infections.

This study aims to assess the antifungal activity of 10 compounds including, (2-Methyldibenzo[f,h]quinoxaline) renamed as 669, (4-Chloro-7-fluoropyrrolo[1,2-a]quinoxaline)renamed as 802, (2-(Thiophen-2-yl)quinoxaline) renamed as 071, (Quinoxaline-2-carboxamide) renamed as 003, (Methyl quinoxaline-2-carboxylate) renamed as 647, (Quinoxaline) renamed as 557, (Quinoxaline-2,3-diamine), renamed as 843, (2-Chloro-7-(trifluoromethyl)quinoxaline) renamed as 279, (2-Chloro-3-(trifluoromethyl)quinoxaline) renamed as 780, (2-Chloro-6-(trifluoromethyl)quinoxaline) renamed as 872.

METHOD

Fungal strains and growth condition

Twenty Test organisms used in the study comprised either reference strains obtained from the American Type Culture Collection (ATCC; Manassas, VA) or clinical isolates sourced from the Micromyx collection (MMX, Kalamazoo, MI). Upon arrival at Micromyx, the isolates were streaked onto agar medium suitable for each organism and were then incubated at 35ºC. Colonies obtained from these growth plates were suspended in an appropriate medium containing a cryoprotectant. Portions of each suspension were subsequently frozen at -80ºC. Before testing, yeast isolates were streaked from frozen vials onto Sabouraud Dextrose Agar (Becton Dickenson BD/BBL; Sparks, MD; Lot No. 123819) and were incubated at 35°C for 24 hours. Fungi, on the other hand, were streaked from frozen vials onto potato dextrose agar (Becton Dickenson BD/BBL; Lot No. 9311217) and were incubated at 37°C for 2 weeks. Yeast and fungi underwent testing in RPMI-1640 medium (Hyclone Laboratories; Logan, UT; Lot No. AC29613901A) buffered with MOPS (Millipore; Billerica, MA; Lot No. 3706650).

Antimicrobial agents

Ten antimicrobial compounds sourced from AmBeed, USA were stored under appropriate conditions until they were ready to be tested. Comparator drugs were obtained from Micromyx. DMSO from Sigma (St. Louis, MO; Lot No. SHBJ1785) served as the solvent and diluent for all the drugs. Each compound was prepared as a stock solution at 101 times the final testing concentration. These stock solutions were allowed to stand for at least 1 hour before use to ensure auto-sterilization. Any remaining stock solutions were stored at -80°C. The suppliers, catalog/lot numbers, solvents, and testing ranges for each compound were recorded as follows.

Broth microdilution MIC procedure

MIC values were determined using a broth microdilution technique outlined by CLSI guidelines [14]. Automated liquid handlers (Multidrop 384, Helsinski; Biomek 2000 and Biomek FX, Beckman Coulter, Fullerton CA) facilitated serial dilutions and liquid transfers. In a standard 96-well microdilution plate, columns 2 through 12 were filled with 150 µL of the appropriate diluent, while column 1 received 300 µL of the tested agents at 101X the highest final concentration. Serial two-fold dilutions were made across rows through column 11 using the Biomek 2000. Column 12 served as the growth control wells. Daughter plates were prepared by loading each well with 190 µL of media using the Multidrop 384. The Biomek FX then transferred 2 µL of drug solution from each well of the mother plate to corresponding wells of the daughter plate in a single step. A standardized inoculum of each test organism, prepared per CLSI methods, was added to the daughter plates using the Biomek 2000. Final concentrations ranged from approximately 0.5 to 2.5 x 10^3 CFU/mL for yeast and 0.2 to 2.5 x 10^4 CFU/mL for filamentous fungi. The plates were incubated aerobically at 35ºC for 24 and 48 hours. For yeast, MIC values based on complete inhibition and 50% inhibition were recorded at both time points, while for filamentous fungi, MIC values were recorded at 24 hours based on least concentration can inhibit microorganism growth. This experiment was performed in triplicate and the average was calculated [15].

RESULT

The effectiveness of various compounds against fungal infections was evaluated using the broth microdilution assay. Our investigation revealed notable differences in the effectiveness of these compounds. Specifically, compounds 669 and 802 displayed good activity against *Candida glabrata* MMX 7285 (not 802) and *Candida krusei* ATCC 6258.

Interestingly, compound 872 and 279 exhibited good efficacy against *Candida glabrata* MMX 7285, *Candida tropicalis* MMX 7525, and *Candida glabrata* ATCC 90030.

Despite their effectiveness against certain specific *Candida* strains mentioned earlier, none of these compounds exhibited any activity against several other *Candida* species, including *Candida albicans* ATCC 90028, *Candida albicans* ATCC MYA-573, *Candida albicans* MMX 7424, *Candida tropicalis* ATCC 90874, *Candida auris* MMX 9867, and *Candida parapsilosis* ATCC 22019. Moreover, they displayed no activity against various strains of *Aspergillus* species, including *Aspergillus fumigatus* ATCC MYA-3626, *Aspergillus fumigatus* ATCC 204305, *Aspergillus fumigatus* ATCC MYA-4609, *Aspergillus fumigatus* ATCC 32820, *Aspergillus niger* ATCC 9508, *Aspergillus niger* MMX 5953, *Aspergillus flavus* ATCC 22546, *Aspergillus flavus* ATCC 64025, *Aspergillus brasiliensis* ATCC 16404, and *Aspergillus terreus* ATCC 3628. In contrast, it was observed that compounds numbered 071, 003, 647, 557, 843, and 780 exhibited no discernible activity against the fungal infections tested in our study. Despite our thorough evaluation, these particular compounds did not demonstrate any efficacy in inhibiting the growth or proliferation of the tested fungal strains. This indicates a lack of antifungal potential for these specific compounds against the fungal species examined in our experimental setup. Furthermore, they showed no activity against various strains of *Aspergillus* species, including *Aspergillus fumigatus* ATCC MYA-3626, *Aspergillus fumigatus* ATCC 204305, *Aspergillus fumigatus* ATCC MYA-4609, *Aspergillus fumigatus* ATCC 32820, *Aspergillus niger* ATCC 9508, *Aspergillus niger* MMX 5953, *Aspergillus flavus* ATCC 22546, *Aspergillus flavus* ATCC 64025, *Aspergillus brasiliensis* ATCC 16404, and *Aspergillus terreus* ATCC 3628 (Table 1).

This comprehensive assessment highlights the varying susceptibilities of different fungal species to these compounds. This variation underscores the diverse responses of different fungal strains to these compounds.

DISCUSSION

For the first time, we showed the effectiveness of numerous compounds against fungal infections using the broth microdilution assay, revealing significant variations in their efficacy. Compounds of 2-Methyldibenzo[f,h]quinoxaline and 4-Chloro-7-fluoropyrrolo[1,2-a] quinoxaline exhibited notable activity against *Candida glabrata* MMX 7285 and *Candida krusei* ATCC 6258, while compounds 2-Chloro-6-(trifluoromethyl)quinoxaline) and 2-Chloro-7-(trifluoromethyl) quinoxaline) demonstrated efficacy against *Candida glabrata* MMX 7285, *Candida tropicalis* MMX 7525, and *Candida glabrata* ATCC 90030. However, none of these compounds displayed activity against *Candida albicans* ATCC 90028, *Candida albicans* ATCC MYA-573, *Candida albicans* MMX 7424, *Candida tropicalis* ATCC 90874, *Candida auris* MMX 9867, *Candida parapsilosis* ATCC 22019, or various strains of *Aspergillus* species.

Conversely, compounds 2-(Thiophen-2-yl)quinoxaline), (Quinoxaline-2-carboxamide), (Methyl quinoxaline-2-carboxylate), (Quinoxaline), (Quinoxaline-2,3-diamine), and (2-Chloro-3-(trifluoromethyl) quinoxaline), showed no activity against the tested fungal infections, indicating a lack of antifungal potential against the examined fungal species. These compounds also exhibited no efficacy against multiple strains of *Candida* or *Aspergillus* species. This thorough evaluation underscores the varying susceptibilities of different fungal species to the tested compounds, highlighting the diverse responses of different fungal strains. This comprehensive assessment provides valuable insights into the antifungal properties of these compounds and their potential applications in fungal infection management.

The observed disparity in antifungal activity among the tested compounds can be attributed to several factors, including differences

Table 1. Minimum inhibitory concentration (MIC) in microgram per ml of the 10 compounds against tested organisms.

Isolate	Time (Hours)	100%percent inhibition	669	802	071	003	647	557	843	279	780	872
***Candida albicans* ATCC 90028**	24	100	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
***Candida albicans* ATCC MYA573**	24	100	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
***Candida albicans* MMX 7424**	24	100	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
***Candida tropicalis* ATCC 90874**	24	100	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
***Candida tropicalis* MMX 7525**	24	100	>64	>64	>64	>64	>64	>64	>64	32	>64	32
***Candida glabrata* ATCC 90030**	24	100	>64	>64	>64	>64	>64	>64	>64	32	>64	32
***Candida glabrata* MMX 7285**	24	100	32	>64	>64	>64	>64	>64	>64	32	>64	32
***Candida parapsilosis* ATCC 22019**	24	100	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
***Candida krusei* ATCC 6258**	24	100	16	8	>64	64	>64	>64	>64	64	>64	>64
***Candida auris* MMX 9867**	24	100	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
***Aspergillus fumigatus* ATCC MYA3626**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus fumigatus* ATCC 204305**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus fumigatus* ATCC MYA4609**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus fumigatus* ATCC 32820**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus niger* ATCC 9508**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus niger* MMX 5953**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus flavus* ATCC 22546**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus flavus* ATCC 64025**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus brasiliensis* ATCC 16404**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64
***Aspergillus terreus* ATCC 3628**	24	100	>16	>32	>32	>64	>64	>64	>64	>64	>64	>64

in chemical structure, mode of action, and target specificity [16]. Compounds 669, 802, and 071 demonstrated significant antifungal activity against specific Candida strains, likely due to their structural features that enable effective interaction with fungal targets or cellular components essential for growth and survival. These compounds may possess molecular characteristics that facilitate their penetration into fungal cells, interference with vital cellular processes, or inhibition of key enzymes involved in fungal metabolism or cell wall synthesis [17].

On the other hand, compounds such as 003, 647, 557, 843, and 780 exhibited no discernible antifungal activity against the tested fungal infections. This lack of efficacy could be attributed to various factors, including inadequate chemical properties for effective fungal targeting, insufficient potency against fungal targets, or poor solubility or bioavailability, which may hinder their ability to exert antifungal effects in vitro. Various antifungal approaches rely predominantly on small molecule antifungal drugs. Nonetheless, these drugs face constraints due to inadequate solubility and bioavailability [18]. Additionally, these compounds may lack specificity for fungal targets, leading to non-selective interactions or ineffective inhibition of essential fungal processes. Research has shown that rifampin, an antibacterial agent, does not exhibit activity against fungal infections [19].

Furthermore, differences in the genetic makeup or physiological characteristics of the fungal strains tested may also contribute to the variable response to the tested compounds. Research has demonstrated that fungi capable of melanin production, such as Aspergillus niger and Cladosporium herbarum, exhibit a wide range of functions attributed to this pigment. Melanins, found in these fungi, contain enduring free radical centers that enable them to function as antioxidants, scavenging free radicals and shielding the fungi from oxidative stress and damage caused by ionizing radiation in various environments. Additionally, melanin serves as a crucial virulence factor and contributes to drug resistance [20], which may partially elucidate why certain fungal organisms withstand the action of antifungal agents.

Fungal species and strains exhibit inherent variations in drug susceptibility profiles, reflecting differences in their virulence factors, drug efflux mechanisms, or mutations in drug target sites. Consequently, compounds that effectively target specific fungal vulnerabilities or exploit particular cellular pathways may demonstrate potent antifungal activity against susceptible strains but fail to exert significant effects against resistant or less susceptible strains [21]. It has been shown that two noteworthy compounds exhibiting antifungal properties warrant discussion in the context of chitin synthetase inhibitors. Firstly, FR-900403, characterized as a peptide-nucleoside antibiotic, differs from the polyoxin-nikkomycin complex by featuring adenosine as the nucleoside and a peptide linked to the nucleoside via the C-3' residue [22]. This compound demonstrates activity against Candida albicans but lacks efficacy against filamentous fungi. Secondly, FR-900848 is a nucleoside-fatty acid compound with uridine linked to a monounsaturated fatty acid containing five cyclopropane rings [23]. Notably, FR-900848 exhibits antifungal activity against filamentous fungi but shows no efficacy against yeasts [17 24].

On the other hand, compounds that showed no activity may lack the necessary structural features or biochemical properties required for effective interaction with fungal cells. They may also target non-essential fungal components or pathways, rendering them ineffective against fungal growth or proliferation [25]. Despite the lack of activity observed for some compounds, it is important to explore alternative strategies to enhance their efficacy. Combination therapy involving two or more compounds with complementary mechanisms of action could potentially overcome fungal resistance mechanisms and improve overall efficacy. By combining compounds that target different fungal cellular components or pathways, synergistic interactions may be achieved, leading to enhanced antifungal activity. Although rifampin, an antibacterial RNA polymerase inhibitor, lacks intrinsic activity against fungi, it has demonstrated notable effectiveness against several fungal species when paired with amphotericin B [19]. Therefore, we hypothesize that an agent with no antifungal activity, along with other active agents that may show efficacy against the tested organisms, could potentially synergize to enhance antifungal activity. Therefore, further studies are warranted to investigate the efficacy of combining different compounds and assess their potential synergistic effects against fungal infections. This approach may offer new insights into developing more effective antifungal therapies and combating fungal resistance.

Overall, the observed variation in antifungal activity highlights the complexity of fungal-host interactions and the challenges inherent in developing effective antifungal agents. Further research is needed to elucidate the molecular mechanisms underlying the observed differences in compound efficacy and to identify novel targets for antifungal drug development, ultimately paving the way for the discovery of more potent and broad-spectrum antifungal therapies.

CONCLUSION

Overall, our findings demonstrate the varying susceptibilities of different fungal species to the tested compounds. While some compounds exhibited promising activity against specific Candida strains, others showed limited or no efficacy against both Candida and Aspergillus species. These results emphasize the importance of continued research to identify and develop effective antifungal agents capable of combating a broad spectrum of fungal infections.

Abbreviation

MIC, Minimum inhibitory concentration, (2-Methyldibenzo[f,h] quinoxaline), 669, (4-Chloro-7-fluoropyrrolo[1,2-a]quinoxaline), 802, (2-(Thiophen-2-yl), 071, (Quinoxaline-2-carboxamide), 003, (Methyl quinoxaline-2-carboxylate), 647, (Quinoxaline), 557, (Quinoxaline-2,3-diamine), 843, (2-Chloro-7-(trifluoromethyl)quinoxaline) 279, (2-Chloro-3-(trifluoromethyl)quinoxaline) 780, (2-Chloro-6-(trifluoromethyl)quinoxaline), 872.

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