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ORIGINAL ARTICLE

Investigation of the antifungal activity of panobinostat, tamoxifen, and miltefosine alone and in combination with some conventional antifungal drugs against fluconazole-resistant *Candida* species

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ABSTRACT

Background and Objectives: The increasing incidence of antifungal-resistant *Candida* infections, particularly among cancer patients, emphasizes the urgency of exploring alternative therapeutic strategies. This study aimed to assess the in vitro antifungal efficacy of three anticancer agents—tamoxifen, panobinostat, and miltefosine—both individually and in combination with the antifungals fluconazole and itraconazole, against fluconazole-resistant *Candida* strains.

Materials and Methods: A total of 21 clinical *Candida* isolates (*C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. auris*) were evaluated. Antifungal susceptibility testing was conducted following the microdilution protocol outlined by CLSI.

Results: The combination of panobinostat with fluconazole exhibited full synergistic activity against *C. albicans* and *C. tropicalis*. Conversely, antagonistic effects were observed with *C. parapsilosis* and *C. glabrata*, while *C. auris* displayed an indifferent response. Panobinostat paired with itraconazole showed synergy exclusively against *C. albicans*. Similarly, miltefosine combined with itraconazole demonstrated synergism with *C. albicans*, but no interaction was found with fluconazole. Tamoxifen in conjunction with itraconazole revealed a synergistic response against *C. albicans*, antagonism with *C. tropicalis*, and indifference with other species.

Conclusion: Certain combinations of antifungals and anticancer agents could potentiate antifungal activity against resistant *Candida* isolates. Therefore, precise species-level identification is vital for tailoring effective combination therapies, particularly in immunocompromised individuals.

Keywords: Panobinostat; Tamoxifen; Miltefosine; Fluconazole; Itraconazole; *Candida*

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INTRODUCTION

Over recent years, the frequency of candidiasis has risen markedly, primarily due to the increasing population of immunocompromised individuals. Although *Candida albicans* remains the leading causative species, non-*albicans Candida*—including *C. parapsilosis, C. glabrata, C. tropicalis,* and *C. krusei*—are being reported with growing regularity as significant pathogens (1, 2).

Currently, the main antifungal agents available for candidiasis treatment fall into three categories: polyenes, azoles, and echinocandins. However, amphotericin B, a widely used polyene, is associated with considerable toxicity, restricting its broader application (3). Compounding the problem, resistance to azoles and echinocandins is becoming increasingly common, particularly among certain non-*albicans* species that exhibit intrinsic resistance to azoles (4).

The identification of *Candida auris*—a recently emerged species notable for its resistance to multiple antifungal classes—has further intensified therapeutic concerns on a global scale (5). These issues highlight the need to explore new antifungal strategies, including drug repurposing and combination therapies that may enhance efficacy while mitigating resistance development.

Nevertheless, not all drug combinations are beneficial. Some, such as azoles combined with polyenes, may result in antagonistic interactions (6). Consequently, recent studies have investigated the synergistic potential of combining fluconazole with non-antifungal compounds (7, 8). Among such compounds are tamoxifen (9), panobinostat (10), and miltefosine (11), which are frequently administered to cancer patients and have shown promise in enhancing antifungal effects when used in combination.

Considering that cancer patients are particularly vulnerable to fungal infections due to their immunosuppressed status, this study aims to evaluate the in vitro interactions of tamoxifen, panobinostat, and miltefosine with azole antifungals—fluconazole and itraconazole—against fluconazole-resistant clinical isolates of *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. auris.* Given the limited existing data on such combinations, the findings may inform future research and clinical strategies for managing invasive candidiasis in immunocompromised populations.

MATERIALS AND METHODS

Microorganisms. In this study, fluconazole-resistant *Candida* species were isolated from clinical specimens collected from patients diagnosed with candidiasis. The samples included blood cultures, biopsy tissues, and bronchoalveolar lavage fluids. All isolates originated from the biobank of the Tehran Medical Mycology Laboratory (TMML), Tehran, Iran. The isolates represented several *Candida* species: *C. parapsilosis* (n=6), *C. albicans* (n=5), *C. glabrata* (n=5), *C. tropicalis* (n=4), and *C. auris* (n=1). Species identification had been previously performed using a multiplex 21-plex PCR assay and internal transcribed spacer (ITS) region sequencing (12). Due to the clinical relevance of fluconazole resistance, only isolates resistant to this azole antifungal were included in the experiments.

Antifungal susceptibility testing (AFST). The in vitro antifungal susceptibility testing was conducted following the Clinical and Laboratory Standards Institute (CLSI) guideline M27-A3, which outlines broth microdilution methods for yeast (13). AFST included fluconazole (128-0.125 µg/mL), itraconazole (64-0.063 µg/mL), tamoxifen (256-0.5 µg/mL), panobinostat (512-1µg/mL), and miltefosine (256-0.5 µg/mL), all procured from Sigma-Aldrich (St. Louis, MO, USA).

Reference strains of *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were utilized for the purposes of quality control (13). These strains have established MIC ranges for standard antifungal drugs (like fluconazole and itraconazole). If the test results for these strains fall within the accepted MIC range, we can trust the results for our clinical isolates. Tests were performed in triplicate to confirm reproducibility.

In vitro combination testing using the checkerboard method. Synergistic effects between anticancer agents (tamoxifen, panobinostat, miltefosine) and azole antifungals (fluconazole, itraconazole) were assessed using the checkerboard microdilution technique based on CLSI protocols (14). The assay was performed in 96-well microplates (Suzhou Conrem Biomedical Technology Co., China).

Concentration ranges for each drug were selected according to MIC values determined for the individual isolates. In the assay setup, 50 µL of each con-

centration of the anticancer drugs was added across columns 1 to 10, and 50 µL of azoles was dispensed along rows A to G. Row H contained azoles alone, while column 11 contained anticancer drugs alone. In addition, column 12 was used as the drug-free growth control. It is a well that contains no antifungal or anticancer drug—only the growth medium and the inoculum (yeast cells). This control represents 100% growth. Each well was inoculated with 100 µL of a standardized yeast suspension, prepared from fresh colonies and adjusted to 1-3 × 10^3 CFU/mL based on optical transmittance at 530 nm set between 75-77%. Plates were incubated at 35°C for 24 hours before reading the results.

MIC endpoints were determined visually using a mirror reader and defined as the lowest drug concentration causing a ≥50% growth reduction relative to the growth control. To characterize the interaction type between drugs, the fractional inhibitory concentration index (FICI) was calculated as follows:

$$\text{FIC of drug A} = \frac{\text{MIC drug A when tested in combination with drug B}}{\text{MIC of drug A alone}}$$

$$\text{FIC of drug B} = \frac{\text{MIC drug B when tested in combination with drug A}}{\text{MIC of drug B alone}}$$

$$\mathbf{FIC = FIC_A + FIC_B}$$

The interaction was considered synergistic when the FICI was ≤ 0.5, indifferent when > 0.5 to ≤ 4.0, and antagonistic when > 4.0 (14). All experiments were performed independently at least three times.

RESULTS

Table 1 presents the results of antifungal activity testing of panobinostat, tamoxifen, and miltefosine, both individually and in combination with conventional antifungal agents, against fluconazole-resistant *Candida* species.

Based on the FICI interpretation, the most notable synergistic interactions were observed for panobinostat combined with fluconazole against *C. albicans* and *C. tropicalis* isolates (100% synergy, FICI ≤ 0.5). However, antagonistic effects were observed in *C. parapsilosis* and *C. glabrata* (100%, FICI > 4.0). In case of *C. auris*, the combination of panobinostat with fluconazole showed an indifferent interaction (FICI > 0.5 to ≤ 4.0).

Furthermore, the combination of itraconazole with panobinostat exhibited 100% synergistic effects against *C. albicans* (FICI ≤ 0.5). In other species, the interaction was indifferent (FICI > 0.5 to ≤ 4.0), except for *C. glabrata*, which showed 100% antagonism (FICI > 4.0).

In addition, the highest rate of synergy (100%) was observed for miltefosine combined with itraconazole against *C. albicans* isolates (FICI ≤ 0.5). In contrast, miltefosine showed no synergistic or antagonistic interaction with fluconazole in any of the tested isolates (FICI > 0.5 to ≤ 4.0).

Similarly, the combination of tamoxifen with itraconazole demonstrated 100% synergistic interaction against *C. albicans* isolates (FICI ≤ 0.5). Conversely, it showed antagonistic effects (100%) against *C. tropicalis* (FICI > 4.0), and indifferent interactions with *C. auris, C. glabrata*, and *C. parapsilosis* (FICI > 0.5 to ≤ 4.0).

DISCUSSION

The rising incidence of *Candida* infections, coupled with the emergence of both acquired and intrinsic resistance in certain non-*albicans* species to commonly used antifungal drugs, presents a significant clinical challenge. The recent global emergence of *Candida auris*, known for its cross-resistance to multiple antifungal classes, and the limited arsenal of approved antifungal medications—particularly for cancer patients facing antifungal-resistant *Candida* infections—underscore the urgent need for novel therapeutic approaches. Given that the discovery and development of new antifungal agents are often costly and time-intensive, current research increasingly focuses on uncovering synergistic effects between existing antifungals and other therapeutic agents.

This study evaluated the combined antifungal effects of three anticancer drugs—tamoxifen, panobinostat, and miltefosine—in combination with azole antifungals (fluconazole and itraconazole) against fluconazole-resistant *Candida* isolates. These particular anticancer drugs were selected due to their frequent use in oncology patients, who are notably vulnerable to candidiasis (9-11).

Our findings highlight the importance of species-specific identification for guiding combination therapy. For instance, tamoxifen paired with itraconazole exhibited synergistic activity against *C. albicans*, yet the same drug combination demonstrated

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Table 1. The results of antifungal activity testing of panobinostat, tamoxifen, and miltefosine alone and in combination with fluconazole and itraconazole against each tested fluconazole-resistant *Candida* species.

		MIC alone (µg/ml)					MIC in combination (µg/ml)						FICI/INTERPRETATION					
Strain Num.	**SPP.**	**FLC**	**ITC**	**TAM**	**MIL**	**PAN**	**FLC/TAM**	**FLC/MIL**	**FLC/PAN**	**ITC/TAM**	**ITC/MIL**	**ITC/PAN**	**FLC/TAM**	**FLC/MIL**	**FLC/PAN**	**ITC/TAM**	**ITC/MIL**	**ITC/PAN**
TMML 1290	*C. albicans*	128	0.5	128	2	64	16/64	16/1	16/4	0.125/8	0.125/0.25	0.125/8	0.625/IND	0.625/IND	0.187/SYN	0.3125/SYN	0.375/SYN	0.375/SYN
TMML 1291	*C. albicans*	128	64	32	4	256	16/0.5	0.5/2	1/ 64	4/ 2	8/ 1	0.5/16	0.503/IND	0.503/IND	0.257/SYN	0.125/SYN	0.375/SYN	0.063/SYN
TMML 1292	*C. albicans*	128	64	32	4	256	16/0.5	0.5/2	1/ 64	4/ 2	8/ 1	0.5/16	0.503/IND	0.503/IND	0.257/SYN	0.125/SYN	0.375/SYN	0.063/SYN
TMML 1293	*C. albicans*	128	64	128	8	256	16/64	16/4	1/ 64	4/32	8/2	0.5/16	0.625/IND	0.625/IND	0.257/SYN	0.312/SYN	0.325/SYN	0.063/SYN
TMML 1294	*C. albicans*	128	0.063	32	4	256	16/0.5	0.5/2	1/ 64	0.031/4	0.031/0.5	0.031/16	0.503/IND	0.503/IND	0.257/SYN	0.31/SYN	0.375/SYN	0.375/SYN
TMML 1296	*C. parapsilosis*	32	0.063	32	16	32	8/16	16/4	128/16	0.031/16	0.031/8	0.031/16	0.75/IND	0.75/IND	4.5/ANT	1/IND	1/IND	1/IND
TMML 1297	*C. parapsilosis*	16	0.25	64	4	32	8/32	8/1	64/16	0.125/32	0.125/2	0.125/16	1/IND	0.75/IND	4.5/ANT	1/IND	1/IND	1/IND
TMML 1298	*C. parapsilosis*	16	0.125	128	2	128	8/16	8/1	64/16	0.0625/64	0.0625/1	0.0625/64	0.625/IND	1/IND	4.125/ANT	1/IND	1/IND	1/IND
TMML 1299	*C. parapsilosis*	16	0.125	128	4	128	32/32	16/2	64/64	0.0625/64	0.0625/1	0.0625/64	2.25/IND	1.5/IND	4.5/ANT	1/IND	1/IND	1/IND
TMML 1300	*C. parapsilosis*	16	0.063	128	2	128	8/16	8/1	64/16	0.031/64	0.031/1	0.031/64	0.625/IND	1/IND	4.125/ANT	1/IND	1/IND	1/IND
TMML 1301	*C. parapsilosis*	32	0.063	128	0.5	8	8/64	16/0.5	16/32	0.031/64	0.031/0.25	0.031/64	0.75/IND	1.5/IND	4.5/ANT	1/IND	1/IND	1/IND
TMML 1305	*C. auris*	128	0.25	128	4	128	64/32	128/2	32/64	0.125/1	0.125/1	0.5/2	1/IND	1.5/IND	0.75/IND	0.507/IND	0.75/IND	2.015/IND
TMML 1307	*C. glabrata*	64	16	64	1	8	4/32	4/0.5	16/32	8/8	8/0.5	2/32	0.562/IND	0.5625/IND	4.25/ANT	0.625/IND	1/IND	4.125/ANT
TMML 1309	*C. glabrata*	64	16	32	8	8	1/16	16/8	4/32	8/8	0.5/4	1/32	0.5625/IND	1.5/IND	4.25/ANT	0.75/IND	0.531/IND	4.031/ANT
TMML 1310	*C. glabrata*	64	1	128	4	128	16/64	16/4	32/512	0.5/64	1/2	0.5/512	0.75/IND	1.25/IND	4.5/ANT	1/IND	1.5/IND	4.5/ANT
TMML 1311	*C. glabrata*	128	64	32	4	4	64/8	32/2	32/16	16/16	16/2	8/16	0.75/IND	0.75/IND	4.25/ANT	0.75/IND	0.75/IND	4.125/ANT
TMML 1312	*C. glabrata*	128	16	32	1	8	32/16	64/0.5	4/32	8/8	2/0.5	4/32	0.75/IND	1/IND	4.031/ANT	0.75/IND	0.625/IND	4.25/ANT
TMML 1313	*C. tropicalis*	8	0.25	128	32	128	2/64	2/16	0.5/16	0.125/512	0.5/16	0.5/64	0.75/IND	0.75/IND	0.127/SYN	4.5/ANT	2.05/IND	2.05/IND
TMML 1314	*C. tropicalis*	8	0.031	128	32	128	2/16	2/16	1/16	0.015/128	0.015/16	0.015/64	0.625/IND	1/IND	0.375/SYN	4.5/ANT	1/IND	1/IND
TMML 1315	*C. tropicalis*	128	1	16	32	128	32/8	32/16	8/16	0.5/64	0.5/16	0.5/64	0.75/IND	0.75/IND	0.187/SYN	4.5/ANT	1/IND	1/IND
TMML 1316		128	2	32	16	128	32/8	32/8	4/32	1/128	0.5/8	0.5/64	1/IND	0.75/IND	0.28/SYN	4.5/ANT	0.75/IND	0.75/IND

FLC: Fluconazole, ITC: Itraconazole, TAM: Tamoxifen, MIL:Miltefosine, PAN: Panobinostat, MIC: minimum inhibitory concentration, FICI: fractional inhibitory concentration index, SYN: synergistic, IND: indifferent, ANT: antagonistic.

antagonism when tested against *C. tropicalis.*

To date, there is limited research on the joint application of anticancer drugs and antifungals against fluconazole-resistant *Candida* species. Barreto et al. reported that miltefosine effectively inhibited *C. auris* in both planktonic and biofilm forms, with enhanced activity when combined with alginate nanoparticles (15). Contrarily, in our study, miltefosine did not show interaction with fluconazole or itraconazole against *C. auris* isolates.

In agreement with our results, Su et al. demonstrated that panobinostat combined with fluconazole exerted synergistic antifungal effects against fluconazole-resistant *C. albicans* strains (7). However, many studies emphasize that drug interactions can range from antagonistic to synergistic outcomes depending on drug concentrations and host factors (16, 17).

Similarly, Muthular et al. found that tamoxifen inhibited the growth of both fluconazole-sensitive and fluconazole-resistant *C. albicans* in vitro, which aligns with our observations (8).

Overall, this research provides initial evidence supporting the potential utility of these drug combinations against resistant *Candida* species. Since all drugs examined are already FDA-approved for other indications, these findings may facilitate further investigations and clinical trials, particularly in cancer patients who are simultaneously managing fungal infections.

Moreover, the rise of resistant non-*albicans* species with diverse susceptibility patterns highlights the limitations of traditional mycological methods for *Candida* identification. Precise species-level diagnosis and tailored combination therapy are essential to effectively address antifungal resistance.

Ultimately, the long-term goal of this work is to encourage large-scale clinical studies to evaluate the efficacy of combination therapies for resistant *Candida* infections in cancer patients, with the potential to influence treatment guidelines both nationally and internationally.

CONCLUSION

The study could provide evidence supporting the efficacy of antifungal and anticancer drug combinations against resistant *Candida* species. Given that the drugs under investigation are already approved, the results could serve as a basis for further advanced studies on the treatment of invasive *Candida* infections caused by resistant strains, particularly in cancer patients undergoing treatment with these medication.

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